# The effects of climate change on the growth and physiology of reef snappers

Thesis submitted by

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# Statement of the contributions of others

This thesis included collaborative work with my supervisors Dr. Jennifer Donelson and Prof. Philip Munday, as well as Dr. Darren Parsons. While undertaking these collaborations I was responsible for project design, data collection, analysis, and interpretation of my results. My co-authors provided intellectual guidance, editorial assistance, financial support, and technical assistance. Financial support was provided by the ARC Centre of Excellence for Coral Reef Studies (P. Munday and J. Donelson), New Zealand Institute of Water and Atmospheric Research (D. Parsons), GBRMPA Reef Guardians (S. McMahon), and The Australian Coral Reef Society (S. McMahon). During my degree I was supported by a James Cook University Postgraduate Research Scholarship.

# Declaration of Ethics

The research presented in this thesis was conducted in accordance with the national Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th Edition (2013) and the Queensland Animal Care and Protection Act (2001). The research was conducted under the animal ethics approval from the JCU Animal Ethic Committee (Ethics permit: A2482 and A2522)

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# General abstract

Anthropogenic emissions of carbon dioxide ($CO_2$) are causing the global climate to change. In the ocean, the average sea surface temperature has increasing by ~0.5°C since the 1950s and is projected to increase 2-4°C by the end of the century. Additionally, marine heatwaves (MHWs), while not a new phenomenon, have been occurring more frequently and intensely, and are predicted to become even more widespread and extreme as climate change advances. Increasing atmospheric $CO_2$ is also affecting the chemistry of the world's oceans in the form of ocean acidification. Since the industrial revolution, the uptake of additional $CO_2$ from the atmosphere has caused the average pH of the ocean to decrease by 0.1 pH units and is predicted to decrease a further by 0.3-0.4 units by the end of the century. These rapid changes in $CO_2$ and pH, and the increased frequency and intensity of MHWs pose a significant threat to marine life.

The early life stages of marine organisms are especially sensitive to environmental change because they typically have high energetic demands while simultaneously having low energetic reserves. Small increases in water temperature have a range of significant effects on larval fishes, including changes in growth rate and mortality. Similarly, increased environmental $CO_2$ levels are thought to increase the energy required to maintain pH homeostasis, which could affect the energy available for other important physiological and life history functions. Most marine fish are broadcast spawners, but the difficulty of breeding and rearing the early life stages of these species in the laboratory has limited experimental research into the effects of elevated temperature and $CO_2$ levels on their populations. A key trophic group of fishes on coral and rocky reefs are the intermediate-sized mesopredators, which prey on smaller fish, but are themselves prey to larger

predatory fishes. The majority of mesopredatory reef fishes are broadcast spawners, which has limited experimental studies into the effects of climate change impacts on their early life stages, due to inadequate knowledge and capacity for completing the life cycle in captivity. This thesis set out to investigate how predicted future climate change scenarios affect the growth and physiology of reef mesopredators, primarily focusing on the early life stages.

Two species were used to investigate the questions in this thesis. Firstly, a temperate reef mesopredator, Australasian snapper (*Chrysophrys auratus),* was used in ***Chapters 2 and 3*** to investigate the effects of temperature and $CO_2$ on the larval and juvenile stages of a mesopredator. Being a commercially important species the husbandry techniques for spawning and larval rearing have been established, which makes them a prime candidate for early life history experiments. In contrast, the life cycle has not been closed in captivity for the majority of coral reef mesopredators. Therefore, the husbandry techniques used in ***Chapters 2 and 3*** were modified and transferred to the Spanish flag snapper (*Lutjanus carponotatus*), in order to close their life cycle in captivity and use them as a model species for the questions surrounding thermal sensitivity in ***Chapters 4 and 5***.

Rising water temperature and ocean acidification are especially relevant for species that utilize nearshore environments, where the magnitude of environmental change and extreme events is predicted to be greatest. In ***Chapter 2*** I investigated how elevated temperature and $CO_2$ levels relevant to climate change and MHWs in coastal waters (+4°C: 22°C & ~1000 $pCO_2$) affect the growth and survival of larval Australasian snapper, *Chrysophrys auratus*. Elevated temperature was found to have a strong effect on larval growth rate, but elevated $CO_2$ did not. At 1 day post hatch (dph) larvae in the elevated temperature treatments were longer and had deeper muscle blocks. Conversely, these fish

had smaller amounts of yolk and oil suggesting an accelerated use of their endogenous reserves. This trend held at 16 dph where larvae in the elevated temperate treatment were larger in all morphometric measurements, while elevated $CO_2$ had no effect. By contrast, larvae had higher survivorship in the elevated $CO_2$ treatment compared with control. These findings suggest that *C. auratus* may not be currently living close to their thermal maximum and could possibly see beneficial effects from elevated water temperature and $CO_2$; however, this would need to be weighed against negative effects that higher temperature and $CO_2$ might possibly have on other physiological traits.

MHWs and high $pCO_2$ in coastal habitats have the potential to impact the early life stages of reef fishes as they transition from pelagic larvae to benthic juveniles in coastal habitats. Therefore, ***Chapter 3*** investigated how a simulated heatwave (+4$^oC$) and elevated $pCO_2$ (1000 µatm) effected the aerobic physiology and swimming performance of juvenile Australasian snapper during the ontogenetic stage when they settle to coastal habitats. Elevated temperature and $CO_2$ increased resting metabolic rate of juvenile snapper by ~22% and ~10% respectively. Maximum metabolic rate was similarly affected by elevated temperature, with an increase of ~17%, but elevated $CO_2$ decreased maximum metabolic rate by ~15%. Maximum sustained swimming speed also increased with elevated temperature and decreased with elevated $CO_2$, matching the results for maximum metabolic rate. These findings suggest that elevated $CO_2$ could have greater impact than MHWs on the metabolic rates and swimming performance of juvenile snapper. This has the potential to reduce their overall performance and potentially have negative flow on effects to the population.

How MHWs affect the early life stages of broadcast spawning coral reef fishes is largely unknown, because of the challenges in completing the life cycle under experimental

conditions. I used knowledge gained in my research with Australasian snapper to develop and refine methods for breeding a common mesopredatory coral reef fish, the Spanish flag snapper (Lutjanidae), *Lutjanus carponotatus*. In ***Chapter 4*** I set out to investigate how elevated temperatures, similar to recent MWHs, effect the energy utilization, growth, and survival of larval Spanish flag snapper. Elevated temperatures (+1.5°C: 30°C and +3°C: 31.5°C) nearly doubled the rate at which endogenous reserves were depleted, compared with control fish (28.5°C). By 14 dph all growth metrics were 55-90% larger in both elevated temperature treatments, however, survival in these treatments was significantly lower. This chapter highlights the sensitivity of Spanish flag snapper larvae to MWHs. While there may be some benefits, such as increased growth rate, ultimately MHWs may push larvae past their physiological limits, causing increased mortality, with potential implications for juvenile recruitment.

The adult spawning stage, which is energetically demanding, has been identified as a thermal bottleneck in the life cycle of marine fishes. Yet little is known about how breeding populations of coral reef fish will cope with MWHs which typically occur during, or soon after, the breeding season. Therefore, in ***Chapter 5*** I investigated how simulated MWH conditions affected the physiological performance of reproductively mature *Lutjanus carponotatus*. Spawning adults were exposed to MWH intensities of +1°C (29.5°C) and +2°C (30.5°C) and the effects on their physiological performance tested after two and four weeks exposure, and again at two weeks post-exposure. The MHW treatments increased metabolic rate, stress recovery time, blood lactate, and haemoglobin levels. Interestingly, the aerobic physiological costs of simulated fishing catch-and-return were not increased by the MHW treatments, but lactate levels were significantly higher, suggesting that anaerobic energy production is increased to meet the energy demands in MHWs. Further, post-MHW

exposure individuals from MHW treatments had a higher aerobic cost during the capture events and lower lactate levels, which indicates that their aerobic capacity, or efficiency, for aerobic energy production had increased. These findings show that MWHs place significant physiological costs on coral reef mesopredators, however they appear to possess the ability to alter their physiology processes to maintain homeostasis.

This research is among the first to test the effects of climate change scenarios and MHWs on rocky reef and coral reef mesopredatory fishes. The results demonstrate that while there may be some early life history traits that may benefit from higher temperature and $CO_2$ levels, other traits are negatively affected. Although this indicates that the overall effect of climate change may be negative to these species it also highlights the complexity of understanding exactly how larval fish are likely to fare under different climate change scenarios. Similarly, the findings of how short-term temperature increases affect adult spawning fish sheds new insight into the physiological costs and residual effects of MWHs. These new findings further our understanding of how reef mesopredators will cope with climate change and MHWs and allow us to better manage populations of these ecologically and economically important fishes into the future.

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# Chapter 1: General introduction

## 1.1 Climate change and our oceans

The Earth is experiencing an anthropogenic driven change in climatic conditions due to a buildup of carbon dioxide ($CO_2$) in the atmosphere. Atmospheric $CO_2$ has risen from ~280 ppm to over 400 ppm since the industrial revolution (Solomon et al., 2009; NOAA, 2021), higher than any point in at least the last 800,000 years (Luthi et al., 2008). Projected $CO_2$ levels by the end of the century range from anywhere between 500 to 1000 ppm, depending on the action taken to reduce emissions (Meinshausen et al., 2011; Pörtner et al., 2019). Increasing atmospheric $CO_2$ has caused a range of climate change effects in our oceans including warming (Levitus et al., 2001; Wernberg et al. 2011), sea level rise (Cahoon & Guntenspergen, 2010; Nerem et al., 2018), changes in storm (Michener et al. 1997; Bromirski et al. 2003) and precipitation patterns (Dore, 2005; Trenberth, 2011), altered ocean currents (Rahmstorf et al., 2015; Voosen, 2020), and the acidification of seawater (Doney et al. 2009; Hönisch et al. 2012). However, ocean warming and acidification are thought to be of primary concern for marine ecosystems due to their wide geographical impact (Brierley & Kingsford, 2009; Hoegh-Guldberg & Bruno, 2010; Doney et al. 2012).

The increased $CO_2$ in the atmosphere enhances the greenhouse effect and as a result the temperature of the atmosphere has increase by ~1°C over the last 150 years (IPCC, 2014; NOAA, 2020). This temperature increase would be much greater except that the oceans act as a heat sink that absorbs heat from the atmosphere (Trenberth & Solomon, 1994; Pörtner et al., 2019). The average sea surface temperature (SST) has

increased by 0.5°C since the 1950s (Rhein et al., 2013), with the rate of warming doubling in the past 30 years (Gleckler et al., 2016; Hoegh-Guldberg et al., 2018). If the present rate of anthropogenic $CO_2$ emission is maintained, average SST is projected to increase by approximately 4$^{o}$C by the end of the century (Sheppard & Rioja-Nieto 2005; Collins et al., 2013; Hoegh-Guldberg et al., 2018). However, if global communities are able to sufficiently reduce emissions and meet agreed targets, such as those in UNFCC's Paris Agreement, is it possible that global warming could be kept below 2$^{o}$C (Rogelj et al., 2018).

In addition to ocean warming, higher atmospheric $CO_2$ concentrations increase the $CO_2$ content of seawater by increasing the uptake of $CO_2$ at the ocean's surface (Doney et al., 2009). It is estimated that the ocean absorbs between 25-30% of the anthropogenic $CO_2$ released into the atmosphere (Calderia & Wickett, 2003; Gruber et al., 2019). The increased uptake of $CO_2$ causes a number of chemical reactions in seawater which ultimately leads to a decrease in seawater pH and a change in the relative abundance of carbonate and bicarbonate ion (Doney et al., 2009; Zeebe et al., 2016). This process is referred to as ocean acidification. Since the industrial revolution the average ocean pH has decreased by 0.1 units and, depending on emissions scenarios, ocean pH could be further decreased by 0.3-0.4 pH units (Calderia & Wickett, 2003; Collins et al., 2013). However, in coastal habitats this additional $CO_2$ exacerbates periods of high $pCO_2$ and low pH seawater that already occur in some nearshore environments due to upwelling of $CO_2$-rich water and nutrient inputs that stimulate biological activity (Feely et al., 2008, Hofmann et al., 2011; Green and Zeldis, 2015). Consequently, coastal habitats may already be subjected to episodes of high $CO_2$ that exceed predictions for the open ocean by the end of the century and these episodes may be further exacerbated by the ongoing uptake of $CO_2$ from the

atmosphere (Shaw et al., 2013; Hoegh-Guldberg et al., 2014; Waldbusser & Salisbury, 2014).

In addition to steady changes in average ocean temperature, global warming is also causing an increase in short-term extreme shifts in environmental conditions (e.g. marine heatwaves: MHWs). Specifically, MWHs have been defined as anonymously warm events, exceeding the 90$^{th}$ percentile of a 30 year average that lasts for at least 5 days (Hobday et al., 2016). MHWs are caused by a combination of processes, with common drivers including persistent high-pressure systems, and ocean currents, which can create a build-up of warm water, and air-sea heat flux which is the transfer of atmospheric heat into the sea surface (Hobday et al., 2016; Holbrook et al., 2019; Gupta et al., 2020). Abnormal temperatures are already experienced in many locations with up to 30% of the world's coastlines experiencing temperature spikes and heatwaves (Lima & Wethey, 2012). Of particular concern is the fact that over the last century MWHs have increased in both frequency (by 34%) and duration (17%), increasing the number of marine heatwave days by 54% (Oliver et al., 2018). The frequency, intensity, and duration of MHWs are expected to further increase as anthropogenic climate change continues, even if SST warming is kept below 2$^{o}$C (King et al., 2016; Frölicher et al., 2018). As the majority of marine organisms are ectotherms, a sudden increase in water temperature can result in thermal stress, with potential flow on affects to individual performance (Nguyen et al., 2011; Kingsolver et al., 2013; Pinsky et al., 2019). Consequently, MHWs are a more imminent threat to marine species and populations than the average increase of sea surface temperate.

MHWs have been shown to have a range of effects on marine ecosystems, including increased mortalities in invertebrates (Garrabou et al., 2009), loss of seagrass meadows

(Marba & Duarte, 2010), mass bleaching and mortality in corals (Hughes et al., 2017), and reductions in fish population biomass (Cheung & Frolicher, 2019). Due to the wide range of organisms effected by MWHs they have the capacity to significantly alter the structure of marine ecosystems (Wernberg et al., 2013; Cavole et al., 2016; Smale et al., 2019). Generally, the effects of MHWs have been more acutely observed in tropical systems, like coral reefs, with coral bleaching and mortality due to anomalous temperatures seen in many locations, including Hawaii (1996, 2002, 2004, 2015), Okinawa (1998, 2001, 2007, 2016), Maldives (2016), Western Australia (1999, 2011), and on the Great Barrier Reef (1980, 1982, 1992, 1998, 2002, 2006, 2016, 2017, 2020). The high thermal sensitivity of corals results in a break-down of the symbiotic relationship between photosynthetic symbionts and the host coral, leading to the symbionts being expelled from the host coral, turning the corals pale, and ultimately causing mortality of the coral host if the bleaching is severe or prolonged (Baird et al., 2002; Baker et al., 2008). During recent MWHs on the Great Barrier Reef a number of reefs have experienced sustained temperatures in the range of +1-2°C above the summer average for several weeks (AIMS, 2017; Spinks et al., 2019) however the effects of MHWs on other marine organisms, such as fish, are less well understood.

Generally, tropical marine species are expected to be highly sensitive to extreme temperatures because they have evolved in thermally stable environments and, therefore, are predicted to have a narrower thermal tolerance range compared with species from higher latitudes (Deutsch et al., 2008; Tewksbury et al., 2008; Rummer et al., 2014). Indeed, recent comparative analyses indicate that tropical marine species are living closer to their upper thermal maximum than similar species in temperate waters which makes them at greater risk from ocean warming and MHWs (Sunday et al., 2014; Sunday et al., 2019).

However, while temperate species may have a wider thermal tolerance they are still threatened by MWHs. Previous MHWs have been shown to change fish community structure (Freedman et al., 2020), exceed cardiac thermal limits of adult fish (van der Walt et al. 2021), and shift species distributions (Smith et al. 2019). These events show that while tropical species may be more sensitive to thermal changes MHWs may pose significant threats to both tropical and temperate fish species.

## 1.2 Fish in a changing ocean

Since most marine organisms are ectothermic, environmental warming potentially means they will have to endure conditions that fall outside their optimal physiological range, which has ramifications for physiological performance, survival, and population sustainability (Mora & Maya, 2006; Peck et al., 2009; Pörtner, 2012; Paaijmans et al., 2013). Temperature is one of the most important physical factors in any biochemical reaction as it generally increases the rate of reaction and because cellular processes tend to be optimised to certain temperature ranges (Gillooly et al., 2001; Pörtner et al., 2006; Angilletta, 2009). High temperature can affect a range of physiochemical mechanisms within an individual and disrupt homeostasis (Reynolds & Casterlin, 1980). Increasing water temperature has an array of effects on the physiology of fish, from the direct thermodynamic effect on biochemical reaction rates (Somero, 2004) and increased metabolic rate (Clarke & Johnston, 1999; Gillooly et al., 2001), through to whole organism traits such as swimming performance (Johansen & Jones, 2011; Little et al., 2020). For instance, the mitochondria, which plays a central role in all ATP production, becomes less efficient above the optimal temperature, compromising its ability to meet energy demands

(Brand & Nicholls, 2011; O'Brien et al., 2018). Reduced mitochondrial efficiency at high temperature can flow on to affect whole organism traits such as metabolic rate, growth rate and swimming performance (Pörtner, 2010; Salin et al., 2019).

Thermal sensitivity can vary greatly between taxonomically related species (Edwards & Richardson, 2004; Peck et al., 2012) and differ across locations for the same species (Gardiner et al., 2010; Rummer et al., 2014; Donelson & Munday, 2012). Current knowledge suggests that thermal sensitivity of ectotherms is related to the range of thermal conditions experienced, with those species or populations that experience a narrow thermal range being more sensitive to warming above normal conditions (Tewksbury et al., 2008; Rummer et al., 2014). Sensitivity also differs between life stages, with reproductive adults and early life stages generally the most sensitive to temperatures outside the optimal range (Hofman & Todgeham, 2010; Pörtner & Farrell, 2008; Melzner et al., 2009a). Due to the potential sensitivity of reproductive and early life stages to higher temperature, ocean warming is predicted to have significant effects on marine fishes (Perry et al., 2005; Munday et al., 2008; Cheung et al., 2009), with shifts in species distributions, change in timing of life history events and alterations to population sizes. Many of these ecological effects have already been observed in response to warming that has occurred since 1960 (Parmesan & Yohe, 2003; Poloczanska et al., 2013) and are predicted to increase as the rate of climate change intensifies towards the end of the century (IPCC, 2014).

In addition to warming, increased atmospheric $CO_2$ is being absorbed into the surface ocean representing a concurrent challenge for marine organisms as climate change progresses. High seawater $pCO_2$ can affect physiological functions of marine organisms because it raises plasma $pCO_2$, which in turn acts to acidify the organism's blood and tissue

(Pörtner et al., 2004). As most cellular processes function optimally within a narrow pH range, many marine organisms actively regulate their acid-base balance to prevent acidosis in high $CO_2$ conditions (Pörtner et al., 2005; Heuer & Grosell, 2014). While the process of ion exchange to maintain a stable pH can be energetically costly (Melzner et al., 2009b; Heuer & Grosell, 2016) early physiological studies on adult fishes found they were able to fully compensate sudden pH changes in relatively short periods (hours to days), even at extremely high levels (up to 10,000 $pCO_2$), without significant effects on survival (Cameron, 1978; Claiborne et al., 2002; Heuer & Grosell, 2014). This pioneering physiological research demonstrated that marine fish are efficient acid-base regulators and possessed a broad $CO_2$ tolerance range (Kroeker et al., 2010; Melzner et al., 2009). However, this research was done on adults which are more robust to environmental changes and possess larger energy reserves, than the sensitive early life stages of marine fishes.

## 1.3 Early life history and environmental change

Embryonic development is one of the most thermally sensitive periods in the life cycle of fish. Most temperate species have a tolerance window of only ±3$^{o}$C from the temperature of fertilization (Rombough, 1997) and, while tropical species are thought to have a smaller tolerance window, it remains to be tested. Even within the embryonic phase, the earlier stages (cleavage, gastrulation, and blastopore closure) tend to be more sensitive than later stages due to the thermal sensitivity of mitotic division (Kazuyuki et al., 1988). Developmental rates increase with rising temperature, which significantly reduces embryonic duration (Houde, 1989; O'Connor et al., 2007). For example, Lesser Sandeel eggs hatched up to 17 days earlier at 11$^{o}$C, compared to 6$^{o}$C, almost halving the incubation time

(Regnier et al., 2018). In contrast, for the tropical spiny chromis damselfish a 3$^{o}$C temperature increase resulted in only a 1.5-day reduction in embryogenesis time (Donelson et al., 2010). This potentially suggests that embryonic duration of temperate species may be more sensitive to temperature increases: however, embryonic development in temperate species tends to be longer than in tropical species (Pauly & Pullin, 1988). Alteration of metabolic and development rate can flow on to the size and condition of newly hatched larvae. Specifically, elevated temperature can both increase (Gillooly et al., 2002; Martell et al., 2005) or decrease (Laurel et al., 2008) standard length, increase the usage of yolk reserves (Rombough, 1997; Fuiman et al., 1998; Ojanguren et al., 1999), and increase developmental deformations (Abdel et al., 2004; Georgakopoulou et al., 2010; Pimentel et al., 2014). The challenge in drawing general conclusions on the likely impact of ocean warming during embryogenesis is that the current literature is limited regarding the total number of studies and the variety of species investigated, with commercially valuable species making up the majority, and of these most are temperate species. Tropical species have significantly fewer studies examining the effects of temperature on the embryonic phase, and conversely most of the studied species are demersal spawners, which leaves tropical broadcasting species underrepresented in the literature. However, the known effects of elevated temperature during embryogenesis can alter the fitness of individuals at hatching, which can have flow on effects into the larval stages warranting further research attention.

As in the embryonic phase, rising water temperature increases the development rate for larval fish, accelerating growth and reducing the time to metamorphosis (Green & Fisher 2004; O'Connor 2007). For benthic marine fishes, the pelagic larval duration (PLD), which is the period from hatching to metamorphosis and settlement, can range from 7 to

~174 days (Leis, 1991; Shanks, 2009). Typically, there is a clear relationship between temperature increase and reduced PLD, with similar trends in tropical, temperate, demersal, and broadcast spawning species (McCormick & Molony, 1995; Green & Fisher, 2004; Sponaugle et al., 2006; Laurel et al., 2014). The length of the PLD and the developmental transitions that occur during the period can be important in determining survival (Armsworth, 2002; Paris & Cowen, 2004; Leis, 2007). For example, the developmental timing of critical swimming transitions, such as flexion where larvae transition from passive to active swimmers, is important as it enables larvae to modify their dispersal trajectory (Leis, 2015), increase their ability to avoid predators (Hunter, 1981; Yin & Blaxter, 1987), and increase foraging ability to secure energetic resources (Munk & Kiorboe, 1985). While increased developmental rates might not be inherently negative, rising water temperature can increase larval mortality, regardless of latitude and reproductive strategy (Houde, 1989; Pepin, 1991). Furthermore, in an analysis of PLD and larval growth rate across latitudes, McLeod et al., (2015) observed that lower latitude populations may already be at, or possibly beyond, their thermal optimum. This suggests that larval development is optimized to present-day thermal conditions for equatorial and low latitude populations and that future warming could have negative impacts to this life stage.

Larval developmental is energetically demanding and is a period associated with exceptionally high mortality (Post & Parkinson, 2001; Stallings et al., 2010). As with the embryonic phase, the larval stages are highly sensitive to environmental temperature (Pörtner & Peck, 2010; Dahlke et al., 2020). The growth and developmental rate of larval fish is typically seen to increase as temperature rises (Pepin, 1991; Steinarsson & Bjornsson, 1999; Dou et al., 2005) however increased mortality has also been seen at higher

temperatures (Houde, 1989; Pope et al., 2014; Watson et al., 2018). The heightened thermal sensitivity during larval development is partially attributed to the fact that larval fish have limited energetic reserves with which to buffer effects of higher temperature (Hoar et al., 1983; Blaxter, 1991). Another reason for heightened sensitivity to environmental change during larval development is because larvae have incomplete morphological and physiological development, and thus may be less capable of dealing with environmental stressors compared with fully developed juveniles and adults (Pörtner et al., 2006; Miller & Kendal 2009). In combination with high thermal sensitivity, early life stages have the highest metabolism and growth rates in addition to limited energetic reserves compared to later life stages (Cunha et al., 2007; Killen et al., 2007). As water temperature increases there could be a shift in the energy distribution within an individual, potentially limiting available energy for growth, as more critical basic energy demands are prioritized (Stallings et al., 2010; Mogensen & Post, 2012). Yet ecologically, many fish species may not see temperatures high enough to reduce larval growth rates, as the literature shows positive correlation between growth and temperature for many species (Houde, 1989; Blaxter, 1991; Pepin, 1991). However, several lower latitude species have shown reductions in growth under ecologically relevant, elevated temperatures (McLeod et al., 2015). The impact of temperature on larval growth can flow on to later juvenile stages, where larger juveniles, or those in better condition have a greater chance of survival (McCormick et al., 1995; Shima & Findlay, 2002; Almany & Webster, 2006)

Although adult fish have well developed acid-base regulation (Janssen & Randall, 1975; Claiborne et al., 2002), larval fishes are more sensitive to elevated $CO_2$ as their regulatory mechanisms are still developing (Ishimatsu et al., 2008; Melzner et al., 2009; Brauner et al., 2019). Nevertheless, there appears to be considerable variation among

species in the sensitivity of early life stages to elevated $CO_2$ (Munday et al., 2019; Bauman, 2019). In laboratory experiments, larval growth rate has been observed to increase (Munday et al., 2009; Chambers et al., 2014; Bignami et al., 2014), decrease (Murray et al., 2014; Silva et al., 2016) or be unaffected (Hurst et al., 2013; Pope et al., 2014) by elevated $CO_2$. Larval survivorship has typically been found to be unaffected by elevated $CO_2$ (Frommel et al., 2013; Hurst et al., 2013), however, some species have exhibited increased (Pope et al., 2013) and decreased survivorship (Baumann et al., 2012; Pimentel et al., 2014; Murray et al., 2019) at $CO_2$ levels predicted to occur in the ocean by the end of this century. Such effects have the potential to significantly impact population sustainability. For example, Stiasny and colleagues (2016) estimated that decreased survival of cod larvae under projected future $CO_2$ levels could reduce recruitment to adult populations to just 8-30% of current day levels. While the exact mechanisms responsible for these variable responses to elevated $CO_2$ are still unknown, they highlight that some species could potentially benefit from projected future $CO_2$ conditions, whereas others will be negatively affected. The performance of early life stages influences the dynamics of most fish populations (Caley et al., 1996; Jones & McCormick, 2002; Petitgas et al., 2013); therefore, any effects that elevated $CO_2$, or elevated temperature, have on the growth, survival and individual performance of larvae could impact population abundance and biomass.

Marine habitats are dynamic environments and we can expect multiple environmental parameters to change simultaneously as climate change progresses. Water temperature and $CO_2$ levels are two important parameters expected to change in many marine habitats as climate change progresses (Doney et al. 2012; Henson et al., 2017). Therefore it is important to understand how these simultaneous changes could impact marine species as there is the possibility for additive, synergistic, or antagonistic effects

(Côté et al., 2016). Larval fish are likely to be the most susceptible to these environmental changes, as they have relatively low physiological buffering capacity (Pörtner & Farrell 2008; Wittman & Pörtner, 2013), however few studies have looked at the combined effects of elevated temperature and $CO_2$ on larval fish (Lefevre, 2016). Research to date has found that elevated temperature and $CO_2$ can have varying effects on the early life stages of fish depending on whether they occur individually or simultaneously. For instance, the survival of European bass larvae was increased when exposed to either elevated temperatures or elevated $CO_2$, but there was no change in survival when they occurred together (Pope et al. 2014). Additionally, there is evidence for interactive effects of elevated temperature and $CO_2$ on behaviour (Nowicki et al. 2012) and growth (Sswat et al. 2018) during the early life stages of marine fish. However, these same attributes appear to be robust and unaffected by the combination of elevated temperature and $CO_2$ in other species (Bignami et al. 2017), which highlights how species may be differentially effected as climate change progresses. With this in mind, it will be important to incorporate both elevated temperature and $CO_2$ into future experiments, where ecologically appropriate, to identify any interactive effects that they may have on the early life stages of marine fish.

## 1.4 Marine heatwaves and adult reef fish

While adult fish are generally not as sensitive to temperature changes as the early life stages, physiological processes are still influenced by temperature change. Specifically, increasing water temperature has a direct thermodynamic effect on biochemical reaction rates and enzymatic processes (King et al., 2003; Pankhurst & Munday, 2011; Miranda et al., 2013), which can flow on to whole organism traits such as growth, swimming

performance, and reproduction (Pörtner et al., 2001; Neuheimer et al., 2011; Little et al., 2020). For instance, since metabolic rates in fish increases with temperature (Gillooly et al., 2001; Neuheimer et al., 2011), fish will require more energy to sustain the basic costs of cellular maintenance (Pörtner et al., 2004; Melzner et al., 2009; Heuer & Grosell, 2016) potentially limiting energy available for other activities (Melzner et al., 2009; Pedersen et al., 2014). Studies have shown that increases in water temperature above summer averages can reduce aerobic capacity (Nilsson et al., 2009; Sandblom et al., 2014; Rummer et al., 2014), increase mortality rate (Genin et al., 2020), and reduce swimming performance and general activity of adult fishes (Johansen & Jones, 2011; Johansen et al., 2014). Reproduction is one of the most energetically expensive activities for adult fishes and thus may be especially sensitive to changes in energy allocation. Reproduction occurs within a narrow window of the overall thermal tolerance range of most marine fishes and is therefore especially sensitive to ocean warming. (Pankhurst & Munday, 2011). Indeed, a recent meta-analysis of observational, experimental, and phylogenetic data has predicted that elevated temperatures may present a thermal bottleneck for up to 60% of marine fish populations by 2100 (Dalke et al., 2020).

To date, the vast majority of the research into the effects of ocean warming on coral reef fishes has focused on smaller bodied, site-attached species, with larger bodied, more mobile fish receiving less attention (exceptions on coral trout: Johansen et al 2014; Johansen et al., 2015; Messmer et al., 2017; Pratchett et al., 2017). While larger mobile species may be able to partially mitigate the effect of short-term warming events, like MWHs, by moving to deeper and/or cooler waters, many are dependent on the reefs for food and shelter, meaning that thermal avoidance may still affect individual performance, condition and survival (du Plessis et al., 2012: Kleckova et al., 2014). Therefore, to further

our understanding of how reef systems will fare under future MHWs it is important to understand how larger and more mobile reef fish with be affected. In addition, there has been evidence that MWHs can have indirect effects on coral reef fish by making them more susceptible to fishing efforts (Brown et al., 2020). This is thought to be due a number of factors elicited by warmer waters including elevated metabolic demands resulting in increased feeding rates (Johansen et al., 2015), reduced swimming ability (Johansen et al., 2014), and smaller home ranges (Scott et al., 2019). The direct and indirect effects of MWHs on fish are likely to have negative effects on individuals and populations where temperatures are pushed above optima. However, we know little about whether the physiological effects and the rate of recovery following MHWs differ depending on the magnitude and duration. Understanding these aspects would greatly increase our ability to predict the consequences of stronger and longer MHWs in the future.

## 1.5 Reef mesopredators

To date, the effects of elevated water temperature and $CO_2$ levels on marine fish has predominantly considered either small benthic species, such as damselfish (Domenici et al., 2011; Donelson et al., 2014) and anemonefish (Nilsson et al., 2012; Miller et al., 2013) due to the ease maintaining them in a laboratory setting, or larger pelagic species such as kingfish (Munday et al., 2015), cobia (Bignami et al., 2013) and dolphinfish (Pimentel et al., 2014) due to their commercial importance. These focused efforts on lower and higher trophic species have left a gap in our understanding in how the intermediate trophic levels will be affected by climate change. Mesopredatory fishes have an important ecological role in connecting energy transition between the lower and upper trophic levels (Polovina et

al., 1984; Link et al., 2005). They are predators that prey on many smaller fishes and invertebrates but are themselves preyed on by larger predatory fishes. Mesopredators are diverse and abundant in most temperate and tropical reef fish assemblages and are critical to food web functioning. Due to this, and their intermediate trophic position, shifts in the abundance of mesopredators have the potential to cause significant changes to ecosystem health and function (Estes & Palmisano, 1974; Prugh et al., 2009). For example, introduction of a coral reef mesopredator to patch reefs has been shown to alter the fish assemblage (Hempson et al., 2018), and changes in the predatory behaviour of mesopredators (e.g. from apex predation) can alter the abundance and behavior of prey species (Palacios et al., 2016). In addition to being ecologically important to rocky and coral reef ecosystems, many mesopredatory fishes, such as species from the Sparidae, Lutjanidae and Serranidae, are important to recreational and commercial fishing (MPI 2013; GBRMPA, 2014). Although mesopredators are ecologically and commercially important they remain substantially understudied in respect to the impacts of climate change.

## 1.6 Study species

In this thesis I investigate the effects of elevated temperature and ocean acidification on two reef mesopredators, the Australasian snapper, *Chrysophrys auratus* (Fig.1.1), from temperate and subtropical rocky reefs and the Spanish Flag snapper, *Lutjanus carponotatus*, from tropical corals reefs. *Chrysophrys auratus*, supports important commercial and recreational fisheries in New Zealand and Australia (MPI, 2013; Wortmann et al., 2018). They have a wide geographic range from sub-tropical to temperate waters,

with different populations experiencing summer temperatures between 18-26°C (Scott & Pankhurst, 1992; Sheaves, 2006; Wakefield, 2010). Spawning occurs at temperatures between 18-21°C, depending on the population, and juveniles settle to nearshore habitats at ~30 dph (Saunders et al., 2012; Parsons et al., 2016; Lohrer et al., 2018). As the species is commercially valuable there have been significant efforts to close the life cycle in captivity and refine husbandry techniques to make them an economically viable species for aquaculture. Consequently, *C. auratu*s is a prime candidate for research into the effects of ocean warming and acidification on the early life stages of reef mesopredators as they can be successfully reared under laboratory conditions.

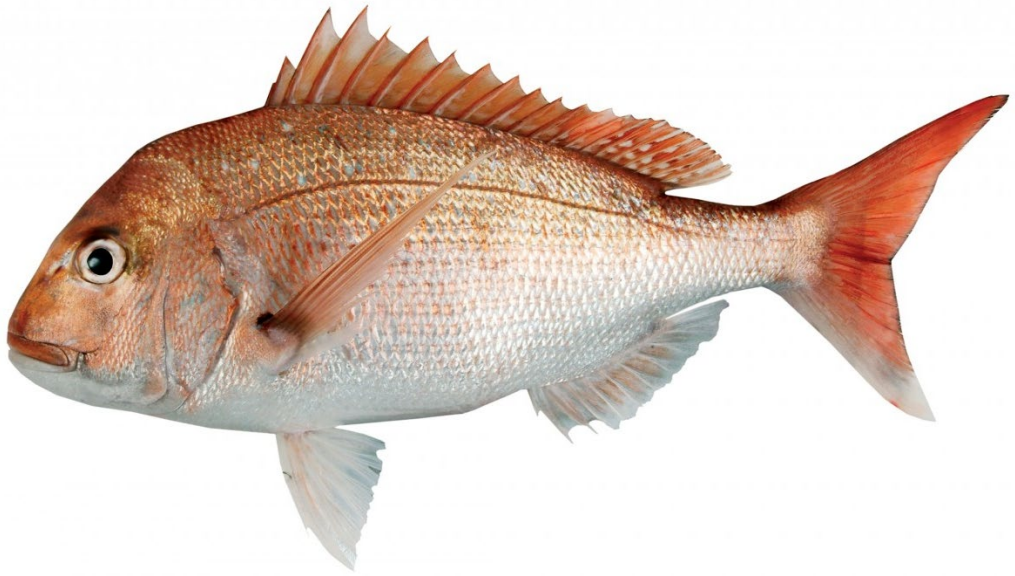

**Figure 1.1** *Chrysophrys auratus*, Australasian snapper, adult. Image from New Zealand Geographic.

The Spanish Flag snapper, *Lutjanus carponotatus* (Fig.1.2), is a tropical mesopredator that inhabits coral reefs throughout the Great Barrier Reef (GBR) and Indo-Pacific region (Allen, 1985). They are gonochoristic serial spawners with the peak spawning for the GBR population occurring between October and December (Kritzer, 2004). The

larvae have a pelagic duration that can last 33-38 days where they are found relatively close to the surface (Quéré & Leis, 2010), after which they settle to benthic reef habitat. As *L. carponotatus* are often abundant on coral reefs, commercially important, and closely reef associated they make a promising candidate for a model coral reef mesopredator in which to test the effects of ocean warming and acidification. However, it has been historically challenging to rear the larvae of most coral reef fishes due to limited capacity and knowledge of reproductive requirements and the environmental requirements of the sensitive pelagic larval stage. This has inhibited experimental research on the early life stages of coral reef fishes, with the exception of demersal spawning anemonefishes and the direct developing spiny chromis damselfish, which are easily reared in captivity. Yet, there are similarities in the ontogenetic development of early life stages of tropical and temperate perciform fishes (Blaxter, 1988; Miller & Kendall, 2009), and consequently, there should be several transferable aquaculture and husbandry practices. Therefore, this thesis aimed to take the practical skills and knowledge of *C. auratus* aquaculture to close the lifecycle of *L. carponotatus* and utilize them for experimental research into the effects of climate change on coral reef mesopredators.

**Figure 1.2** *Lutjanus carponotatus*, Spanish Flag snapper, adult. Image from the Government of Western Australia.

## 1.7 Aims and thesis outline

This thesis focuses on how temperate rocky reef and coral reef mesopredator fishes are affected by ocean warming and acidification with an emphasis on the early life stages. While there is increasing understanding of how some reef fish will fare under predicted climate change scenarios, the majority of the research to date has focused on smaller bodied, demersal species. Consequently, there is a major knowledge gap in understanding how larger reef fish, such as mesopredators, and broadcast spawning species will be affected by climate change. The primary reason for the absence of literature on the effects of climate change on reproduction and the early life stages of tropical mesopredators fishes is that it has proven difficult to breed and rear them in captivity. However, there are some ecologically similar temperate water species, such as *C. auratus*, which have had their lifecycle closed in captivity, making them good candidates for this research and well suited to knowledge transfer for experiments with tropical reef mesopredators. Specifically, this thesis investigates how elevated temperatures and $pCO_2$ levels, similar to projected climate change scenarios, including marine heatwaves, affect the growth, physiology and swimming performance of two common reef mesopredators.

***Chapter 2*** tests the effects of elevated temperature and $CO_2$ on the growth and survival of larval *C. auratus*, a temperate and subtropical reef mesopredator. This study used current summer averages relevant for the population (18°C and 400 $pCO_2$) and projected future climate change scenario levels (+4°C and 1000 $pCO_2$) in a fully-crossed experiment to determine the independent and interactive effects of elevated temperature and elevated $CO_2$ on the early life history traits of larval snapper. The elevated temperature

and $CO_2$ treatment used is projected to occur as the normal summer conditions by the end of the century, but will likely also occur intermittently during present-day summers when anomalous climate and oceanographic events occur (Feely et al., 2008; Green & Zeldis, 2015; NIWA, 2018). Following this, ***Chapter 3*** investigates how elevated $CO_2$ and thermal conditions affect the aerobic and swimming performance of juvenile *C. auratus.* This study tested whether aerobic metabolic traits (oxygen consumption) and swimming capacity were affected by early development in elevated temperature and $CO_2$ conditions for a temperate reef mesopredator. The traits measured in both ***Chapters 2*** and ***3*** are fundamental to the individual performance and fitness of marine fishes and have been predicted to be impacted by both ocean warming and acidification.

The skills gained from the successful experiments on the temperate snapper *C. auratus* allowed me to break new ground in this research field by completing the life cycle in captivity for the coral reef mesopredator, *L. carponotatus*. In turn, this allowed me in ***Chapter 4*** to investigate how the earliest life stages of *L. carponotatus* are affected by elevated temperatures. The study tested how MHWs (+1.5°C and +3°C above the summer average, 28.5°C) affected energy utilization, growth, and development of a larval coral reef snapper from fertilization to 14 days post hatching.

Finally, ***Chapter 5*** explores how the physiology of reproductively mature *L. carponotatus* is affected by MHWs during the summer spawning season. In this chapter I put adults through two heatwave treatments (+1°C and +2°C above the summer average 28.5°C) and tested how their metabolic rates, capture swimming costs, and blood chemistry were affected by two and four weeks exposure to the MHW conditions and, at two weeks post-exposure.

This thesis addressed key knowledge gaps and advances understanding of how the early life stages, and sensitive adult stages, of reef mesopredators may be affected by climate change, including extreme events like MHWs that are already occurring in many marine ecosystems. Understanding the sensitivity of the reproductive and early life stages to environmental change is critical for predicting the possible effects of ocean warming, ocean acidification, and heatwaves on fish populations. The knowledge gained in this thesis will enable better management of these functionally, socially, and economically important species and their role in both temperate and tropical marine ecosystems.

# Chapter 2: Elevated temperature and $CO_2$ have positive effects on the growth and survival of larval Australasian snapper

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## 2.1 Abstract

Rising water temperature and increased uptake of $CO_2$ by the ocean are predicted to have widespread impacts on marine species. However, the effects are likely to vary, depending on a species' sensitivity and the geographical location of the population. Here, I investigated the potential effects of elevated temperature and $pCO_2$ on larval growth and survival in a New Zealand population of the Australasian snapper, *Chrysophrys auratus*. Eggs and larvae were reared in a fully cross-factored experiment (18°C and 22°C / $pCO_2$ 440 and 1040 µatm) to 16 days post hatch (dph). Morphologies at 1 dph and 16 dph were significantly affected by temperature, but not $CO_2$. At 1 dph, larvae at 22°C were longer (7%) and had larger muscle depth at vent (14%) but had reduced yolk (65%) and oil globule size (16%). Reduced yolk reserves in recently hatched larvae suggests higher metabolic demands in warmer water. At 16 dph, larvae at elevated temperature were longer (12%) and muscle depth at vent was larger (64%). Conversely, survival was primarily affected by $CO_2$ rather than temperature. Survivorship at 1 dph and 16 dph was 24% and 54% higher, respectively, under elevated $CO_2$ compared with ambient conditions. Elevated temperature increased survival (24%) at 1 dph, but not at 16 dph. These results suggest

that projected climate change scenarios may have an overall positive effect on early life history growth and survival in this population of *C. auratus*. This could benefit recruitment success but needs to be weighed against negative effects of elevated $CO_2$ on metabolic rates and swimming performance observed in other studies on the same population.

## 2.2 Introduction

Global warming has increased the average surface temperature of the oceans by 0.5°C since the 1950s (Rhein et al., 2013), with the rate of warming doubling in the past 30 years (Gleckler et al., 2016; Hoegh-Guldberg et al., 2018). Extreme temperatures are also occurring with increased frequency and intensity. Since the 1980s over a third of the world's coastal environments have experienced heatwave conditions, characterised by anomalously high water temperature (Lima & Wethey, 2012). Such heatwaves are expected to increase in frequency and intensity as climate change advances (Frolicher et al., 2018; Hoegh-Guldberg et al., 2018). Additionally, increased concentrations of atmospheric $CO_2$ have led to the increased uptake of $CO_2$ at the ocean's surface, which has caused a decline in the oceans pH (Doney et al., 2009). Without significant reduction in the rate of anthropogenic $CO_2$ emission, average sea surface temperature could increase by 2-4°C (Collins et al., 2013) and $pCO_2$ at the ocean's surface could regularly exceed 900 µatm (McNeil & Sasse, 2016; McNeil & Matsumoto, 2019) by the end of the century. These changes will directly affect marine organisms and alter the structure and productivity of marine ecosystems (Bell et al., 2013; Wittmann & Pörtner 2013; Hoegh-Guldberg et al., 2018).

While there is abundant observational evidence that marine organisms are already being affected by environmental changes occurring in the ocean (Edwards & Richardson, 2004; Hoegh-Guldberg & Bruno, 2010; Poloczanska et al., 2016), and experimental evidence indicates they can be negatively affected by predicted future ocean warming and acidification (Kroeker et al., 2013; Cattano et al., 2018), not all marine species are equally sensitive (Somero, 2010; Kroeker et al., 2013; Wittmann & Pörtner, 2013). Differences in sensitivity to environmental change can be linked to the natural variation experienced by the population or species. For example, tropical species on average are thought to be more sensitive to increasing temperature compared to temperate species (Deutsch et al., 2008; Tewksbury et al., 2008; Huey et al., 2009), because they have evolved in more thermally stable conditions. In fact, in some locations temperate species are currently living below their thermal optimum and might even benefit from the same amount of warming that is detrimental to tropical species (Tewksbury et al., 2008). Differences in thermal sensitivity can also occur among populations of the same species, with thermal tolerance ranges typically increasing towards higher latitudes (Sunday et al., 2014; Rodgers et al., 2018). Consequently, the impacts of climate change are expected to vary geographically (Poloczanska et al., 2016; Hoegh-Guldberg et al., 2018).

The early life stages of marine fish are sensitive to thermal change (Blaxter 1991; Pörtner & Peck 2010; Llopiz et al., 2014). Growth rates of larval fish typically increase with rising temperature, for both temperate (Pepin, 1991; Steinarsson & Bjornsson, 1999; Dou et al., 2005) and tropical species (Munday et al., 2008), with increased growth maintained far closer to lethal thermal thresholds than observed in adults (Houde, 1989). As water temperature rises, survival has been found to increase for some species (Pope et al., 2014) and decrease in others (Sswat et al., 2018; Watson et al., 2018). Similarly, a positive

correlation between temperature and survival has been observed for some wild populations, with higher recruitment following warmer spawning seasons (Fowler & Jennings, 2003; Cheal et al., 2007), but not in others (Lo-Yat et al, 2011), and even opposite effects for different stocks of the same species (Ottersen et al., 2013). This variation in the effects of temperature on growth and survival could be associated with species specific differences in thermal tolerance, or the thermal safety margin of the population tested, with some populations living further from their thermal limits than others (Sunday et al., 2014; McLeod et al., 2015; Rodgers et al., 2018).

The early life history of fishes is also the stage most sensitive to elevated $CO_2$ (Cattano et al., 2018), but there appears to be considerable variation in species sensitivity (Munday et al., 2019; Bauman, 2019). Under elevated $CO_2$ conditions in laboratory experiments, larval growth rate has been observed to increase (Munday et al., 2009; Chambers et al., 2014; Bignami et al., 2014), decrease (Baumann et al., 2012; Silva et al., 2016; Murray et al., 2019) or be unaffected (Miller et al., 2013; Hurst et al., 2013; Pope et al., 2014). Larval survivorship has typically been unaffected by elevated $CO_2$ (Frommel et al., 2013; Hurst et al., 2013), however, some species have exhibited increased (Pope et al., 2014) or decreased survivorship (Baumann et al., 2012; Pimentel et al., 2016; Murray et al., 2019). While the exact mechanisms that are responsible for these different responses to elevated $CO_2$ are still uncertain, they highlight that some species could potentially benefit from projected future $CO_2$ conditions, whereas others will be negatively impacted.

Multiple drivers, such as warming and elevated $CO_2$, may have additive, synergistic, or antagonistic effects (Côté et al., 2016). To date, few studies have investigated the combined effects of elevated temperature and $CO_2$ on larval fishes, yet they have highlighted the importance of considering both drivers in combination (Baumann, 2019).

For example, survival of larval European bass (*Dicentrarchus labrax*) increased at higher temperature and in elevated $CO_2$, but there was no change in survivorship when they both occurred together, suggesting an antagonistic effect (Pope et al., 2014). Conversely, survival of larval Senegalese sole (*Solea senegalensis*) was reduced by higher temperature and in elevated $CO_2$, and decreased further when both drivers were present, suggesting an additive effect (Pimentel et al., 2014). These results indicate that the combined effects of elevated temperature and $CO_2$ can vary markedly among species, but as yet the underlying reasons for such variation are unknown.

In this study I tested the independent and combined effects of elevated temperature and elevated $CO_2$ on the early life stages of the Australasian snapper, *Chrysophrys auratus*, from northern New Zealand. The study population experiences mean water temperature of 18$^{o}$C during the peak spawning period of November and December (Evans and Atkins, 2013; NIWA 2018). I used elevated temperatures (+4$^{o}$C) and $CO_2$ levels (~1040 µatm) consistent with model projection for 2100 under IPCC's Representative concentration pathway (RCP) 8.5 (IPCC, 2014). These elevated temperature and $CO_2$ levels are also consistent with heatwave conditions and extreme $CO_2$ events that can occur within coastal embayments in northern New Zealand (Law et al., 2018; McMahon et al., 2020), and which are predicted to increase in frequency and duration in the future (Frolicher et al., 2018; Hoegh-Guldberg et al., 2018). Specifically, I used a cross-factored experiment with four treatments: 1) current-day average temperature during peak spawning (18$^{o}$C) and ambient $CO_2$ (432 µatm), 2) current-day average temperature during peak spawning (18$^{o}$C) and elevated $CO_2$ (1035 µatm), 3) projected future warming of +4$^{o}$C (22$^{o}$C) and ambient $CO_2$ (457 µatm), and 4) projected future warming of +4$^{o}$C (22$^{o}$C) and elevated $CO_2$ (1043 µatm) (Table 2.1). Eggs and larvae were reared in these treatments to investigate how

growth and survival were affected by elevated temperature and $CO_2$ at 1 and 16 days post-hatching (dph), as they are key fitness-associated metrics and important parameters in population-dynamics models.

## 2.3 Materials and methods

### 2.3.1 Study species

The Australasian snapper, *Chrysophrys auratus*, supports important commercial and recreational fisheries in New Zealand and Australia (MPI, 2013; Wortmann et al., 2018). They have a wide geographic range from sub-tropical to cool temperate waters, with different populations experiencing summer temperatures between 18-26°C (Scott & Pankhurst, 1992; Sheaves, 2006; Wakefield, 2010). Spawning occurs at temperatures between 18-21°C, depending on the population, and juveniles settle to nearshore habitats at ~30 dph (Saunders et al., 2012; Parsons et al., 2016; Lohrer et al., 2018). A recent study found that metabolic rates and swimming performance of juveniles from northern New Zealand were negatively affected by elevated $CO_2$ (McMahon et al., 2020), but whether there are corresponding effects on growth rates and survival at elevated $CO_2$, and possible interacting effects with elevated temperature, are unknown.

### 2.3.1 Breeding and larval rearing

This study was conducted at the National Institute of Water and Atmospheric Research Northland Marine Research Centre (NMRC), in Ruakaka, New Zealand. Adult brood stock *C. auratus* (n=39) were captured from the wild population by longline fishing in Bream Bay, adjacent to the NMRC, during May of 2017. Adults were split between two 20 $m^3$ circular tanks at the ambient temperature when they were collected (c. 16˚C). Each tank was supplied with 130 L $min^{-1}$ ambient seawater, filtered to 10 µm and UV sterilized, and followed the natural temperature increase up to the summer average (18˚C), at which point

it was maintained for spawning (Table 2.1). Spawning was induced during January 2018 by implanting slow release GnRH pellets at 200 ug/kg (Galvin et al., 2003). Two spawns, from consecutive days, were used in this experiment. To maximize genetic variation in the experiment, eggs were collected from both brood stock tanks in even proportions. Eggs were collected using an external egg collector as described by Moran ***et al.,*** (2007), with a 500 µm mesh net to retain eggs from the surface overflow of each tank. An equal proportion of floating eggs from both contributing tanks were mixed, rinsed with oxygenated sea water for 5 min, and disinfected with Tosylchloramide (chloramine-T) at 50 ppm for 15 min. Eggs were then rinsed with seawater and evenly distributed between sixteen 400 L conical hatching tanks. Each tank was stocked with approximately 50,000 fertilized eggs and received flow-through seawater at ambient temperature (18˚C) at a flow rate of 4 L $min^{-1}$. Incoming water was filtered to 5 µm and UV sterilized. Immediately after eggs were stocked the incoming water flow to each tank was changed to one of the four water treatments and allowed to slowly adjust over 12 hours. Photoperiod was maintained at 14 h light: 10 h dark.

Snapper eggs hatch in 24-48 hours at ambient summer temperatures at Bream Bay. Newly hatched larvae remained in the conical rearing tanks until 1 dph. Larvae were not fed during this period as they rely on their endogenous reserves (Battaglene & Talbot, 1992). Any dead eggs, larvae, and egg shells were removed daily by draining from an outlet at the bottom of the rearing tank. At 1 dph larvae from each tank were transferred to a reciprocal 200 L rearing tank located in the same facility for grow-out. From 2 dph green-water treatment was used at a concentration of ~230,000 cells per ml (Nano 3600, Reed Mariculture) and larvae were fed enriched rotifers (INVE, Selco S.Presso) three times a day (0800, 1200, and 1600).

**Table 2.1** Mean (± 1 SD) of seawater chemistry parameters for Australasian snapper (*Chrysophrys auratus*) brood stock tank and larval treatments. Brood stock tanks were measured during the week of spawning at the start, middle, and end of the week. Temperature and $pH_{total}$l were measured daily in each rearing tank over the experiment. Total alkalinity and salinity were measured at the start of the experiment and then every 4 days from 1 day post hatching. $pCO_2$ was estimated from these parameters in CO2SYS.

| Treatment | Salinity (ppt) | Temperature (°C) | Total Alkalinity (mmol/kgSW) | pH (Total) | $pCO_2$ (µatm) |
|---|---|---|---|---|---|
| **Brood stock** | 35.60 ±0.05 | 18.03 ±0.04 | 2154 ±6 | 7.88 ±0.01 | 583 ±14 |
| **Ambient Temperature Ambient $CO_2$** | 35.39 ±0.29 | 18.12 ±0.18 | 2310 ±7 | 8.02 ±0.02 | 432 ±26 |
| **Ambient Temperature Elevated $CO_2$** | 35.27 ±0.28 | 18.14 ±0.23 | 2317 ±8 | 7.68 ±0.01 | 1035 ±37 |
| **Elevated Temperature Ambient $CO_2$** | 35.34±0.21 | 21.95 ±0.23 | 2317 ±9 | 8.00 ±0.02 | 457 ±21 |
| **Elevated Temperature Elevated $CO_2$** | 35.29 ±0.16 | 21.92 ±0.20 | 2319 ±9 | 7.69 ±0.01 | 1043 ±31 |

### 2.3.3 Experimental design

Eggs and larval fish were reared in a 2 x 2 experimental design comprising two temperature and two $CO_2$ treatments. The temperatures used were 18°C, which is the temperature where spawning is at its peak for this species (Scott & Pankhurst, 1992; Wakefield, 2010) and 22°C, which is close to the maximum sea surface temperature (SST) recorded in the region during heatwave events (Evans & Atkins, 2013; NIWA, 2018) and also the approximate average SST (+4°C) projected for the region under RCP8.5. The $CO_2$ treatments were an unmanipulated ambient of 430-460 µatm and an elevated treatment of 1030-1050 µatm (Table 2.1). The elevated $CO_2$ treatment is consistent with the upper range of $pCO_2$ that can occur in embayments inhabited by larval and juvenile snapper in northern New

Zealand (Law et al., 2018) and $pCO_2$ levels projected to become frequent in the open ocean by 2100 under RCP8.5 (McNeil & Sasse, 2016). Temperature was controlled by 1 Kw bar heaters in 8 x 200 L sumps tanks and maintained to ±0.1°C. Each sump contained two submerged pumps, one for delivery of treatment water to the rearing tanks and one to mix the water in the sump to maintain homogenous temperature. The second pump also served as the location for $CO_2$ dosing in the elevated $CO_2$ treatment. The desired $pCO_2$ was achieved by dosing $CO_2$ gas to maintain the appropriate pH set point. $CO_2$ dosing was regulated by a pH computer (Aquamedic) connected to a pH probe and a solenoid valve, which slowly dosed $CO_2$ through a needle value into the inlet of the submerged pump whenever pH deviated above the set point. The pump immediately dissolved the $CO_2$ and circulated it evenly throughout the sump. Each treatment was duplicated (i.e. 4 treatments each with 2 sumps). Treatment water from each of the 8 sumps was supplied to 2 egg hatching tanks, and subsequently to 2 larval rearing tanks, at a flow rate of 4 L $min^{-1}$. Consequently, the experimental design consisted of 4 tanks per treatment (2 tanks per duplicate system), with fish transferred from hatching to larval rearing tanks at 1 dph.

The pH on the total hydrogen scale ($pH_{total}$) of each rearing tank was measured daily by spectrophotometry (Hach, DR3900) with cresol purple dye (Clayton & Bryne, 1993). Temperature was measured daily with a digital thermometer (Comark C22). Water samples were taken from each tank at the start of the experiment and then every 4 days from 1 dph (i.e. 1, 4, 8, 16 dph) for total alkalinity (TA) analysis. Water samples were immediately poisoned with a saturated solution of mercuric chloride (0.05% of the sample volume) and later analysed at the University of Otago Research Centre for Oceanography, Dunedin, New Zealand. Alkalinity was determined by potentiometric titration in a closed cell (Dickson et al., 2007) using a Metrohm Dosimat burette (model 765, Metrohm, Switzerland), a Fluke

model 8846A voltmeter, and with 0.2M HCl (nominal concentration, fortified with NaCl to the ionic strength of seawater) added in 0.1 mL steps. Samples were water-jacketed at 25°C. TA was determined from the titration data using a least squares minimisation technique and calibrated with certified reference material (Prof. A.G. Dickson, Scripps Institution of Oceanography, U.S.). The salinity of each sample was measured with a YSI Pro30 salinity probe. The daily $pCO_2$ of each rearing tank was then calculated in CO2SYS (Pierrot et al., 2006) from the measured values of $pH_{total}$, temperature, TA and salinity and using the constants of Mehrbach et al., (1973), refit by Dickson & Millero (1987) (Table 2.1).

### 2.3.4 Sampling protocols for life history traits

The numbers of eggs stocked at the start of the experiment and the numbers of larvae present in each tank at 1 dph and 16 dph were estimated to calculate survivorship. At each time point, five 500 ml water samples were taken from each tank, strained, screened on 200 (eggs) or 500 µm mesh (larvae) and the number of eggs or larvae counted. To ensure accurate sampling, each tank was mixed with gentle aeration and mechanical mixing before sampling. The average of the five counts was used to estimate number of eggs or larvae in each tank, using sample volume to tank volume ratio. Samples were not returned to tanks. Survival was calculated for 1 dph by using the proportion of eggs at stocking to larvae at 1 dph, while survival at 16 dph was calculated by the proportion of larvae from 1 dph to 16 dph. For morphometric traits, a subset of 30 random fish were collected from each tank at 1 dph and 16 dph, sedated with Aqui-S and individually photographed using a Leica DFC 420 camera fitted to a Leica MZ7.5 stereo microscope.

A range of metamorphic traits, that are indicators of growth and performance (Watson et al., 2018), were measured from the photographs: standard length (SL), total length (TL), body length (BL), muscle depth at vent (MDV), fin depth at vent (FDV), eye diameter (ED), mandible length (ML), yolk area (YA), oil globule diameter (OGD), oil globule length (OGL), head length (HL), head depth (HD) (Fig. 2.1). An observer, who was blinded to the treatments, recorded the morphological data from the images using Image-J software and a high resolution screen.

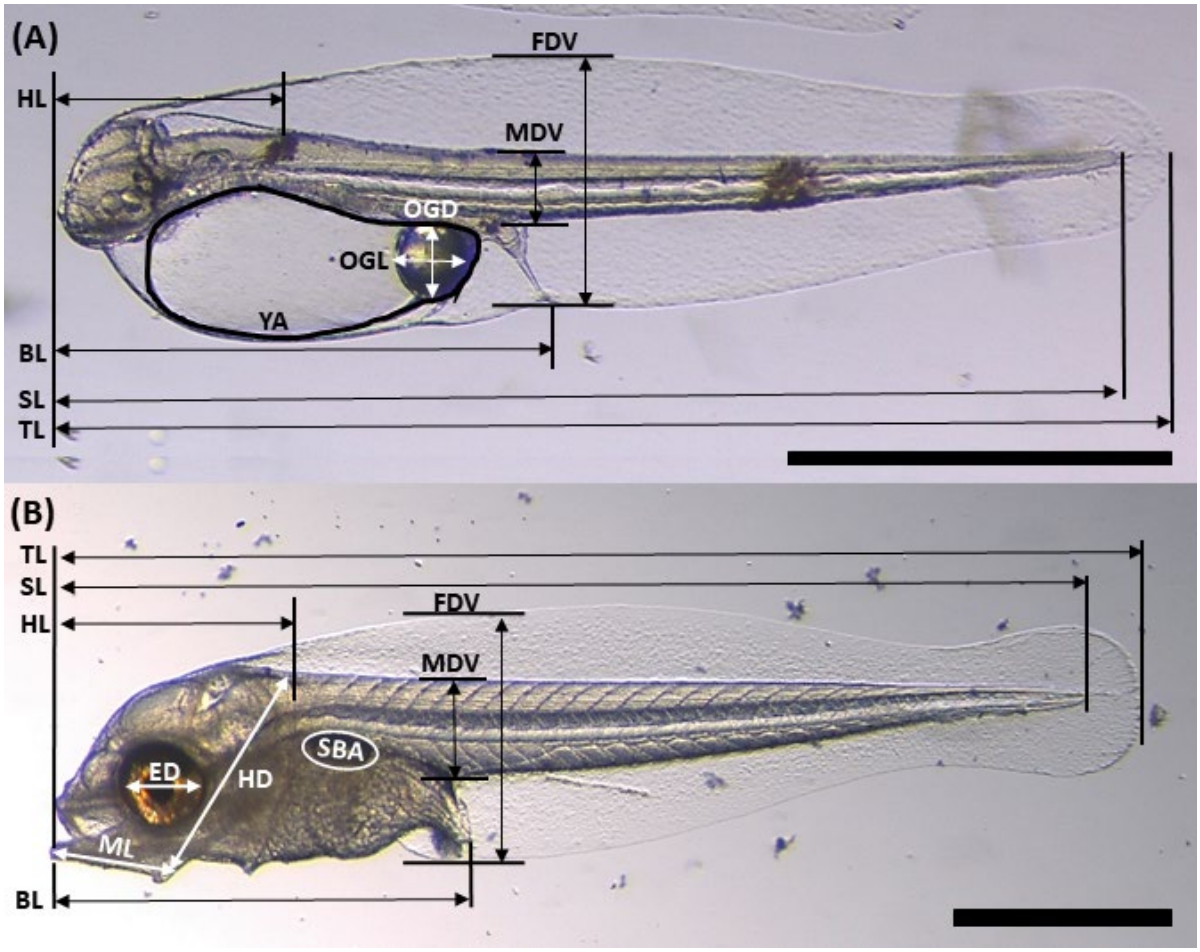


**Figure 2.1**. Morphological traits measured from larval *Chrysophrys auratus* at (a) 1 dph and (b) 16 dph. Scale bars represent 1mm. Standard length (SL), total length (TL), body length (BL), muscle depth at vent (MDV), fin depth at vent (FDV), eye diameter (ED), mandible length (ML), yolk area (YA), oil globule diameter (OGD), oil globule length (OGL), head length (HL), head depth (HD).

### 2.3.5 Statistical analysis

Separate Principle Component Analyses (PCA) were conducted on the morphological data from larvae at 1 dph and 16 dph. As growth metrics can be highly correlated the PCA was conducted to reduce the complexity of multivariate data and to identify the variables that accounted for the largest variation. The traits used in the PCA for 1 dph were BL, SL, TL, HL, FDV, MDV, OGL, OGD, and YA. In the PCA for 16 dph the traits used were BL, SL, TL, HL, MDV, FDV, ML, ED, HD, and SBA. For both 1 dph and 16 dph only two principle components were required to explain over 70% of the variation. Following the PCA, linear mixed effects models (LME) were used to test for significant effects of temperature and $CO_2$ on PC1 and PC2. Temperature and $CO_2$ were treated as fixed factors, and rearing tank was included as a random factor. In addition to the LME on PC scores, each morphological trait was tested separately as a dependent variable to further explore the potential effects of temperature or $CO_2$ on specific morphometric traits. A generalised linear model (GLM) was used to compare survival data among treatments. Temperature and $CO_2$ were fixed factors. Stocking density was used as a weight in the model, where numbers of eggs stocked in each tank were used for survival at 1 dph and numbers of individuals at 1 dph transfer were used for survival at 16dph. Survival at 1 dph and from 16 dph were tested as dependant variables. A Gaussian distribution was used for both GLMs. All analysis was conducted in R (R Core Team, 2014) using the LME4 package (Bates et al., 2015) for the LMEs and the GLM package was used for the GLMs. All models met the assumptions of the relevant tests. This was confirmed by accessing the residuals, goodness of fit, and checking dispersion.

## 2.4 Results

### 2.4.1 Morphology

Temperature was the primary driver of morphological differences at 1 dph (Fig. 2.2). There was a clear separation in individuals reared at either 18°C or 22°C, but no division related to $CO_2$ treatment. The division between temperatures was seen on the PC1 axis ($t_{12}$=11.527, P=<0.001), which described 65% of the variation. By viewing both the vectors, and the individual LMEs per traits, it was observed that fish reared at 22°C were larger in TL, HL, SL, MDV and FDV, but were smaller in BL, OGL, OGD and YA compared to fish reared at 18°C. Specifically, SL was 7% longer, but body length was 5% shorter, in elevated temperature. Elevated temperature also produced increases in MDV (14%), HL (12%), and FDV (11%). Conversely, there were decreases in YA (65%) and OGL (16%) at elevated temperature (See Table A2.1 for complete statistical results).

At 16 dph morphological differences between larval fish were only observed depending on water temperature (Fig. 2.2). Over 81% of the variation could be explained on the PC1 axis ($t_{12}$=-7.887, P=<0.001). By viewing both the vectors, and the individual LMEs per traits, it was observed that fish reared at 22°C were larger in every trait measured. Specifically, SL and TL were 12% longer. All other traits (MD, ML, ED, FDV, BL, HL, and SBA) were 21-24% larger with the exception of MDV, which was 63% larger, and HD, which was 35% larger. Elevated $CO_2$ had no significant effects on morphological traits and there were no significant interactions between $CO_2$ and temperature (See Table A2.2 for complete statistical results).

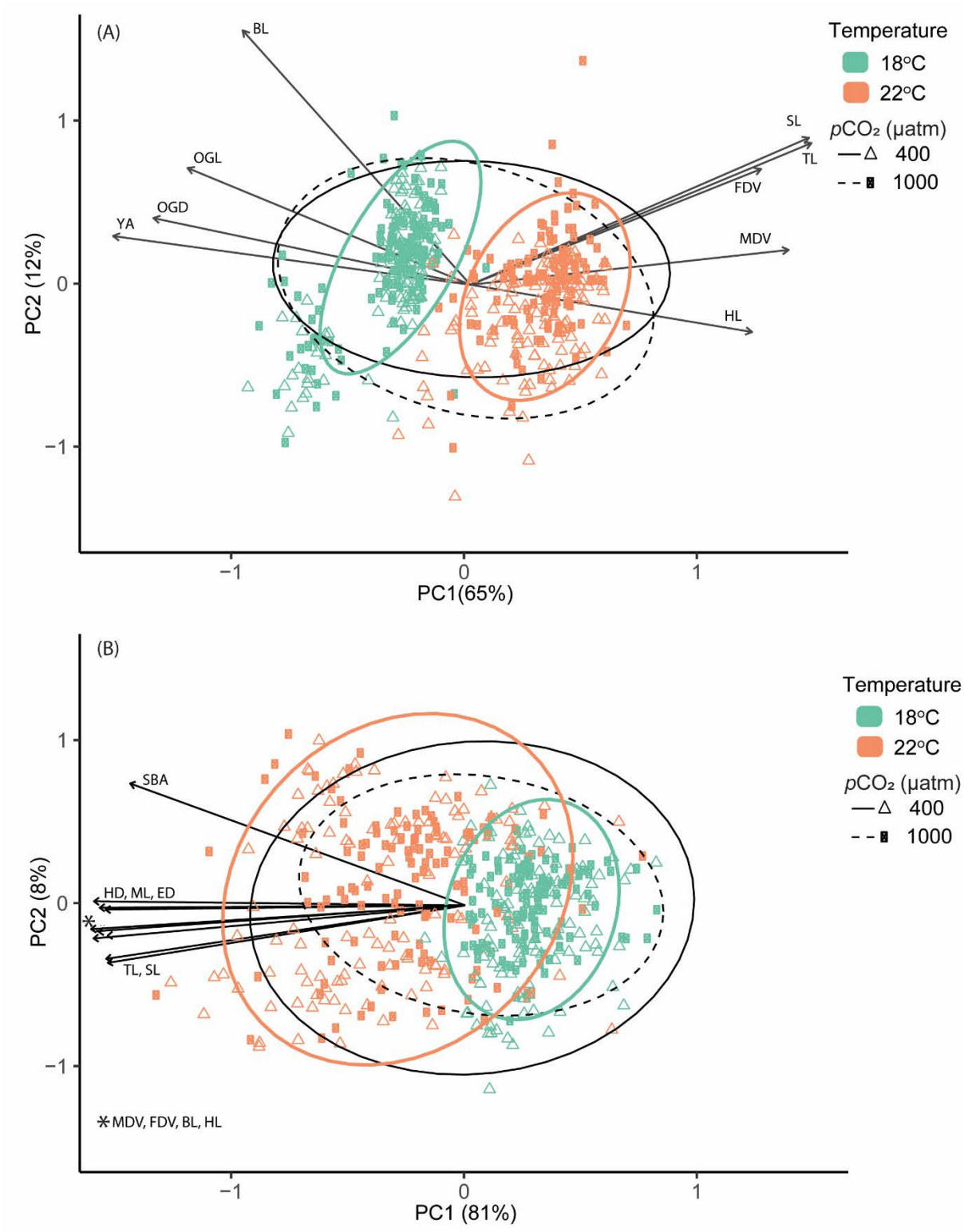


**Figure 2.*2*** PCA of morphometric data for larval *Chrysophrys auratus* at (A) 1 dph and (B) 16 dph. Rings represent 95% confidence that data fall with for 18°C (green), 22°C (orange), 400 $pCO_2$ (solid black), and 1000 $pCO_2$ (dashed black). Morphological traits are indicated by arrows. Standard length (SL), total length (TL), body length (BL), muscle depth at vent (MDV), fin depth at vent (FDV), eye diameter (ED), mandible length (ML), yolk area (YA), oil globule diameter (OGD), oil globule length (OGL), head length (HL), head depth (HD)

## 2.4.2 Survival

Survival of larvae was significantly affected by elevated $CO_2$ at both 1 dph ($F_{1,11}$=5.845, P=0.034) and 16 dph ($F_{1,11}$=6.791, P=0.024) (Fig.2.3). Survivorship was increased by 27% and 56% in elevated $CO_2$ at 1 dph and 16 dph, respectively. Survivorship was not significantly affected by elevated temperature at either 1 dph or 16 dph, nor were there any significant interactions (See Table A2.3 for complete statistical results).

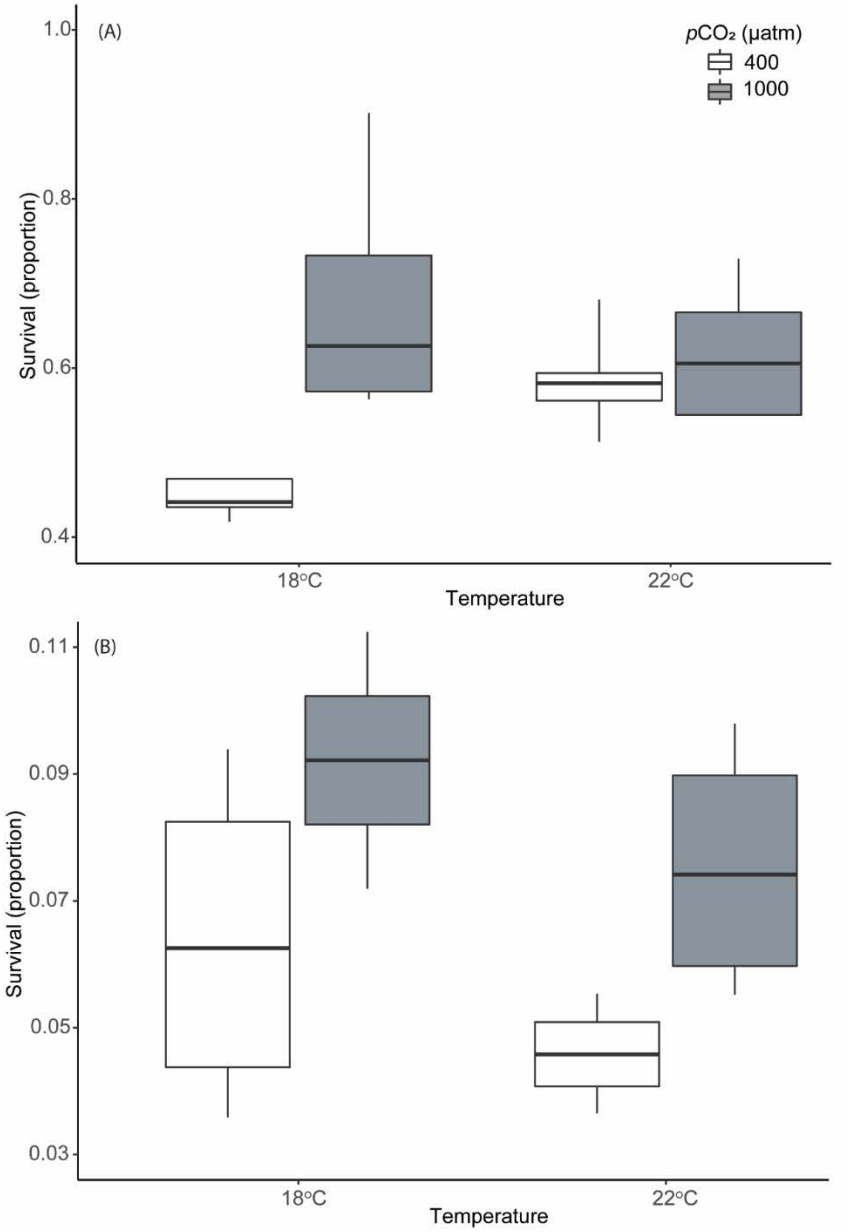


**Figure 2.3** Proportional survival of *Chrysophrys auratus* from hatching to 1 dph (A) and from 1 dph to 16 dph (B) depending on water temperature and $CO_2$.

## 2.5 Discussion

As climate change advances, the sensitive early life stages of fish will likely be the first to be affected (Houde, 1989; Pankhurst &Munday, 2011), and these effects could flow through and influence the dynamics of adult populations (Rijnsdorp et al., 2009; Pörtner &Peck, 2010; Petitgas et al., 2013). I found that elevated temperature significantly increased the growth of larval snapper in the first 16 days post-hatching, whereas elevated $CO_2$ had no detectable effects on morphological development. Conversely, elevated $CO_2$ significantly increased the survival of larval snapper, at both 1 dph and 16 dph, while elevated temperature had no significant effects. These results highlight how these two environmental drivers can affect different fitness-associated traits during early life, and that the effects of higher temperature and elevated $CO_2$ need not be detrimental. For this population of *C. auratus*, it seems that exposure to predicted future temperatures and $CO_2$ levels could have positive effects on individual fitness, at least under experimental conditions, with increased growth at elevated temperature and positive effects on survival under elevated $CO_2$.

Elevated temperature has been shown to have a range of effects on larval fish. Typically, the growth rate of larvae increases in warmer water (Houde, 1989; Pepin, 1991) due to faster rates of chemical reactions and metabolism. This is reflected in our findings where elevated temperature produced larger individuals at both 1 and 16 dph. Reduced yolk area and oil globule size in 1 dph larvae at elevated temperature indicate that this increased growth rate is initially fuelled by endogenous reserves. Presumably, increased growth is supported by a higher feeding rate at elevated temperature once endogenous reserves are exhausted. Interestingly, while elevated temperature increased overall length

in 1 dph larvae, they had a significantly shorter body length (BL). This may be due to a change in energy allocation (Stallings et al., 2010; Mogensen & Post, 2012), where caudal peduncle development is prioritised in elevated temperature, or it could also be due to yolk consumption bringing forward the measurement landmark (anus); however, I cannot distinguish between these alternatives.

In this study I found that a relatively large increase in temperature, modelled off a "worst case" climate change scenario of +4$^{o}$C, had a positive effect on larval growth. This accelerated growth would reduce the time spent in the most vulnerable stages and potentially reduce predation pressure (Almany & Webster, 2006), as well as expanding their prey diversity and ability to compete for habitat (Shulman, 1985; Clifton, 1990; McCormick & Weaver, 2012). However, a caveat is that larvae in this experiment had unlimited access to food, which would unlikely be experienced in the wild. Higher metabolic rate (McMahon et al., 2020) and higher growth rates must be fuelled by additional food resources, which for larval fishes (e.g. zooplankton) in nature are patchy in space and time (Owen, 1989; MacKenzie & Leggett, 1991). Therefore, faster growing larvae may also be more susceptible to starvation if they cannot locate sufficient food (Munday et al., 2009). Whether faster growth would lead to higher survivorship (escape from predation) or lower survivorship (risk of starvation) will depend on how the distribution and abundance of food resources for larval fishes are affected by elevated temperature. Nevertheless, these results suggest that elevated temperature, up to +4$^{o}$C above the average summer temperature, is not necessarily detrimental to larval growth and development in this population of *C. auratus*.

Elevated $CO_2$ had a significant effect on the survival of larvae, but no effect on morphological traits. These results are consistent with previous research where similar

elevated $CO_2$ levels had no effect on the growth of larval fish (Bignami et al., 2013; Frommel et al., 2012; Hurst et al 2013; Munday et al., 2015) and induced increased survival (Pope et al., 2013, Sswatt et al., 2018). However, there is a wide variety of responses to elevated $CO_2$ and not all early life stages of marine organisms respond positively as I found here (Baumann et al., 2012; Pimentel et al., 2014; Stiasny et al., 2016; Murray et al., 2019). One potential reason why elevated $CO_2$ could have a positive or benign effect on *C. auratus* is that this species tends to spawn in inshore waters and live around rocky reefs, which may naturally see wide environmental fluctuations in pH (Law et al., 2018). Indeed, careful experimentation has shown that survivorship of larval Atlantic silverside (*Menidia menidia*) under elevated $CO_2$ conditions depends on the spawning conditions experienced by adults, with larvae resilient to elevated $CO_2$ when adults spawn at periods of high environmental $CO_2$ (Murray et al., 2014; Bauman et al., 2018). Regardless of the reason why survivorship was higher in larval snapper, an increase of 25 to 55% (at 1 dph and 16 dph respectively) in the elevated $CO_2$ treatment is important because shifts in larval survival can have a direct effect on the rate of recruitment to the adult population. A potential caveat for our results is that larvae in this experiment had unlimited access to food, which could potentially offset any increase in the energetic cost of developing in a high $CO_2$ environment, and might thus influence survivorship under laboratory conditions.

While we are beginning to develop our understanding of the effects that warming and ocean acidification have on fish early life stages, the reality is that these drivers will occur simultaneously. Here, both drivers produced positive effects, but on different traits. Growth rate was higher under elevated temperature and survivorship was higher under elevated $CO_2$, which would likely have additive effects on the overall fitness of larvae. Different outcomes have been observed for other traits. For example, elevated

temperature (+4°C) increases the swimming ability of larval *C. auratus,* but elevated $CO_2$ (1000 µatm) decreased swimming ability by a similar amount (McMahon et al., 2020). In another example, larval cod mortality was ~3 times higher in elevated $CO_2$ treatments, but this was reversed when temperature was also increased (Kunz et al., 2016). This highlights the importance of investigating the effects of multiple environmental changes in order to better understand how climate change may affect marine organisms. For larval *C. auratus*, the positive effects on both growth and survival from these two environmental drivers would likely translate to higher individual fitness and possibly flow on to benefit population recruitment.

This study found primarily positive effects on larval growth and survival under a strong climate change scenario (RCP8.5: +4°C temperature and >1000 µatm $CO_2$) in a northern New Zealand population of *C. auratus*. Growth increased in the elevated temperature treatment, which would likely translate to increased survival in the wild as they would pass through the most vulnerable life stages faster. Additionally, survival increased by 55% at 16 dph under elevated $CO_2$, which would have a positive effect on recruitment to the adult population. Other populations of *C. auratus* in New Zealand, at higher latitudes, experience cooler average temperatures than those in the far north of the North Island. Therefore, they may be equally tolerant to elevated temperature, especially as thermal tolerance ranges typically increase towards higher latitudes (Sunday et al., 2014). Although larvae may tolerate warmer conditions, it should be noted that *C. auratus* typically spawn in spring-summer between 18-20°C (Scott & Pankhurst, 1992; Francis, 1994; Wakefield, 2010), suggesting that the timing of spawning may be more sensitive to temperature increases than the larval stage of this species. On the east coast of Australia, the timing of *C. auratus* spawning is tightly linked to temperature, with the lowest latitude

populations spawning in the coolest part of the year, whereas higher latitude populations spawn later in the year at a similar temperature (Sheaves, 2006). Consequently, it is likely that the timing of snapper spawning in New Zealand will shift towards the cooler months, earlier in the year, as the ocean continues to warm. This information, combined with the specific knowledge about how elevated temperature and $CO_2$ affect larval *C. auratus,* gained from this study will help inform population-dynamics models to more accurately predict how this economically and ecologically important species will cope as climate change advances.

# Chapter 3: Elevated $CO_2$ and heatwave conditions affect the aerobic and swimming performance of juvenile Australasian snapper

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## 3.1 Abstract

As climate change advances, coastal marine ecosystems are predicted to experience increasingly frequent and intense heatwaves. At the same time, already variable $CO_2$ levels in coastal habitats will be exacerbated by ocean acidification. High temperature and elevated $CO_2$ levels can be stressful to marine organisms, especially during critical early life stages. Here, I used a fully cross-factored experiment to test the effects of simulated heatwave conditions (+4°C) and elevated $CO_2$ (1000 µatm) on the aerobic physiology and swimming performance of juvenile Australasian snapper, *Chrysophrys auratus*, an ecologically and economically important mesopredatory fish. Both elevated temperature and elevated $CO_2$ increased resting metabolic rate of juvenile snapper, by 21-22% and 9-10% respectively. By contrast, maximum metabolic rate was increased by elevated temperature (16-17%) and decreased by elevated $CO_2$ (14-15%). The differential effects of elevated temperature and elevated $CO_2$ on maximum metabolic rate resulted in aerobic scope being reduced only in the elevated $CO_2$ treatment. Critical swimming speed also increased with elevated temperature and decreased with elevated $CO_2$, matching the results for maximum metabolic rate. Periods of elevated $CO_2$ already occur in the coastal

habitats occupied by juvenile snapper, and these events will be exacerbated by ongoing ocean acidification. Our results show that elevated $CO_2$ has a greater effect on metabolic rates and swimming performance than heatwave conditions for juvenile snapper and could reduce their overall performance and potentially have negative consequences for population recruitment.

## 3.2 Introduction

Accumulation of anthropogenic carbon dioxide in the atmosphere is causing rapid warming at the earth's surface. In the oceans, average sea surface temperature has increased by ~0.5˚C since the 1950's (Rhein et al., 2013) and is projected to increase up to 4˚C by the end of the century (Collins et al., 2013). Climate change is also increasing the frequency, intensity, and duration of marine heatwaves, which are defined as prolonged periods of anomalously high sea surface temperature (Oliver et al., 2018). Thirty five percent of coastal marine environments have already experienced more intense and frequent extreme temperatures since the start of the 20$^{th}$ century (Lima and Wethey 2012), with heatwaves expected to further increase in severity and frequency as climate change advances (King et al., 2016; Frölicher et al., 2018). In addition to causing rapid warming, higher atmospheric $CO_2$ concentrations increase the $CO_2$ content of seawater, by increasing the uptake of $CO_2$ at the ocean's surface (Doney et al., 2009). In coastal habitats, this additional $CO_2$ exacerbates periods of high $pCO_2$ and low pH seawater that already occur in some nearshore environments due to upwelling of $CO_2$-rich water and nutrient inputs that stimulate biological activity (Feely et al., 2008, Hofmann et al., 2011; Green & Zeldis, 2015). Consequently, marine organisms in coastal habitats may already be subjected to episodes of high $CO_2$ that exceed predictions for the open ocean by the end of the century, and which

will be exacerbated by the ongoing uptake of $CO_2$ from the atmosphere (Shaw et al., 2013; Hoegh-Guldberg et al., 2014; Waldbusser & Sailsbury 2014).

Most marine organisms are ectotherms, whose physiological functions are affected by temperature change, especially when outside the range usually experienced. Higher temperature increases biochemical reactions and cellular processes, and consequently increases metabolic rate (Gillooly et al 2001; Schulte, 2015). High seawater $pCO_2$ can also affect physiological functions of marine organisms because it raises plasma $pCO_2$, which acts to acidify the organism's blood and tissue (Pörtner et al., 2004). As most cellular processes function optimally within a narrow pH range, many marine organisms actively regulate their acid-base balance to prevent acidosis in high $CO_2$ conditions (Pörtner et al., 2004; Heuer & Grosell, 2014). However, the process of ion exchange to maintain a stable pH can be energetically costly (Melzner et al., 2009b; Heuer & Grosell, 2016). Therefore, high temperature and elevated $CO_2$ have the potential to increase the basal energy requirements an organism needs to survive (Pörtner & Farrell 2008; Enzor et al., 2013).

Understanding how environmental stressors, such as elevated temperature and $CO_2$, affect the metabolic rates of marine organisms is key to determining the impact of climate change on their populations (Pörtner & Knust, 2007; Rijnsdorp et al., 2009; Pörtner & Peck, 2010). As metabolism is difficult to directly measure, the rate of oxygen consumption is commonly used as a proxy for metabolic rate (Roche et al., 2013; Rummer et al., 2016). Typically, resting metabolic rate (RMR) of an organism is estimated by measuring resting oxygen consumption ($MO_{2rest}$) and the maximum metabolic rate (MMR) is estimated by measuring maximum oxygen consumption ($MO_{2max}$). The difference between maximum and minimum oxygen consumption is used to calculate aerobic scope ($MO_{2max}$ - $MO_{2rest}$ = absolute aerobic scope). Aerobic scope is a proxy for an individual's

capacity to undertake aerobic activities, such a swimming and foraging, therefore reductions in aerobic scope can reduce physical performance (Priede, 1985; Pörtner & Farrell, 2008; Johansen & Jones, 2011). For example, aerobic scope in salmon was positively correlated with swimming performance needed during migration to natal spawning sites (Eliason et al., 2011). Generally, increased water temperature raises $MO_{2rest}$, whereas $MO_{2max}$ increases to an optimal temperature and then declines at higher temperatures as the cardiovascular system is no longer able meet oxygen demands (Pörtner & Knust, 2007; Pörtner, 2010). Therefore, aerobic scope may initially increase with rising temperature, but then declines above the optimum temperature for $MO_{2max}$ (Farrell et al., 2008; Eliason et al., 2013). On the other hand, elevated $CO_2$ has displayed variable effects on $MO_{2rest}$, $MO_{2max}$ and aerobic scope. While there appears to be no overall effect of elevated $CO_2$ on aerobic scope when all studies are considered together (Lefevre, 2016; Cattano et al., 2018), different studies have reported increases ($MO_{2rest}$: Enzor et al., 2013; Laubenstein et al., 2018; $MO_{2max}$: Silva et al., 2016; Aerobic Scope: Rummer et al., 2013; Grans et al., 2014), decreases ($MO_{2rest}$: Rummer et al., 2013; Pimental et al., 2014; Aerobic Scope: Munday et al., 2009), and no effect ($MO_{2rest}$: Strobel et al., 2012; Couturier et al., 2013; $MO_{2max}$: Pope et al., 2013; Aerobic Scope: Laubenstein et al., 2018) on $MO_{2rest}$ , $MO_{2max}$ and aerobic scope. Thus, it seems that the effects of elevated $CO_2$ on fish metabolic rates can be species-specific.

Coastal habitats are more susceptible to heatwaves and elevated $CO_2$ events than the open ocean (Hoegh-Guldberg et al., 2014). Additionally, nearshore environments are expected to experience more severe and frequent heatwaves in the future, even under the most conservative $CO_2$ emission scenarios (King et al., 2016; Frölicher et al., 2018). In the Indo-Pacific region, notable heatwaves have occurred on Ningaloo Reef, Western Australia

in 2011 (+3$^{o}$C average over five weeks) (Pearce & Feng, 2013), the northeast Pacific in 2013-2015 (reaching +2.5$^{o}$C, February 2014) (Di Lorenzo & Mantua, 2016) and across northern Australia in 2016-2017 (reaching +2$^{o}$C and lasting for 3 months) (Benthuysen et al., 2018). The 2016-2017 heatwave caused unprecedented back-to-back mass coral bleaching and mortality on the Great Barrier Reef (+1-2$^{o}$C for over 4 weeks) (Hughes et al., 2017; Spinks et al., 2019). In the summer of 2017/2018, New Zealand recorded one of the most pronounced and extensive marine heatwaves on record, with water temperatures up to 4$^{o}$C higher than average during three distinct peaks, each lasting more than 5 days, and an average 2$^{o}$C higher temperature throughout January and February (NIWA, 2018; Salinger et al., 2019). Importantly, these heatwave events are becoming more frequent during the critical recruitment times for many fish species (Caley et al., 1996), which could have serious consequences for the replenishment of fish populations.

In contrast to the open ocean, some coastal habitats experience substantial fluctuations in pH, often reaching levels equivalent to worst case climate change scenarios (Hofmann et al., 2011; Price et al., 2012; Law et al., 2018). These fluctuations can be caused by upwelling of $CO_2$ rich seawater (Feely et al., 2008; Fassbender et al., 2011), nutrient input that enhances biological activity and respiration (Borgesa & Gypensb, 2010; Cai et al., 2011; Duarte et al., 2013) and limited water exchange in bays and shallow habitats (Middelboe & Hansen, 2007; Feely et al., 2010; Challener et al., 2016). For example, pH (total hydrogen scale $pH_{total}$) may range between 7.6-8.4 (~100-1200 µatm $pCO_2$) over 24 hours in some shallow reef environments (Shaw et al., 2012) and can range between 7.7-8.3 (~200-1000 µatm $pCO_2$) in bays and estuaries with low pH events lasting for weeks and, in some cases, becoming persistent over multiple months (Hofmann et al., 2011, Law et al., 2018). The fluctuation in pH and $pCO_2$ of coastal habitats are also expected to be exacerbated as

climate change advances due to the change in the buffering capacity of seawater as it takes up additional $CO_2$ from the atmosphere (Shaw et al., 2013; McNeil & Sasse, 2016). Therefore, marine organisms recruiting into these coastal habitats are expected to be confronted with significantly more extreme pH and $pCO_2$ in the future.

The Australasian snapper, *Chrysophrys auratus*, is a commercially and recreationally important reef mesopredator found in Australian and New Zealand waters (MPI 2013; GBRMPA, 2014). It inhabits nearshore and estuarine environments during its early life stages, utilizing complex habitat structure for protection (Parsons et al., 2016; Lohrer et al., 2018). Early life stages of fish are generally more sensitive to environmental stressors than adults as the demands of growth and maintenance are highest during this stage (Post & Parkinson 2001; Stallings et al., 2010). Additionally, the early juvenile stage is a time when aerobic performance and swimming ability are critically important for migrating to settlement habitat and avoiding predators (Dudley et al., 2000; Almany & Webster, 2006). Since juvenile snapper use nearshore and estuarine environments there is a high chance that they will increasingly be exposed to high temperature and elevated $pCO_2$ levels in the future.

This study aimed to determine how the aerobic capacity and swimming performance of juvenile *C. auratus* is affected by elevated temperature and $CO_2$ levels that are already occurring in some coastal habitats, and which are predicted to become more frequent and extreme as climate change progresses. For example, the Firth of Thames is a major recruitment habitat for juvenile snapper in northern New Zealand (Parsons et al., 2014). Average summer water temperature is approximately 18$^{o}$C in this region but can reach 22$^{o}$C under extreme conditions (Evans & Atkins, 2013; NIWA, 2018). Seawater pH in the Firth of Thames varies from a high around 8.3 down to at least 7.7 (Law et al., 2018),

equivalent to a $pCO_2$ range of approximately 200-1000 µatm. Importantly, while elevated temperature or $CO_2$ can each affect fish performance, they can have additive, synergistic, or antagonistic effects when they occur together (Côté et al., 2016). Therefore, I used a fully crossed 2 x 2 experiment where I exposed juvenile snapper from the northern New Zealand population to current-day average summer conditions (18$^{o}$C and 400 µatm $CO_2$) and levels possible in near-shore environments during a heatwave (22$^{o}$C and 1000 µatm $CO_2$) for 21 days, from 21-42 days post hatching (dph). The exposure period was chosen to start from 21 dph as this is when juveniles being to settle from the pelagic environment into near-shore habitats (Parsons et al., 2014). I then tested the metabolic performance of juveniles, by measuring oxygen consumption as a proxy for RMR, MMR and absolute aerobic scope. Metabolic rates indicate the energy requirements of an individual that can underpin a number of fitness related traits (Burton et al., 2011). For instance, swimming performance is positively correlated with $MO_{2max}$ and aerobic scope in a range of fishes (Brett et al., 1964; Eliason et al., 2011; Johansen and Jones, 2011). Therefore, I also tested the maximum swimming performance of juveniles, using a *U*crit test, to determine whether any effects on aerobic performance affected key aspects of the fish's physical performance.

## 3.3 Methods

### 3.3.1 Aquarium setup

This study was conducted at the National Institute of Water and Atmospheric Research Northland Marine Research Centre (NMRC), in Ruakaka, New Zealand. Brood stock fish (n=39) were captured from the wild population of *C. auratus* by longline fishing in Bream Bay, adjacent to the NMRC, during September of 2017. The brood stock were split between two 20 $m^3$ circular tanks at the ambient temperature when they were collected (~16˚C). Each tank was supplied with 130 L $min^{-1}$ ambient seawater, filtered to 10 µm, and followed the natural temperature increase up to the summer average (18˚C), at which point it was maintained for spawning (Table 3.1). Spawning occurred naturally within brood stock tanks during January, 2018. To maximize genetic variation in the experiment, eggs were collected from both brood stock tanks in even proportions. Eggs were collected using an external egg collector as described by Moran et al., (2007), with a 500 µm mesh net to retain eggs from the surface overflow of each tank. An equal proportion of floating eggs from both contributing tanks were mixed, rinsed with oxygenated sea water for 5 min, and disinfected with Tosylchloramide (chloramine-T) at 50 ppm for 15 min. Eggs were then rinsed with seawater and evenly distributed between two 400 L conical hatching tank. Each tank was stocked with approximately 100,000 fertilized eggs and received flow-through seawater at ambient temperature (18˚C) at a flow rate of 4 L $min^{-1}$. Photoperiod was maintained at 14 h light 10 h dark. Snapper eggs hatch in 24-48 hours at ambient summer temperatures at Bream Bay. Newly hatched larvae remained in the conical rearing tanks until 2 days post-hatching (dph). Larvae were not fed during this period as they rely on their endogenous

reserves (Battaglene & Talbot, 1992). Any dead eggs, larvae, and egg shells were removed daily by draining from an outlet at the bottom of the rearing tank. At 2 dph larvae were transferred to two 1500 L tanks located in the same facility for grow-out. These tanks received flow-through ambient seawater (18˚C, 400 µatm$CO_2$) at a maximum flow of 20 L $min^{-1}$ per tank. Larvae were grown out at ambient conditions until 21 dph, at which point they were transferred to the experimental treatments. Larvae were fed enriched rotifers (INVE, Selco S.Presso) three times a day (0800, 1200, and 1600) from 2dph. Larvae were transitioned onto enriched artemia between 20-26dph with feeding twice a day (0800 and 1600) until 42dph.

**Table 3.1** Mean (± SD) of experimental seawater chemistry parameters for Australasian snapper (*Chrysophrys auratus*) brood stock tank and juvenile treatments. Brood stock tanks were measured during the week of spawning at the start, middle, and end of the week. Temperature and $pH_{total}$ were measured daily in each rearing tank over the 21 day experiments. Total alkalinity and salinity were measured at the start of the experiment and then every 7 days. *p*$CO_2$ was estimated from these parameters in CO2SYS.

| Treatment | Salinity (ppt) | Temperature (°C) | Total Alkalinity (mmol/kgSW) | pH (Total) | *p*$CO_2$ (µatm) |
|---|---|---|---|---|---|
| **Brood stock** | 35.60 ±0.05 | 18.03 ±0.04 | 2154 ±6 | 7.88 ±0.01 | 583 ±14 |
| **Ambient Temperature Ambient $CO_2$** | 35.39 ±0.29 | 18.05 ±0.06 | 2299 ±9 | 8.02 ±0.02 | 425 ±19 |
| **Ambient Temperature Elevated $CO_2$** | 35.27 ±0.28 | 18.05 ±0.07 | 2308 ±8 | 7.69 ±0.02 | 1011 ±47 |
| **Elevated Temperature Ambient $CO_2$** | 35.34±0.21 | 22.03 ±0.07 | 2313 ±9 | 7.99 ±0.02 | 465 ±26 |
| **Elevated Temperature Elevated $CO_2$** | 35.29 ±0.16 | 21.96 ±0.20 | 2316 ±10 | 7.69 ±0.01 | 1027 ±32 |

### 3.3.2 Experimental design

At 21 dph larval fish were transferred into a fully crossed experimental design with 2 x temperatures and 2 x $CO_2$ levels. The temperature treatments were 18°C, which is the temperature where spawning is at its peak for this species (Scott & Pankhurst, 1992; Sheaves, 2006; Wakefield, 2010) and 22°C, which is close to the maximum temperature recorded in the region (Evans & Atkins, 2013) and matching heatwave conditions in 2017/2018 (NIWA, 2018). The $CO_2$ treatments were an unmanipulated ambient of ~400 µatm and an elevated treatment of ~1000 µatm, which is within the current range of pH fluctuations in habitats used by juvenile *C. auratus* in New Zealand (Law et al., 2018). Treatments were duplicated (four treatments each with two replicate rearing tanks) and each rearing tank had independent temperature and $CO_2$ control as per best practice (Cornwall & Hurd, 2016). Temperature was controlled by 1Kw bar heaters in 200 L sumps tanks, mixed with recirculating submersible pumps, and maintained to ±0.1$^{o}$C. The elevated ~1000 µatm treatment was achieved by dosing $CO_2$ to the appropriate pH set point in the same 200 L sump tanks. $CO_2$ dosing was regulated by a pH computer (Aquamedic) connected to a pH probe and a solenoid valve, which maintained the desired pH by slowly dosing $CO_2$ when pH deviated above the set point. Water was delivered at 4 L min$^{-1}$ from the sump tank to the respective rearing tank.

Approximately 1000 larval snapper (21 dph) were stocked to each rearing tank at ambient conditions. The high temperature and $CO_2$ treatments were then turned on to produce a gradual change over a 24 hour period. Larvae were held under these treatments for a further 21 days to 42 dph, during which they metamorphose into juveniles, at which point they underwent physiological assays. The $pH_{total}$ of each rearing tank was measured daily by spectrophotometry (Hach, DR3900) with cresol purple dye (Clayton & Bryne, 1993).

Temperature was measured daily with a digital thermometer (Comark C22). Water samples were taken from each tank at the start of the experiment and then every seven days throughout the experiment (21, 28, 35, 42dph) for total alkalinity (TA) analysis. Water samples were immediately poisoned with a saturated solution of mercuric chloride (0.05 % of the sample volume) and later analysed at the University of Otago Research Centre for Oceanography, Dunedin, New Zealand. Alkalinity was determined by potentiometric titration in a closed cell (Dickson et al*.,* 2007) using a Metrohm Dosimat burette (model 765, Metrohm, Switzerland), a Fluke model 8846A voltmeter, and with 0.2M HCl (nominal concentration, fortified with NaCl to the ionic strength of seawater) added in 0.1 mL steps. Samples were water-jacketed at 25 °C. TA was determined from the titration data using a least squares minimisation technique and calibrated with certified reference material (Prof. A.G. Dickson, Scripps Institution of Oceanography, U.S.). The salinity of each sample was measured with a YSI Pro30 salinity probe. The daily $pCO_2$ of each rearing tank was then calculated in CO2SYS (Pierrot et al., 2006) from the measured values of $pH_{total}$, temperature, TA and salinity and using the constants of Mehrbach et al., (1973), refit by Dickson and Millero (1987) (Table 3.1).

### 3.3.3 Respirometry

Aerobic performance was measured using intermittent flow respirometry (Clark et al., 2013; Svendsen et al., 2016) in 15 fish from each treatment (60 fish total). Fish were fasted for 18 hours before testing and were tested in their respective rearing treatment. $MO_{2rest}$ was used as a proxy for RMR and $MO_{2max}$ was used to estimate MMR, whilst absolute aerobic scope was calculated by subtracting $MO_{2rest}$ from $MO_{2max}$ for each fish.

Respirometry was conducted in purpose-built intermittent-flow respirometry chambers for juvenile fish (between 13 and 14.5 ml per closed system), submerged in aquaria within the fish's respective experimental treatment water. Submersible pumps fitted to each chamber supplied a continuous water flow from the surrounding water bath through the chambers. Activity was reduced in the respiration chambers by using appropriately sized chambers to minimise movement and by shading each chamber from visual simulants. A purpose built python program, AquaResp V3.0, was used to control the timing measurement cycle. This consisted of a four minute measurement period, two minute flushing period, and a one minute wait period, which was repeated over a 4 hour trial duration. The $O_2$ consumption rates were measured during the intervals of interrupted water flow with a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany), which the AquaResp program recorded during the measurement periods. The entire measurement period was used to calculate MO2 provided that the slope $R^2$ was >0.95. Over 98% for measured slopes across all treatments were above this threshold. Immediately before respirometry commenced, each fish was swum for 5 minutes at 10 body lengths per second in a swimming flume (see below). Five minutes was enough to illicit unsteady swimming and anaerobic muscle use. Fish were then placed immediately into respirometry chambers allowing for $MO_{2max}$ to be measured immediately following exercise. Fish then remained in the chambers while recovering back to $MO_{2rest}$ over 4 hours, with the majority of juveniles reaching stable $MO_{2rest}$ within 1-2 hours (Fig A1.1). At the end of each trial, wet mass was taken for each individual to adjust the $MO_2$ calculations for the individual's specific weight. $MO_{2max}$, $MO_{2rest}$ and total aerobic scope of individuals in mg $O_2$ $kg^{-1}$ $h^{-1}$ were calculated using the equation:

$$\mathbf{MO_2 = K*V*\beta / M}$$

where *K* is the linear rate of decline ($kPah^{-1}$) in the oxygen content over time (h) in the respirometer; *V* is the volume of the respirometer in L, which is adjusted for the volume of the fish; β is the solubility of oxygen in water at a specific temperature and salinity (mg $O_2$ $L^{-1}$ $kPa^{-1}$); and *M* is the body mass of the fish (kg). Blank measurements were taken for each chamber at the start and end of each trail to calculate any background respiration. Background respiration did not exceed 45 mg $O_2$ $kg^{-1}$ $h^{-1}$ in any trail. Linear regressions were then used to calculate background respiration over the trail, which was used to adjust the $MO_2$ measurements for each fish. $MO_{2rest}$ was determined by using the mean of the lowest normal distribution for $MO_2$ values (Behrens & Steffensen, 2006; Chabot et al., 2016).

### 3.3.4 Critical swimming speed

Critical swimming speed (*U*crit) was measured to compare swimming performance among treatments. Individual fish (13 fish per treatment, 52 fish total) were swum against a water current in a Brett type swimming tunnel (Brett, 1964). *U*crit was measured in different individuals to those used in respirometry trials (above). The water was maintained at the desired temperature and $pCO_2$ by constant flow-through of the respective treatment water of each fish from the main system. A single fish was placed into the swim tunnel and allowed 10 minutes to habituate at a water speed of one body length per second (bl $s^{-1}$). Following standard procedure (Brett, 1964), the water flow was then increased by increments of ~2 bl $s^{-1}$ (~3 cm $s^{-1}$). Each flow speed was maintained for 10 minutes, after which the water speed was increased by another 2 bl $s^{-1}$. These 10 minute intervals, increasing by 2 bl $s^{-1}$ each time, were conducted until the fish was no longer able to

maintain its position in the water current. The trial was stopped when an individual rested against the rear screen of the flume for 5 seconds, because fish have the potential to rest momentarily and then burst back into swimming. After fatiguing the water flow was stopped and the fish was allowed 10 minutes to recover before it was removed from the swim tunnel. *U*crit was calculated following Brett (1964):

$$U_{crit} = U + U_i * (t/t_i)$$

where $U$ is the penultimate speed before the fish stopped swimming; $U_i$ is the flow speed increment; $t$ is the time elapsed in the final increment during which the fish stopped swimming; and $t_i$ is the amount of time individuals were maintained at each speed.

### 3.3.5 Statistical analysis

Separate linear mixed effects models (LMEs) were used to test for differences in $MO_{2rest}$, $MO_{2max}$, aerobic scope and *U*crit across the experimental treatments. Temperature and $CO_2$ were fixed factors in the models. Rearing tank and testing day were included as random factors. An additional random factor of respiration chamber was used in $MO_{2rest}$, $MO_{2max}$, aerobic scope LMEs. Standard length was included as a covariate in the LME for *U*crit. All assumptions for the LMEs were met. Statistical analysis was conducted with a statistical significance of $\alpha = 0.05$. Analysis were done in SPSS V.25 (IMB).

## 3.4 Results

### 3.4.1 Aerobic performance

$MO_{2rest}$ was significantly higher at 22°C compared with 18°C ($F_{1,53}$=313.01, P=<0.001) (Fig. 3.1). $MO_{2rest}$ was ~100 mg $O_2$ $kg^{-1}$ $h^{-1}$ higher at 22°C, which represented a 21-23% increase from 18°C. Elevated $CO_2$ also significantly increased $MO_{2rest}$ ($F_{1,55}$=68.61, P=<0.001), by ~50 mg $O_2$ $kg^{-1}$ $h^{-1}$ or 9-10% regardless of temperature. There was no interaction between temperature and $CO_2$ on $MO_{2rest}$ ($F_{1,55}$=1.35, P=0.249).

$MO_{2max}$ was significantly higher at 22°C compared with 18°C ($F_{1,54}$=86.43 P=<0.001) (Fig. 3.1). $MO_{2max}$ was 148-187 mg $O_2$ $kg^{-1}$ $h^{-1}$ higher at 22°C, which represented a 16-18% increase at the higher temperature. Conversely, elevated $CO_2$ significantly reduced $MO_{2max}$ by 144-183 mg $O_2$ $kg^{-1}$ $h^{-1}$ or 14-15% regardless of temperature ($F_{1,55}$,=80.21, P=<0.001). There was no interaction between temperature and $CO_2$ on $MO_{2max}$ ($F_{1,55}$,=0.11, P=0.739).

Elevated temperature significantly increased aerobic scope by 35-77 mg $O_2$ $kg^{-1}$ $h^{-1}$, or 8-13% ($F_{1,53}$=18.73, P=<0.001). The absolute aerobic scope of juvenile snapper was significantly reduced by elevated $CO_2$ with fish in the 400 µatm treatment having an aerobic scope of 591-674 mg $O_2$ $kg^{-1}$ $h^{-1}$, compared 405-440 mg $O_2$ $kg^{-1}$ $h^{-1}$ for fish in the 1000 µatm treatment ($F_{1,55}$=261.36, P=<0.001) (Fig. 3.2). Consequently, there was a 31-35% reduction in aerobic scope among fish in the elevated $CO_2$ treatment. There was no interaction between temperature and $CO_2$ ($F_{1,55}$=2.69, P=0.106).

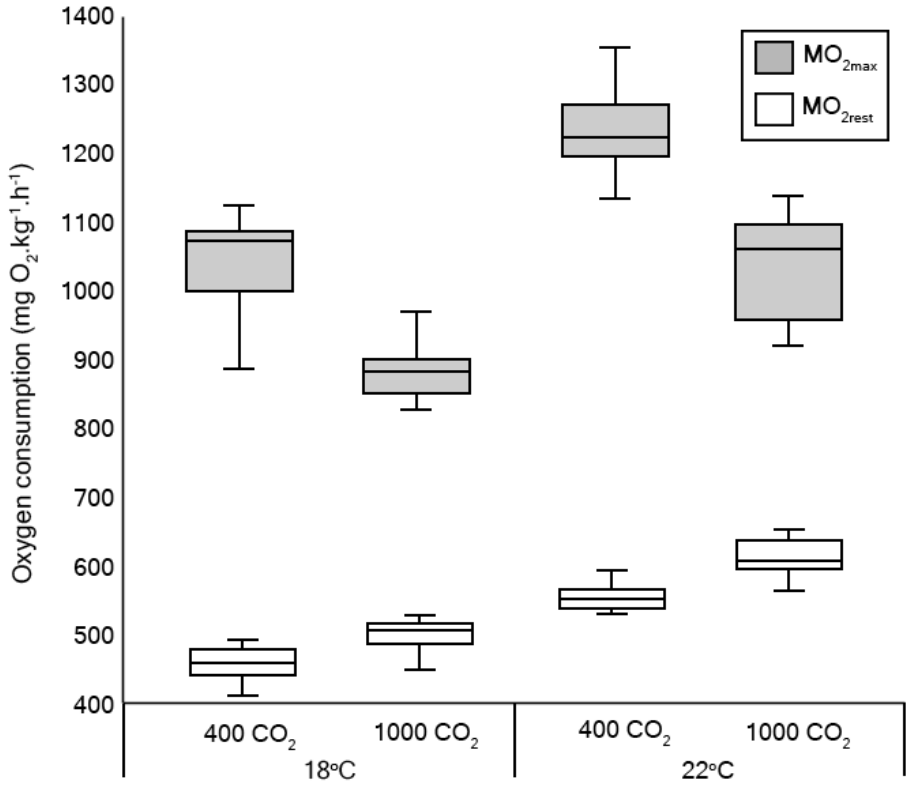


**Figure 3.1** Oxygen consumption of larval *C. auratus* maintained at ambient and elevated $CO_2$ (400 and 1000 µatm) and temperature conditions (18 and 22˚C) for 21 days (21 to 42 dph).

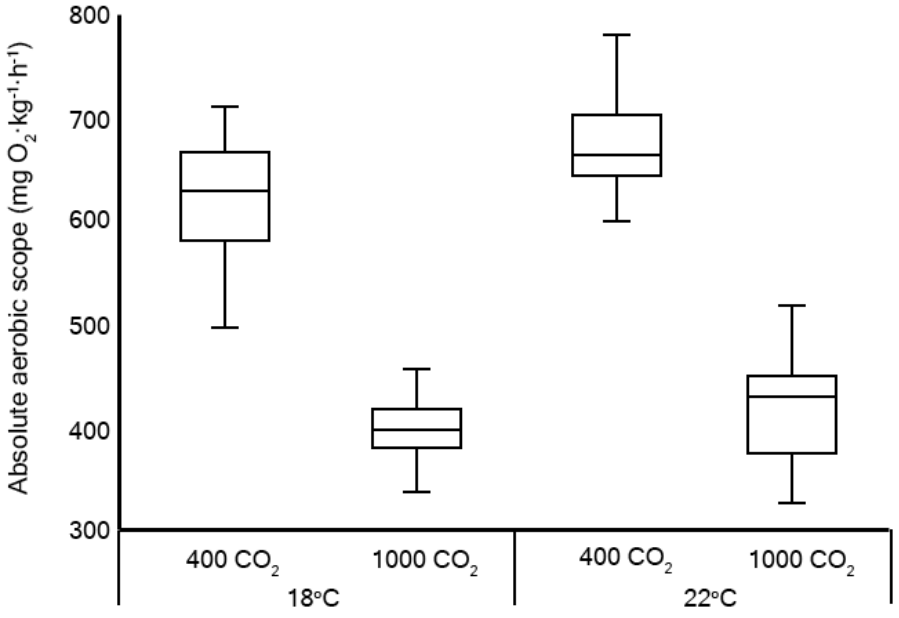


**Figure 3.2** Absolute aerobic scope of larval *Chrysophrys auratus* maintained under ambient and elevated $CO_2$ (400 and 1000 *µatm*) and temperature conditions (18 and 22˚C) for 21 days (21 to 42 dph).

### 3.4.2 Critical swimming speed

The *U*crit of juvenile snapper ranged from 14 to 21 cm $s^{-1}$ (7 to 10 body lengths $s^{-1}$) and speed was dependent on both temperate and $CO_2$, as well as body length (Fig. 3.3) (Table A3.1). Specifically, *U*crit significantly increased by 8-12% or 1.2 to 2.1 cm $s^{-1}$, at 22°C compared with 18°C ($F_{1,46}$=7.12, P=0.010) (Fig. 3.3). Conversely, elevated $CO_2$ significantly decreased *U*crit, by 8-11% or 1.3 to 2.1 cm $^{s-1}$ ($F_{1,46}$=30.60, P=<0.001). There was no interaction between elevated temperature and $CO_2$ ($F_{1,46}$ =0.67, P=0.414).

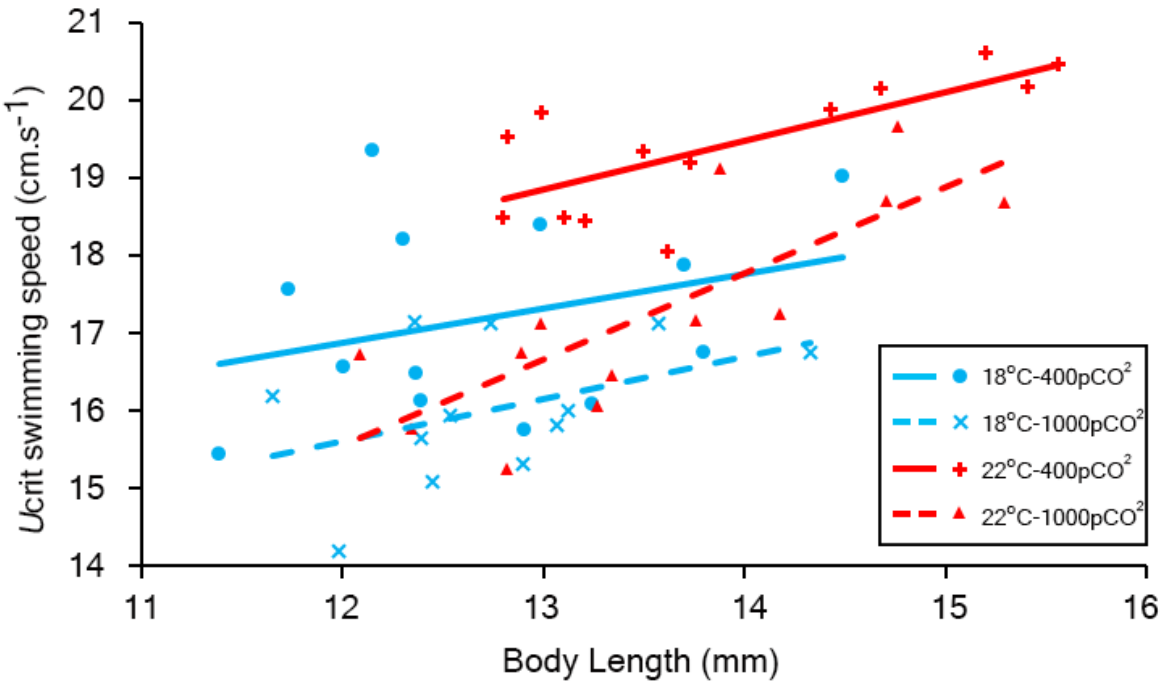


**Figure 3.3** *U*crit swimming speed of larval *Chrysophrys auratus*, depending on standard length, in fish exposed for 21 days (21 to 42 dph) to ambient and elevated $CO_2$ (400 and 1000 µatm) and temperature conditions (18 and 22˚C).

## 3.5 Discussion

Coastal environments are critical habitats for the early life stages of marine fish, yet they are also susceptible to extreme environmental conditions (Grantham et al., 2004; Hofmann et al., 2011; Lima & Wethey, 2012). I found that simulated heatwave temperature and elevated $CO_2$ both had significant, but non-interacting, effects on the metabolic rates and swimming performance of juvenile Australasian snapper, an iconic fish species of high ecological, economic, and social importance. Both high temperature and elevated $CO_2$ increased $MO_{2rest}$ in juvenile snapper, whereas $MO_{2max}$ was increased by elevated temperature and decreased by elevated $CO_2$. Consequently, aerobic scope decreased in fish exposed to elevated $CO_2$, but not elevated temperature. *U*crit swimming followed a similar trend to $MO_{2max}$, where it increased at high temperature and decreased in elevated $CO_2$. These results suggest that the aerobic capacity and swimming performance of juvenile snapper in New Zealand are likely to be more vulnerable to elevated $CO_2$ events (up to 1000 $pCO_2$) than heatwave events (+4°C above ambient summer temperature).

$MO_{2rest}$ is indicative of the daily energy expenditure for basic maintenance. As expected, $MO_{2rest}$ increased in the high temperature treatment. $MO_{2rest}$ was also higher in elevated $CO_2$, which has implications for juvenile snapper living in locations that naturally experience periods of high $pCO_2$, especially if they coincide with heatwave conditions. The ramifications of 20% higher $MO_{2rest}$ at 22°C and 10% higher $MO_{2rest}$ in 1000 µatm $CO_2$ would be substantial, considering juveniles have limited stored energy available (Post & Parkinson, 2001; Stallings et al., 2010) and food resources can be naturally patchy (Link et al., 2005; Brown et al., 2010). In cases where both stressors are experienced in tandem, acquiring 30% more energetic resources to support daily energetic costs might be difficult and could result in reduced growth and survival in nature (Morgensen & Post, 2012).

$MO_{2max}$ also increased at 22°C compared with 18°C, suggesting that 22°C is still within the optimal range for maximal aerobic capacity in juvenile *C. auratus*. This is not surprising considering the temperature range experienced by this species across its distributional range is from 16-25°C during summer (Sheaves, 2006; Wakefield et al., 2015). This result is also consistent with the observation that the thermal niche of temperate species is often wider than tropical species, and that populations of many temperate ectotherms are not living close to their upper thermal limits (Tewksbury et al., 2008; Sunday et al., 2010). Since the increase in $MO_{2max}$ was greater than $MO_{2rest}$ increase, the absolute aerobic scope of these juvenile fish was increased by ~11% from 18°C to 22°C. However, the decrease in $MO_{2max}$ and increase of $MO_{2rest}$ under elevated $CO_2$ ultimately reduced the aerobic scope of juvenile snapper by approximately 30%. Therefore, while an increase in aerobic scope at higher temperature may prove beneficial for juvenile fish, any positive effects would likely be overshadowed by the effects of elevated $CO_2$ when they occur together. A lower aerobic scope in juvenile fish can potentially affect growth rates, dispersal, settlement rates, and survival (Pörtner & Peck, 2010), which would be detrimental to the population, even if later life stages of these species are robust to elevated $CO_2$. While the link between aerobic scope and fitness-related activities is still debated (Grans et al., 2014; Norin et al., 2014; Farrell, 2016; Pörtner et al., 2017), reduced $MO_{2max}$ and, thus aerobic scope, could affect swimming performance (see below) and foraging ability, and thus have implications for juvenile snapper living in nearshore habitats.

Swimming performance can be critical to the success of juvenile fish especially near the time of settlement to benthic habitats (May, 1974; Fisher, 2005). As with other traits, the effect of any particular temperature on swimming speed will depend on where that temperature sits within the thermal performance curve (Wardle, 1980; Green & Fisher,

2004; Johansen et al., 2014). Increased swimming performance has been observed in a number of temperate fish species at temperatures above their natural ambient conditions (Burst swimming: Batty & Blaxter, 1992; *U*crit: Schurmann & Steffensen, 1997; Lee et al., 2003; Routine swimming: Peck et al., 2006), again suggesting that populations of many species may be living below their optimal temperature, at least for maximum swimming performance. As the $MO_{2max}$ of an individual plays a pivotal role in determining the maximum performance of aerobic activities (Metcalfe et al., 2016; Norin & Clarke, 2016), it is unsurprising that the trend for $MO_{2max}$ and *U*crit matched in juvenile snapper. Similarly, while increases in aerobic scope do not always represent a benefit to an individual's overall performance (Clarke et al., 2013; Grans et al., 2014), I found that the increase in aerobic scope up at 22$^{o}$C correlated with increased swimming performance for juvenile *C. auratus*. It should be noted that juveniles from the elevated temperature treatment were ~1mm longer (7-9%) on average, which also gave them an advantage in swimming performance, but this was accounted for by using size as a covariate in the model. Increasing swimming ability within the temperature range tested could be beneficial during the juvenile phase, as it may increase survival and settlement rates (Letcher et al., 1996; Hamilton et al., 2008). However, elevated $CO_2$ reduced swimming performance in juvenile snapper. The decrease in *U*crit at 1000 µatm $CO_2$ was of a similar magnitude to the increase in *U*crit from 18-22 $^{o}$C; therefore, any benefits of higher temperature on swimming performance would be offset by elevated $CO_2$ if both occur simultaneously. Moreover, juvenile snapper may often experience periods of high $CO_2$ in their natural habitats, even when water temperature is not elevated. Our results suggest that these high $CO_2$ events, or habitats that sustain elevated $CO_2$ for long periods of time, such as bay and estuarine systems like the Firth of

Thames (Green & Zeldis, 2015; Law et al., 2018), could already reduce the swimming performance of juvenile snapper in the wild.

Our observation that elevated $CO_2$ increased $MO_{2rest}$ in juvenile snapper differs from most previous studies and similarly, our observed reduction of $MO_{2max}$ has only been seen in a few other species (reviewed in Lefevre et al., 2016, Cattano et al., 2018). Interestingly, previous studies that have detected a decline in $MO_{2max}$ under elevated $CO_2$ have tested juvenile stages of pelagic spawning predatory species (Pope et al., 2014; Laubenstein et al., 2018), akin to this study. In addition, I found that elevated $CO_2$ reduced swimming performance in juvenile snapper, which is comparable to studies on larval (Pimentel et al., 2014) and juvenile (Bignami et al., 2014) dolphin fish, and juvenile yellowtail kingfish (Watson et al., 2018). However, other studies have found no effect of elevated $CO_2$ on the swimming performance of juvenile fish (Atlantic cod: Melzner et al., 2009a; sand smelt: Silva et al., 2016; cobia: Bignami et al., 2017), suggesting that sensitivity to elevated $CO_2$ may be highly species specific. Indeed, Hamilton et al., (2018) showed that elevated $CO_2$ reduced the swimming performance and aerobic scope of one species of rockfish (*Sebastes caurinus*) but had no effect on a closely related species (*Sebastes mystinus*), showing that sensitivity to elevated $CO_2$ may differ even between closely related species.

Another consideration is the possibility of varying sensitivity to elevated $CO_2$ at different life stages. In a meta-analysis of all studies in fish conducted at the time, Lefevre (2016) found no overall effect of elevated $CO_2$ on $MO_{2rest}$, $MO_{2max}$, or aerobic scope. However, when meta-analysis have separated studies into life stages there have been varying effects of elevated $CO_2$ depending on the life stage at which they were tested (Cattano et al., 2018). This could be due to differences in energetic demands and acid-base regulation capacity between particular life stages. The majority of studies that have

reported no effects of elevated $CO_2$ on oxygen consumption have used more developed stages, from late juveniles to adults, which are better equipped to deal with elevated $CO_2$ as they have fully developed acid-base regulatory mechanisms (Ishimatsu et al., 2008; Melzner et al., 2009b). There are now a number of species where metabolic rates have been found to be affected by elevated $CO_2$ during early life history stages (Miller et al., 2013; Pimentel et al., 2014; this study), suggesting that this may be a more susceptible ontogenetic stage. However, there are also a number of species which are not affected by elevated $CO_2$, even during early life (Lefevre, 2016) and the underlying reasons for apparent species-specific differences in sensitivity are unknown.

Changes in aerobic and swimming performance in response to short-term elevated temperature and $CO_2$, such as those reported in this study, have the potential to affect population dynamics. Adult populations of most fishes are strongly influenced by patterns of growth and mortality in the larval and juvenile phases (Doherty & Williams, 1988; Caley et al., 1996; Parsons et al., 2014). I found no interactive effects between temperature and $CO_2$ on the traits measured, so in this case their combined effect was predictable from each one independently. Additionally, elevated $CO_2$ (1000 µatm) would likely have more significant negative ecological effects than high temperature on these juvenile snapper, as the higher temperature appeared to be within the optimal performance range for the traits measured here. However, further increases in temperature may push this species past their optimal range which could add to, or compound, the negative effects of elevated $CO_2$ on aerobic scope and swimming performance. It is also important to note that mature *C. auratus* have been shown to have their peak spawning in early summer where SST is between 18-20°C, with a marked decline in spawning above this range (Scott & Pankhurst, 1992; Francis, 1994; Wakefield, 2010), suggesting that snapper reproduction may be

susceptible to heatwaves. Therefore, the independent effects of higher temperatures and elevated $CO_2$ may be felt on snapper populations during different parts of their life history, but with both potentially having a negative effect on long-term viability of snapper populations. The next step will be to incorporate our results into numerical population models to evaluate how the independent and combined effects of elevated temperature and $CO_2$ may influence the dynamics and sustainability of snapper populations.

# Chapter 4: Effects of elevated temperature on the energy use and growth of larval coral reef snapper

This chapter is prepared for submission to Coral Reefs

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## 4.1 Abstract

The success of individuals during the pelagic larval phase is critical to maintaining healthy and viable populations of coral reef fishes, however it is also the most environmentally sensitive and energetically demanding life stage. Ocean warming from climate change, along with the increasing frequency and intensity of marine heatwaves, could have significant effects on the development and survival of fishes during their larval phase. In this study I tested how elevated temperatures, similar to those occurring during a heatwave, effected the yolk utilization, growth, and survival of larvae of a coral reef mesopredator, *Lutjanus carponotatus*. Eggs and larvae were reared at a current-day average summer temperature (28.5°C) and two elevated temperatures (30°C & 31.5°C) until 14 days post hatch (dph). Larvae in the elevated temperatures depleted their yolk reserves 39% faster than at the control temperature. The standard length of larvae in the elevated temperature treatments was 55% (30°C) and 92% (31.5°C) longer than at the control temperature at 14 dph. A similar pattern occurred in all other morphological traits that were measured. Conversely, survival of larvae was 54% (30°C) and 68% (31.5°C) lower at elevated temperatures compared with the control temperature. This study provides new insights as to how the early life stages of coral reef fishes could be affected by ocean warming and marine heatwaves, with implications for their population dynamics.

## 4.2 Introduction

The viability of a population is dependent on the successful production of offspring via reproduction to offset losses of individuals through mortality (Hughes et al., 1990; Caswell, 2000). Most marine species possess a pelagic larval stage that is important for population connectivity, but which tends to have a high and variable rate of mortality (Armsworth, 2002; Cowen & Sponaugle, 2009; Jones et al., 2009). Consequently, successful recruitment into benthic marine populations is heavily dependent on processes occurring during the larval phase (Caley et al., 1996). This early life period is typically characterised of rapid development, large energy demands, and high sensitivity to environmental conditions (biotic and abiotic) (May, 1974; Houde, 1989; Blaxter 1991; Stallings et al., 2010; Post & Parkinson, 2001). Thus, any changes in environmental conditions during early development can be stressful, energetically costly, and lead to a bottleneck for population replenishment (Doherty et al., 2004; Dahlke et al., 2020). As the majority of marine organisms are ectotherms, environmental temperature is a critical factor during development, with higher temperatures increasing the rates of cellular processes and metabolism (Gillooly et al., 2001; Neuheimer et al., 2011; Schulte, 2015). These physiological changes can accelerate developmental rate and increase energetic demands (Houde, 1989; Blaxter, 1991: Pepin, 1991), with implications for the size, condition and number of individuals that survive the pelagic larval stage (O'Connor et al., 2007, Munday et al., 2009).

The larval phase is the fastest developmental period for marine fish and is the most sensitive to environmental conditions (Post & Parkinson, 2001; Stallings et al., 2010). However, varying effects of temperature increase during this early life stage have been reported. For example, increased larval growth and survivorship have been reported in

some studies (Green & Fisher, 2004; Fielder et al., 2005; Sponaugle et al., 2006; O'Conner et al., 2007), whereas decreased larval growth and survival have been reported in others (McCormick & Molony, 1995; Grorud-Colvert & Sponaugle, 2011; Pope et al., 2014; Watson et al., 2018). Much of the diversity in results is attributed to varying thermal sensitivity and differences in the optimal temperature range of different populations and species (Rummer et al., 2014; McLeod et al., 2015). For instance, numerous species from higher latitudes exhibit positive effects in growth (Pepin, 1991; Steinarsson & Bjornsson, 1999; Dou et al., 2005) and survival (Fowler & Jennings, 2003; Pope et al., 2014) when cohorts experience warmer than normal temperatures. Conversely, tropical reef fish, which have evolved in relatively warm and stable temperature at lower latitudes, often experience negative effects when they are exposed to higher temperature. For example, an increase of just 1-2 °C increased energetic demands (Johansen & Jones, 2011; McLeod et al., 2013; McLeod & Clark, 2016), decreased growth rate (Munday et al., 2008; McLeod et al., 2015; Spinks et al., 2019), and reduced survivorship (Houde, 1989; Rankin & Sponaugle, 2011), during the early life stage of coral reef fishes. However, much of the research to date has focused on smaller bodied reef fish, such as damselfish that are frequently caught in light traps or easily bred in captivity (McCormick et al., 1995; Munday et al., 2008; Donelson et al., 2010; Rankin & Sponaugle, 2011; Spinks et al., 2018). Thus, for most coral reef fishes we don't know whether increased environmental temperatures would benefit or hinder early life history development and survival.

The majority of coral reef fish spawn in the warmer months, from late spring and through summer (Domeier & Colin, 1997; Russell, 2001), which are also when marine heatwaves are most likely to occur. Marine environments have seen an increase in abnormally warm days of ~50% over the last century (Oliver et al., 2018) and the duration

and frequency of these events are expected to increase as climate change progresses (King et al., 2016; Frolicher et al., 2018; Hoegh-Guldberg et al., 2018). Moreover, average sea surface temperature is projected to increase up to 2-4$^{o}$C by the end of the century (Collins et al., 2013; Hoegh-Guldberg et al., 2018). With the Great Barrier Reef having experienced heatwaves in 3 out of the last 5 summers (2016, 2017, and 2020: AIMS, 2017; Hughes et al., 2017; BOM, 2020), there is an urgency to understand how extreme temperatures affect the early life stages of coral reef fish. In particular, with some notable exceptions (e.g. Pratchett et al., 2017) there is a paucity of research on the effects of elevated temperature on larger predatory and pelagic spawning species.

In this study, I investigated how elevated temperatures, effect the early life history of the coral reef snapper, *Lutjanus carponotatus*, a mesopredatory fish that is ecologically important to coral reef ecosystems, and important to recreational and commercial fisheries (GBRMPA, 2014). If elevated temperature significantly increases the metabolic rate of these larval fish then I would expect individuals to utilise endogenous resources at a faster rate and experience accelerated growth. To test this I exposed gametes from fertilization to 14 days post hatch (dph) to either 28.5°C (the ambient summer conditions), 30°C or 31.5 °C. The 30°C and 31.5°C temperatures were chosen as they are projected to be average reef temperature in the future (mid and end of century) and because these temperatures have already occurred for days to weeks during recent heatwaves (Hughes et al., 2018; Spinks et al., 2019). During this experiment I assessed how elevated temperatures: 1) effected the utilization of endogenous energetic reserves from 0-51 hours post-hatching (hph), 2) influenced growth and development at 1 dph and 14 dph by measuring key morphometric and developmental traits, and 3) effected hatching and larval survival at 1

dph and 14 dph to better understand the potential impacts of ocean warming on the early life of coral reef fishes.

## 4.3 Methods

### 4.3.1 Study species

The Spanish Flag snapper, *Lutjanus carponotatus,* is a tropical mesopredator that inhabits coral reefs throughout the Great Barrier Reef (GBR) and Indo-Pacific reefs (Allen, 1985). They are gonochoristic serial spawners with the peak spawning for the GBR population occurring between October and December (Kritzer, 2004). Over this period the upper SST range is ~26.5 - 28.5°C for the northern GBR where our broodstock fish were collected (AIMS data using Lizard Island and One Tree as a north south range; AIMS, 2017). The larvae have a pelagic phase where they are found relatively close to the surface which can last 33-38 days (Querer & Leis, 2010), after which point they settle to the reef.

### 4.3.2 Broodstock and spawning

Adult snapper were caught by hand-line fisherman from the northern GBR between November 2018 and September 2019 and transported to the Marine and Aquaculture Research Facility at James Cook University, Townsville. Between six to eight adult fish (28-40 cm standard length) were housed together in ten 2,500 L tanks (n=72 adults). The temperature cycle for the adults followed a pattern similar to the average monthly temperatures for the northern GBR, where the winter and summer temperatures were 23°C and 28.5°C respectively. Adults were fed daily a mixture of Skretting pellets (Spectra SS) and pilchards at ~2% body weight. Spawning was allowed to occur naturally without any hormonal stimulus, with spawns for this experiment being produced at 28.5°C. Spawning occurred late at night and eggs were collected in an overflow egg collector

(Moran et al., 2007). For this experiment 4 adult tanks were used (n=32 adults total), as they spawned simultaneously during December-January, 2019. The spawns were mixed before being placed into larval rearing tanks to maximize genetic diversity. Spawns collected from the same 4 adults tanks a week later were used in the yolk utilization experiment.

### 4.3.3 Experimental design

The study comprised two experiments, one focused on the yolk utilization of larvae between 0-51 hph, and another on larval development from hatching to 14 dph. For both the yolk utilization and larval development experiments, the eggs and larvae were reared in three stable temperature treatments of 28.5°C, 30°C, and 31.5°C (Table 4.1). At ~6 hours (0600) post fertilization, eggs mixed from the 4 adult tanks where spawning occurred were transferred to 20 L larval rearing tanks (n=6 per treatment) set at 28.5°C. Larval rearing tanks were initially stocked at ~1000 eggs per tank. At this point the two elevated temperature treatments (30°C and 31.5°C) were increased gradually over two hours until they reached target temperature. Samples were then taken 6 hours after initial stocking (1200), by counting the eggs in three 50 ml samples from each tank, to accurately estimate the number of eggs per tank and account for any mortality during handling. The average stocking density at this point varied between 3-5%. Hatching occurred between ~14-16 hours post fertilization (1400-1600). The same process was used for the yolk utilization experiment, except that tanks were stocked at ~500 eggs for that component.

Each tank received water flow of ~100 ml $min^{-1}$ allowing for a complete water volume exchange every ~3 hours. Each temperature treatment received water from a 1000

L sump connected by partial exchange to a central 5000 L sump where filtration (Mechanical bag filters to 25 micron and 400 L protein skimmer) and UV sterilization occurred (Wedeco, B32-PE). Salinity, alkalinity, pH, and oxygen saturation were all maintained at natural levels (Table 4.1). Salinity and oxygen (Hach, HD40u), and pH (Mettler, Seven2Go Pro) were measured daily. Alkalinity was measured weekly (Metrohm, 888 Titrando). The photo period was set at 12:12 light/dark (0700-1900) with simulated sunrise and sunset through an hour gradual increase from 0-100% (0700-0800) and an hour decrease from 100-0% (1900-2000).

Standard green-water methodology was used for rearing larval fishes (Palmer et al., 2007; McMahon et al., 2020). From 1 day post hatching (dph), larval rearing tanks were treated with inert algae (Nano 3600, Reed Mariculture) at a concentration of ~230,000 cells per ml. Larval were fed enriched rotifers (INVE, Selco S.parkle) three times a day (0800, 1200, and 1600) from 1 dph. From 7 dph larvae were fed enriched Artemia nauplii (INVE, Selco S.presso) at a density of 1 $ml^{-1}$ once per day (0800). Rotifer feeding and green-water treatment were gradually reduced between 9-11 dph. Larvae used for the yolk utilisation experiment were reared in green-water, but not fed.

**Table 4.1** Mean (± SD) of experimental seawater chemistry parameters for larval Spanish flag snapper (*Lutjanus carponotatus*). Temperature and $pH_{nbs}$ were measured daily. Total alkalinity and salinity were measured at the start of the experiment and then every 7 days.

| Treatment | Salinity (ppt) | Temperature (°C) | Total Alkalinity (mmol/kgSW) | pH (nbs) |
|---|---|---|---|---|
| **28.5°C** | 34.90 ±0.10 | 28.48 ±0.04 | 2282 ±35 | 8.10 ±0.05 |
| **30.0°C** | 34.93 ±0.25 | 30.02 ±0.10 | 2311 ±54 | 8.14 ±0.02 |
| **31.5°C** | 35.01 ±0.20 | 31.55 ±0.23 | 2278 ±42 | 8.11 ±0.06 |

### 4.3.4 Sampling

In the yolk utilization experiment, eggs were initially sampled from each treatment at stocking (n=20). Subsequently, larvae were sampled from each treatment (n=20) at hatching (0-1 hph) and then every 12 hours until 51 hph, when endogenous energy reserves were depleted. Initial survival, in both experiments, was measured once the vast majority of eggs in each tank were either hatched or had died. During the larval development experiment, larvae were sampled from the three temperature treatments at 1 dph and 14 dph. To sample each tank the water was gently stirred to homogenize the larvae and three 50 ml samples were taken. Larvae in each sample were counted and the total larvae in the tank were extrapolated from the samples. Survival was calculated as a percentage using the number of individuals in each tank at a sampling point compared to initial stocking. Larvae were then placed gently back into the tank with the exception of 10 individuals (n=60 per treatment), which were euthanized and photographed using a digital camera (Olympus, SC50) fitted to a stereo microscope (Olympus, SZX7). All larvae were euthanized and photographed within 30 minutes of sampling.

Morphometric data was extracted from digital photographs using ImageJ2 (Rueden et al., 2017) by an observer (SJM) who was blind to the treatments. In the yolk utilization experiment, standard length (SL), muscle depth (MD), yolk area (YA), and oil globule area (OGA) were measured. In the larval development experiment, the larval traits measured were: standard length (SL), total length (TL), body length (BL), muscle depth (MD), fin depth (FD), eye diameter (ED), mandible length (ML), yolk area (YA), oil globule diameter (OGD), head length (HL), head depth (HD) (Fig.4.1). In the larval development experiment, oil globule diameter, rather than area, was measured as the oil globule shape changes from spherical to asymmetrical during consumption.

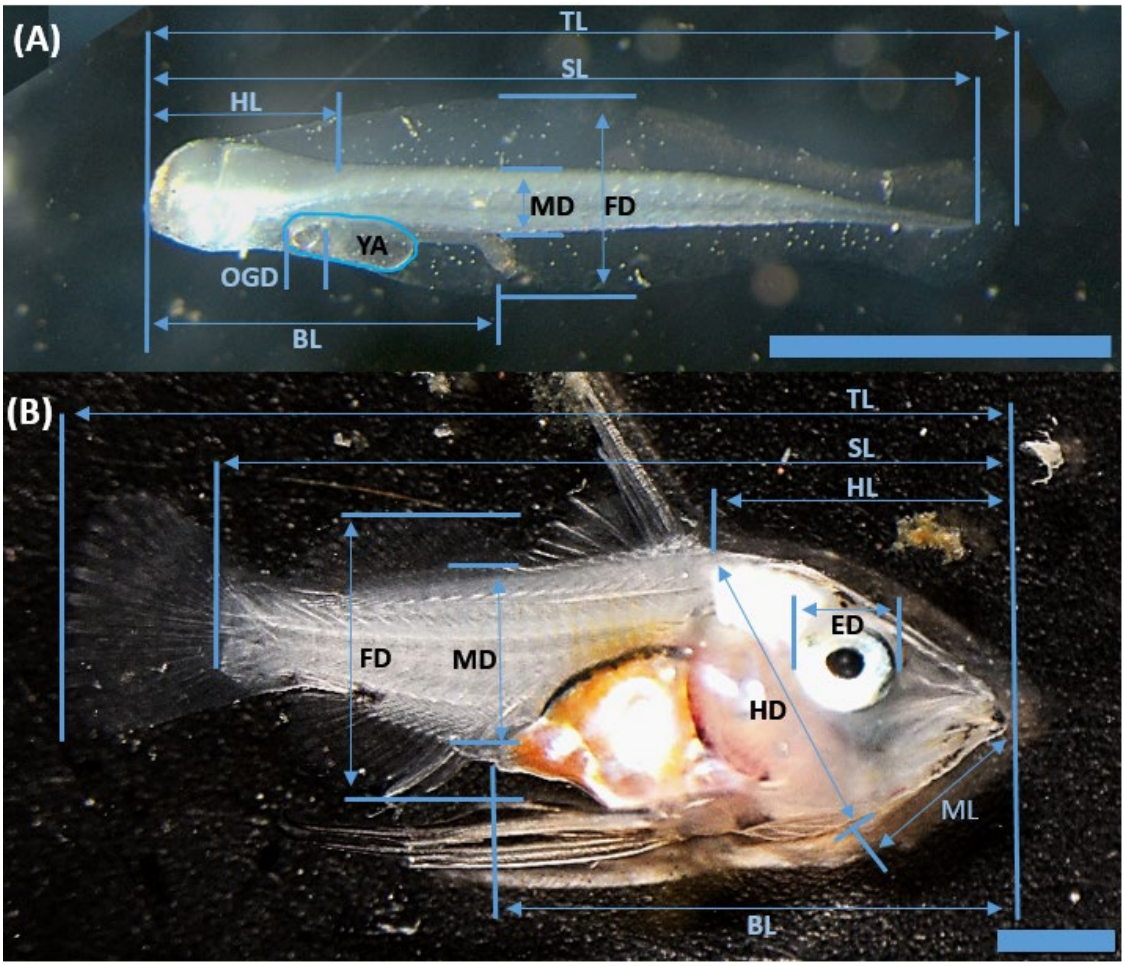


**Figure 4.1** Morphological traits measured from larval *Lutjanus carponotatus* at (a) 1 dph and (b) 14 dph. Scale bars represent 1mm. Standard length (SL), total length (TL), body length (BL), head length (HL), head depth (HD), mandible length (ML), muscle depth (MD), fin depth (FD), eye diameter (ED), yolk area (YA), and oil globule diameter (OGD).

### 4.4.4 Statistical analysis

Linear mixed effects models (LME) were conducted on the morphometric data from the yolk utilization experiment (standard length & muscle depth). Temperature and hours post hatch (hph) were fixed factors, and tank was included as a random effect. An ANOVA was used on each model to test whether the fixed effects were statistically significant. A Tukey's post-hoc test was then used to check for significant differences among levels within the treatments. To determine if temperature had an effect on the consumption of yolk and oil globules (YA and OGA), Kruskal-Wallis tests, followed by Dunn tests, were conducted at each time point to determine differences between the temperature treatments. Specifically, comparisons of YA were conducted from 0-27 hph due to YA being depleted in all fish at 39 hph (i.e. data was all zeros), while comparisons of OGA were conducted from 0-51 hph.

Since growth metrics can be highly correlated Principle Component Analyses (PCA) were used on the morphological data from larvae at 1 dph and 14 dph. Using PCA reduced the complexity of the multivariate data and identified the variables accounting for the largest variation in the data. The PCA for 1 dph larvae included the traits: TL, SL, BL, HL, MDV, FD, OGD, and YA. For the PCA of the 14 dph larvae the traits used were: TL, SL, BL, HL, HD, ML, ED, MD, and FD. In the 1 dph PCA three principle components were needed to explain over 70% of the variation, while in the 14 dph PCA 97% of the variation was explained by the first principle component.

To further examine the effects of temperature on morphological traits, LMEs were applied to the first two principle components and to each of the morphological traits separately. Temperature was included as a fixed factor and rearing tank was used as a

random effect in these LMEs. ANOVAs were used to analyse each of the models and significant effects were further analysed using a Tukey's post-hoc test. Individual generalized linear models (GLM) were used to compare the survival of larvae at 1, 7 and 14 dph between treatments. Initial stocking density was used as a covariate in the model and proportional survival was tested as the dependant variable. A post-hoc multiple comparison test (Tukey's Test) was used on significant LMEs to determine the specific differences between treatments.

All analyses was conducted in R (R Core Team, 2014) using the LME4 and GLM packages (Bates et al., 2012). Post hoc tests were conducted using ghlt function in Multcomp package (Hothorn et al., 2016). All models met the assumptions of the relevant tests. This was confirmed by accessing the residuals, goodness of fit, and checking dispersion.

## 4.4 Results

Overall, I found that temperature had strong effects on the early life stages of *L. carponotatus*. Yolk and oil globule reserves were used at a significantly faster rate at elevated temperatures when compared to larvae reared in the control temperature. The growth of larvae was also significantly increased by elevated temperature at 1 dph, however, it was far more pronounced at 14 dph with growth significantly different between each temperature treatment. Conversely, survival was found to be reduced by elevated temperatures.

### 4.4.1 Yolk utilization

There was no difference in yolk area among treatments at 0 hph ($X^2$=3.56, p=0.169) and yolk reserves were consumed by 39 hph (Fig.4.2a). However, water temperature had a significant effect on yolk area from 3 to 27 hph (3 hph: $X^2$=29.95, p<0.001; 15 hph: $X^2$=40.50, p<0.001; 27 hph: $X^2$=38.05, p<0.001). Larvae from both warmer treatments had significantly less yolk at each time point between 3-27 hph compared with the 28.5°C control, and at 27 hph there was a smaller yolk area remaining in the 31.5°C than the 30°C larvae (Fig.4.2a; all p=<0.001, Table A4.1). The oil globule of larvae that developed at 28.5°C was consumed by 63 hph, whereas larvae from both warmer treatments consumed their oil globule by 39 hph (Fig.4.2b). There was a significant effect of temperature on oil globule area (Table A4.2), with larvae from the warm treatments using their oil globule ~39% faster that the 28.5°C control. Specifically, larvae that developed in 31.5°C had a smaller oil globule area at all hph, while the oil globule of larvae in 30°C was smaller at all times except 3 hph (Fig.4.2b)(Table A4.2).

The standard length of larvae in all treatments increased by ~30% between hatching and 15 hph, after which time length remained at around 2.8 mm (Fig.4.2c). While standard length differed with both temperature ($F_{2,6}$=17.7, p=0.003) and time ($F_{5,337}$,=1495.90 p=<0.001), there was a significant interaction between temperature and hours post-hatching ($F_{10,337}$=29.3, p<0.001). This was largely driven by differences between temperature treatments at 3 hph (28.5 vs 31.5°C: t=-9.37, p=0.003; 30 vs 31.5°C: t=-8.75, p=0.004) and 15 hph (28.5 vs 30°C: t=-8.68, p=0.004; 28.5 vs 31.5°C: t=-11.81, p<0.001), where larvae were longer in warmer treatments. After 15 hph there was a trend that standard length decreased slightly (~3-5%) in the warmer treatments while the control fish did not (Fig.4.2c, 31.5°C 15 hph significant from 31.5°C 27, 39 and 51 hph: t=4.47, t=4.50, t=5.44, respectively, p≤0.001 all; 30°C 27 vs 51 hph: t=4.44, p=0.002). There was also a significant interaction between temperature and time for muscle depth ($F_{10,337}$=3.56, p=<0.001). This was largely driven by the warmer treatments tending to have greater muscle depth at 3-15 hph and reduced muscle depth at 51 hph, however, this did not result in any significant temperature differences within a time-point. A general pattern of decreased muscle depth from 0 to 3 hph (~20%; 28.5°C 0 vs 3 hph: t=6.80, p<0.001; 30°C 0 vs 3 hph: t=8.65, p<0.001) followed by an increase of ~30% between 3 and 15 hph (28.5°C 3 vs 15 hph: t=-15.27, p<0.001; 30°C 3 vs 15 hph: t=-15.13, p<0.001, 31°C 3 vs 15 hph: t=-14.55, p<0.001) was observed. Muscle depth then steadily decreased by ~10% between 15-51 hph (Fig.4.2d; 28.5°C 15 vs 51 hph: t=4.04, p=0.008; 30°C 15 vs 51 hph: t=6.37, p<0.001, 30°C 15 vs 51 hph: t=-8.44, p<0.001).

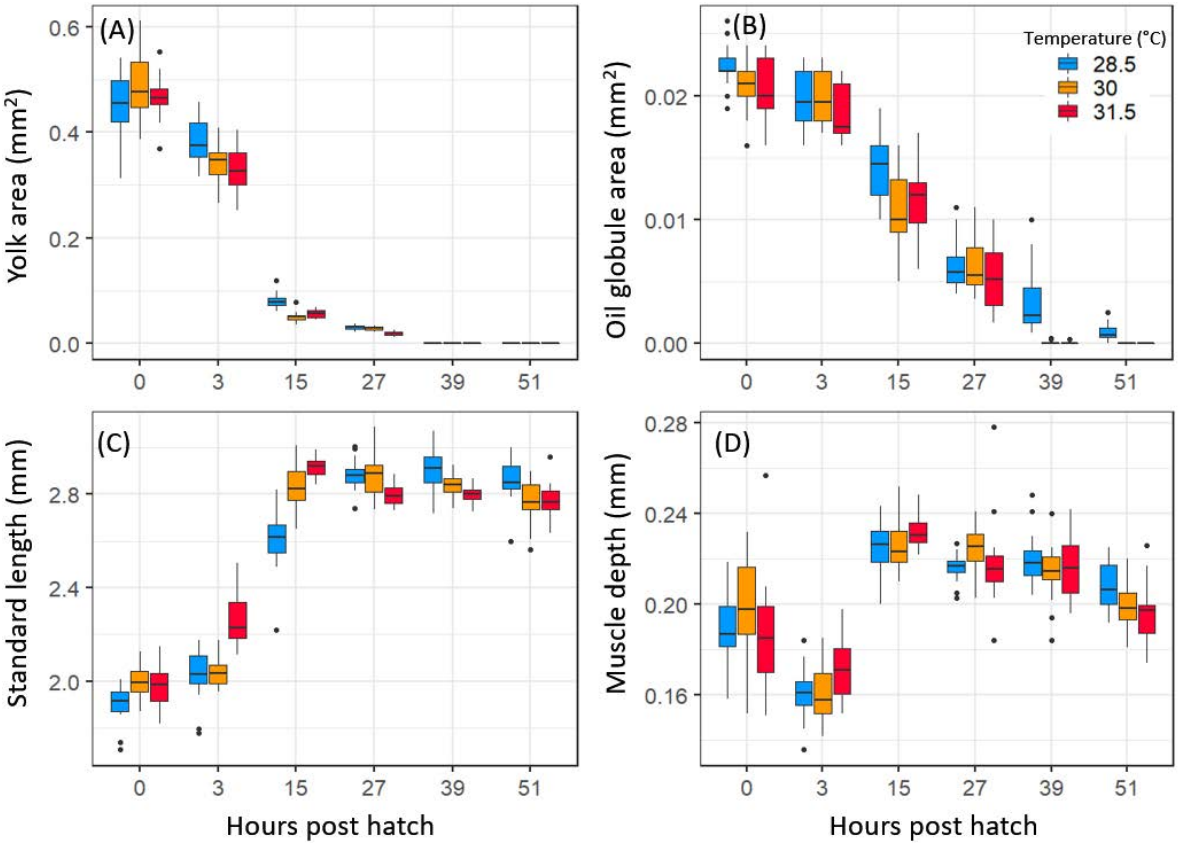


**Figure 4.2**. The area of Yolk (A) and oil globule (B), the standard length (C), and muscle depth (D) of larval *Lutjanus carponotatus* measured between hatching and 51 hours post hatching.

## 4.4.2 Growth morphometrics

At 1 dph PC1 (46% of total variation) was largely described by differences in all traits (standard length, total length, body length, head length, muscle depth, and fin depth) except yolk area and oil globule diameter which were associated with the PC2 axis (19%; Fig. 4.3a). There was a significant difference between the control and elevated temperatures (28.5°C vs 30°C: t=-4.55, p=0.009; 28.5°C vs 31.5°C: t=-5.38, p=0.004), but not between the two elevated temperatures (30 vs 31.5°C: t=-0.82, p=0.702). There were significant differences between temperatures for PC2 ($F_{2,6}$=6.12, p=0.036), however, this was found to be driven by the difference between the 30°C and 31.5°C treatments (t=3.49, p=0.001). The effect of temperature was further supported by the individual analysis of

each trait finding that 30°C larvae were significantly larger in total length (8%), standard length (7%), body length (9%), and head length (14%) compared to control larvae (Fig. 4a, Table 4.3). Larvae from the 31.5°C treatment also were significantly larger in TL (9%), standard length (6%), body length (7%), head length (15%), and muscle depth (12%) compared with control fish (Fig. 4.4a, Table A4.3). In addition, oil globule diameter was significantly smaller (14%) in 31.5°C treated fish than the other two treatments (Fig. 4.4a, Table A4.3).

At 14 dph there was clear morphological distinction between larvae from 28.5°C and the warmer groups, seen by the lack of overlap in 95% confidence ellipses for PC1, which described 97% of all trait variation (Fig. 4.3b). All morphometric traits measured, and their combination described by PC1, were significantly larger with increasing water temperature (Fig. 4.3b; All p=<0.001, Table A4.4). This resulted in all traits being significant between all treatment groups (All p=<0.001, Table A4.4). Specifically, standard length, total length and body length were ~55% longer in the 30°C treatment and ~92% longer in the 31.5°C treatment, compared to 28.5°C (Fig.4b). Head depth was 86% bigger in 30°C treatment and 146% bigger in the 31.5°C treatment compared to 28.5°C (Fig.4.4b). Muscle depth was 192% bigger in the 30°C treatment and 283% bigger in the 31.5°C treatment compared with the 28.5°C control (Fig.4.4b). In addition, at 14 dph all fish in both the 30°C and 31.5°C treatments had undergone flexion while no individuals had commenced flexion in the 28.5°C control.

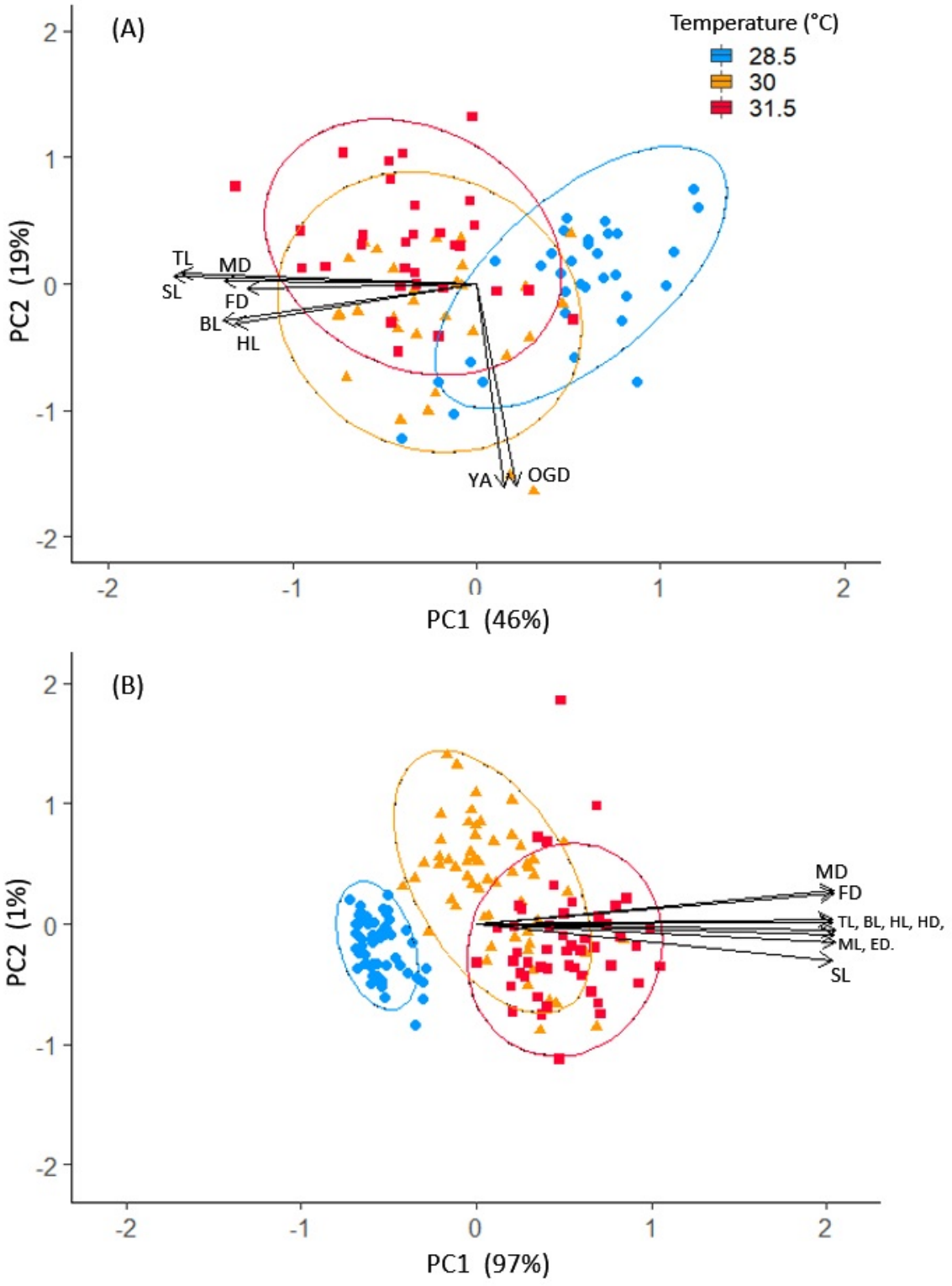


**Figure 4.3** PCA of morphometric data for larval *Lutjanus carponotatus* at (A) 1 dph and (B) 14 dph. Rings represent 95% confidence that data fall with for 28.5°C (blue), 30°C (orange) and 31.5°C (red). Morphological traits are indicated by arrows. Standard length (SL), total length (TL), body length (BL), muscle depth (MD), fin depth (FD), eye diameter (ED), mandible length (ML), yolk area (YA), oil globule diameter (OGD), head length (HL), and head depth (HD).

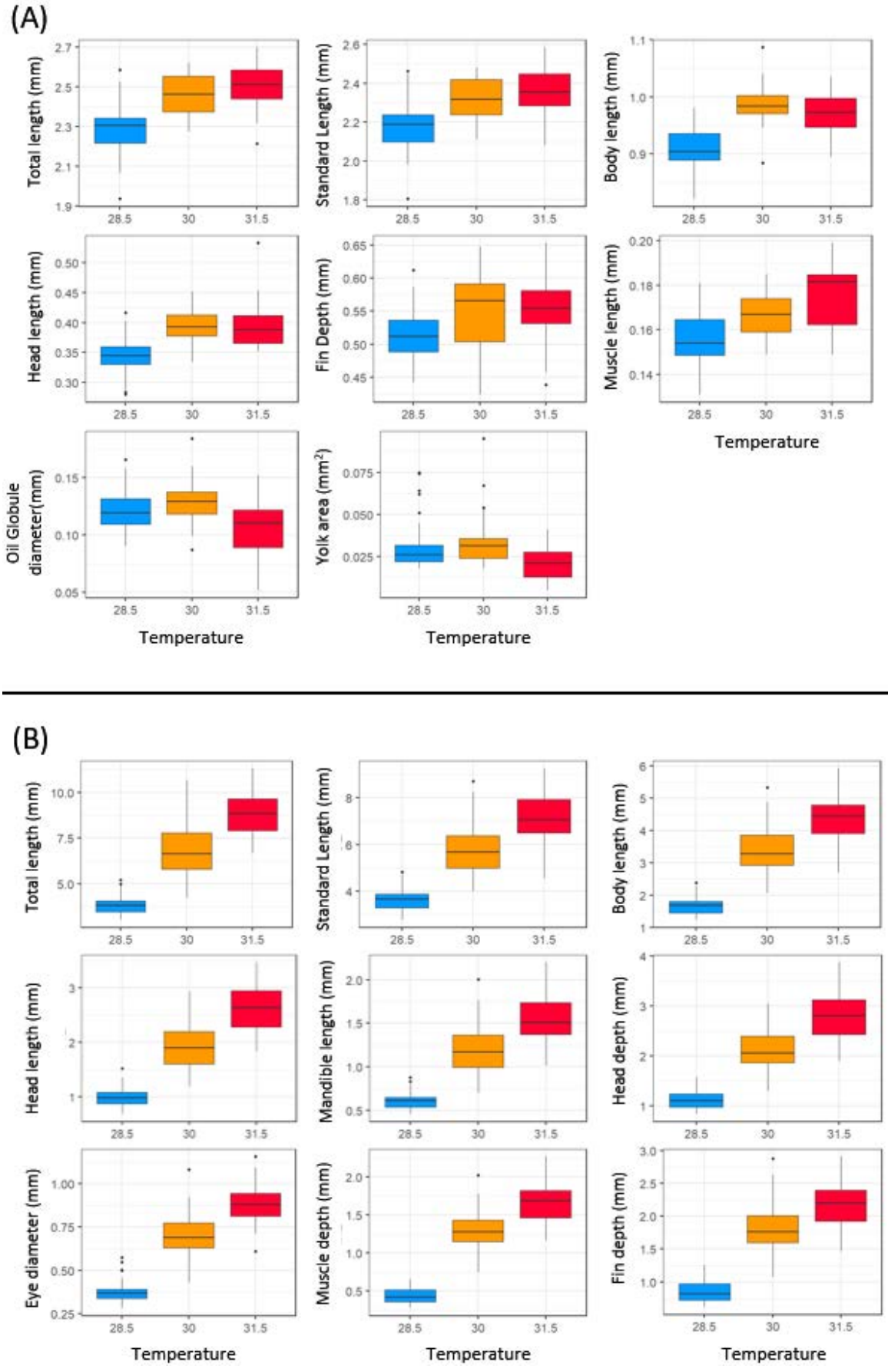


**Figure 4.4** Morphometric data for larval *Lutjanus carponotatus at* 1 dph (A) and 14 dph (B).

### 4.4.3 Survival

The hatching success of eggs was significantly higher in the 30°C (+10%) and 31.5°C (+9%) treatments, compared to the 47% hatching success at 28.5°C (Fig.4.5; 28.5 vs 30°C: z=-3.586, p=0.010; 28.5 vs 31.5°C: z=-3.478, p=0.015). However, this pattern was reversed in larvae at 7 dph, where survival at 28.5°C (39%) which was 3 and 4 times higher than in the 30°C and 31.5°C treatments, respectively (28.5 vs 30°C: z=9.659, p<0.001; 28.5 vs 31.5°C: z=11.147, p<0.001). This pattern was maintained at 14 dph, with 15% survival in the control treatment being 2 and 3 times higher than in the 30°C and 31.5°C treatments, respectively (28.5 vs 31.5°C: z=3.881, p=0.003).

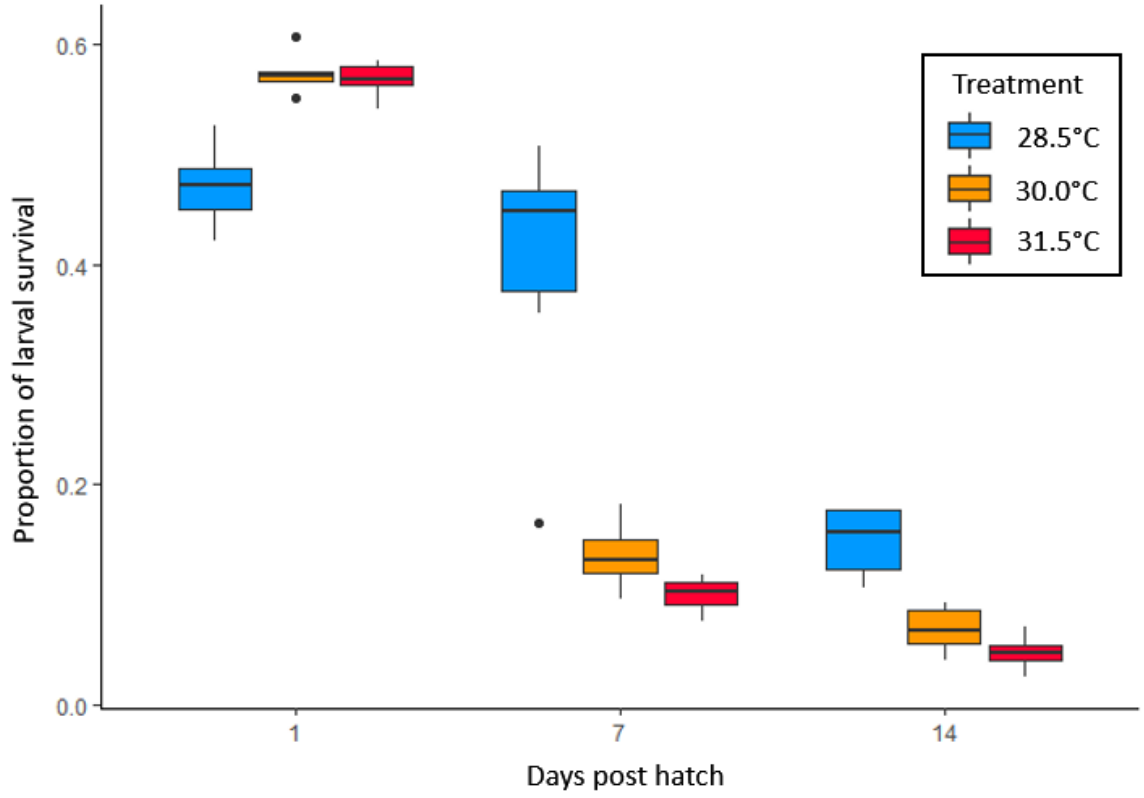


**Figure 4.5** Proportional survival of *Lutjanus carponotatus* from at 1 dph, 7 dph, and 14 dph.

## 4.5 Discussion

Environmental effects on fish larval stages can alter the cohort's recruitment success, and therefore influence variability in the size and demographic structure of the adult population. I found that under elevated temperatures (30 and 31.5°C) consistent with climate change projections, and heatwave conditions that are already occurring, larval *L. carponotatus*, had higher hatching success, developed at a faster rate, and grew up to twice as large by 14 dph compared to the 28.5°C current-day control conditions. Faster growth would normally be expected to enhance survival in a natural setting if larger size provides a refuge from predation (Almany & Webster, 2006; Gagliano et al., 2007). However, survival during the 14 day experiment was ~50-70% lower in the elevated temperature treatments. Larvae in warmer conditions consumed their endogenous energy reserves ~20% faster, which could potentially explain reduced survivorship if larvae had a reduced time window for which to begin feeding. In this population of *L. carponotatus* the benefits detected in growth and development at elevated temperature may be negated by decreased survival, highlighting the complexity of determining the effects of environmental stressors on fish early life stages.

Elevated water temperature increased the rate of growth and development during the first 14 days of life. By 14 dph all morphometric traits measured were larger in larvae that developed at 30 and 31.5°C compared to larvae reared at the current-day control temperature of 28.5°C. Importantly, it was not just morphological size that was increased, but also the rate of development, because all fish in elevated temperatures had undergone flexion at 14 dph whereas no fish had entered flexion at 28.5°C. The combination of enhanced growth and development indicate that the rate of cellular processes and metabolism were increased due to elevated temperature (Fry, 1971; Johnston & Dunn,

1987; Angilletta et al., 2004). Furthermore, the consistent response between elevated temperature and the change in traits measured (i.e. the increase in 31.5°C larvae was approximately 2 times the 30°C response) further support that observed shifts are a direct results of cellular metabolism. These findings also suggest that this population of *L. carponotatus* (from the central region of the species range) may be living below its thermal optimum for the larval period, and small increases in water temperature (≤3°C) may enhance growth and development during early life. However, due to the increased levels of mortality observed at elevated temperatures, it is unlikely that ocean warming will be entirely beneficial. Interestingly, these findings are in contrast to previous work on demersal spawning reef fish where warmer temperatures (>29°C) reduced larval and juvenile growth (Zarco-Perello et al. 2012; McLeod et al. 2015) suggesting that broadcast spawning species may not be as negatively affected. While the reason to this is unknown it is possible that the different spawning strategies (i.e. demersal spawning producing fewer but more highly provisioned eggs) are differentially effected as temperature increases. It is important to note that in this experiment larvae were provided with ample food to fuel increased metabolism and developmental rate at elevated temperatures, whereas this may not be available in nature (Owen, 1989; MacKenzie & Leggett, 1991; Hays et al., 2005). Limited and/or patchy food supply in nature could limit the benefits of higher temperature on larval growth and developmental rate (Munday et al., 2009). It is also possible that the enhanced growth observed in the two elevated treatments could be due in part to selection for faster growing phenotypes (Meekan & Fortier, 1996). However, this seems unlikely from our PCA results at 14 dph with 97% of all variation in morphology described by PC1 and all trait vectors clustering together, which implies that larvae are following an ontogenetic developmental path that is simply accelerated in warmer conditions.

Endogenous provisions are critical to the survival of larval fish as they supply the energy needed to grow, develop, and successfully start feeding. They are the only energetic resources available to larval fish until they can develop the organs and structurers (e.g. develop the gut, stomach, jaw, eyes, etc.) to successfully capture and digest their prey (Kamler, 2008). Due to this, the amount of endogenous resources provided by mothers, and the rate at which larvae consume them, can be critical to successfully surviving through the early life pre-feeding stages (Kamler, 2002; Tocher, 2010). I found that warmer treatments had a faster rate of yolk utilization from 3 to 27 hph, however, all temperature groups consumed their yolk by 39 hpf. I also observed that oil globule was used ~39% faster and was exhausted by 39 hpf in the elevated treatments, while control 28.5°C fish consumed their oil globules by 63 hpf. This accelerated usage of yolk and oil is likely due to the additional costs of cellular chemical reactions and metabolism at higher temperatures, therefore resulting in faster consumption of energetic resources (Houde et al., 1993; Rombough. 1997; Kamler, 2002). Consequently, it appears that in this species the faster usage of energy reserves at elevated temperatures was used to maintain homeostasis, since there was no observed increase in growth between temperature treatments over this timeframe. In fact, I observed a slight reduction in length and muscle depth for warmer treatments at 51 hph, although this was not statistically significant. Interestingly, at ~1 dph temperature appeared to have contrasting effects on the standard length of larvae in the yolk utilization experiment versus the growth experiment. While the reason for this is unknown, it may have been caused by the yolk utilization experiment methods not including green water or live feed, or possibly by the use of different spawns, which may have been of different quality (growth experiment spawn was prior to yolk utilization) or differed slightly in thermal sensitivity. Additionally, the largest change in all growth and

development metrics, and between treatments, was seen between 3 and 15 hph, which suggests that this window may envelop a key metamorphic milestone for this species warranting further investigation. Critically for larvae, elevated temperature and the increased consumption rate of energetic reserves would result in a shorter window in which to successfully begin feeding, which could reduce survival in a natural setting where food availability can be patchy (Owen, 1989; MacKenzie & Leggett, 1991; Hays et al., 2005).

Survival during larval development is low for the majority of marine fish, less than 1% on average (Pepin, 1991; Houde, 1994), and small changes in survival during this life stage can impact successful recruitment of different cohorts to the adult population (Rijnsdorp et al., 2009; Pörtner & Peck, 2010; Petitgas et al., 2013). The point in larval development where endogenous reserves are depleted, and individuals are required to successfully acquire energetic resources via feeding, is thought to be a critical period where environmental conditions and successful feeding can significantly effect a cohort's survival (Hjort, 1914; May, 1974; Houde, 1997). I observed that elevated water temperature significantly reduced the survival of larval *L. carponotatus* at 7 to 14 dph. Survival in the elevated temperature groups declined markedly within the first week of life to just 10-14% survival in warm conditions compared to ~40% survival in the current-day control temperature. This increase in mortality would coincide with endogenous resources being used (observed at 63 hph in this study) and energy having to be acquired through feeding. This pattern of mortality is likely due to the additional metabolic costs at elevated temperature as larvae begin to feed, resulting in larvae needing to acquire more food at a time when they are known to be highly inefficient in food acquisition (China & Holzman, 2014). Therefore, at elevated temperatures it is possible that the window for successful first feeding may be reduced, which in combination with increased metabolic demands

would likely reduce larval survival. For the control fish the largest drop in mortality was observed from 7 to 14 dph, again likely associated with transition to feeding and this being delayed at 28.5°C; however, the reduced survival in elevated temperature treatments was still present at 14 dph. Similar reductions in survival and higher temperatures have been observed in the early life stage of other coral reef fishes (Houde 1989; Rankin & Sponaugle, 2011), suggesting this could be a general pattern. A reduction in survival, as seen in this study, could prove to have detrimental effects on a population if these patterns where to hold in the natural environment.

This study found that elevated temperatures consistent with recent heatwave conditions on coral reefs and projected to become average summer water temperatures in the future, had both positive and negative effects on the early life history of larval *L. carponotatus*. Increased growth, along with accelerated attainment of developmental milestones, is typically seen as advantageous, because it means that larvae more quickly reach a size where they gain some refuge from predation (Almany & Webster, 2006), however, this came at the cost of lower cohort survival. Consequently, there is a potential trade-off between more rapid attainment of predatory size thresholds at higher temperature and the direct effects of higher temperature on mortality rate. Additionally, it seems unlikely that the increased growth observed would occur in all natural settings since food availably is often patchy and limited (Owen, 1989; MacKenzie & Leggett, 1991; Hays et al., 2005). Additionally, the finding that endogenous energy reserves were used faster at elevated temperature, up to 12-24 hours faster, is a considerable change when the time window between gut development and feeding can be less than 24 hours in coral reef fish (Bagarinao, 1986). However, it is worth noting that natural survival of larvae to settlement in most marine fish is typically no greater than 1%, therefore it may be that

elevated temperature accelerates the selection of surviving cohort and does not ultimately affect the overall number of surviving individuals. Further research focusing the long term effects of heatwaves and warming on larval fish, monitoring growth, fitness, and survival into the juvenile to sub-adult stages, would benefit our understanding of how ocean warming will effect coral reef fish. This study has provided new insights as to how the early life stages of a coral reef mesopredator could be affected by a warming ocean, which will allow us to better understand how these populations will fare as climate change progresses.

# Chapter 5: The effects of marine heatwaves on the physiology of a coral reef snapper

This chapter is prepared for submission to Global Change Biology

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## 5.1 Abstract

Marine heatwaves (MHWs) are increasing in frequency and intensity. Coral reefs are particularly susceptible to MWHs, which cause mass coral bleaching and mortality. However, little is known about how MWHs directly affect the fishes living on coral reefs. In this study I investigated how MWH conditions affect the physiology of a coral reef mesopredator, *Lutjanus carponotatus*. Specifically, I exposed mature adults to two different MWH intensities, +1°C (29.5°C) and +2°C (30.5°C) and tested how their physiological performance was affected at two and four weeks exposure, and recovery at two weeks post-exposure. At these time points I measured whole-organism physiological attributes (resting and capture oxygen consumption, and recovery time) and associated biochemical markers in the blood (baseline lactate, post-capture lactate, glucose, haemoglobin levels and haematocrit proportion). I found that two weeks exposure to MWH conditions increased resting oxygen consumption (+1°C = 23%, +2°C = 37%), recovery time (+1°C = 62%, +2°C = 77%), baseline lactate (+1°C = 27%, +2°C = 28%), post-capture lactate (+1°C = 62%, +2°C = 109%) and haemoglobin levels (+1°C = 13%, +2°C = 28%). This pattern was maintained at four weeks exposure except for post-capture lactate which was reduced (+1°C = -37%, +2°C = -27%), which resulted in only +2°C lactate levels being significantly higher than control. In combination, these results suggest that fish had a greater reliance

on anaerobic glycolysis to maintain homeostasis in MHW conditions. Some effects were observed at two weeks post-exposure, where capture oxygen consumption was significantly increased (+1°C = 25%, +2°C = 26%) and haemoglobin was still significantly higher (+1°C = 15%, +2°C = 21%) than control fish. These results show that MWH conditions have direct physiological costs on adult coral reef snapper and that ecologically relevant residual effects can last for at least two weeks afterwards. Nevertheless, the preferential temperature of adult *L. carponotatus* was found to be within the +1°C MHW range used in this study, which suggests they are adapted to these temperatures. This provides new insight into the effects of MWHs on the physiological performance of coral reef fishes and possible interactions with recreational and commercial fishing capture.

## 5.2 Introduction

Continued anthropogenic release of $CO_2$ and enhancement of the greenhouse effect is causing Earth's average temperature to increase and causing an increase in the frequency and intensity of extreme climatic events (Meehl et al., 2000; Allen et al., 2018; Hoegh-Guldberg et al., 2018). The oceans are estimated to have taken up more than 90% of the excess heat in the climate system, leading to unabated ocean warming since the 1970s (Hoegh-Guldberg et al., 2018). Both the increasing average temperature of the ocean, and anomalous warming events called marine heatwaves (MHWs), are of concern to marine ecosystems and the biodiversity they support. MHWs are caused by a combination of atmospheric and oceanographic processes with common drivers including persistent high-pressure systems, ocean currents that create a build-up of warm water, and air-sea heat flux that transfers atmospheric heat into the sea surface (Hobday et al., 2016; Holbrook et

al., 2019; Gupta et al., 2020). Specifically, MWHs have recently been defined as an anonymously warm event, exceeding the 90$^{th}$ percentile of a 30 year average that lasts for at least 5 days (Hobday et al., 2016). Of particular concern is the fact that over the last century MWHs have increased in both frequency (by 34%) and duration (17%) resulting in a 54% increase in the number of marine heatwave days (Oliver et al., 2018). The frequency, intensity, and duration of MHWs are expected to further increase as anthropogenic climate change continues (King et al., 2016; Frölicher et al., 2018). Consequently, MHWs are considered a more imminent threat to marine organisms than the more gradual average increase of sea surface temperate (Oliver et al., 2019; Smale et al., 2019).

Most marine organisms are ectotherms, therefore, an increase in water temperature can result in thermal stress when it exceeds their thermal optima (Somero, 1995; Mora & Maya, 2006; Pinsky et al, 2019). Recent MHWs have caused significant mortalities in invertebrates (Garrabou et al., 2009), loss of seagrass meadows (Marba & Duarte, 2010), mass bleaching and mortality in corals (Hughes et al., 2017) and are predicted to reduce the biomass of some fish populations (Cheung & Frolicher, 2020). While many marine ecosystems are affected by MHWs (Wernberg et al., 2013; Cavole et al., 2016; Smale et al., 2019) the impacts have been most acutely observed on coral reefs where mass coral bleaching and mortality due to anomalous temperatures have been observed with increasing frequency, magnitude, and geographical extent over the past 30 years (Heron et al., 2016; Donner et al., 2017; Le Nohaic et al., 2017; Hughes et al., 2018; Dietzel et al., 2020). For example, mass coral bleaching events occurred on the Great Barrier Reef in the summers of 2016 and 2017 where coral mortality was estimated to exceed 50% when averaged over the entire reef (Hughes et al., 2018; Stuart-Smith et al., 2018). However, documented effects of MHWs on other coral reef organisms are more

restricted (exceptions Spinks et al., 2019; Bernal et al., 2020; Le et al., 2020). Tropical marine species are predicted to be more sensitive to extreme temperatures than higher latitude species because they have evolved in more thermally stable environments (Tewksbury et al., 2008; Sunday et al., 2012; Comte & Olden, 2017) and are often living close to their thermal optimum in summer (Rummer et al., 2014, Rodgers at al. 2018). Yet, the direct effects of MHWs on most coral reef organisms remains unknown.

Increased water temperature can have broad physiological effects on fish from direct thermodynamic effects on biochemical reaction rates through to changes in whole organism traits such as swimming performance (Clarke & Johnston, 1999; Farrell et al., 2009; Little et al., 2020). Increasing rates of cellular processes in warmer water results in rising basal metabolic rates (Clarke & Johnston, 1999; Gillooly et al., 2001) and elevated energetic costs for physical activities (Johansen & Jones, 2011; Hein & Keirsted, 2012), resulting in increased recovery time post-exercise (Lee et al., 2003; Nofrizal & Arimoto, 2009). Furthermore, the additional energy requirement for physical activities may not be always possible through aerobic processes. For instance, the mitochondria, which have a key role in all ATP production, can decrease in efficiency due to enzyme thermal sensitivity, compromising their ability to meet ATP demands when optimal temperatures are surpassed (Brand & Nicholls, 2011). When oxidative metabolism is insufficient, individuals can increase anaerobic metabolism to meet energetic demands (Jacobs, 1986; Omlin & Weber, 2010; Iftikar et al., 2014) but at the cost of accumulation of byproducts like blood lactate (Jain & Farrell, 2003; Zakhartsev et al., 2004). Water temperature above the thermal optimum can also reduce aerobic capacity (Nilsson et al., 2009; Rummer et al., 2014; McMahon et al., 2019) through an inability of the cardio-vascular system to keep pace with maximum oxygen demands (Farrell et al., 2009; Pörtner et al., 2017). While research to

date provides some understanding of the likely impacts of elevated temperature on tropical marine fish (Donelson et al., 2010; Johansen & Jones, 2011; Rummer et al., 2014) much of the work has been designed to explore the effects of longer-term increase in average water temperature rather, than shorter duration warming events and potential recovery trajectories afterwards (Hallowed et al., 2013; Lefevre, 2016).

Additionally, the vast majority of research to date on the effects of elevated water temperature on coral reef fishes has focused on smaller bodied, site-attached species, leaving larger bodied, more mobile fish, relatively understudied. High thermal sensitivity has been observed in coral trout, a large predatory coral reef fish, with high water temperature affecting both their behavior and physiology (Johansen et al., 2014; Messmer et al., 2017; Pratchett et al., 2017). For example, coral trout were found to have decreased survivorship, activity, and aerobic scope under elevated temperatures (Pratchett et al., 2017). Maximal physiological performance is particular important for fisheries species as capture stress can lead to mortality in reef fish (Diggles & Ernst, 1997; Frisch & Anderson, 2010; Sumpton et al. 2010) and wild populations have also been found to be more susceptible to fishing efforts during MHWs (Brown et al., 2020). Further research into the effects of MHWs on larger coral reef fishes is essential because the expectation they can mitigate the negative effects of MHWs by moving to cooler and/or deeper waters may not hold for reef associated species. Research into the effects of MHWs on mesopredatory fishes, which prey on smaller coral reef organisms, but are also the prey of apex predators, is particularly lacking. Tropical snappers from the family Lutjanidae are among the most abundant mesopredators on coral reefs (Newman & Williams, 1996), they play an important role in ecosystem function (Ritchie & Johnson, 2009; Hixon, 2015; Hempson et al., 2017) and are components of both commercial and recreation fishing catches. The

Spanish flag snapper, *Lutjanus carponotatus* is one of the most abundant species of lutjanid on coral reefs and is often bycatch from line fishers who are targeting more desirable species such as coral trout. While the majority of line bycatch is thrown back alive the sudden and intense stress or capture can have significant physiological impacts (Davis, 2002; Wilson et al., 2014; Raby et al., 2018). Due to this, there could be increased capture stress during MHWs and consequently exacerbating the overall effect of MHWs on mesopredators.

To test the physiological effects of MHWs on a coral reef mesopredator, I subjected adult *L. carponotatus* to two different magnitudes of simulated heatwave conditions, +1°C (29.5°C) and +2°C (30.5°C) above summer average, for a period of four weeks. At two weeks exposure, four weeks exposure, and two weeks post-exposure I measured resting oxygen consumption, capture oxygen consumption, recovery time and associated blood chemistry responses (lactate, glucose, haematocrit and haemoglobin). In a subset of fish not exposed to the MHW treatments, I explored the thermal preference temperature and avoidance temperature to determine how their behavioural thermal optimum range relates to the simulated MHWs temperatures. This experimental design allowed me to measure the physiological effects of MHW conditions on adult *L. carponotatus*, both during and following the period of elevated temperature, and to determine if the magnitude and duration of the warming event have substantive effects as well as investigate potential lag effects on capture stress after a MHW.

## 5.3 Methods

### 5.3.1 Study species and collection

*Lutjanus carponotatus,* the Spanish Flag snapper, is a mesopredator that lives in coral reef habitat throughout the Great Barrier Reef (GBR) and Indo-Pacific region (Allen, 1985). On the GBR they are often one of the most abundant and widespread species of Lutjanidae on inshore and mid-shelf reefs (Newman & Williams, 1996). They are ecologically important to coral reef ecosystems (Polovina, 1984; Hempson et al., 2017), and valuable to recreational and commercial fisheries (GBRMPA, 2014). Mature adults for the experiment were collected by hook and line from multiple locations in the northern GBR between Cairns and Cape Melville from November 2018 to September 2019. The fish were transferred from holding facilities in Cairns to the Marine and Aquaculture Research Facility at James Cook University, Townsville within 10 days of capture. The average summer SST for the northern GBR, where the fish were collected, is approximately 28.5°C, with a seasonal range of 24-30°C (AIMS, 2017).

### 5.3.2 Aquaria and husbandry

Between six to eight adult fish (28-40 cm standard length) were housed together in twelve 2,500 L tanks (n=80 adults). Each tank was connected to a 10,000 L sump, where water was filtered through a 1 $m^3$ sand filter, 25 micron bag filters, and a 400 L protein skimmer. Before being delivered to the tanks the water passed through UV sterilization (~250 mJ $cm^3$). Dissolved oxygen and salinity were monitored continually with a permanent emersion probe and maintained at 6.1-6.6 mg/L. Adults were fed daily a mixture of

Skretting pellets (Spectra SS) and pilchards at ~2% body weight. The temperature cycle for all tanks followed a pattern similar to the average monthly temperatures for the northern GBR, where the winter and summer temperatures were 24°C and 28.5°C respectively.

### 5.3.3 Heatwave simulation

Average summer SST is approximately 28.5°C on the northern GBR, but periods of warmer water, up to 31°C, occur for short periods of time (24-48 hr) in most years (AIMS, 2017). Longer episodes of warmer water, lasting longer than five days, are classed as marine heatwaves (MWHs; Hobday et al., 2018). To simulate two different intensities of MHW conditions I chose treatments of +1°C (29.5°C) and +2°C (30.5°C) for a four week period, which similar in magnitude and duration to recent GBR MHWs (Spinks et al. 2019). Control water temperature was maintained at 28.5°C with a heat pump (Toyesi, Titan 20 kW) in a 10,000 L sump. Elevated temperature treatments were achieved with two 5,000 L ballast tanks controlled with titanium heat exchangers, one set at 45°C and the other at 13°C. These two water sources were mixed to create accurate delivery water temperatures for the +1°C (29.5°C) and +2°C (30.5°C) treatments via an automated controller (Innotech, Omni). The temperature treatments were automatically monitored and logged for the duration of the experiment and individual tank temperatures were checked daily. The MHW treatments began in February 2020 after adult fish had spawned. Four tanks were allocated to control, +1°C and +2°C treatments (12 tanks in total). The warming rate was 1°C per 24 hr for both heatwave treatments, which resulted in the +1°C treatment reaching the required temperature in 24 hr and the +2°C treatment reaching required temperature in 48 hr. Once the MHW treatments were achieved, tanks were maintained at that

temperature for four weeks. At the end of the 4 weeks, MHW treatments underwent cooling of -1°C per 24 hrs, until they reached 28.5°C.

### 5.3.4 Experimental design

A range of experimental assays were conducted over this study at 2 weeks exposure, 4 weeks exposure, and 2 weeks post-exposure (Fig.5.1). At each of these 3 time points there were two testing groups per temperature treatment, and all fish were fasted for 24 hrs prior to testing. The same individuals were tested at each time point. The first group were baseline controls, which comprised of 6 fish per treatment that were caught directly from their tank and a blood sample was taken. Blood was drawn from the caudal vein (~150 µl blood, <1% total blood volume) using a hypodermic needle pre-coated in lithium heparin (21-G, 1 ml syringe). 15 µl of this sample was used to determine baseline blood lactate (mmol per ml) with the Accutrend Plus (Roche Diagnostics Australia). These individuals were not used in any other subsequent assays for that time point. The second testing group was comprised of 12 individuals from each temperature treatment. This testing group was put through a simulated capture event, which was immediately followed by blood collection (<1% total blood volume), followed by respirometry to collect $MO_2$ over the subsequent ~20 hours (details below). Following the 6 week MHW exposure period experimental temperatures was brought down from 28.5°C to 26°C, over a four week period, at which point 11 individuals from the control treatment were used to determine preferential temperature.

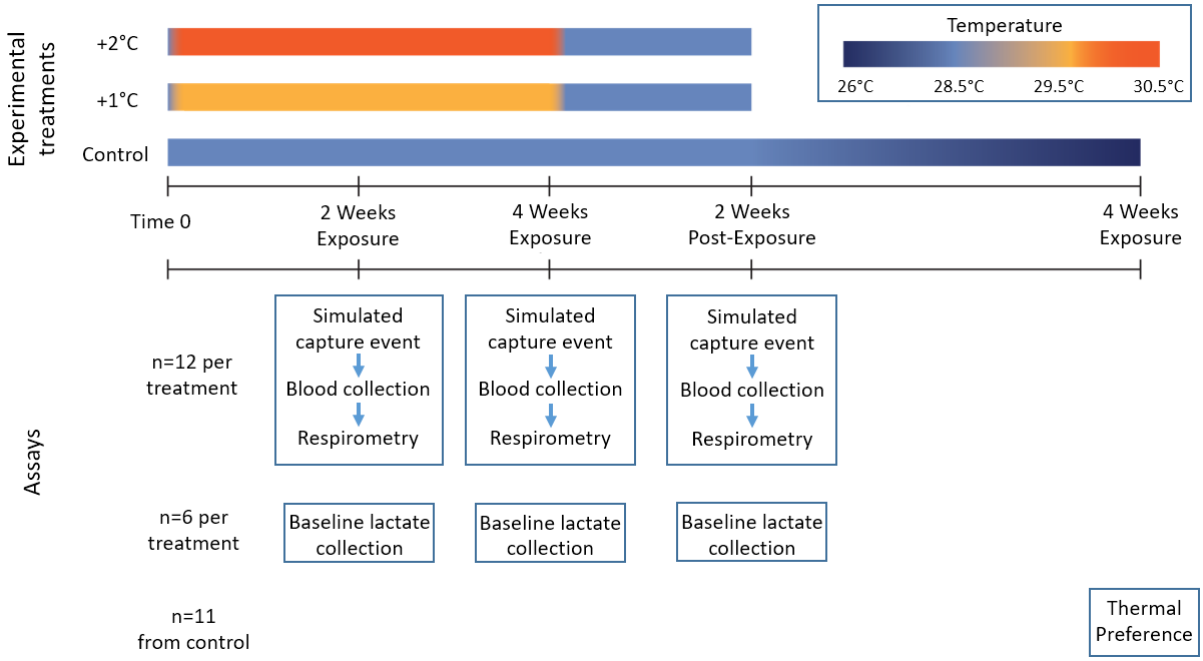


**Figure 5.1** Experimental timeline and assay time points testing the effect of marine heatwaves on adult Spanish flag snapper (*Lutjanus carponotatus*).

## 5.3.5 Simulated capture event

To simulate a hook and line capture event I used a 90 L swim tunnel (Loligo, fitted with a TechTop 1 hp, 2880 rpm motor) to elicit sudden burst swimming. Twelve fish from each treatment (36 fish total, standard length = 310 mm ± 31 mm, weight= 588 g ± 87 g, mean ± SE) were placed in the swimming chamber at a slow water speed of 5 cm/s. Once the individual had orientated into the water flow (~5-10 seconds on average) the water speed was immediately increased to full speed (from 5 cm/s to 200 cm/s in 5 seconds), equating to approximately 6-7 body lengths per second, which elicited bursting and erratic swimming. This was sustained for 60 seconds after which point the water flow was stopped and the fish removed. As the species are typically found in shallow reef environments (<20m depth) a fishing capture event would typically be between 1-2 minutes and the species is known to swim intensely for the duration of the event. This simulation is intended

to be more ecologically accurate measurement of the upper oxygen consumption for the species than using a traditional chase methods.

### 5.3.6 Blood collection and analysis

Blood was drawn from all fish following the capture stress assay, prior to respirometry testing (Wood & Munger, 1994). A minimal amount of blood was drawn from the caudal vein (~150 µl blood, <1% total blood volume) using a hypodermic needle pre-coated in lithium heparin (21-G, 1 ml syringe). The blood drawing site was cleaned with betadine wipes and the procedure took no longer than 60 seconds. Each blood sample was immediately used to measure the concentration of lactate (mmol per ml) and glucose (mg per L) from 15 µl samples using the Accutrend Plus (Roche Diagnostics Australia). Haemoglobin was then calculated by Drabkin's method (Drabkin & Austin, 1935; Balasubramaniam & Malathi, 1992) using a spectrophotometer (Thermo Scientific, Spectronic 200) to calculate the haemoglobin (g per L). Finally, haematocrit proportion was calculated by centrifuging three micro capillary tubes with ~30 µl of blood for 3 minutes, then averaging the proportion of packed red blood cells of the three tubes.

### 5.3.7 Intermittent flow respirometry

Aerobic performance was measured using intermittent flow respirometry and fish were fasted for 24 hrs before respirometry (Clark et al., 2013; Svendsen et al., 2016) (n=12 per treatment). Fish were tested at their respective MHW treatments during the exposure period and all treatments at control temperature 2 week post-exposure (Figure 1; control: 28.5°C, +1°C: 29.5°C and +2°C: 30.5°C). Respirometry was conducted in purpose-built

intermittent-flow respirometry chambers (35.5 L per closed system), submerged in aquaria within the individuals respective experimental treatment water. Submersible pumps fitted to each chamber supplied a continuous water flow from the surrounding water bath through the chambers. Activity was reduced in the respiration chambers by using appropriately sized chambers to minimise movement and by shading each chamber from visual simulants. A purpose built python program, AquaResp v3.0, was used to control the measurement cycle timing. This consisted of a 10 minute measurement period, 7 minute flushing period, and a 1 minute wait period, which was repeated over 24 hr trial duration. The $O_2$ consumption rates were measured during the intervals of interrupted water flow with a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany), which the AquaResp program recorded during the measurement periods. The entire measurement period was used to calculate $MO_2$ provided that the slope $R^2$ was >0.90. Over 93% of measured slopes across all treatments were above this threshold. The 7% of slopes that were under 0.90 $R^2$ were not used. Fish were immediately placed into respirometry chambers following the capture assay and 1 minute air exposure where blood was drawn (<1% total volume) and the fish were weighed. Measurement started once the chamber was closed allowing for capture $MO_2$ to be measured. Fish then remained in the chambers while recovering back to resting $MO_2$ over 20 hours.

Capture $MO_2$ and resting $MO_2$ of individuals was calculated in mg $O_2$ $kg^{-1}$ $h^{-1}$ using the equation:

$$\mathbf{MO_2} = \boldsymbol{K}*\boldsymbol{V}*\beta \,/\, \boldsymbol{M}$$

where $K$ is the linear rate of decline ($kPah^{-1}$) in the oxygen content over time (h) in the respirometer; $V$ is the volume of the respirometer in L, which is adjusted for the volume of the fish; β is the solubility of oxygen in water at a specific temperature and salinity (mg $O_2$

$L^{-1}$ $kPa^{-1}$); and *M* is the body mass of the fish (kg). Blank measurements were taken for each chamber at the start and end of each trail to calculate any background respiration.

Background respiration did not exceed 5 mg $O_2$ $kg^{-1}$ $h^{-1}$ in any trial. Linear regressions were then used to calculate background respiration over the trail, which was used to adjust the $MO_2$ measurements for each fish. Capture $MO_2$ was determined from the first $O_2$ reading, directly after the capture assay was completed. Resting $MO_2$ was determined by using the mean of the lowest normal distribution for $MO_2$ values (Behrens & Steffensen, 2007; Chabot et al., 2016). Recovery time was determined by amount of time it took for oxygen consumption to decline from capture $MO_2$ to the intercept of the resting $MO_2$. Resting $MO_2$ was used as a proxy for resting metabolic rate and capture $MO_2$ was used to estimate the metabolic cost of the capture event.

### 5.3.8 Temperature preference

Thermal preference is expected to indicate the optimal temperature for physiological functions (Martin &Huey, 2008; Angilletta, 2009), and in some species has been correlated to thermal optima for growth (Killen, 2014). To determine the temperature preference in this population of *L. carponotatus*, I used a custom-built shuttle box arena (Chambers= diameter 80 cm/total arena volume of 330 L) in conjunction with commercial tracking software and controllers (Loligo, ShuttleSoft), to conduct a preferential temperature test (Killen, 2014). All individuals were held at 26°C before the experiment began as it the approximate mid-point of this species temperature range. The experiment was automated to determine the preferred temperature and avoidance temperature of adult *L. carponotatus*. Before the test an individual (from control treatment only) was placed in the

arena at 1600, with one chamber set at 25°C and the other at 27°C. Fish were then allowed to habituate to the arena overnight until the preference trial started at 0800. All fish settled into the 27°C chamber overnight, consequently the automated system started all trails by increasing the temperature of both sides, at a rate no faster than 2°C per hour, while maintaining 2°C differential. Avoidance temperature was determined once the individual left the warmer side of the arena for sufficient time to start cooling both sides. The preferential temperature was determined when the individual positioned itself in the thoroughfare between the two sides or swam continuously between both sides, thereby stopping any further shift in temperature.

### 5.3.9 Statistical analysis

Linear mixed effects models (LME) were used for all measured physiological responses to identify significant differences between treatments, except for Haematocrit, which was analysed with a generalized linear mixed model (GLMM). In all LMEs, and in the GLMM for Haematocrit, temperature and exposure time were fixed factors and tank a random effect. An ANOVA was then run on each model to test the main effects. Following this, Tukey's post-hoc tests were conducted on any models with significant effects. All analyses were conducted in R (R Core Team, 2014) using the LME4 and GLMM packages (Bates et al., 2015). Tukey's post-hoc tests were conducted on significant factors while estimated marginal means tests, adjusted with Tukey's. All models met the assumptions of the relevant tests. This was confirmed by accessing the residuals, goodness of fit, and checking dispersion.

## 5.4 Results

### 5.4.1 Aerobic performance

Resting oxygen consumption was significantly higher in the MHW treatments ($F_{2,97}$=66.52, p<0.001) compared to the controls, varied between sampling time points ($F_{2,97}$=18.15, p<0.001), and there was an interaction between the effects of temperature differed across times ($F_{4,97}$=7.92, p<0.001)(Fig. 5.2a). In fish exposed to the +1°C MHW treatment resting $MO_2$ increased on average by 22-23% at two (220 mg $O_2$ kg $h^{-1}$; t=-5.96, p<0.001) and four (216 mg $O_2$ kg $h^{-1}$; t=-5.63, p<0.001) weeks exposure compared to control fish (180 mg O2 kg $h^{-1}$)(Fig. 5.2a). A similar pattern was seen in the +2°C MHW treatment where resting $MO_2$ was significantly increased on average by 31-33% after two (240 mg O2 kg $h^{-1}$; t=-9.44, p<0.001) and four (237 mg O2 kg $h^{-1}$; t=-7.99, p<0.001) weeks exposure compared to control fish (Fig. 5.2a). At two-weeks post-exposure, resting $MO_2$ in both MHW treatments was lower than during the warming exposure (12% and 23% reduction in +1°C and +2°C fish respectively) and was no longer higher than in control fish (+1°C: t=-2.32, p= 0.337; +2°C: t=-1.95, p=0.583). The resting $MO_2$ of control fish remained at approximately 180 mg $O_2$ kg $h^{-1}$ throughout the 6 week experiment (Fig. 5.2a; all p>0.05, Table A5.1).

The capture $MO_2$ of individuals was effected by MHW treatments ($F_{2,97}$=6.01, p=0.004), heatwave exposure duration ($F_{2,97}$=6.36, p=0.003), and there was also an interaction between warming and sampling time point ($F_{2,97}$=4.65, p=0.002)(Fig. 5.2b). Specifically, capture $MO_2$ was similar across treatments for both two and four weeks exposure at ~ 640 mg $O_2$ kg $h^{-1}$ (all post-hocs p>0.05; Table A5.2). However, at two weeks post-exposure capture $MO_2$ was increased by 25% in fish that had experienced the +1°C

treatment (~764 mg $O_2$ kg $h^{-1}$; $t_{93}$=-4.68, p<0.001) and the +2°C treatment (~765 mg $O_2$ kg $h^{-1}$; $t_{93}$=-4.70, p<0.001) compared to control fish (~594 mg $O_2$ kg $h^{-1}$)(Fig. 5.2b).

Recovery time following the simulated capture event was significantly higher in MHW treatments ($F_{2,97}$=24.27, p<0.001), differed between sampling time points ($F_{2,97}$=33.71, p<0.001) and there was a significant interaction between MHW exposure and time point ($F_{2,97}$=9.56, p<0.001)(Fig. 5.2c). In the +1°C MWH treatment, recovery time at two weeks exposure was approximately 140 minutes longer (77%; t=-5.74, p<0.001) than control fish, and remained significantly longer at four weeks exposure (120 min longer, 66%; t=-4.90, p<0.001). For fish in the +2°C MHW treatment, recovery took approximately 170 minutes longer at two weeks exposure (94%; t=-6.95, p<0.001) and 120 minutes longer at four weeks exposure (64%; t=-4.70, p<0.001) than control fish (Fig. 5.2c). While this was a 30% reduction between two and four weeks exposure it was not significantly different (t=2.28, p=0.363). At two weeks post-exposure the recovery time for fish that had been exposed to both MHW treatments was significantly lower than during the MHW phase, with recovery only taking 180 minutes on average (all p<0.001; Table A5.3). Post-exposure recovery times were comparable to the control fish (control vs +1°C: t=0.470, p=1.000; control vs +2°C: t=0.940, p=0.990). Recovery time was similar between the two MHW treatments at each of the exposure timings (all post-hocs p>0.05; Table A5.3).

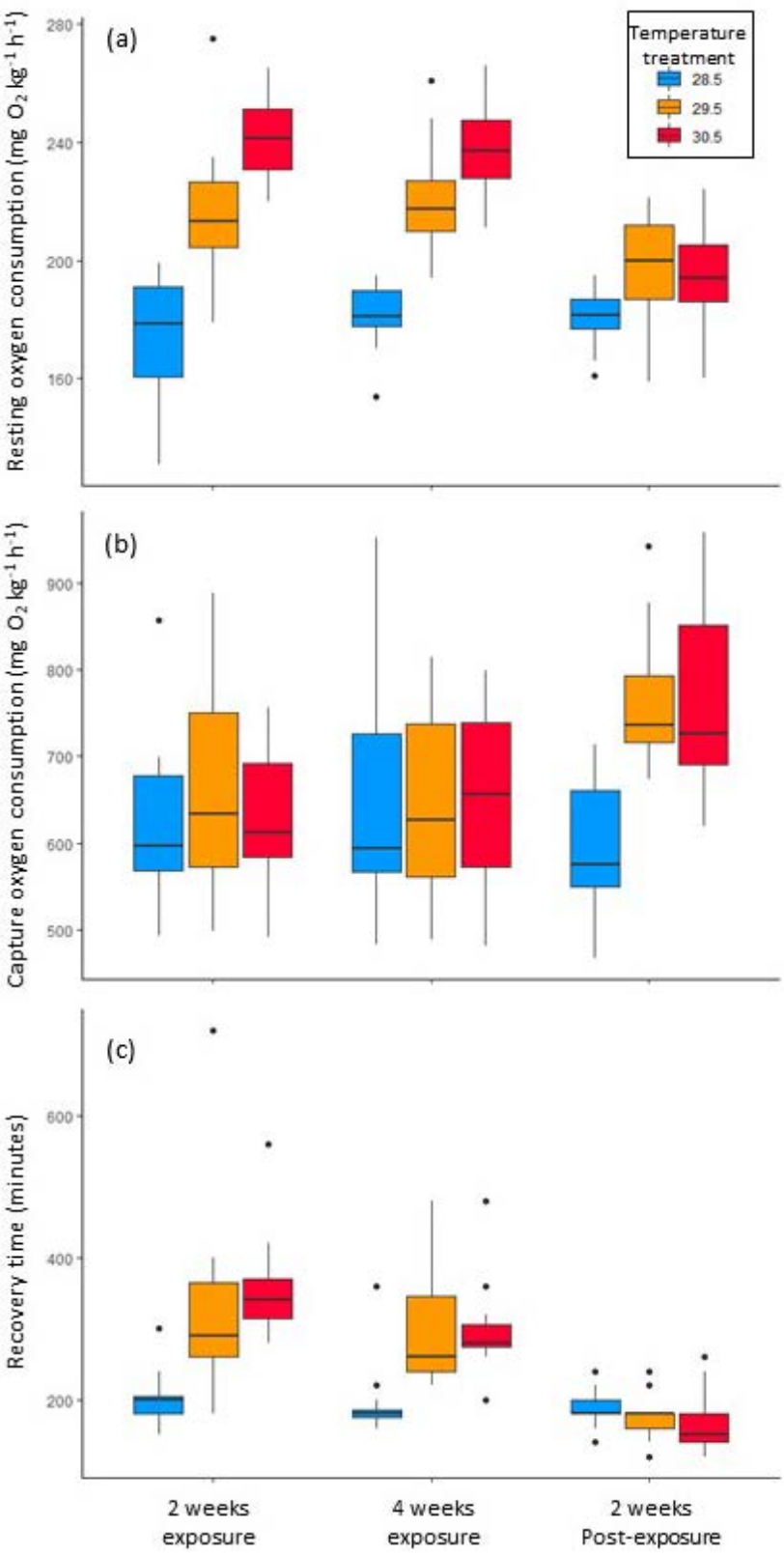


**Figure 5.2** Resting oxygen consumption (a) capture oxygen consumption (b), and recovery time (c) of adult *Lutjanus carponotatus* under ambient (28.5°C) conditions and two marine heatwave treatments of +1°C (29.5°C) and +2°C (30.5°C). Individuals were tested at 2 weeks exposure, 4 weeks exposure and 2 weeks post-exposure.

## 5.4.2 Blood parameters

Baseline blood lactate was significantly affected by MHW treatments ($F_{2,34}$=7.64, p=0.002) and sampling time point ($F_{2,34}$=3.32, p=0.048)(Fig. 5.3a), but there was no interaction between MHW treatments and sampling time point ($F_{4,34}$=0.65, p=0.628). Baseline lactate in control fish was 1.68 µmol $ml^3$ at two weeks (Fig. 5.3a). Baseline blood lactate was 27% and 28% higher than control levels in the +1°C and +2°C MHW treatments respectively (Fig. 5.3a). At four weeks exposure, the baseline blood lactate levels in the MWH treatments were 36% (+1°C) and 48% (+2°C) higher than in the control fish (1.66 µmol $ml^3$). At two weeks post-exposure the blood lactate of MHW treated fish had reduced to levels closer to control fish, albeit still ~15% higher (Fig. 5.3a). This pattern resulted in significant differences in blood lactate between both +1°C and +2°C MHW treatments compared with control fish (control vs +1°C: t=-3.07, p=0.012; control vs +2°C: t=-3.63, p=0.003) and between four weeks exposure and two week post-exposure (t=2.55, p=0.04).

Post-capture blood lactate was significantly affected by MHW treatment ($F_{2,97}$=26.17, p<0.001), exposure sampling time point ($F_{2,97}$=32.59, p<0.001) and the interaction between temperature and sampling time point ($F_{4,97}$=5.15, p<0.001)(Fig. 5.3b). Specifically, post-capture lactate was higher at two weeks exposure in fish from both the +1°C (8.3 mmol $L^{-1}$, 62% higher; t=-4.12, p=0.002) and the +2°C MHW treatment (10.7 mmol $L^{-1}$, 109% higher; t=-7.26, p<0.001) compared with control fish (5.1 mmol $L^{-1}$). Post-capture blood lactate was significantly lower after four weeks compared with the two week sampling point for both the +1°C (5.29 mmol $L^{-1}$ , 37% reduction; 2 weeks vs 4 weeks: t=3.92, p=0.005) and +2°C MHW treatments (7.84 mmol $L^{-1}$ , 27% reduction; 2 weeks vs 4 weeks: t=3.76, p=0.009)(Fig.5.3b). This resulted in only the +2 °C MHW treatment fish

having higher post-capture lactate than control (t=-4.03, p=0.003) and +1°C MHW fish (t=-3.29, p=0.036) at the four week sampling point. At two weeks post-exposure, fish from the +2°C MHW treatment had reduced post-capture lactate levels compared with the 4 week MHW sampling point (38% reduction; t=3.78, p=0.008), such that no treatments were different from each other at two weeks post-exposure (28.5°C vs 29.5°C: t=-0.76, p=0.976; 28.5°C vs 30.5°C: t=-1.22, p=0.949; 29.5°C vs 30.5°C: t=-0.45, p=0.999). Blood glucose levels were not significantly affected by MHW treatments ($F_{2,97}$=2.90, p=0.06), sampling time point ($F_{2,97}$=2.51, p=0.09) and there was no interaction between MHW treatment and time point ($F_{4,97}$=1.83, p=0.129)(Fig. 5.3c).

MHW treatments significantly increased haemoglobin ($F_{2,97}$=43.36, p<0.001) and haematocrit ($X^2$=139.00, df=2,97, p<0.001) (Fig 5.4). Haematocrit and haemoglobin concentration was highest in the +2°C treatment (Haematocrit: 44.5%, Haemoglobin: 114 g $L^{-1}$), followed by the +1°C (Haematocrit: 40.3%, Haemoglobin: 101 g $L^{-1}$) and lowest in control fish (Haematocrit: 35.5%, Haemoglobin: 89 g $L^{-1}$; all p<0.05, Tables A5.4 & A5.5). On average there was ~13% more RBCs for every 1°C temperature increase. Both haematocrit and haemoglobin exhibited a pattern of generally decreasing with time, however, this was only significant for haematocrit ($X^2$=13.44, df=2,97, p=0.001; Fig. 5.4a) and not for haemoglobin ($F_{2,97}$=2.89, p=0.06; Fig. 5.4b). Furthermore, this difference was only significant between two weeks exposure and two weeks post-exposure (t=3.67, p=0.001).

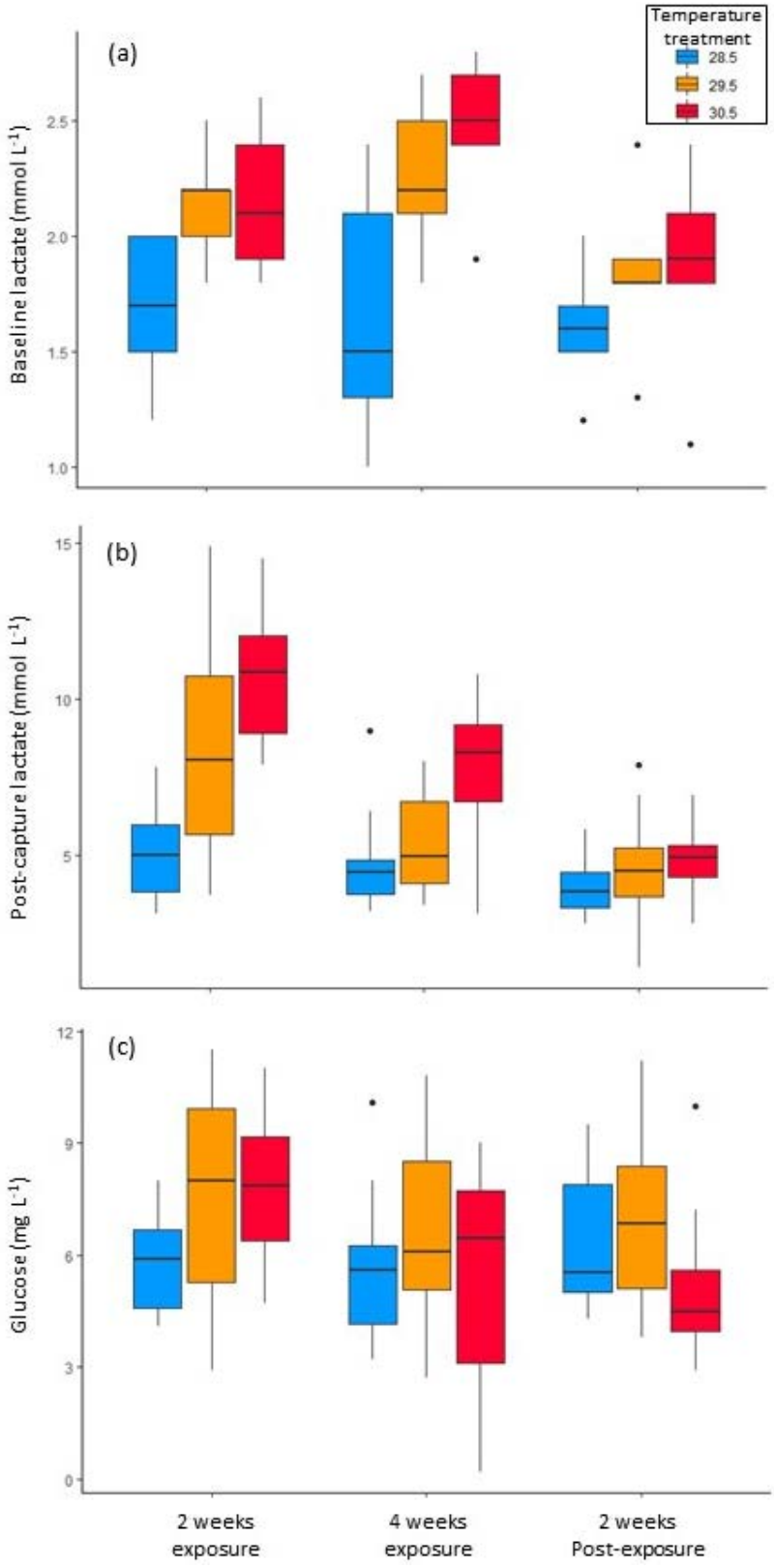


**Figure 5.3** Baseline lactate (a), post-capture lactate (b), and glucose (c) of adult *Lutjanus carponotatus* under ambient (28.5°C) conditions and two marine heatwave treatments of +1°C (29.5°C) and +2°C (30.5°C). Individuals were tested at 2 weeks exposure, 4 weeks exposure and 2 weeks post-exposure.

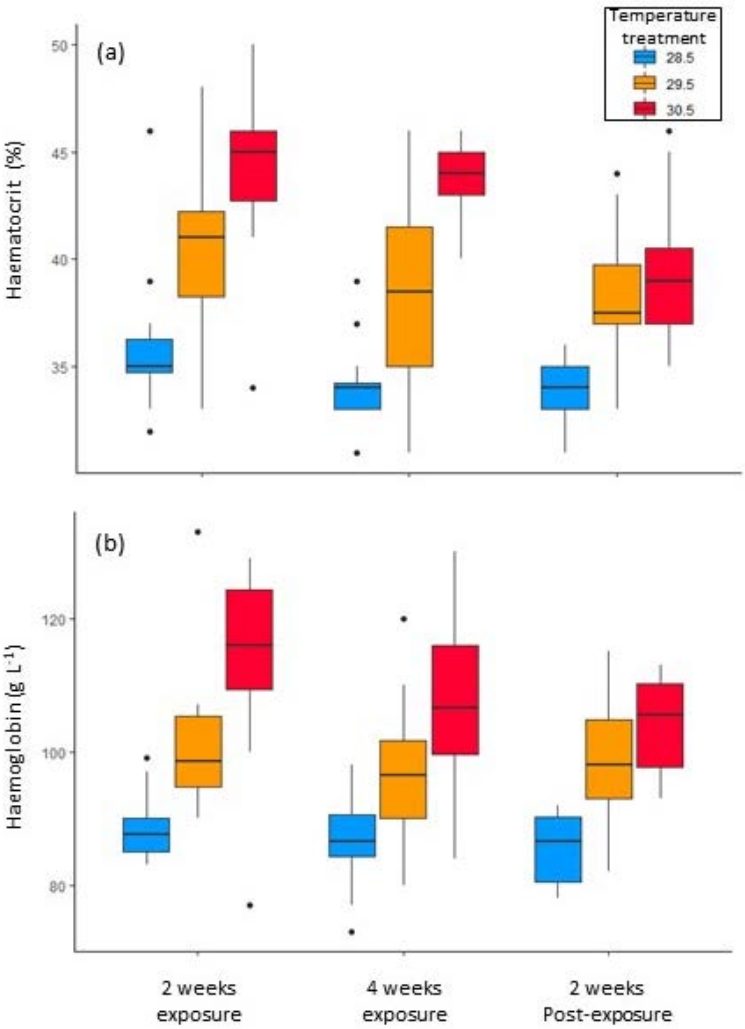


**Figure 5.4** Haematocrit proportion in whole blood (a) and haemoglobin (b) of adult *Lutjanus carponotatus* under ambient (28.5°C) conditions and two marine heatwave treatments of +1°C (29.5°C) and +2°C (30.5°C). Individuals were tested at 2 weeks exposure, 4 weeks exposure and 2 weeks post-exposure.

### 5.4.3 Temperature preference

The preferred temperature of *L. carponotatus* was found to be 29.81°C ± 0.25 (SE) (range 28 to 31.2°C). The avoidance temperature was approximately 2°C warmer than the preferred temperature at 31.93°C ± 0.22 (range 29.5°C to 33.1°C). All individuals started the trial by increasing the arena temperature, by staying in the warmer side, until they reached their avoidance temperature at which point, they moved to the cooler side and then swam evenly between the warm and cool side to stabilize the arena temperatures.

## 5.5 Discussion

The increased frequency and intensity of MWHs pose a significant threat to marine organisms, especially those evolved to live in relatively stable thermal environments such as coral reefs. I found that adult *L. carponotatus* displayed a range of physiological responses at simulated MHW conditions only +1 and +2°C above the summer average. During the MWHs, fish had higher metabolic costs and showed evidence of higher stress but mostly recovered within 2 weeks post-exposure. The increased basal cellular costs increased recovery time following a capture event and elevated blood lactate indicated negative physiological effects of MHW conditions. Depending on the length of a MHW in nature this could potentially lead to a decrease in body condition and reduced capacity to escape predators or forage for prey (Killen et al., 2015; von Biela et al., 2019; Brown et al., 2020; Piatt et al., 2020). Interestingly, haemoglobin /haematocrit and capture oxygen consumption were still elevated at two weeks post-exposure which, in combination with post-capture lactate, indicates evidence for a shift in the relative aerobic vs anaerobic energy production. These findings suggest that while some physiological processes may be pushed to their limit this species has the capacity to alter its physiology to meet the required energy demands.

Thermal stress was observed in several physiological attributes during MHW exposure. Resting oxygen consumption, recovery time, and baseline blood lactate levels were all significantly higher in the two MWH treatments during the 4 week exposure period, but showed recovery after two weeks back at control temperature (i.e. post-exposure). Resting oxygen consumption, and consequently basic metabolic costs, are generally observed to increase anywhere in the range of 2-14% ($Q_{10}$: 1.48 – 3.71) for every

degree of warming during summer for tropical coral reef fish (Nilsson et al., 2009; Johansen & Jones, 2011; Rummer et al., 2014; Messmer et al., 2016; Pratchett et al., 2017). Resting $MO_2$ of *L. carponotatus* increasing by 10-20% per degree Celsius ($Q_{10}$: 2.86 - 7.93) which suggests a high degree of thermally sensitivity compared to other coral reef species but is similar to another larger bodied mesopredator, the coral trout (10-14% increase per °C) (Messmer et al., 2016; Pratchett et al., 2017). I also observed costs in the time it took for fish to reach resting $MO_2$ following simulated capture stress, with recovery time of individuals around 2 hours longer in both +1°C and +2°C MHW conditions. Not only is this a cost in terms of elevated metabolic rate for the extended period but it could also leave them at risk from predators for a longer period due to reduced aerobic escape capacity (Killen et al., 2015). Fish from both MHW treatments also had higher baseline lactate levels (27-48% higher than controls) suggesting that a greater amount or proportion of energy was being produced through anaerobic glycolysis. This is perhaps due to negative thermal effects on mitochondrial efficiency or simply an inability to make all the required energy aerobically since resting oxygen consumption also increased (Jacobs, 1986; Omlin & Weber, 2010; Iftikar et al., 2014). There was also a trend that blood lactate, and perhaps stress, was accumulating through time in MHW fish due to the general increase (15-20%) in lactate between two and four weeks exposure. However, these fish were able to process this lactate once temperatures returned to normal.

During exercise, blood lactate levels can rise quickly when there is increased energy demands for swimming (Jones, 1982; Weber et al., 2016). Blood lactate was elevated by MHW conditions to a far greater extent due to simulated capture stress (67-109%) relative to the baseline elevation (27%) at that same MHW exposure time point. Since capture oxygen consumption was the same across all treatments during MHW exposure it is

probable that aerobic capacity was insufficient in elevated MHW treatments and therefore anaerobic energy production was increased to compensate (Drucker & Jensen, 1996; Svendsen et al., 2010). The additional lactate would be a contributor to the longer recovery times observed as aerobic metabolism would remain elevated to process lactate from the blood via oxidation (Wells et al., 2009; Ohlendieck, 2010). Interestingly, when comparing the baseline and post-capture lactate there were slightly different patterns through time. Baseline lactate tended to increase at two week exposure and remained high at four weeks, while in contrast post-capture lactate decreased between 2 and 4 week exposure. This seems to indicate that no physiological mechanisms were induced to compensate the basic cellular costs of functioning in the elevated conditions. However, other physiological mechanisms not measured directly here may have been initiated and consequently resulted in a reduction of lactate following the capture event (e.g. upregulation of lactate dehydrogenase; Larios-Soriano et al., 2020).

Haemoglobin and haematocrit were elevated during MHW treatments and there were residual effects post-exposure. Increased production of red blood cells (RBCs), as indicated by increased haematocrit, may have been induced to meet the increased respiration and energy demands elicited by elevated temperatures (Gillooly & Zenil-Ferguson, 2014). Additional RBCs would likely result in greater capacity to transport oxygen, and greater efficiency in diffusion at the gills, when dissolved oxygen concentration is reduced at higher temperatures (Wells & Baldwin, 1990; Gallaugher & Farrell, 1998). Consequently, we might expect to observe a similar shift in aerobic performance, including elevated resting and capture respiration. The pattern for resting oxygen consumption somewhat followed expectations, with increased oxygen demand during MHW, however, there was no increase in oxygen consumption during simulated capture stress despite

additional RBCs. While it is possible that capture swimming cost did not rise with in MHW treatments (i.e. if fish were still swimming within their thermal optimal range), the increased lactate levels and recovery time indicate an elevated energy demand of swimming in MHW temperatures. Therefore, it is more likely that another aspect of swimming physiology, not oxygen delivery, was limiting capture oxygen consumption (Pörtner, 2010; Pörtner et al., 2017). For example, capture oxygen consumption may represent the maximum capacity of aerobic swimming (Johnston & Dunn, 1987; Schulte, 2015) which is relatively uniform between our three treatments during a MHW. Alternatively, our measurements may represent the limit of aerobically produced energy (i.e. Krebs cycle: Krebs, 1950). While I am unable to elucidate the physiological mechanism within this study, there does appear to be a limit to aerobic capacity that is not influenced by MHW treatment.

While the additional RBCs did not alter capture oxygen consumption during MHW conditions, they may have once temperature returned to normal in the two weeks following. Capture oxygen consumption was 25% higher than control, and higher than these fish during the MHW, in both +1°C and +2°C MHW treatments at two week post-exposure while haemoglobin and haematocrit remained elevated. The lifespan of a RBC is thought to be ~60-120 days (Franco, 2012; Shrestha et al., 2016) and if the initial temperature increase induced the production of additional RBCs, they would not be destroyed or discarded if healthy. Thus, the elevated proportion of RBCs (haematocrit) post-exposure are likely to be a legacy of MHW exposure rather than an active response. Interestingly, alongside this elevated aerobic response, higher lactate levels and recovery time returned to control levels in MHW fish post-exposure. This suggests that the relative production of aerobic to anaerobic energy during swimming capture has shifted, and

perhaps the increase in RBC is assisting the increase of aerobic energy production (Mairburl, 2013; Saunders et al., 2013).

Although I observed some persistence of physiological effects post MHW exposure the fish generally showed limited stress and costs two weeks post-MWHs. The only physiological attribute that suggested a continuing cost was resting oxygen consumption (~9% higher) however this was not statistically significant. Baseline lactate and recovery time, while significantly higher during the MHW exposure phase, both returned to control levels within two weeks of fish returning to control conditions. There may have been other energetic or physiological costs associated with MHWs that were not measured here, such as the release and replacement of hormones (Iwama et al., 1998; Iwama et al., 1999; Alfonso et al., 2020), production of proteins (Foster et al., 1992; Jonassen et al., 1999; Johansen et al., 2020), and cell repair from oxidative stress (Lepock, 2005; Lushchak & Bagnyukova, 2006; Madeira et al., 2013; Birnie-Gaivin et al., 2017) that may still impose an energetic cost on fish following a MWH. It is also probable that I would have observed costs in the metrics I investigated had I measured closer to the end of exposure (e.g. 1 week post-exposure). However, it is encouraging that while *L. carponotatus* is sensitive to MHWs, there is a relatively rapid recovery of the physiological system within two weeks. This suggests that MHWs similar to the length and duration used in this study may not have any significant, long lasting effects on these fish.

Physiological changes measured in this study are expected to be a result of thermal effect to molecular processes, cellular stress response, and cellular homeostasis response (Kültz, 2003; Kültz, 2005). When stressful conditions persist, genes may be up or down regulated to adjust cellular and whole organism phenotype in response to the altered environmental conditions. Only one study so far has investigated the molecular response

of coral reef fish (damselfish and cardinalfish) to a natural MHW (Bernal et al., 2020). Interestingly, Bernal and colleagues (2020) found that during a MHW fish exhibited gene expression changes that related to metabolic processes, cell damage, and cell repair. At the peak of the MHW gene expression differences were associated with processes including mitochondrial activity, adenosine triphosphate activity, and cholesterol and fatty acid metabolism, which may indicate genomic level effects to the higher-level metabolic processes in the present study.

The average thermal preference temperature of 29.8°C observed for *L. carponotatus* was similar to the +1°C MHW treatment (29.5°C) used in this study, at which I observed indicators of stress (baseline lactate) and increased energetic costs (resting $MO_2$ and recovery time). This preferred temperature is only slightly warmer than what has been recorded for other reef fish species, collected from similar regions of the GBR, including the five-lined cardinalfish (29.5°C, Nay et al., 2015) and the blue green damselfish (28.9°C, Habary et al., 2017). It is possible that there are other physiological responses, that were not measured here (e.g. reproduction, enzyme activity), that may be enhanced in warmer conditions and therefore at 29.8°C the overall fitness of this species is optimised. However, it is important to remember that preference temperature is not always what the individual will select in nature with other aspects of ecology (territory, shelter, food, etc.) influencing body temperature (Hugie & Dill, 1994; Lindberg et al., 2006; Eurich et al., 2018). Interestingly, this preferred temperature may indicate that shorter warming events than tested here (<2 weeks) might not be a physiological challenge for this species, but further work would need to explore this. The avoidance temperature for *L. carponotatus* was found to be only 3.4°C above the summer average and only 1.1°C above preference, indicating a relatively narrow thermal window. Importantly, GBR reefs have exceeded this avoidance

temperature during MHWs in 2016, 2017, 2020 (AIMS, 2017; Hughes et al., 2018; BOM, 2020), suggesting that existing MHWs have already come close to the thermal limits for this species. Alternatively, as this species has evolved in shallow tropical reefs they may simply remain in their established home ranges during MHWs and endure the physiological responses they elicit. This hypothesis may be supported by the relative speed at which they can recover from MWHs. A similar coral reef mesopredator, *Plectropomus leopardus,* was shown to reduce its activity but increase feeding rate as temperature rose above the summer average (Scott et al. 2019), which could be a strategy for *L. carponotatus*. However, regardless of which strategy the species employs to maintain homeostasis during MWHs there will be energetic requirements that could be difficult to manage.

Interestingly, the physiological effects of the two different magnitudes of simulated MHW (+1°C and +2°C) was often similar. The temperature increase of +1°C elicited a significant response in baseline lactate, post-capture lactate (exception four weeks exposure), resting metabolic rate, and recovery time, which was not proportionally increased further at +2°C (i.e. there was not an additive effect of each 1°C temperature increase in the MHW treatments). This suggests that not all physiological processes will be effected linearly by MHWs which supports the hypothesis that tropical species are sensitive to relatively small temperature increases and are living close to their thermal optimum during summer (Tewksbury et al., 2008; Sunday et al., 2012; Rummer et al., 2014; Comte & Olden, 2017; Rodgers et al., 2018). The complexity of these various thermal physiological responses indicates the importance of understanding a range of physiological traits when investigating the effects of future MHWs on wild populations, as no single metric is sufficient to comprehend the whole animal physiological response to elevated temperature.

The increasing frequency and intensity of MHWs is an immediate concern for thermally sensitive marine organisms. This study shows that while short-term (2-4 week) exposure to MWHs conditions has significant effects on the physiology of a coral reef mesopredator there is a relatively rapid restoration to baseline levels post-exposure. Nevertheless, while fish appear able to alter their physiological processes to cope with MWHs, the elevated resting metabolic rate suggests that fish still need to obtain about 20% more energetic resources to sustain basic maintenance during MHWs. For mesopredators, this would ultimately mean increased predation of smaller reef organisms which could flow on to affect abundance and assemblage composition of lower trophic levels. Conversely, these increased energy demands could be met with a trade-off by decreasing other activities like growth or reproduction, which might influence population dynamics of *L. carponotatus*. While individuals may be able to mitigate the effects of MWHs by moving to cooler, deeper reefs this will not be possible for all individuals and will have ramifications in terms of food availability and predation risk. Additionally, while the thermal preference of this species was shown to be in the range of MHWs treatments used, the avoidance temperature was only around 1°C warmer than the preferred temperature and within 3-4°C of the current-day summer temperatures, which suggests that this species is currently living close to its thermal maximum in summer. Further research into how the length and intensity of MWHs effect the physiology of *L. carponotatus,* and other coral reef mesopredators, would help us identify the physiological limits and costs that future MHWs may impose on these fish. Overall, my findings shown that *L. carponotatus* is negatively affected by predicted future MHW conditions but possess capacity to recover within a few weeks of these events.

# Chapter 6: General discussion

Understanding the effect of climate change on mesopredators is important because of their essential trophic position and functional role in marine ecosystems. Yet, there is a paucity of research on how sensitive life stages of rocky and coral reef mesopredatory fishes will cope with warming and acidifying oceans. This thesis investigated how future climate change scenarios, including marine heatwaves (MWHs), affect rocky and coral reef mesopredatory fish, focusing on the critical early life stages. My findings demonstrated that increased water temperatures and elevated $CO_2$ can have potentially beneficial effects on some early life history traits, whereas other traits are negatively affected. Similarly, the physiology of mature adults exhibited a range of potentially beneficial and detrimental effects under MHW scenarios. These new findings further our insight and understanding of how two mesopredators will fare under future climate change allowing us to better manage these important species.

## *Ocean warming and larval mesopredators*

In ***Chapter 2*** I found that larvae of rocky reef mesopredator, *Chrysophrys auratus*, exhibited increases in length, muscle depth, head size, and all other measured morphological traits when developed at elevated temperature. Additionally, at 1 dph elevated temperature had a positive effect on survival but this did not extend to later stages (16 dph). Similarly, in ***Chapter 3*** the swimming ability of juveniles was found to be significantly improved by elevated temperature which suggests that the net overall effect of elevated temperature may be positive for the early life stages of *C. auratus*. One potential negative outcome,

however, was the elevation in metabolic rate. While increasing metabolism with temperature is typical of ectotherms (Clarke & Johnston, 1999; Gillooly et al., 2001) this has the potential to cause problems for larval fish as it is already the most energetically demanding part of the life cycle (Post & Parkinson 2001; Stallings et al., 2010). If individuals can obtain sufficient energetic resources, such as they were able to in these laboratory experiments in ***Chapters 2 and 3***, then the overall effect of elevated temperature on performance could be positive. However, food resources in the wild are usually patchy and individuals rarely have an unlimited food supply, therefore the magnitude of positive effects may be reduced in a natural setting. Moreover, there is the potential for increased patchiness, and changes in prey assemblage, as climate change advances (Behrenfeld et al., 2006; Hoegh-Guldberg & Bruno 2010) which may exacerbate the effects of increased energetic demands from higher metabolic rate.

In contrast to ***Chapter 2***, the findings of elevated temperature on coral reef mesopredators in ***Chapter 4*** are more complex. While there were positive effects on the development of all morphological traits measured, there was a significant decline in survival under elevated temperature. The question is, does faster growth and development compensate for direct effects on survivorship? For instance, numerical models suggest that faster development through the high-mortality larval stage can lead to higher survivorship overall (O'Connor, 2007). Being larger at point of settlement is also advantageous and likely to translate to increased survival (Shima & Swearer, 2009; Murphy et al., 2014). What ultimately needs to be determined is whether the benefits of faster growth and development offset the direct effects of higher temperature on survivorship. This is a question that would require further research to estimate how these results translate into recruitment to the population. Nevertheless, from the findings of ***Chapter 4,*** it would

appear that elevated temperatures could push larvae of the tropical *Lutjanus carponotatus* past their thermal optimum, which contrasts to the findings for larvae of the temperate *C. auratus,* in ***Chapter 2***. Considering the populations of both species studied were collected from the middle of their thermal ranges, my findings support the hypothesis that tropical species are currently living closer to their thermal optimums (Deutsch et al., 2008; Tewksbury et al., 2008; Rummer et al., 2014). Therefore, while the rocky reef mesopredators may see benefits from increasing water temperatures, it is likely that coral reef mesopredators will suffer negative effects as water temperatures increase.

## *Dual climate change stressors and larval mesopredators*

Coastal marine habitats are physically dynamic environments. As climate change progresses, we expect to not only see increases in water temperature but also increased $pCO_2$ and decreased seawater pH. This is especially relevant for coastal and near shore environments that already experience significant variation in $pCO_2$ and pH on a range of temporal and spatial scales. In ***Chapters 2 and 3*** elevated temperature and $CO_2$ were used in a fully cross factored experiment to assess how these abiotic conditions effected the growth and physiology of larval and juvenile Australasian snapper when experienced individually and simultaneously. Interestingly, there were a range of effects, positive and negative, elicited by each abiotic factor. As previously mentioned above, elevated temperature increased growth and development in larval fish, but did not have a significant effect on survival. Conversely, elevated $CO_2$ did not affect growth and development, but it significantly improved survival. There were no interactions detected, showing that for growth, development, and survival both elevated temperature and $CO_2$ had differing but positive effects on larval *C. auratus*. This suggests that this species is still within its physical

environmental niche even at temperature and $CO_2$ levels predicted by the end of this century.

Yet, the physiology and swimming performance of juvenile *C. auratus* was differentially affected by elevated temperature and $CO_2$. In ***Chapter 3***, elevated temperature had both positive and negative effects on juveniles. While elevated temperature increased resting oxygen consumption ($MO_2$) and therefore increased the amount of base energy required, it also increased maximum $MO_2$, thereby maintaining aerobic scope. On the other hand, elevated $CO_2$ induced negative effects by increasing resting $MO_2$, decreasing maximum $MO_2$, and therefore resulted in reduced aerobic scope. Therefore, when experienced together elevated temperature and $CO_2$ increased resting metabolic rate to 30% more than that of control fish, and consequently, reduced aerobic capacity. The increase in resting metabolic rate is an interesting finding as the majority of previous research has found negligible effects of $CO_2$ on metabolic rates (Lefevre, 2016; Cattano et al., 2018). This may be due to the fact that much of this previous work has focused on more developed juveniles or adult fish, which are more robust and capable of handling the additional energetic demands of dealing with elevated $CO_2$ (e.g. acid base regulation). The impact on metabolic rate and aerobic scope could have significant implications for juvenile fish in respect to settlement, as well as growth and condition if energy demands are not met. An example of flow on effects to other attributes of performance was seen in swimming ability where, at elevated $CO_2$ individuals had a lower sustained swimming speed. In contrast, elevated temperature increased the sustained swimming speed of individuals. When experienced together the enhanced swimming performance under elevated temperature was essentially cancelled out by the negative effect of elevated $CO_2$. Therefore, when experienced simultaneously elevated temperature

and $CO_2$ could have an overall negative effect on juvenile *C. auratus*. This is a novel finding because, while previous research has shown improved swimming ability with elevated temperature (Wardle, 1980; Dickson et al., 2002; Claireaux et al., 2006). My findings that elevated $CO_2$ essentially negates the positive effect of elevated temperature on swimming ability implies that these previous findings could be an overestimation when secondary environmental factors are included.

My results highlight the complexity of estimating the impact of climate change on larval fish. There are several positive and negative effects produced under elevated temperature and $CO_2$ which reduces clarity in projecting the overall future impacts. However, this data is indispensable in broadening our knowledge and can be used in population models, along with data for multiple other studies, to help project the overall effects of climate change on these species. Indeed, we have been able to do exactly that in a population dynamics fisheries model for New Zealand Australasian snapper. Parsons et al., (2020), using data from ***Chapters 2 and 3***, found that, depending on the specific climate change scenario, *C. auratus* could see fishery yield decline by 29% or increase by 44%. This highlights the sensitivity of this species to multiple stressors and illustrates the difficulty in making definitive predictions about the impacts of ocean warming and acidification on important fisheries resources. This also reinforces the importance of the precautionary principle in managing fisheries in the face of climate change. Preventative and precautionary management changes will allow populations a greater buffer against negative impacts, both known and unforeseen, of climate change (Garcia, 1994; Lauck et al., 1998).

## *Marine heatwaves and adult coral reef mesopredators*

The increasing frequency and intensity of MHWs in coral reef ecosystems has spawned a significant amount of research into how corals will cope with MHWs. However, there has been far less focus on how fish may be affected by MWHs, especially large bodied species, as they were thought to be more resilient to temperature increases. Yet, the majority of this earlier research, and therefore the assumption of thermal resilience, has been conducted on temperate species. Another compounding factor is that MHWs typically occur during summer months when tropical reef fish are spawning which is a sensitive part of the life cycle and therefore likely to be impacted by temperature increase. In ***Chapter 5*** I found that resting $MO_2$, recovery time, lactate and haemoglobin were all increased under the MHW treatments and tended to be higher than control at two weeks post-exposure. Interestingly, the increase in resting $MO_2$ is relatively high, even for coral reef fish (Johansen & Jones, 2011; Pratchett et al., 2017; Rodgers et al., 2019). Due to this, adult *L. carponotatus* would have to either acquire significantly more energetic resources or alter their behaviour and activity to reduce energetic costs (Johansen et al., 2014; Pratchett et al., 2017). If able to acquire necessary additional resources then the effects of MHWs may be negligible, however, prey assemblages on reefs can be variable (Pratchett et al., 2011; Richardson et al., 2018) which could present a problem for this species during MHWs. Additionally, the close association of this species with coral reef habitat may limit their likelihood of moving to deeper, cooler water to avoid the energetic costs of MHWs.

As there is evidence that energy demands will increase during MHWs, how fish are able to meet these demands is an aspect that deserves consideration. My results suggest *L. carponotatus* shifts the amount of energy produced by aerobic metabolism towards anaerobic metabolism during MHWs. This was indicated by a stable capture $MO_2$

consumption across treatments, but far higher blood lactate levels in MHW treatment fish following the capture event. The ability to meet energetic demands via two production pathways, aerobic and anaerobic, are advantageous during events such as MHWs, but this presents two aspects that may have been historically overlooked. One is the narrow focus on aerobic metabolism to indicate energetic demands in previous studies. If other species previously studied were also increasing the amount of anaerobic energy production, but it was not recorded, then previous experiments may have underestimated the demands that warming could impose on coral reef fish. Secondly, few studies test recovery time, which can be a twofold issue, as it underestimates overall energy demands, and neglects the ecological importance of recovery in the health and survival of individuals (Killen et al., 2015a, 2015b). In future research a greater focus should be on understanding energetic demands and production pathways, including incorporating more traits to help describe anaerobic energy production, such as lactate levels and recovery time, to better comprehend the overall costs MHWs place on these fish.

The preferred temperature of *L. carponotatus* was found to be above the summer average and within the MHW range used in the experiments. The fact that the species preferred temperature in a range that elicits what would typically be thought of as negative effects (e.g. increased metabolic rates, higher blood lactate) highlights a potential weakness in physiological experiments. While experiments aim to obtain as much data, and as clear a picture of animal physiology, there are undoubtedly metrics that are not measured. These unmeasured traits may be enhanced in warmer environments and therefore increase an individual's overall fitness. This highlights the importance of multi-metric gathering in physiological experiments to try fully capture overall fitness.

## *Concluding remarks*

This thesis demonstrates the sensitivity of larval, juvenile, and spawning adult mesopredators to elevated temperature and $CO_2$ and highlights the complexity of their physiological responses. By utilizing controlled laboratory experiments this research has provided new insights to how the growth, development, and physiology of rocky and coral reef mesopredators may be impacted by predicted future climate change. The experimental approach used throughout this thesis has enabled a greater understanding of the sensitive early life stages of these two mesopredators, which would be incredibly difficult, if not impossible, in a natural setting. The reality of increasing water temperature, decreasing ocean pH, and more intense and frequent MHWs pose a significant challenge for these species. While these abiotic stressors elicited a range of differential responses, both positive and negative, it appears that the overall net effect could be negative.

As future research is conducted in this field, a focus on testing a wider range physiological responses would provide us with a better understanding of how environmental changes may impact individuals. Additionally, it will be important moving forward to incorporate multiple environmental parameters (e.g. temperature, $CO_2$, salinity, sediment, nutrient loads, etc.) within laboratory experiments to best mimic the changing natural environment and future scenarios. Another interesting avenue of research would be to incorporate physiology with research areas such as energetic modeling to give us a clearer picture of how wild populations will manage their energetic demands under future scenarios. By designing more comprehensive future experiments to best represent a changing ocean we will further our understanding of how marine life may cope as climate change progresses.

The findings from this thesis highlight the challenges we face in trying to predict the impact of climate change on fish populations, even for relatively well studied species such as *C. auratus*. The contrasting effects of different environmental drivers on the performance of marine fish indicates that there is a wide envelope of possible future impacts, which could be overall beneficial or detrimental to the population dynamics of these species. This reinforces the need to apply the precautionary principle in managing fish populations in order to provide them the best chance at coping with further climate change.

# References


Abdel, I, Abellán, E, López-Albors, O, Valdés, P, Nortes, M, & García-Alcázar, A. (2004). Abnormalities in the juvenile stage of sea bass (*dicentrarchus labrax l.*) reared at different temperatures: Types, prevalence and effect on growth. *Aquaculture International,* 12, 523-538.

AIMS. (2017). AIMS sea water temperature observing system (AIMS temperature logger program). doi.Org/10.25845/5b4eb0f9bb848

Alfonso, S, Gesto, M, & Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology,* 1-13.

Allen, GR. (1985). Snappers of the World*.* FAO Fisheries Synopsis, no. 125, vol. 6. Food and Agriculture Organization, Rome.

Allen, M, Babiker, M, Chen, Y, de Coninck, H, & Conors, S. (2018). Global warming of 1.5 c. An ipcc special report on the impacts of global warming of 1.5 c above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. *IPCC Special Report.*

Almany, GR, & Webster, MS. (2006). The predation gauntlet: Early post-settlement mortality in reef fishes. *Coral Reefs,* 25, 19-22.

Angilletta, MJ, Jr., Steury, TD, & Sears, MW. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology,* 44, 498-509.

Angilletta, MJ, Jr. (2009). Thermal adaptation: A theoretical and empirical synthesis. Oxford University Press.

Armsworth, PR. (2002). Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology,* 83, 1092-1104.

Bagarinao, T. (1986). Yolk resorption, onset of feeding and survival potential of larvae of three tropical marine fish species reared in the hatchery. *Marine biology,* 91, 449-459.

Baird, A, Marshall, P, & Wolstenholme, J. (2002). Latitudinal variation in the reproduction of acropora in the coral sea. *Proceedings of the 9th International Coral Reef Symposium*, 385–389.

Baker, AC, Glynn, PW, & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science,* 80, 435-471

Balasubramaniam, P, & Malathi, A. (1992). Comparative study of hemoglobin estimated by drabkin's and sahli's methods. *Journal of postgraduate medicine*, 38, 8-9.

Bates, D, Maechler, M, Bolker, B, Walker, S, Christensen, RHB, Singmann, H,… Scheipl, F. (2012). Package 'lme4'. CRAN. R Foundation for Statistical Computing, Vienna, Austria

Battaglene, S, & Talbot, R. (1992). Induced spawning and larval rearing of snapper, *pagrus auratus* (pisces: Sparidae), from australian waters. *New Zealand Journal of Marine and Freshwater Research,* 26, 179-185.

Batty, R, & Blaxter, J. (1992). The effect of temperature on the burst swimming performance of fish larvae. *Journal of Experimental Biology,* 170, 187-201.

Baumann, H, Talmage, SC, & Gobler, CJ. (2012). Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change,* 2, 38-41.

Baumann, H, Cross, EL, & Murray, CS. (2018). Robust quantification of fish early life $CO_2$ sensitivities via serial experimentation. *Biology Letters,* 14, 20180408.

Baumann, H. (2019). Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: How far have we come? *Canadian Journal of Zoology,* 97, 399-408.

Behrenfeld, MJ, O'Malley, RT, Siegel, DA, McClain, CR, Sarmiento, JL, Feldman, GC,… Boss, ES. (2006). Climate-driven trends in contemporary ocean productivity. *Nature,* 444, 752-755.

Behrens, JW, & Steffensen, JF. (2007). The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, *Ammodytes tobianus* (linnaeus, 1785). *Marine Biology,* 150, 1365-1377.

Bell, JD, Ganachaud, A, Gehrke, PC, Griffiths, SP, Hobday, AJ, Hoegh-Guldberg, O, … Lough, JM. (2013). Mixed responses of tropical pacific fisheries and aquaculture to climate change. *Nature Climate Change,* 3, 591-599.

Benthuysen, JA, Oliver, EC, Feng, M, & Marshall, AG. (2018). Extreme marine warming across tropical Australia during austral summer 2015–2016. *Journal of Geophysical Research: Oceans,* 123, 1301-1326.

Bernal, MA, Schunter, C, Lehmann, R, Lightfoot, DJ, Allan, BJM, Veilleux, HD,… Ravasi, T. (2020). Species-specific molecular responses of wild coral reef fishes during a marine heatwave. *Science Advances,* 6, eaay3423.

Bignami, S, Sponaugle, S, & Cowen, RK. (2013). Response to ocean acidification in larvae of a large tropical marine fish, *Rachycentron canadum*. *Global Change Biology,* 19, 996-1006.

Bignami, S, Sponaugle, S, & Cowen, RK. (2014). Effects of ocean acidification on the larvae of a high-value pelagic fisheries species, mahi-mahi *Coryphaena hippurus*. *Aquatic Biology,* 21, 249-260.

Bignami, S, Sponaugle, S, Hauff, M, & Cowen, RK. (2017). Combined effects of elevated $pCO_2$, temperature, and starvation stress on larvae of a large tropical marine fish. *ICES Journal of Marine Science,* 74, 1220-1229.

Birnie-Gauvin, K, Costantini, D, Cooke, SJ, & Willmore, WG. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries,* 18, 928-942.

Blaxter, JHS. (1988). Pattern and variety in development. In WS Hoar & DJ Randall (Eds.), *Fish physiology*. Academic Press, 1-58.

Blaxter, JHS. (1991). The effect of temperature on larval fishes. *Netherlands Journal of Zoology,* 42, 336-357.

BOM. (2020). 2020 marine heatwave on the Great Barrier Reef. Bureau of meteorology. Doi: 10.13140/rg.2.2.12494.48969.

Borgesa, AV, & Gypensb, N. (2010). Carbonate chemistry in the coastal zone responds more strongly to eutrophication than ocean acidification. *Limnology and Oceanography,* 55, 346-353.

Brand, MD, & Nicholls, DG. (2011). Assessing mitochondrial dysfunction in cells. *Biochemical Journal,* 435, 297-312.

Brauner, CJ, Shartau, RB, Damsgaard, C, Esbaugh, AJ, Wilson, RW, & Grosell, M. (2019). Acid-base physiology and $CO_2$ homeostasis: Regulation and compensation in response to elevated environmental $CO_2$. In M Grosell, PL Munday, AP Farrell, & CJ Brauner (Eds.), *Fish physiology* (Vol. 37, pp. 69-132): Academic Press.

Brett, JR. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada,* 21, 1183-1226.

Brierley, AS, & Kingsford, MJ. (2009). Impacts of climate change on marine organisms and ecosystems. *Current Biology*, 19, 602-614

Bromirski, PD, Flick, RE, & Cayan, DR. (2003). Storminess variability along the California coast: 1858–2000. *Journal of Climate*, 16, 982-993.

Brown, CJ, Fulton, EA, Hobday, AJ, Matear, RJ, Possingham, HP, Bulman, C,... Richardson, AJ. (2010). Effects of climate-driven primary production change on marine food webs: Implications for fisheries and conservation. *Global Change Biology,* 16, 1194-1212.

Brown, CJ, Mellin, C, Edgar, GJ, Campbell, MD, & Stuart-Smith, RD. (2020). Direct and indirect effects of heatwaves on a coral reef fishery. *Global Change Biology,* 27, 1214-1225.

Brown, JS, & Kotler, BP. (2004). Hazardous duty pay and the foraging cost of predation. *Ecology Letters,* 7, 999-1014.

Cahoon, DR, & Guntenspergen, GR. (2010). Climate change, sea-level rise, and coastal wetlands. *National Wetlands Newsletter*, 32, 8-12.

Cai, WJ, Hu, X, Huang, W-J, Murrell, MC, Lehrter, JC, Lohrenz, SE,… Wang, Y. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience,* 4, 766-770.

Caldeira, K, & Wickett, ME. (2003). Oceanography: Anthropogenic carbon and ocean pH. *Nature,* 425, 365-365.

Caley, M, Carr, M, Hixon, M, Hughes, T, Jones, G, & Menge, B. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics,* 27, 477-500.

Cameron, JN. (1978). Regulation of blood pH in teleost fish. *Respiration Physiology,* 33, 129-144.

Cattano, C, Claudet, J, Domenici, P, & Milazzo, M. (2018). Living in a high $CO_2$ world: A global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecological Monographs,* 88, 320-335.

Cavole, LM, Demko, AM, Diner, RE, Giddings, A, Koester, I, Pagniello, CM,… Yen, NK. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the northeast pacific: Winners, losers, and the future. *Oceanography,* 29, 273-285.

Chabot, D, Steffensen, JF, & Farrell, AP. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology,* 88, 81-121.

Challener, RC, Robbins, LL, & McClintock, JB. (2016). Variability of the carbonate chemistry in a shallow, seagrass-dominated ecosystem: Implications for ocean acidification experiments. *Marine and Freshwater Research,* 67, 163-172

Chambers, RC, Candelmo, AC, Habeck, EA, Poach, ME, Wieczorek, D, Cooper, KR,… Phelan, BA. (2014). Effects of elevated $CO_2$ in the early life stages of summer flounder, *paralichthys dentatus*, and potential consequences of ocean acidification. *Biogeosciences,* 11, 1613-1626.

Cheal, A, Delean, S, Sweatman, H, & Thompson, A. (2007). Spatial synchrony in coral reef fish populations and the influence of climate. *Ecology,* 88, 158-169.

Cheung WW, Lam VW, Sarmiento JL, Kearney K, Watson R, & Pauly D. (2009). Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries,* 10, 235-251.

Cheung, WW, & Frölicher, TL. (2020). Marine heatwaves exacerbate climate change impacts for fisheries in the northeast pacific. *Scientific Reports,* 10, 1-10.

China, V, & Holzman, R. (2014). Hydrodynamic starvation in first-feeding larval fishes. *Proceedings of the National Academy of Sciences,* 111, 8083-8088.

Claiborne, JB, Edwards, SL, & Morrison-Shetlar, AI. (2002). Acid-base regulation in fishes: Cellular and molecular mechanisms. *Journal of Experimental Zoology,* 293, 302-319.

Claireaux, G, Couturier, C, & Groison, A-L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *Journal of Experimental Biology,* 209, 3420-3428.

Clark, TD, Sandblom, E, & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *The Journal of Experimental Biology,* 216, 2771-2782.

Clarke, A, & Johnston, NM. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology,* 68, 893-905.

Clayton, TD, & Byrne, RH. (1993). Spectrophotometric seawater pH measurements: Total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. *Deep Sea Research Part I: Oceanographic Research Papers,* 40, 2115-2129.

Clifton, KE. (1990). The costs and benefits of territory sharing for the caribbean coral reef fish, scarus iserti. *Behavioral Ecology and Sociobiology,* 26, 139-147.

Collins, M, Knutti, R, Arblaster, J, Dufresne, J-L, Fichefet, T, Friedlingstein, P,… Krinner, G. (2013). Long-term climate change: projections, commitments and irreversibility. In *Climate Change 2013-The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1029-1136). Cambridge University Press.

Comte, L, & Olden, JD. (2017). Climatic vulnerability of the world's freshwater and marine fishes. *Nature Climate Change,* 7, 718-722.

Cornwall, CE, & Hurd, CL. (2016). Experimental design in ocean acidification research: Problems and solutions. *ICES Journal of Marine Science,* 73, 572-581.

Côté, IM, Darling, ES, & Brown, CJ. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences,* 283, 20152592.

Couturier, CS, Stecyk, JAW, Rummer, JL, Munday, PL, & Nilsson, GE. (2013). Species-specific effects of near-future $CO_2$ on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* 166, 482-489.

Cowen, RK, & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1, 443-466.

Cunha, I, Conceiçao, LE, & Planas, M. (2007). Energy allocation and metabolic scope in early turbot, *scophthalmus maximus*, larvae. *Marine Biology,* 151, 1397-1405.

Dahlke, FT, Wohlrab, S, Butzin, M, & Pörtner, H-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science,* 369, 65-70.

Davis, MW. (2002). Key principles for understanding fish bycatch discard mortality. *Canadian Journal of Fisheries and Aquatic Sciences,* 59, 1834-1843.

Deutsch, CA, Tewksbury, JJ, Huey, RB, Sheldon, KS, Ghalambor, CK, Haak, DC, & Martin, PR. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences,* 105, 6668-6672.

Di Lorenzo, E, & Mantua, N. (2016). Multi-year persistence of the 2014/15 north pacific marine heatwave. *Nature Climate Change,* 6, 1042-1047.

Dickson, AG, & Millero, FJ. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers,* 34, 1733-1743,

Dickson, KA, Donley, JM, Sepulveda, C, & Bhoopat, L. (2002). Effects of temperature on sustained swimming performance and swimming kinematics of the chub mackerel *Scomber japonicus*. *Journal of Experimental Biology,* 205, 969-980.

Dietzel, A, Bode, M, Connolly, SR, & Hughes, TP. (2020). Long-term shifts in the colony size structure of coral populations along the Great Barrier Reef. *Proceedings of the Royal Society B: Biological Sciences,* 287, 20201432.

Diggles, BK, & Ernst, I. (1997). Hooking mortality of two species of shallow-water reef fish caught by recreational angling methods. *Marine and Freshwater Research*, 48, 479-483.

Doherty, PJ, & Williams, DM. (1988). The replenishment of coral reef fish populations. *Oceanography and Marine Biology: An Annual Review*, 26, 487-551.

Domeier, ML, & Colin, PL. (1997). Tropical reef fish spawning aggregations: Defined and reviewed. *Bulletin of Marine Science,* 60, 698-726.

Domenici, P, Allan, B, McCormick, MI, & Munday, PL. (2012). Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biology Letters,* 8, *78-81.*

Donelson, JM, Munday, PL, McCormick, MI, Pankhurst, NW, & Pankhurst, PM. (2010). Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. *Marine Ecology Progress Series,* 401, 233-243.

Donelson, JM, & Munday, PL. (2012). Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *Journal of Animal Ecology,* 81, 1126-1131.

Donelson, JM, McCormick, MI, Booth, DJ, & Munday, PL. (2014). Reproductive acclimation to increased water temperature in a tropical reef fish. *PLOS One,* 9, E97223.

Doney, SC, Fabry, VJ, Feely, RA, & Kleypas, JA. (2009). Ocean acidification: The other $CO_2$ problem. *Annual Review of Marine Science,* 1, 169-192.

Doney, SC, Ruckelshaus, M, Emmett Duffy, J, Barry, JP, Chan, F, English, CA, . . . & Knowlton, N. (2012). Climate change impacts on marine ecosystems*. Annual Review of Marine Scienc*e, 4, 11-37

Donner, SD, Rickbeil, GJM, & Heron, SF. (2017). A new, high-resolution global mass coral bleaching database. *PLOS One,* 12, e0175490.

Dou, S, Masuda, R, Tanaka, M, & Tsukamoto, K. (2005). Effects of temperature and delayed initial feeding on the survival and growth of Japanese flounder larvae. *Journal of Fish Biology,* 66, 362-377,

Drabkin, DL, & Austin, JH. (1935). Spectrophotometric studies: Ii. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *Journal of Biological Chemistry,* 112, 51-65.

Drucker, E, & Jensen, J. (1996). Pectoral fin locomotion in the striped surfperch. II. Scaling swimming kinematics and performance at a gait transition. *Journal of Experimental Biology,* 199, 2243-2252.

du Plessis, KL, Martin, RO, Hockey, PA, Cunningham, SJ, & Ridley, AR. (2012). The costs of keeping cool in a warming world: Implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Global Change Biology,* 18, 3063-3070.

Duarte, CM, Hendriks, IE, Moore, TS, Olsen, YS, Steckbauer, A, Ramajo, L,… McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater ph. *Estuaries and Coasts,* 36, 221-236.

Dudley, B, Tolimieri, N, & Montgomery, J. (2000). Swimming ability of the larvae of some reef fishes from New Zealand waters. *Marine and Freshwater Research,* 51, 783-787.

Edwards, M, & Richardson, AJ. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature,* 430, 881-884.

Eliason, EJ, Clark, TD, Hague, MJ, Hanson, LM, Gallagher, ZS, Jeffries, KM,… Farrell, AP. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science,* 332, 109-112.

Eliason, EJ, Wilson, SM, Farrell, AP, Cooke, SJ, & Hinch, SG. (2013). Low cardiac and aerobic scope in a coastal population of sockeye salmon *Oncorhynchus nerka* with a short upriver migration. *Journal of Fish Biology,* 82, 2104-2112.

Enzor, LA, Zippay, ML, & Place, SP. (2013). High latitude fish in a high $CO_2$ world: Synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of antarctic notothenioids. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 164, 154-161.

Estes, JA, & Palmisano, JF. (1974). Sea otters: Their role in structuring nearshore communities. *Science,* 185, 1058-1060.

Eurich, J, McCormick, M, & Jones, G. (2018). Habitat selection and aggression as determinants of fine-scale partitioning of coral reef zones in a guild of territorial damselfishes. *Marine Ecology Progress Series,* 587, 201-215,

Evans, J, & Atkins, J. (2013). Seawater temperature dataset at Goat Island, Leigh New Zealand from 1967 to 2011. University of Auckland. hdl.handle.net/2292/20612. Accessed 8 Nov 2018.

Farrell, A. (2016). Pragmatic perspective on aerobic scope: Peaking, plummeting, pejus and apportioning. *Journal of Fish Biology*, 88, 322-343.

Farrell, A, Eliason, E, Sandblom, E, & Clark, T. (2009). Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology,* 87, 835-851.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, & Mathes MT. (2008). Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology,* 81, 697-709.

Fassbender, AJ, Sabine, CL, Feely, RA, Langdon, C, & Mordy, CW. (2011). Inorganic carbon dynamics during Northern California coastal upwelling. *Continental Shelf Research,* 31, 1180-1192.

Feely, RA, Alin, SR, Newton, J, Sabine, CL, Warner, M, Devol, A,… Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on ph and carbonate saturation in an urbanized estuary. *Estuarine Coastal and Shelf Science,* 88, 442-449.

Feely, RA, Sabine, CL, Hernandez-Ayon, JM, Ianson, D, & Hales, B. (2008). Evidence for upwelling of corrosive" acidified" water onto the continental shelf. *Science,* 320, 1490-1492.

Fielder, DS, Bardsley, WJ, Allan, GL, & Pankhurst, PM. (2005). The effects of salinity and temperature on growth and survival of Australian snapper, *Pagrus auratus* larvae. *Aquaculture,* 250, 201-214.

Foster AR, Houlihan DF, Hall SJ, & Burren LJ. (1992). The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua*). *Canadian Journal of Zoology,* 70, 2095-2102.

Fowler, A, & Jennings, P. (2003). Dynamics in 0+ recruitment and early life history for snapper (*Pagrus auratus*, sparidae) in South Australia. *Marine and Freshwater Research,* 54, 941-956.

Francis, MP. (1994). Growth of juvenile snapper, *Pagrus auratus*. *New Zealand Journal of Marine and Freshwater Research,* 28, 201-218.

Franco, RS. (2012). Measurement of red cell lifespan and aging. *Transfusion Medicine and Hemotherapy,* 39, 302-307.

Freedman, RM, Brown, JA, Caldow, C, & Caselle, JE. (2020). Marine protected areas do not prevent marine heatwave-induced fish community structure changes in a temperate transition zone. *Scientific Reports*, 10, 21081.

Frisch, AJ, & Anderson, TA. (2000). The response of coral trout *(Plectropomus leopardus*) to capture, handling and transport and shallow water stress. *Fish Physiology and Biochemistry*, 23, 23-34.

Frölicher, TL, Fischer, EM, & Gruber, N. (2018). Marine heatwaves under global warming. *Nature,* 560, 360-364.

Frommel, AY, Schubert, A, Piatkowski, U, & Clemmesen, C. (2013). Egg and early larval stages of Baltic cod, *Gadus morhua*, are robust to high levels of ocean acidification. *Marine Biology,* 160, 1825-1834.

Fry, F. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiology,* 1-98.

Fuiman, LA, Poling, KR, & Higgs, DM. (1998). Quantifying developmental progress for comparative studies of larval fishes. *Copeia*, 5, 602-611.

Gagliano, M, McCormick, MI, & Meekan, MG. (2007). Survival against the odds: Ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proceedings of the Royal Society B: Biological Sciences,* 274, 1575-1582.

Gallaugher, P, & Farrell, A. (1998). Hematocrit and blood oxygen-carrying capacity. *Fish Physiology,* 17, 185-227.

Garcia, S. (1994). The precautionary principle: Its implications in capture fisheries management. *Ocean & Coastal Management,* 22, 99-125.

Gardiner, NM, Munday, PL, & Nilsson, GE. (2010). Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLOS One,* 5, E13299.

Garrabou, J, Coma, R, Bensoussan, N, Bally, M, Chevaldonné, P, Cigliano, M,… Kersting, D. (2009). Mass mortality in northwestern mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Global Change Biology,* 15, 1090-1103.

GBRMPA. (2014). Great Barrier Reef outlook report 2014. *Great Barrier Reef Marine Park Authority*. http://hdl.handle.net/11017/2855

Genin, A, Levy, L, Sharon, G, Raitsos, DE, & Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proceedings of the National Academy of Sciences,* 117, 25378-25385.

Georgakopoulou, E, Katharios, P, Divanach, P, & Koumoundouros, G. (2010). Effect of temperature on the development of skeletal deformities in gilthead seabream (*Sparus aurata* linnaeus, 1758). *Aquaculture,* 308, 13-19.

Gillooly, JF, & Zenil-Ferguson, R. (2014). Vertebrate blood cell volume increases with temperature: Implications for aerobic activity. *PeerJ,* 2, e346

Gillooly, JF, Charnov, EL, West, GB, Savage, VM, & Brown, JH. (2002). Effects of size and temperature on developmental time. *Nature,* 417, 70-73.

Gillooly, JF, Brown, JH, West, GB, Savage, VM, & Charnov, EL. (2001). Effects of size and temperature on metabolic rate. *Science,* 293, 2248-2251.

Gleckler, PJ, Durack, PJ, Stouffer, RJ, Johnson, GC, & Forest, CE. (2016). Industrial-era global ocean heat uptake doubles in recent decades. *Nature Climate Change,* 6, 394-398.

Gleiber, MR, Sponaugle, S, & Cowen, RK. (2020). Some like it hot, hungry tunas do not! Implications of temperature and plankton food web dynamics on growth and diet of tropical tuna larvae. *ICES Journal of Marine Science,* 77, 3058-3073.

Goolish, EM. (1991). Aerobic and anaerobic scaling in fish. *Biological Reviews*, 66, 33-56.

Goyer, K, Bertolo, A, Pépino, M, & Magnan, P. (2014). Effects of lake warming on behavioural thermoregulatory tactics in a cold-water stenothermic fish. *PLOS One,* 9, e92514.

Grans, A, Jutfelt, F, Sandblom, E, Jonsson, E, Wiklander, K, Seth, H,… Axelsson, M. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated $CO_2$ in Atlantic halibut. *Journal of Experimental Biology,* 217, 711-717.

Grantham, BA, Chan, F, Nielsen, KJ, Fox, DS, Barth, JA, Huyer, A,… Menge, BA. (2004). Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast pacific. *Nature,* 429, 749-754.

Green, BS, & Fisher, R. (2004). Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. *Journal of Experimental Marine Biology and Ecology,* 299, 115-132.

Green, MO, & Zeldis, JR. (2015). Firth of Thames water quality and ecosystem health. NIWA Client Report No. HAM2015-016, prepared for Waikato Regional Council and DairyNZ. http://www.waikatoregion.govt.nz/services/publications/technical-reports/tr/tr201523. Accessed 12 Dec 2018

Grorud-Colvert, K, & Sponaugle, S. (2011). Variability in water temperature affects trait-mediated survival of a newly settled coral reef fish. *Oecologia,* 165, 675-686.

Gruber, N, Clement, D, Carter, BR, Feely, RA, Van Heuven, S, Hoppema, M,… Lauvset, SK. (2019). The oceanic sink for anthropogenic $CO_2$ from 1994 to 2007. *Science,* 363, 1193-1199.

Habary, A, Johansen, JL, Nay, TJ, Steffensen, JF, & Rummer, JL. (2017). Adapt, move or die–how will tropical coral reef fishes cope with ocean warming? *Global Change Biology,* 23, 566-577.

Hamilton, SL, Kashef, NS, Stafford, DM, Mattiasen, EG, Kapphahn, LA, Logan, CA,… Sogard, SM. (2019). Ocean acidification and hypoxia can have opposite effects on rockfish otolith growth. *Journal of Experimental Marine Biology and Ecology,* 521, 151245.

Hamilton, SL, Regetz, J, & Warner, RR. (2008). Post-settlement survival linked to larval life in a marine fish. *Proceedings of the National Academy of Sciences,* 105, 1561-1566.

Haschemeyer, A, Persell, R, & Smith, M. (1979). Effect of temperature on protein synthesis in fish of the Galapagos and Perlas islands. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry,* 64, 91-95.

Hays, GC, Richardson, AJ, & Robinson, C. (2005). Climate change and marine plankton. *Trends in Ecology & Evolution,* 20, 337-344.

Hein, AM, & Keirsted, KJ. (2012). The rising cost of warming waters: Effects of temperature on the cost of swimming in fishes. *Biology Letters,* 8, 266-269.

Hempson, TN, Graham, NA, MacNeil, MA, Hoey, AS, & Almany, GR. (2018). Mesopredator trophodynamics on thermally stressed coral reefs. *Coral Reefs,* 37, 135-144.

Hempson, TN, Graham, NAJ, MacNeil, MA, Williamson, DH, Jones, GP, & Almany, GR. (2017). Coral reef mesopredators switch prey, shortening food chains, in response to habitat degradation. *Ecology and Evolution,* 7, 2626-2635.

Henson, SA, Beaulieu, C, Ilyina, T, John, JG, Long, M, Séférian, R, . . . Sarmiento, JL. (2017). Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nature Communications*, 8, 14682.

Heron, SF, Maynard, JA, Van Hooidonk, R, & Eakin, CM. (2016). Warming trends and bleaching stress of the world's coral reefs 1985–2012. *Scientific Reports,* 6, 1-14.

Heuer, RM, & Grosell, M. (2016). Elevated $CO_2$ increases energetic cost and ion movement in the marine fish intestine. *Scientific Reports,* 6, 1-8.

Heuer, RM, & Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 307, 1061-1084.

Hixon, MA. (2015). Predation: Piscivory and the ecology of coral-reef fishes. *Ecology of fishes on coral reefs*, 41-53.

Hjort, J. (1914). Fluctuations in the great fisheries of northern europe viewed in the light of biological research. *ICES*

Hoar, WS, Randall, DJ, & Donaldson, E. (1983). *Fish physiology*. Academic Press.

Hobday, AJ, Alexander, LV, Perkins, SE, Smale, DA, Straub, SC, Oliver, EC,… Feng, M. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography,* 141, 227-238.

Hobday, AJ, Oliver, EC, Gupta, AS, Benthuysen, JA, Burrows, MT, Donat, MG,… Wernberg, T. (2018). Categorizing and naming marine heatwaves. *Oceanography,* 31, 162-173.

Hoegh-Guldberg, O, & Bruno, JF. (2010). The impact of climate change on the world's marine ecosystems. *Science,* 328, 1523-1528.

Hoegh-Guldberg, O, Cai, R, Poloczanska, ES, Brewer, PG, Sundby, S, Hilmi, K,… Stone, DA. (2014). The Ocean. *Climate Change 2014: Impacts, Adaptation, and Vulnerability, Part B: Regional Aspects*, 30, 1655-1731.

Hoegh-Guldberg, O, Jacob, D, Bindi, M, Brown, S, Camilloni, I, Diedhiou, A,… Guiot, J. (2018). Impacts of 1.5°C global warming on natural and human systems. *Global warming of 1.5°C. An IPCC Special Report,* IPCC Secretariat.

Hofmann, GE, Smith, JE, Johnson, KS, Send, U, Levin, LA, Micheli, F,… Takeshita, Y. (2011). High-frequency dynamics of ocean pH: A multi-ecosystem comparison. *PLOS One,* 6, e28983.

Hofmann, GE, & Todgham, AE. (2010). Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. *Annual Review of Physiology,* 72, 127-145.

Holbrook, NJ, Scannell, HA, Gupta, AS, Benthuysen, JA, Feng, M, Oliver, EC,… Hobday, AJ. (2019). A global assessment of marine heatwaves and their drivers. *Nature Communications,* 10, 1-13.

Hollowed, AB, Barange, M, Beamish, RJ, Brander, K, Cochrane, K, Drinkwater, K,… Ito, S-i. (2013). Projected impacts of climate change on marine fish and fisheries. *ICES Journal of Marine Science,* 70, 1023-1037.

Hönisch, B, Ridgwell, A, Schmidt, DN, Thomas, E, Gibbs, SJ, Sluijs, A, . . . Greene, SE. (2012). The geological record of ocean acidification. *Science*, 335, 1058-1063.

Hothorn, T, Bretz, F, Westfall, P, Heiberger, RM, Schuetzenmeister, A, Scheibe, S, & Hothorn, MT. (2016). Package 'multcomp'. *Simultaneous inference in general parametric models.* Project for Statistical Computing, Vienna, Austria.

Houde, ED. (1989). Comparative growth, mortality, and energetics of marine fish larvae: Temperature and implied latitudinal effects. *Fishery Bulletin,* 87, 471-495.

Huey, RB, Deutsch, CA, Tewksbury, JJ, Vitt, LJ, Hertz, PE, Álvarez Pérez, HJ, & Garland Jr, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B: Biological Sciences,* 276, 1939-1948.

Hughes, TP, Kerry, JT, Álvarez-Noriega, M, Álvarez-Romero, JG, Anderson, KD, Baird, AH,… Berkelmans, R. (2017). Global warming and recurrent mass bleaching of corals. *Nature,* 543, 373-377.

Hughes, TP, Kerry, JT, Baird, AH, Connolly, SR, Dietzel, A, Eakin, CM,… Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature,* 556, 492-496.

Hugie, DM, & Dill, LM. (1994). Fish and game: A game theoretic approach to habitat selection by predators and prey. *Journal of Fish Biology,* 45, 151-169.

Hunter, JR. (1981). Feeding ecology and predation of marine fish larvae. *Fish Behahvior and its Use in the Capture and Culture of Fishes*. 33-77.

Hurst, TP, Fernandez, ER, & Mathis, JT. (2013). Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). *ICES Journal of Marine Science,* 70, 812-822.

Iftikar, FI, MacDonald, JR, Baker, DW, Renshaw, GMC, & Hickey, AJR. (2014). Could thermal sensitivity of mitochondria determine species distribution in a changing climate? *The Journal of Experimental Biology,* 217, 2348-2357.

IPCC. (2014). Climate change 2014: Impacts, adaptation, and vulnerability. *Part a: Global and sectoral aspects. Contribution of working group ii to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge University Press.

Ishimatsu, A, Hayashi, M, & Kikkawa, T. (2008). Fishes in high-$CO_2$, acidified oceans. *Marine Ecology Progress Series,* 373, 295-302.

Iwama, GK, Thomas, PT, Forsyth, RB, & Vijayan, MM. (1998). Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries,* 8, 35-56.

Iwama, GK, Vijayan, MM, Forsyth, RB, & Ackerman, PA. (1999). Heat shock proteins and physiological stress in fish. *American Zoologist,* 39, 901-909.

Jacobs, I. (1986). Blood lactate. *Sports Medicine,* 3, 10-25.

Jacox, MG, Alexander, MA, Bograd, SJ, & Scott, JD. (2020). Thermal displacement by marine heatwaves. *Nature,* 584, 82-86.

Jain, KE, & Farrell, AP. (2003). Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology,* 206, 3569-3579.

Janssen, RG, & Randall, DJ. (1975). The effects of changes in ph and $pCO_2$, in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respiration Physiology,* 25, 235-245.

Johansen, J, & Jones, G. (2011). Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. *Global Change Biology,* 17, 2971-2979.

Johansen, J, Messmer, V, Coker, D, Hoey, A, & Pratchett, M. (2014). Increasing ocean temperatures reduce activity patterns of a large commercially important coral reef fish. *Global Change Biology,* 20, 1067-1074.

Johansen, J, Pratchett, M, Messmer, V, Coker, DJ, Tobin, A, & Hoey, A. (2015). Large predatory coral trout species unlikely to meet increasing energetic demands in a warming ocean. *Scientific Reports,* 5, 13830.

Johnston, I, & Dunn, J. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Society for Experimental Biology,* 41, 67-93.

Jonassen, TM, Imsland, AK, & Stefansson, SO. (1999). The interaction of temperature and fish size on growth of juvenile halibut. *Journal of Fish Biology,* 54, 556-572.

Jones, DR. (1982). Anaerobic exercise in teleost fish. *Canadian Journal of Zoology,* 60, 1131-1134.

Jones, G, Almany, G, Russ, G, Sale, P, Steneck, R, Van Oppen, M, & Willis, B. (2009). Larval retention and connectivity among populations of corals and reef fishes: History, advances and challenges. *Coral Reefs,* 28, 307-325.

Jones, GP, & McCormick, MI. (2002). Numerical and energetic processes in the ecology of coral reef fishes. *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem*, 221-238.

Kamler, E. (2002). Ontogeny of yolk-feeding fish: An ecological perspective. *Reviews in Fish Biology and Fisheries,* 12, 79-103.

Kamler, E. (2008). Resource allocation in yolk-feeding fish. *Reviews in Fish Biology and Fisheries,* 18, 143-200.

Kazuyuki, S, Hisashi, K, & Shogoro, K. (1988). Changes in low temperature tolerance of the eggs of certain marine fish during embryonic development. *Comparative Biochemistry and Physiology Part A: Physiology,* 91, 183-187.

Killen, SS. (2014). Growth trajectory influences temperature preference in fish through an effect on metabolic rate. *Journal of Animal Ecology,* 83, 1513-1522.

Killen, SS, Atkinson, D, & Glazier, DS. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters,* 13, 184-193.

Killen, SS, Costa, I, Brown, JA, & Gamperl, AK. (2007). Little left in the tank: Metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society of London B: Biological Sciences,* 274, 431-438.

Killen, SS, Nati, JJ, & Suski, CD. (2015). Vulnerability of individual fish to capture by trawling is influenced by capacity for anaerobic metabolism. *Proceedings of the Royal Society B: Biological Sciences,* 282, 20150603.

Killen, SS, Reid, D, Marras, S, & Domenici, P. (2015). The interplay between aerobic metabolism and antipredator performance: Vigilance is related to recovery rate after exercise. *Frontiers in Physiology,* 6, 111.

Kindermann, W, Simon, G, & Keul, J. (1979). The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *European Journal of Applied Physiology and Occupational Physiology*, 42, 25-34.

King, AD, Black, MT, Min, SK, Fischer, EM, Mitchell, DM, Harrington, LJ, & Perkins-Kirkpatrick, SE. (2016). Emergence of heat extremes attributable to anthropogenic influences. *Geophysical Research Letters,* 43, 3438-3443.

King, H, Pankhurst, N, Watts, M, & Pankhurst, P. (2003). Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. *Journal of Fish Biology,* 63, 153-167.

Kingsolver, JG, Diamond, SE, & Buckley, LB. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology,* 27, 1415-1423.

Kleckova, I, Konvicka, M, & Klecka, J. (2014). Thermoregulation and microhabitat use in mountain butterflies of the genus erebia: Importance of fine-scale habitat heterogeneity. *Journal of Thermal Biology,* 41, 50-58.

Krebs, H. (1950). Body size and tissue respiration. *Biochimica et Biophysica Acta,* 4, 249-269.

Kritzer, JP. (2004). Sex-specific growth and mortality, spawning season, and female maturation of the stripey bass (*Lutjanus carponotatus*) on the great barrier reef. *Fishery Bulletin, 102*, 94-107.

Kroeker, KJ, Kordas, RL, Crim, R, Hendriks, IE, Ramajo, L, Singh, GS,… Gattuso, JP. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology,* 19, 1884-1896.

Kroeker, KJ, Kordas, RL, Crim, RN, & Singh, GG. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters,* 13, 1419-1434.

Kültz, D. (2003). Evolution of the cellular stress proteome: From monophyletic origin to ubiquitous function. *Journal of Experimental Biology,* 206, 3119-3124.

Kültz, D. (2004). Hyperosmolality triggers oxidative damage in kidney cells. *Proceedings of the National Academy of Sciences,* 101, 9177-9178.

Kunz, KL, Frickenhaus, S, Hardenberg, S, Johansen, T, Leo, E, Pörtner, H-O,… Mark, FC. (2016). New encounters in arctic waters: A comparison of metabolism and performance of polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) under ocean acidification and warming. *Polar Biology,* 39, 1137-1153.

Larios-Soriano, E, Re-Araujo, AD, Díaz, F, Sánchez, CG, López-Galindo, L, Castro, LI, & Ramírez, DT. (2020). Effect of acclimation temperature on thermoregulatory behaviour, thermal tolerance and respiratory metabolism of *Lutjanus guttatus* and the response of heat shock protein 70 (hsp70) and lactate dehydrogenase (ldh-a) genes. *Aquaculture Research,* 51, 1089-1100.

Laubenstein, T, Rummer, J, Nicol, S, Parsons, D, Pether, S, Pope, S,… Munday, P. (2018). Correlated effects of ocean acidification and warming on behavioral and metabolic traits of a large pelagic fish. *Diversity, 10*, 35.

Lauck, T, Clark, CW, Mangel, M, & Munro, GR. (1998). Implementing the precautionary principle in fisheries management through marine reserves. *Ecological Applications,* 8, 72-78.

Laurel, BJ, Danley, C, & Haines, S. (2014). The effects of temperature on growth, development and settlement of northern rock sole larvae (*Epidopsetta polyxystra*). *Fisheries Oceanography,* 23, 495-505.

Law, CS, Bell, JJ, Bostock, HC, Cornwall, CE, Cummings, VJ, Currie, K,… Hurd, CL. (2018). Ocean acidification in New Zealand waters: Trends and impacts. *New Zealand journal of Marine and Freshwater Research,* 52, 155-195.

Le, MH, Dinh, KV, Nguyen, MV, & Rønnestad, I. (2020). Combined effects of a simulated marine heatwave and an algal toxin on a tropical marine aquaculture fish cobia (*Rachycentron canadum*). *Aquaculture Research,* 51, 2535-2544.

Le Nohaïc, M, Ross, CL, Cornwall, CE, Comeau, S, Lowe, R, McCulloch, MT, & Schoepf, V. (2017). Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. *Scientific Reports,* 7, 1-11.

Lee, CG, Farrell, AP, Lotto, A, Hinch, SG, & Healey, MC. (2003). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. Kisutc*h) salmon following critical speed swimming. *Journal of Experimental Biology,* 206, 3253-3260.

Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high $CO_2$ and their interaction. *Conservation Physiology,* 4, cow009.

Leis, JM. (1991). The pelagic stage of reef fishes: The larval biology of coral fishes. *The Ecology of Fishes on Coral Reefs,* Academic Press*,* 183-227.

Leis, JM. (2007). Behaviour as input for modelling dispersal of fish larvae: Behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. *Marine Ecology Progress Series,* 347, 185-193.

Leis, JM. (2015). Is dispersal of larval reef fishes passive? *Ecology of Fishes on Coral Reefs*. Cambridge University Press, 223-227.

Lepock, JR. (2005). How do cells respond to their thermal environment? *International Journal of Hyperthermia,* 21, 681-687.

Letcher, BH, Rice, JA, Crowder, LB, & Rose, KA. (1996). Variability in survival of larval fish: Disentangling components with a generalized individual-based model. *Canadian Journal of Fisheries and Aquatic Sciences,* 53, 787-801.

Levitus, S, Antonov, JI, Wang, J, Delworth, TL, Dixon, KW, & Broccoli, AJ. (2001). Anthropogenic warming of earth's climate system. *Science*, 292, 267-270.

Lima, FP, & Wethey, DS. (2012). Three decades of high-resolution coastal sea surface temperatures reveal more than warming. *Nature Communications,* 3, 1-13.

Lindberg, WJ, Frazer, TK, Portier, KM, Vose, F, Loftin, J, Murie, DJ,… Hart, MK. (2006). Density-dependent habitat selection and performance by a large mobile reef fish. *Ecological Applications,* 16, 731-746.

Link, JS, Stockhausen, WT, & Methratta, ET. (2005). Food-web theory in marine ecosystems. *Aquatic food webs: an ecosystem approach.* Oxford University Press, 98-114.

Little, AG, Loughland, I, & Seebacher, F. (2020). What do warming waters mean for fish physiology and fisheries? *Journal of Fish Biology,* 97, 328-340.

Llopiz, JK, Cowen, RK, Hauff, MJ, Ji, RB, Munday, PL, Muhling, BA,… Sponaugle, S. (2014). Early life history and fisheries oceanography new questions in a changing world. *Oceanography,* 27, 26-41.

Lohrer, AM, McCartain, LD, Buckthought, D, MacDonald, I, & Parsons, DM. (2018). Benthic structure and pelagic food sources determine post-settlement snapper (*Chrysophrys auratus*) abundance. *Frontiers in Marine Science,* 5, 427.

Lo-Yat, A, Simpson, SD, Meekan, M, Lecchini, D, Martinez, E, & Galzin, R. (2011). Extreme climatic events reduce ocean productivity and larval supply in a tropical reef ecosystem. *Global Change Biology,* 17, 1695-1702.

Lushchak, VI, & Bagnyukova, TV. (2006). Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 143, 30-35.

Lüthi, D, Le Floch, M, Bereiter, B, Blunier, T, Barnola, J-M, Siegenthaler, U,… Kawamura, K. (2008). High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature,* 453, 379-382.

MacKenzie, B, & Leggett, W. (1991). Quantifying the contribution of small-scale turbulence to the encounter rates between larval fish and their zooplankton prey: Effects of wind and tide. *Marine Ecology Progress Series,* 73, 149-160.

Madeira, D, Narciso, L, Cabral, HN, Vinagre, C, & Diniz, MS. (2013). Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 166, 237-243.

Mairbäurl, H. (2013). Red blood cells in sports: Effects of exercise and training on oxygen supply by red blood cells. *Frontiers in Physiology,* 4, 332.

Marbà, N, & Duarte, CM. (2010). Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Global Change Biology,* 16, 2366-2375.

Martell, D, Kieffer, J, & Trippel, E. (2005). Effects of temperature during early life history on embryonic and larval development and growth in haddock. *Journal of Fish Biology,* 66, 1558-1575.

Martin, TL, & Huey, RB. (2008). Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *The American Naturalist,* 171, 102-118.

May, R. (1974). Larval mortality in marine fishes and the critical period concept. *The early life history of fish.* Springer, 3-19.

McCormick, MI, & Molony, BW. (1995). Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. *Marine Ecology Progress Series*, 118, 59-68.

McCormick, MI, & Weaver, CJ. (2012). It pays to be pushy: Intracohort interference competition between two reef fishes. *PLOS One,* 7, e42590.

McLeod, IM, & Clark, TD. (2016). Limited capacity for faster digestion in larval coral reef fish at an elevated temperature. *PLOS One,* 11, e0155360.

McLeod, IM, Jones, RE, Jones, GP, Takahashi, M, & McCormick, MI. (2015). Interannual variation in the larval development of a coral reef fish in response to temperature and associated environmental factors. *Marine Biology,* 162, 2379-2389.

McLeod, IM, Rummer, JL, Clark, TD, Jones, GP, McCormick, MI, Wenger, AS, & Munday, PL. (2013). Climate change and the performance of larval coral reef fishes: The interaction between temperature and food availability. *Conservation Physiology,* 1, cot024

McMahon, SJ, Parsons, DM, Donelson, JM, Pether, SM, & Munday, PL. (2020). Elevated temperature and $CO_2$ have positive effects on the growth and survival of larval Australasian snapper. *Marine Environmental Research,* 161, 105054.

McMahon, SJ, Parsons, DM, Donelson, JM, Pether, SMJ, & Munday, PL. (2019). Elevated $CO_2$ and heatwave conditions affect the aerobic and swimming performance of juvenile Australasian snapper. *Marine Biology,* 167, 6.

McNeil, BI, & Matsumoto, K. (2019). The changing ocean and freshwater $CO_2$ system. *Fish Physiology,* 37, 1-32.

McNeil, BI, & Sasse, TP. (2016). Future ocean hypercapnia driven by anthropogenic amplification of the natural $CO_2$ cycle. *Nature,* 529, 383-386.

Meehl, GA, Zwiers, F, Evans, J, Knutson, T, Mearns, L, & Whetton, P. (2000). Trends in extreme weather and climate events: Issues related to modeling extremes in projections of future climate change. *Bulletin of the American Meteorological Society,* 81, 427-436.

Meekan, MG, & Fortier, L. (1996). Selection for fast growth during the larval life of Atlantic cod (*Gadus morhua)* on the scotian shelf. *Marine Ecology Progress Series, 137*, 25-37.

Mehrbach, C, Culberson, CH, Hawley, JE, & Pytkowicx, RM. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography,* 18, 897-907.

Meinshausen, M, Wigley, TML, & Raper, SCB. (2011). Emulating atmosphere-ocean and carbon cycle models with a simpler model. *Atmospheric Chemistry and Physics,* 11, 1457-1471.

Melzner, F, Gutowska, MA, Langenbuch, M, Dupont, S, Lucassen, M, Thorndyke, MC,… Pörtner, HO. (2009a). Physiological basis for high $CO_2$ tolerance in marine ectothermic animals: Pre-adaptation through lifestyle and ontogeny? *Biogeosciences,* 6, 2313-2331.

Melzner, F, Gutowska, M, Hu, M, & Stumpp, M. (2009b). Acid–base regulatory capacity and associated proton extrusion mechanisms in marine invertebrates: An overview. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 153, 2.*

Messmer, V, Pratchett, MS, Hoey, AS, Tobin, AJ, Coker, DJ, Cooke, SJ, & Clark, TD. (2017). Global warming may disproportionately affect larger adults in a predatory coral reef fish. *Global Change Biology,* 23, 2230-2240.

Metcalfe, N, Van Leeuwen, T, & Killen, S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology*, 88, 298-321.

Michener, WK, Blood, ER, Bildstein, KL, Brinson, MM, & Gardner, LR. (1997). Climate change, hurricanes and tropical storms, and rising sea level in coastal wetlands. *Ecological Applications*, 7, 770-801.

Middelboe, AL, & Hansen, PJ. (2007). High pH in shallow-water macroalgal habitats. *Marine Ecology Progress Series,* 338, 107-117.

Miller, B, & Kendall, AW. (2009). Early life history of marine fishes. University of California Press.

Miller, GM, Watson, SA, McCormick, MI, & Munday, PL. (2013). Increased $CO_2$ stimulates reproduction in a coral reef fish. *Global Change Biology,* 19, 3037-3045.

Miranda, LA, Chalde, T, Elisio, M, & Strüssmann, CA. (2013). Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*. *General and Comparative Endocrinology,* 192, 45-54.

Mogensen, S, & Post, JR. (2012). Energy allocation strategy modifies growth–survival trade-offs in juvenile fish across ecological and environmental gradients. *Oecologia,* 168, 923-933.

Mora, C, & Maya, MF. (2006). Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology,* 31, 337-341.

Moran, D, Smith, CK, Gara, B, & Poortenaar, CW. (2007). Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* valenciennes 1833). *Aquaculture,* 262, 95-104.

MPI. (2013). Fisheries assessment plenary, May 2013: Stock assessments and yield estimates: Compiled by the fisheries science group. Ministry for Primary Industries, Wellington, New Zealand. Https://www.Mpi.Govt.Nz/dmsdocument/3884/loggedin.

Munday, PL, Donelson, JM, Dixson, DL, & Endo, GGK. (2009). Effects of ocean acidification on the early life history of a tropical marine fish. *Proceedings of the Royal Society B: Biological Sciences,* 276, 3275-3283.

Munday, PL, Jarrold, MD, & Nagelkerken, I. (2019). Ecological effects of elevated $CO_2$ on marine and freshwater fishes: From individual to community effects. *Fish Physiology*, 37, 323-368.

Munday, PL, Jones, GP, Pratchett, MS, & Williams, AJ. (2008). Climate change and the future for coral reef fishes. *Fish and Fisheries,* 9, 261-285.

Munday, PL, Watson, S-A, Parsons, DM, King, A, Barr, NG, Mcleod, IM,… Pether, SM. (2015). Effects of elevated $CO_2$ on early life history development of the yellowtail kingfish, *Seriola lalandi*, a large pelagic fish. *ICES Journal of Marine Science,* 7, 641-649.

Munk, P, & Kiorboe, T. (1985). Feeding behaviour and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. *Marine ecology progress series. Oldendorf,* 24, 15-21.

Murphy, HM, Warren-Myers, FW, Jenkins, GP, Hamer, PA, & Swearer, SE. (2014). Variability in size-selective mortality obscures the importance of larval traits to recruitment success in a temperate marine fish. *Oecologia,* 175, 1201-1210.

Murray, CS, Malvezzi, A, Gobler, CJ, & Baumann, H. (2014). Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series,* 504, 1-11.

Murray, CS, Wiley, D, & Baumann, H. (2019). High sensitivity of a keystone forage fish to elevated $CO_2$ and temperature. *Conservation Physiology,* 7, coz084.

Nay, TJ, Johansen, JL, Habary, A, Steffensen, JF, & Rummer, JL. (2015). Behavioural thermoregulation in a temperature-sensitive coral reef fish, the five-lined cardinalfish (*Cheilodipterus quinquelineatus*). *Coral Reefs,* 34, 1261-1265.

Nerem, RS, Beckley, BD, Fasullo, JT, Hamlington, BD, Masters, D, & Mitchum, GT. (2018). Climate-change–driven accelerated sea-level rise detected in the altimeter era. *Proceedings of the National Academy of Sciences*, 115, 2022-2025

Neuheimer, A, Thresher, R, Lyle, J, & Semmens, J. (2011). Tolerance limit for fish growth exceeded by warming waters. *Nature Climate Change,* 1, 110-113.

Newman, SJ, Williams, D, & Russ, GR. (1996). Age validation, growth and mortality rates of the tropical snappers (pisces: Lutjanidae) *Lutjanus adetii* (castelnau, 1873) and *l.*

*Quinquelineatus* (bloch, 1790) from the central Great Barrier Reef, Australia. *Marine and Freshwater Research,* 47, 575-584.

Nguyen, KDT, Morley, SA, Lai, C-H, Clark, MS, Tan, KS, Bates, AE, & Peck, LS. (2011). Upper temperature limits of tropical marine ectotherms: Global warming implications. *PLOS One,* 6, e29340.

Nilsson, GE, Crawley, N, Lunde, IG, & Munday, PL. (2009). Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology,* 15, 1405-1412.

Nilsson, GE, Dixson, DL, Domenici, P, McCormick, MI, Sorensen, C, Watson, SA, & Munday, PL. (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change,* 2, 201-204.

NIWA. (2018). The record summer of 2017/18. National institute of water and atmospheric research, Auckland. www.Niwa.Co.Nz/news/the-record-summer-of-2017-18.

NOAA. (2020). State of the climate: Global climate report for March 2020. https://www.ncdc.noaa.gov/sotc/global/202003.

NOAA. (2021). Trends in atmospheric carbon dioxide. www.esrl.noaa.gov/gmd/ccgg/trends.

Nofrizal, Yanase, K, & Arimoto, T. (2009). Effect of temperature on the swimming endurance and post-exercise recovery of jack mackerel trachurus japonicus as determined by ecg monitoring. *Fisheries Science,* 75, 1369-1375.

Norin, T, & Clark, T. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology,* 88, 122-151.

Norin, T, Malte, H, & Clark, TD. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *The Journal of Experimental Biology,* 217, 244-251.

Nowicki, JP, Miller, GM, & Munday, PL. (2012). Interactive effects of elevated temperature and co2 on foraging behavior of juvenile coral reef fish. *Journal of Experimental Marine Biology and Ecology*, 412, 46-51.

O'Brien, KM, Rix, AS, Egginton, S, Farrell, AP, Crockett, EL, Schlauch, K,… Merriman, S. (2018). Cardiac mitochondrial metabolism may contribute to differences in thermal tolerance of red-and white-blooded antarctic notothenioid fishes. *Journal of Experimental Biology,* 221, eb177816.

O'Connor, MI, Bruno, JF, Gaines, SD, Halpern, BS, Lester, SE, Kinlan, BP, & Weiss, JM. (2007). Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences,* 104, 1266-1271.

Ohlendieck, K. (2010). Proteomics of skeletal muscle glycolysis. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics,* 1804, 2089-2101.

Ohlendieck, K. (2011). Proteomic profiling of fast-to-slow muscle transitions during aging. *Frontiers in Physiology,* 2, 105.

Ojanguren, AF, Reyes-Gavilán, FG, & Muñoz, RR. (1999). Effects of temperature on growth and efficiency of yolk utilisation in eggs and pre-feeding larval stages of Atlantic salmon. *Aquaculture International,* 7, 81-87.

Oliver, ECJ, Donat, MG, Burrows, MT, Moore, PJ, Smale, DA, Alexander, LV,… Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, 9, 1324.

Oliver, ECJ. (2019). Mean warming not variability drives marine heatwave trends. *Climate Dynamics,* 53, 1653-1659.

Omlin, T, & Weber, J-M. (2010). Hypoxia stimulates lactate disposal in rainbow trout. *The Journal of Experimental Biology,* 213, 3802-3809.

Ottersen, G, Stige, LC, Durant, JM, Chan, K-S, Rouyer, TA, Drinkwater, KF, & Stenseth, NC. (2013). Temporal shifts in recruitment dynamics of north Atlantic fish stocks: Effects of spawning stock and temperature. *Marine Ecology Progress Series,* 480, 205-225.

Owen, R. (1989). Microscale and finescale variations of small plankton in coastal and pelagic environments. *Journal of Marine Research,* 47, 197-240.

Paaijmans, KP, Heinig, RL, Seliga, RA, Blanford, JI, Blanford, S, Murdock, CC, & Thomas, MB. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology,* 19, 2373-2380.

Palacios, M, Warren, DT, & McCormick, MI. (2016). Sensory cues of a top-predator indirectly control a reef fish mesopredator. *Oikos,* 125, 201-209.

Pankhurst, NW, & Munday, PL. (2011). Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research,* 62, 1015-1026.

Paris, CB, & Cowen, RK. (2004). Direct evidence of a biophysical retention mechanism for coral reef fish larvae. *Limnology and Oceanography,* 49, 1964-1979.

Parmesan, C, & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* 421, 37-42.

Parsons, DM, Buckthought, D, Middleton, C, & MacKay, G. (2016). Relative abundance of snapper (*Chrysophrys auratus*) across habitats within an estuarine system. *New Zealand Journal of Marine and Freshwater Research,* 50, 358-370.

Parsons, DM, Middleton, C, Smith, MD, & Cole, RG. (2014). The influence of habitat availability on juvenile fish abundance in a northeastern New Zealand estuary. *New Zealand Journal of Marine and Freshwater Research,* 48, 216-228.

Pauly, D, & Pullin, RS. (1988). Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes,* 22, 261-271.

Pearce, AF, & Feng, M. (2013). The rise and fall of the “marine heat wave” off Western Australia during the summer of 2010/2011. *Journal of Marine Systems,* 111, 139-156.

Peck, MA, Buckley, LJ, & Bengtson, DA. (2006). Effects of temperature and body size on the swimming speed of larval and juvenile Atlantic cod (*Gadus morhua*): Implications for individual-based modelling. *Environmental Biology of Fishes*, 75, 419-429.

Peck, LS, Clark, MS, Morley, SA, Massey, A, & Rossetti, H. (2009). Animal temperature limits and ecological relevance: Effects of size, activity and rates of change. *Functional Ecology,* 23, 248-256.

Peck, MA, Huebert, KB, & Llopiz, JK. (2012). Intrinsic and extrinsic factors driving match–mismatch dynamics during the early life history of marine fishes. *Advances in Ecological Research*, 47, 177-302.

Pedersen, SA, Hakedal, OJ, Salaberria, I, Tagliati, A, Gustavson, LM, Jenssen, BM,… Altin, D. (2014). Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates. *Environmental Science & Technology,* 48, 12275-12284.

Pepin, P. (1991). Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Canadian Journal of Fisheries and Aquatic Sciences,* 48, 503-518.

Perry, AL, Low, PJ, Ellis, JR, & Reynolds, JD. (2005). Climate change and distribution shifts in marine fishes. *Science,* 308, 1912-1915.

Petitgas, P, Rijnsdorp, AD, Dickey-Collas, M, Engelhard, GH, Peck, MA, Pinnegar, JK,… Nash, RD. (2013). Impacts of climate change on the complex life cycles of fish. *Fisheries Oceanography,* 22, 121-139.

Piatt, JF, Parrish, JK, Renner, HM, Schoen, SK, Jones, TT, Arimitsu, ML,… Duerr, RS. (2020). Extreme mortality and reproductive failure of common murres resulting from the northeast pacific marine heatwave of 2014-2016. *PLOS One,* 15, e0226087.

Pierrot, D, Lewis, E, & Wallace, DWR. (2006). Ms excel program developed for $CO_2$ system calculations *Book ms excel program developed for $CO_2$ system calculations*. City: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy.

Pimentel, MS, Faleiro, F, Dionísio, G, Repolho, T, Pousão, P, Machado, J, & Rosa, R. (2014). Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *Journal of Experimental Biology*, 217, 2062-2070.

Pinsky, ML, Eikeset, AM, McCauley, DJ, Payne, JL, & Sunday, JM. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature,* 569, 108-111.

Pitcher, T, Lang, S, & Turner, J. (1988). A risk-balancing trade off between foraging rewards and predation hazard in a shoaling fish. *Behavioral Ecology and Sociobiology,* 22, 225-228.

Poloczanska, ES, Brown, CJ, Sydeman, WJ, Kiessling, W, Schoeman, DS, Moore, PJ,… Richardson, AJ. (2013). Global imprint of climate change on marine life. *Nature Climate Change,* 3, 919-925.

Poloczanska, ES, Burrows, MT, Brown, CJ, García Molinos, J, Halpern, BS, Hoegh-Guldberg, O,… Schoeman, DS. (2016). Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science,* 3, 62.

Polovina, JJ. (1984). Model of a coral reef ecosystem. *Coral reefs,* 3, 1-11

Pope, EC, Ellis, R, Scolamacchia, M, Scolding, J, Keay, A, Chingombe, P,… Wilson, R. (2014). European sea bass, *Dicentrarchus labrax*, in a changing ocean. *Biogeosciences,* 11, 2519-2530.

Pörtner, H-O. (2010). Oxygen-and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology,* 213, 881-893.

Pörtner, HO, Berdal, B, Blust, R, Brix, O, Colosimo, A, De Wachter, B,… Zakhartsev, M. (2001). Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: Developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Continental Shelf Research,* 21, 1975-1997.

Pörtner, HO, Langenbuch, M, & Reipschläger, A. (2004). Biological impact of elevated ocean $CO_2$ concentrations: Lessons from animal physiology and earth history. *Journal of Oceanography,* 60, 705-718.

Pörtner, HO, Langenbuch, M, & Michaelidis, B. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in $CO_2$ on marine animals: From earth history to global change. *Journal of Geophysical Research: Oceans,* 110, C09S10.

Pörtner, HO, Bennett, AF, Bozinovic, F, Clarke, A, Lardies, MA, Lucassen, M,… Stillman, JH. (2006). Trade-offs in thermal adaptation: The need for a molecular to ecological integration. *Physiological and Biochemical Zoology,* 79, 295-313.

Pörtner, HO, & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science,* 315, 95-97.

Pörtner, HO, & Farrell, AP. (2008). Physiology and climate change. *Science*, 322, 690-692.

Pörtner, HO, & Peck, MA. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology,* 77, 1745-1779.

Pörtner, H-O. (2012). Integrating climate-related stressor effects on marine organisms: Unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series*, 470, 273-290.

Pörtner, H-O, Bock, C, & Mark, FC. (2017). Oxygen- and capacity-limited thermal tolerance: Bridging ecology and physiology. *The Journal of Experimental Biology,* 220, 2685-2696.

Pörtner, H-O, Roberts, DC, Masson-Delmotte, V, Zhai, P, Tignor, M, Poloczanska, E,… Petzold, J. (2019). IPCC special report on the ocean and cryosphere in a changing climate. *IPCC Intergovernmental Panel on Climate Change: Geneva, Switzerland.*

Post, JR, & Parkinson, EA. (2001). Energy allocation strategy in young fish: Allometry and survival. *Ecology,* 82, 1040-1051.

Pratchett, MS, Hoey, AS, Wilson, SK, Messmer, V, & Graham, NAJ. (2011). Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and coral loss. *Diversity,* 3, 424-452

Pratchett, MS, Cameron, DS, Donelson, J, Evans, L, Frisch, AJ, Hobday, AJ,… Munday, PL. (2017). Effects of climate change on coral grouper (plectropomus spp.) and possible adaptation options. *Reviews in Fish Biology and Fisheries,* 27, 297-316.

Price, NN, Martz, TR, Brainard, RE, & Smith, JE. (2012). Diel variability in seawater ph relates to calcification and benthic community structure on coral reefs. *PLOS One,* 7, e43843.

Priede, IG. (1985). Metabolic scope in fishes. *Fish energetics*, Springer, 33-64.

Prugh, LR, Stoner, CJ, Epps, CW, Bean, WT, Ripple, WJ, Laliberte, AS, & Brashares, JS. (2009). The rise of the mesopredator. *Bioscience,* 59, 779-791.

Quéré, G, & Leis, JM. (2010). Settlement behaviour of larvae of the stripey snapper, *Lutjanus carponotatus* (teleostei: Lutjanidae). *Environmental Biology of Fishes,* 88, 227-238.

Raby, GD, Messmer, V, Tobin, AJ, Hoey, AS, Jutfelt, F, Sundin, J,… Clark, TD. (2018). Swim for it: Effects of simulated fisheries capture on the post-release behaviour of four Great Barrier Reef fishes. *Fisheries Research,* 206, 129-137.

Rahmstorf, S, Box, JE, Feulner, G, Mann, ME, Robinson, A, Rutherford, S, & Schaffernicht, EJ. (2015). Exceptional twentieth-century slowdown in Atlantic Ocean overturning circulation. *Nature Climate Change*, 5, 475-480.

Rankin, TL, & Sponaugle, S. (2011). Temperature influences selective mortality during the early life stages of a coral reef fish. *PLOS One,* 6, e16814.

Rebecca, F. (2005). Swimming speeds of larval coral reef fishes: Impacts on self-recruitment and dispersal. *Marine Ecology Progress Series,* 285, 223-232.

Régnier, T, Gibb, FM, & Wright, PJ. (2018). Temperature effects on egg development and larval condition in the lesser sandeel, *Ammodytes marinus*. *Journal of Sea Research,* 134, 34-41.

Reynolds, WW, & Casterlin, ME. (1980). The role of temperature in the environmental physiology of fishes. *Environmental Physiology of Fishes*, Springer, 497-518.

Rhein, Ma, Rintoul, SR, Aoki, S, Campos, E, Chambers, D, Feely, RA,… Kostianoy, A. (2013). Observations: Ocean. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* Cambridge University Press, 255-316.

Richardson, LE, Graham, NAJ, Pratchett, MS, Eurich, JG, & Hoey, AS. (2018). Mass coral bleaching causes biotic homogenization of reef fish assemblages. *Global Change Biology,* 24, 3117-3129.

Rijnsdorp, AD, Peck, MA, Engelhard, GH, Möllmann, C, & Pinnegar, JK. (2009). Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science,* 66, 1570-1583.

Ritchie, EG, & Johnson, CN. (2009). Predator interactions, mesopredator release and biodiversity conservation. *Ecology Letters,* 12, 982-998.

Robinson, JPW, Wilson, SK, Jennings, S, & Graham, NAJ. (2019). Thermal stress induces persistently altered coral reef fish assemblages. *Global Change Biology,* 25, 2739-2750.

Roche, DG, Binning, SA, Bosiger, Y, Johansen, JL, & Rummer, JL. (2013). Finding the best estimates of metabolic rates in a coral reef fish. *Journal of Experimental Biology,* 216, 2103-2110.

Rodgers, G, Donelson, J, McCormick, M, & Munday, P. (2018). In hot water: Sustained ocean warming reduces survival of a low-latitude coral reef fish. *Marine Biology*, 165, 1-10.

Rodgers, GG, Rummer, JL, Johnson, LK, & McCormick, MI. (2019). Impacts of increased ocean temperatures on a low-latitude coral reef fish – processes related to oxygen uptake and delivery. *Journal of Thermal Biology,* 79, 95-102.

Rogelj, J, Shindell, D, Jiang, K, Fifita, S, Forster, P, Ginzburg, V,… Kriegler, E. (2018). Mitigation pathways compatible with 1.5 c in the context of sustainable development. In: Global warming of 1.5 °C. An IPCC Special Report on the Impacts of Global warming of 1.5 °C above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty. Intergovernmental Panel on Climate.

Rombough, PJ. (1997). The effects of temperature on embryonic and larval development. In: *Global Warming: Implications for Freshwater and Marine Fish*, Cambridge University Press, 177–223.

Rueden, CT, Schindelin, J, Hiner, MC, DeZonia, BE, Walter, AE, Arena, ET, & Eliceiri, KW. (2017). Imagej2: Imagej for the next generation of scientific image data. *Bioinformatics,* 18, 1-26.

Rummer, JL, Stecyk, JAW, Couturier, CS, Watson, S-A, Nilsson, GE, & Munday, PL. (2013). Elevated $CO_2$ enhances aerobic scope of a coral reef fish. *Conservation Physiology, 1*, 1.

Rummer, JL, Couturier, CS, Stecyk, JAW, Gardiner, NM, Kinch, JP, Nilsson, GE, & Munday, PL. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology,* 20, 1055-1066.

Rummer, JL, Binning, SA, Roche, DG, & Johansen, JL. (2016). Methods matter: Considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. *Conservation Physiology,* 4, 13.

Russell, M. (2001). Spawning aggregations of reef fishes on the Great Barrier Reef: Implications for management. Great Barrier Reef Marine Park Authority.

Salin, K, Villasevil, EM, Anderson, GJ, Lamarre, SG, Melanson, CA, McCarthy, I,… Metcalfe, NB. (2019). Differences in mitochondrial efficiency explain individual variation in growth performance. *Proceedings of the Royal Society B,* 286, 20191466.

Salinger, MJ, Renwick, J, Behrens, E, Mullan, AB, Diamond, HJ, Sirguey, P,… Fitzharris, BB. (2019). The unprecedented coupled ocean-atmosphere summer heatwave in the new zealand region 2017/18: Drivers, mechanisms and impacts. *Environmental Research Letters,* 14, 044023

Sandblom, E, Gräns, A, Axelsson, M, & Seth, H. (2014). Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: Implications in a thermally extreme future. *Proceedings of the Royal Society B: Biological Sciences,* 281, 20141490.

Saunders, PU, Garvican-Lewis, LA, Schmidt, WF, & Gore, CJ. (2013). Relationship between changes in haemoglobin mass and maximal oxygen uptake after hypoxic exposure. *British Journal of Sports Medicine,* 47, 26-30.

Saunders, RJ, Fowler, AJ, & Gillanders, BM. (2012). The spawning dynamics of snapper (*Chrysophrys auratus*) in northern spencer gulf, South Australia. *New Zealand Journal of Marine and Freshwater Research, 46*, 491-510.

Schulte, PM. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *The Journal of Experimental Biology,* 218, 1856-1866.

Schurmann, H, & Steffensen, JF. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology,* 50, 1166-1180.

Scott, ME, Heupel, MR, Simpfendorfer, CA, Matley, JK, & Pratchett, MS. (2019). Latitudinal and seasonal variation in space use by a large, predatory reef fish, *Plectropomus leopardus*. *Functional Ecology,* 33, 670-680.

Scott, S, & Pankhurst, N. (1992). Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus auratus* (bloch & schneider)(sparidae). *Journal of Fish Biology,* 41, 685-696.

Sen Gupta, A, Thomsen, M, Benthuysen, JA, Hobday, AJ, Oliver, E, Alexander, LV,… Smale, DA. (2020). Drivers and impacts of the most extreme marine heatwaves events. *Scientific Reports,* 10, 19359.

Shanks, AL. (2009). Pelagic larval duration and dispersal distance revisited. *The Biological Bulletin,* 216, 373-385.

Shaw, EC, McNeil, BI, & Tilbrook, B. (2012). Impacts of ocean acidification in naturally variable coral reef flat ecosystems. *Journal of Geophysical Research: Oceans, 117*, C03038.

Shaw, EC, Munday, PL, & McNeil, BI. (2013). The role of $CO_2$ variability and exposure time for biological impacts of ocean acidification. *Geophysical Research Letters,* 40, 4685-4688.

Sheaves, M. (2006). Is the timing of spawning in sparid fishes a response to sea temperature regimes? *Coral Reefs,* 25, 655-669.

Sheppard, C, & Rioja-Nieto, R. (2005). Sea surface temperature 1871–2099 in 38 cells in the Caribbean region. *Marine Environmental Research,* 60, 389-396.

Shima, JS, & Findlay, AM. (2002). Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. *Marine Ecology Progress Series,* 235, 303-309.

Shima, JS, & Swearer, SE. (2009). Larval quality is shaped by matrix effects: Implications for connectivity in a marine metapopulation. *Ecology,* 90, 1255-1267.

Shrestha, RP, Horowitz, J, Hollot, CV, Germain, MJ, Widness, JA, Mock, DM,… Chait, Y. (2016). Models for the red blood cell lifespan. *Journal of Pharmacokinetics and Pharmacodynamics,* 43, 259-274.

Shulman, MJ. (1985). Coral reef fish assemblages: Intra- and interspecific competition for shelter sites. *Environmental Biology of Fishes,* 13, 81-92.

Shultz, AD, Zuckerman, ZC, Stewart, HA, & Suski, CD. (2014). Seasonal blood chemistry response of sub-tropical nearshore fishes to climate change. *Conservation Physiology*, 2, 1.

Silva, CSE, Novais, SC, Lemos, MFL, Mendes, S, Oliveira, AP, Gonçalves, EJ, & Faria, AM. (2016). Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. *Science of The Total Environment, 563*, 89-98.

Smale, DA, Wernberg, T, Oliver, EC, Thomsen, M, Harvey, BP, Straub, SC,… Donat, MG. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nature Climate Change, 9, 306-312.

Smith, KA, Dowling, CE, & Brown, J. (2019). Simmered then boiled: Multi-decadal poleward shift in distribution by a temperate fish accelerates during marine heatwave. *Frontiers in Marine Science*, 6, 407

Solomon, S, Plattner, G-K, Knutti, R, & Friedlingstein, P. (2009). Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences, 106, 1704-1709.

Somero, GN. (1995). Proteins and temperature. *Annual Review of Physiology*, 57, 43-68.

Somero, GN. (2004). Adaptation of enzymes to temperature: Searching for basic "strategies". *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* 139, 321-333.

Somero, G. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology,* 213, 912-920.

Spinks, RK, Munday, PL, & Donelson, JM. (2019). Developmental effects of heatwave conditions on the early life stages of a coral reef fish. *Journal of Experimental Biology,* 222, jeb202713.

Sponaugle Su, Kirsten, G-C, & Deanna, P. (2006). Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish *Thalassoma bifasciatum* in the Florida keys. *Marine Ecology Progress Series,* 308, 1-15.

Sswat, M, Stiasny, MH, Jutfelt, F, Riebesell, U, & Clemmesen, C. (2018). Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and $CO_2$. *PLOS One, 13*, 1.

Stallings, CD, Coleman, FC, Koenig, CC, & Markiewicz, DA. (2010). Energy allocation in juveniles of a warm-temperate reef fish. *Environmental Biology of Fishes,* 88, 389-398.

Steinarsson, A, & Björnsson, B. (1999). The effects of temperature and size on growth and mortality of cod larvae. *Journal of Fish Biology,* 55, 100-109.

Stiasny, MH, Mittermayer, FH, Sswat, M, Voss, R, Jutfelt, F, Chierici, M,… Clemmesen, C. (2016). Ocean acidification effects on Atlantic cod larval survival and recruitment to the fished population. *PLOS One,* 11, e0155448.

Strobel, A, Bennecke, S, Leo, E, Mintenbeck, K, Pörtner, HO, & Mark, FC. (2012). Metabolic shifts in the antarctic fish *Notothenia rossii* in response to rising temperature and p $CO_2$. *Frontiers in Zoology,* 9, 28.

Stuart-Smith, RD, Brown, CJ, Ceccarelli, DM, & Edgar, GJ. (2018). Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. *Nature,* 560, 92-96.

Sumpton, WD, Brown, IW, Mayer, DG, Mclennan, MF, Mapleston, A, Butcher, AR, . . . Begg, GA (2010). Assessing the effects of line capture and barotrauma relief procedures on post-release survival of key tropical reef fish species in Australia using recreational tagging clubs. *Fisheries Management and Ecology,* 17, 77-88.

Sunday, JM, Bates, AE, & Dulvy, NK. (2010). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences,* 278, *1823-1830.*

Sunday, JM, Bates, AE, & Dulvy, NK. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change,* 2, 686-690.

Sunday, JM, Bates, AE, Kearney, MR, Colwell, RK, Dulvy, NK, Longino, JT, & Huey, RB. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences,* 111, 5610-5615.

Sunday, JM, Bennett, JM, Calosi, P, Clusella-Trullas, S, Gravel, S, Hargreaves, AL,… Morales-Castilla, I. (2019). Thermal tolerance patterns across latitude and elevation. *Philosophical Transactions of the Royal Society B,* 374, 20190036.

Svendsen, JC, Tudorache, C, Jordan, AD, Steffensen, JF, Aarestrup, K, & Domenici, P. (2010). Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *The Journal of Experimental Biology,* 213, 2177-2183.

Svendsen, MBS, Bushnell, P, & Steffensen, JF. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology,* 88, 26-50.

Tewksbury, JJ, Huey, RB, & Deutsch, CA. (2008). Putting the heat on tropical animals. *Science,* 320, 1296-1297.

Tocher, DR. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research,* 41, 717-732.

Trenberth, KE, & Solomon, A. (1994). The global heat balance: Heat transports in the atmosphere and ocean. *Climate Dynamics,* 10, 107-134.

Trenberth, KE. (2011). Changes in precipitation with climate change. Climate Research, 47, 123-138.

van der Walt, KA, Potts, WM, Porri, F, Winkler, AC, Duncan, MI, Skeeles, MR, & James, NC. (2021). Marine heatwaves exceed cardiac thermal limits of adult sparid fish (*Diplodus capensis*, smith 1884). *Frontiers in Marine Science,* 8, 801.

von Biela, VR, Arimitsu, ML, Piatt, JF, Heflin, B, Schoen, SK, Trowbridge, JL, & Clawson, CM. (2019). Extreme reduction in nutritional value of a key forage fish during the pacific marine heatwave of 2014-2016. *Marine Ecology Progress Series,* 613, 171-182.

Voosen, P. (2020). Climate change spurs global speedup of ocean currents. *Science,* 367, 612-613

Wakefield, CB. (2010). Annual, lunar and diel reproductive periodicity of a spawning aggregation of snapper *Pagrus auratus* (sparidae) in a marine embayment on the lower west coast of Australia. *Journal of Fish Biology,* 77, 1359-1378.

Waldbusser, GG, & Salisbury, JE. (2014). Ocean acidification in the coastal zone from an organism's perspective: Multiple system parameters, frequency domains, and habitats. *Annual Review of Marine Science*, 6, 221-247.

Wardle, C. (1980). Effects of temperature on the maximum swimming speed of fishes. *Environmental Physiology of Fishes.* Springer, 519-531.

Watson, S-A, Allan, BJM, McQueen, DE, Nicol, S, Parsons, DM, Pether, SMJ,… Munday, PL. (2018). Ocean warming has a greater effect than acidification on the early life history development and swimming performance of a large circumglobal pelagic fish. *Global Change Biology,* 24, 4368-4385.

Weber, J-M, Choi, K, Gonzalez, A, & Omlin, T. (2016). Metabolic fuel kinetics in fish: Swimming, hypoxia and muscle membranes. *Journal of Experimental Biology,* 219, 250-258.

Wells, GD, Selvadurai, H, & Tein, I. (2009). Bioenergetic provision of energy for muscular activity. *Paediatric Respiratory Reviews,* 10, 83-90.

Wells, RMG, & Baldwin, J. (1990). Oxygen transport potential in tropical reef fish with special reference to blood viscosity and haematocrit. *Journal of Experimental Marine Biology and Ecology,* 141, 131-143.

Wernberg, T, Russell, BD, Moore, PJ, Ling, SD, Smale, DA, Campbell, A, . . . Connell, SD. (2011). Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology and Ecology*, 400, 7-16.

Wernberg, T, Smale, DA, Tuya, F, Thomsen, MS, Langlois, TJ, De Bettignies, T,… Rousseaux, CS. (2013). An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Climate Change,* 3, 78-82.

Wilson, SM, Raby, GD, Burnett, NJ, Hinch, SG, & Cooke, SJ. (2014). Looking beyond the mortality of bycatch: Sublethal effects of incidental capture on marine animals. *Biological Conservation,* 171, 61-72.

Wittmann, AC, & Pörtner, H-O. (2013). Sensitivities of extant animal taxa to ocean acidification. *Nature Climate Change,* 3, 995-1001.

Wood, C, Turner, J, & Graham, M. (1983). Why do fish die after severe exercise? *Journal of Fish Biology,* 22, 189-201.

Wood, C, & Munger, R. (1994). Carbonic anhydrase injection provides evidence for the role of blood acid-base status in stimulating ventilation after exhaustive exercise in rainbow trout. *Journal of Experimental Biology,* 194, 225-253.

Wortmann, J, O'Neill, M, Sumpton, W, Campbell, M, & Stewart, J. (2018). Stock assessment of Australian east coast snapper, *Chrysophrys auratus* predictions of stock status and reference points for 2016. Technical Report, State of Queensland. http://era.daf.qld.gov.au/id/eprint/6341

Yin, M, & Blaxter, J. (1987). Escape speeds of marine fish larvae during early development and starvation. *Marine Biology,* 96, 459-468.

Zakhartsev, M, Johansen, T, Pörtner, HO, & Blust, R. (2004). Effects of temperature acclimation on lactate dehydrogenase of cod (*Gadus morhua*): Genetic, kinetic and thermodynamic aspects. *Journ*al of Experimental Biology, 207, 95-112.

Zarco-Perello, S, Pratchett, M, & Liao, V. (2012). Temperature-growth performance curves for a coral reef fish, *Acanthochromis polyacanthus*. *Journal of Coral Reef Studies*, 14, 97-103.

Zeebe, RE, Ridgwell, A, & Zachos, JC. (2016). Anthropogenic carbon release rate unprecedented during the past 66 million years. *Nature Geoscience,* 9, 325-329.

# Appendix

**Table A2.1**. Linear mixed effects models for morphometric traits for 1 dph *Chrysophrsy auratus*. Larvae were reared in either ambient (18°C) or elevated (22°C) temperature cross factored with ambient (400 µatm) or elevated $CO_2$ (1000 µatm). Temperature and $CO_2$ were used as fixed effects. Rearing tank was used as a random effect.

| | **Temperature** | | | **$CO_2$** | | | **Temperature * $CO_2$** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Trait** | **t** | **df** | **p** | **t** | **df** | **p** | **t** | **df** | **p** |
| PC1 | 11.527 | 12 | <0.001 | 0.101 | 12 | 0.921 | 0.781 | 12 | 0.450 |
| PC2 | -4.671 | 12 | <0.001 | 0.375 | 12 | 0.714 | 2.236 | 12 | 0.065 |
| TL | 6.063 | 12 | <0.001 | 0.449 | 12 | 0.661 | 1.120 | 12 | 0.285 |
| SL | 6.027 | 12 | <0.001 | 0.529 | 12 | 0.606 | 1.050 | 12 | 0.314 |
| BL | -12.521 | 12 | <0.001 | -0.629 | 12 | 0.541 | 1.791 | 12 | 0.098 |
| FDV | 4.543 | 12 | <0.001 | 0.305 | 12 | 0.765 | 1.976 | 12 | 0.071 |
| MDV | 6.767 | 12 | <0.001 | -0.734 | 12 | 0.477 | 1.138 | 12 | 0.277 |
| HL | 8.440 | 12 | <0.001 | 1.193 | 12 | 0.256 | -0.663 | 12 | 0.519 |
| OGL | -4.020 | 12 | 0.001 | 1.109 | 12 | 0.289 | -0.705 | 12 | 0.494 |
| OGD | -11.201 | 12 | <0.001 | -1.523 | 12 | 0.154 | 1.643 | 12 | 0.126 |
| YA | -9.963 | 12 | <0.001 | 1.058 | 12 | 0.310 | -0.614 | 12 | 0.551 |

***Table* A2.2.** Linear mixed effects models for morphometric traits for 16dph *Chrysophrys auratus.* Larvae were reared in either ambient (18°C) or elevated (22°C) temperature cross factored with ambient (400 µatm) or elevated $CO_2$ (1000 µatm). Temperature and $CO_2$ were used as fixed effects. Rearing tank was used as a random effect.

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| **Trait** | **Temperature** | | | **$CO_2$** | | | **Temperature * $CO_2$** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **t** | **df** | **p** | **t** | **df** | **p** | **t** | **df** | **p** |
| PC1 | -7.887 | 12 | <0.001 | 0.016 | 12 | 0.987 | 0.951 | 12 | 0.36 |
| PC2 | -0.444 | 12 | 0.665 | -0.32 | 12 | 0.754 | 0.43 | 12 | 0.967 |
| TL | 4.536 | 12 | <0.001 | 0.168 | 12 | 0.869 | -0.64 | 12 | 0.535 |
| SL | 4.286 | 12 | 0.001 | 0.078 | 12 | 0.939 | -0.476 | 12 | 0.642 |
| BL | 7.155 | 12 | <0.001 | 0.275 | 12 | 0.788 | -0.949 | 12 | 0.361 |
| FDV | 6.534 | 12 | <0.001 | 0.331 | 12 | 0.746 | -1.187 | 12 | 0.258 |
| MDV | 15.616 | 12 | <0.001 | 0.129 | 12 | 0.899 | -1.031 | 12 | 0.323 |
| ML | 8.023 | 12 | <0.001 | -0.136 | 12 | 0.894 | -1.075 | 12 | 0.304 |
| HD | 9.489 | 12 | <0.001 | 0.105 | 12 | 0.918 | -0.788 | 12 | 0.446 |
| HL | 6.534 | 12 | <0.001 | 0.115 | 12 | 0.910 | -0.878 | 12 | 0.397 |
| ED | 7.438 | 12 | <0.001 | 0.018 | 12 | 0.986 | -0.504 | 12 | 0.623 |
| SBA | 9.344 | 12 | <0.001 | -0.457 | 12 | 0.656 | -1.146 | 12 | 0.247 |

**Table A2.3.** Generalised linear models for survival at 1 dph and 16 dph *Chrysophrus auratus.* Larvae were reared in either ambient (18°C) or elevated (22°C) temperature cross factored with ambient (400 µatm) or elevated $CO_2$ (1000 µatm). Temperature and $CO_2$ were used as fixed effects. Stocking desnity was used as a weight.

| | **Temperature** | | | **$CO_2$** | | | **Temperature * $CO_2$** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **f** | **df** | **p** | **f** | **df** | **p** | **f** | **df** | **p** |
| 1 dph | 0.242 | 11 | 0.633 | 5.8454 | 11 | 0.034 | 2.738 | 11 | 0.126 |
| 16 dph | 2.460 | 11 | 0.145 | 6.791 | 11 | 0.024 | 0.003 | 11 | 0.958 |

**Table A3.1.** Linear mixed effects model for Ucrit of juvenile *Chrysophrys auratus*. Juveniles were reared in either ambient (18°C) or elevated (22°C) temperature cross factored with *ambient* (400 µatm) or elevated $CO_2$ (1000 µatm). Temperature and $CO_2$ were used as fixed effects. Length was used as a covariate. Testing day and rearing tank were used as random effects. Interactions between length, temperature and $CO_2$ were not significant and therefore were not included in the final model.

| Fixed | Numerator df | Denominator df | F | Sig. |
|---|---|---|---|---|
| Intercept | 1 | 46 | 11.654 | .001 |
| Temperature | 1 | 46 | 7.121 | .010 |
| $CO_2$ | 1 | 46 | 30.606 | .000 |
| Temperature*$CO_2$ | 1 | 46 | 0.678 | .414 |
| Length | 1 | 46 | 19.747 | .000 |

| Random | Estimate | Std.Error | Sig. | Redundant |
|---|---|---|---|---|
| Residual | 1.102 | .229 | .000 | - |
| Testing Day | .000 | .000 | - | Yes |
| Rearing tank | .000 | .000 | - | Yes |

**Table A4.1** Kruskal Wallis, and Dunn tests for Yolk area of larval *Lutjanus carponotatus* between 0-27 hours post hatching. Larvae were held in temperature treatments of either 28.5°C, 30°C, or 31.5°C

| | Yolk Area | | | | | |
|---|---|---|---|---|---|---|
| | Kruskal_Wallis | | | Dunn | | |
| | Chi-Squared | df | p.value | 28.5*30 | 28.5*31.5 | 30*31.5 |
| 0 hph | 3.555 | 2 | 0.169 | 0.420 | 0.060 | 0.290 |
| 3 hph | 29.95 | 2 | <0.001 | <0.001 | <0.001 | 0.520 |
| 15 hph | 40.498 | 2 | <0.001 | <0.001 | <0.001 | 0.092 |
| 27 hph | 38.05 | 2 | <0.001 | 0.039 | <0.001 | <0.001 |

**Table A4.2** Kruskal Wallis, and Dunn tests for oil globule area of larval *Lutjanus carponotatus* between 0-27 hours post hatching. Larvae were held in temperature treatments of either 28.5°C, 30°C, or 31.5°C

| | Oil Globule Area | | | | | |
|---|---|---|---|---|---|---|
| | Kruskal_Wallis | | | Dunn | | |
| | Chi-Squared | df | p.value | 28.5*30 | 28.5*31.5 | 30*31.5 |
| 0 hph | 10.221 | 2 | 0.006 | 0.008 | 0.004 | 0.818 |
| 3 hph | 6.217 | 2 | 0.044 | 0.61 | 0.063 | 0.018 |
| 15 hph | 12.037 | 2 | 0.002 | 0.001 | 0.007 | 0.601 |
| 27 hph | 7.202 | 2 | 0.027 | 0.042 | 0.011 | 0.612 |
| 39 hph | 55.904 | 2 | <0.001 | <0.001 | <0.001 | 1 |
| 51 hph | 52.174 | 2 | <0.001 | <0.001 | <0.001 | 1 |

**Table A4.3** Linear mixed effect models for the metamorphic traits of 1 day post hatch *Lutjanus carponotatus*. Linear mixed effect models for the metamorphic traits of 14 day post hatch *Lutjanus carponotatus*. Anova were used to test the significance of temeprature treatments and those that were significant were further analysied with a Tukey's post-hoc test. Larvae were held in temperature treatments of either 28.5°C, 30°C, or 31.5°C Temperature was used as a fixed effects while rearing tank was a random effect.

| | Anova | | | Tukey's 28.5°C x 30°C | | 28.5°C x 31.5°C | | 30°C x 31.5°C | |
|---|---|---|---|---|---|---|---|---|---|
| 1dph | Degrees of freedom | f-Value | p-Value | t-Value | p-Value | t-Value | p-Value | t-Value | p-Value |
| PC1 | 2,6 | 16.84 | 0.003 | 4.555 | 0.009 | 5.385 | 0.004 | 0.827 | 0.701 |
| PC2 | 2,6 | 6.12 | 0.036 | 1.525 | 0.345 | -1.988 | 0.196 | -3.489 | 0.031 |
| Total length | 2,6 | 31.11 | <0.001 | 6.129 | 0.002 | 7.331 | <0.001 | 1.163 | 0.515 |
| Standard Length | 2,6 | 22.25 | 0.001 | 5.031 | 0.006 | 6.268 | 0.002 | 1.219 | 0.486 |
| Body Length | 2,6 | 42.04 | <0.001 | 8.706 | <0.001 | 6.732 | 0.001 | -1.942 | 0.208 |
| Head Length | 2,6 | 5.37 | 0.045 | 2.741 | 0.016 | 2.926 | 0.009 | 0.184 | 0.981 |
| Fin Depth | 2,6 | 2.86 | 0.133 | - | - | - | - | - | - |
| Muscle Depth | 2,6 | 2.86 | 0.042 | 1.803 | 0.245 | 3.35 | 0.035 | 1.543 | 0.338 |
| Oil Globlue Diameter | 2,6 | 10.05 | 0.012 | 1.284 | 0.453 | 3.14 | 0.045 | 4.353 | 0.011 |
| Yolk Area | 2,6 | 3.78 | 0.086 | - | - | - | - | - | - |

**Table A4.4** Linear mixed effect models for the metamorphic traits of 14 day post hatch *Lutjanus carponotatus*. Anova were used to test the significance of temeprature treatments and those that were significant were further analysied with a Tukey's post-hoc test. Larvae were held in temperature treatments of either 28.5°C, 30°C, or 31.5°C Temperature was used as a fixed effects while rearing tank was a random effect.

| | Anova | | | Tukey's 28.5°C x 30°C | | 28.5°C x 31.5°C | | 30°C x 31.5°C | |
|---|---|---|---|---|---|---|---|---|---|
| 14dph | Degrees of freedom | f-Value | p-Value | t-Value | p-Value | t-Value | p-Value | t-Value | p-Value |
| PC1 | 2,14 | 150.42 | <0.001 | -11.162 | <0.001 | -17.009 | <0.001 | -6.413 | <0.001 |
| PC2 | 2,14 | 11.601 | 0.001 | -4.367 | 0.002 | -0.319 | 0.945 | 3.857 | 0.005 |
| Total length | 2,14 | 121.14 | <0.001 | -9.580 | <0.001 | -15.360 | <0.001 | -6.270 | <0.001 |
| Standard Length | 2,14 | 82.38 | <0.001 | -7.730 | <0.001 | -12.700 | <0.001 | -5.350 | <0.001 |
| Body Length | 2,14 | 157.99 | <0.001 | -11.581 | <0.001 | -17.398 | <0.001 | -6.414 | <0.001 |
| Head Length | 2,14 | 164.23 | <0.001 | -10.827 | <0.001 | -17.953 | <0.001 | -7.694 | <0.001 |
| Mandible Length | 2,14 | 138.88 | <0.001 | -10.760 | <0.001 | -16.337 | <0.001 | -6.134 | <0.001 |
| Head Depth | 2,14 | 142.38 | <0.001 | -10.320 | <0.001 | -16.670 | <0.001 | -6.884 | <0.001 |
| Eye Diameter | 2,14 | 116.21 | <0.001 | -9.909 | <0.001 | -14.919 | <0.001 | -5.499 | <0.001 |
| Fin Depth | 2,14 | 152.51 | <0.001 | -12.542 | <0.001 | -16.693 | <0.001 | -4.784 | <0.001 |
| Muscle Depth | 2,14 | 226.23 | <0.001 | -14.686 | <0.001 | -20.561 | <0.001 | -6.624 | <0.001 |

**Table A5.1** Turkey's contrasts for the Resting oxygen consumption ($RMO_2$) of adult Spanish flag snapper *(Lutjanus carponotatus*). Individuals were held at either 28.5°C (28.5), 29.5°C (29.5), or 30.5°C (30.5) and were tested at 2 weeks exposure (2), 4 weeks exposure (4), and 2 weeks post-exposure (6).

| contrast | estimate | SE | df | t ratio | p.value |
|---|---|---|---|---|---|
| 28.5,2 - 29.5,2 | -42.3 | 7.10 | 97 | -5.96 | <0.001 |
| 28.5,2 - 30.5,2 | -67.0 | 7.10 | 97 | -9.44 | <0.001 |
| 28.5,2 - 28.5,4 | -7.1 | 7.10 | 97 | -1.00 | 0.985 |
| 28.5,2 - 29.5,4 | -47.1 | 7.10 | 97 | -6.63 | <0.001 |
| 28.5,2 - 30.5,4 | -63.8 | 7.10 | 97 | -8.99 | <0.001 |
| 28.5,2 - 28.5,6 | -6.5 | 7.10 | 97 | -0.92 | 0.991 |
| 28.5,2 - 29.5,6 | -23.1 | 7.10 | 97 | -3.25 | 0.041 |
| 28.5,2 - 30.5,6 | -20.4 | 7.10 | 97 | -2.87 | 0.110 |
| 29.5,2 - 30.5,2 | -24.7 | 7.10 | 97 | -3.48 | 0.021 |
| 29.5,2 - 28.5,4 | 35.2 | 7.10 | 97 | 4.96 | <0.001 |
| 29.5,2 - 29.5,4 | -4.7 | 7.10 | 97 | -0.67 | 0.999 |
| 29.5,2 - 30.5,4 | -21.5 | 7.10 | 97 | -3.03 | 0.073 |
| 29.5,2 - 28.5,6 | 35.8 | 7.10 | 97 | 5.04 | <0.001 |
| 29.5,2 - 29.5,6 | 19.3 | 7.10 | 97 | 2.71 | 0.157 |
| 29.5,2 - 30.5,6 | 22.0 | 7.10 | 97 | 3.09 | 0.062 |
| 30.5,2 - 28.5,4 | 59.9 | 7.10 | 97 | 8.43 | <0.001 |
| 30.5,2 - 29.5,4 | 19.9 | 7.10 | 97 | 2.81 | 0.127 |
| 30.5,2 - 30.5,4 | 3.2 | 7.10 | 97 | 0.45 | 1.000 |
| 30.5,2 - 28.5,6 | 60.5 | 7.10 | 97 | 8.52 | <0.001 |
| 30.5,2 - 29.5,6 | 44.0 | 7.10 | 97 | 6.19 | <0.001 |
| 30.5,2 - 30.5,6 | 46.6 | 7.10 | 97 | 6.57 | <0.001 |
| 28.5,4 - 29.5,4 | -40.0 | 7.10 | 97 | -5.63 | <0.001 |
| 28.5,4 - 30.5,4 | -56.7 | 7.10 | 97 | -7.99 | <0.001 |
| 28.5,4 - 28.5,6 | 0.6 | 7.10 | 97 | 0.08 | 1.000 |
| 28.5,4 - 29.5,6 | -15.9 | 7.10 | 97 | -2.24 | 0.386 |
| 28.5,4 - 30.5,6 | -13.2 | 7.10 | 97 | -1.87 | 0.639 |
| 29.5,4 - 30.5,4 | -16.8 | 7.10 | 97 | -2.36 | 0.317 |
| 29.5,4 - 28.5,6 | 40.5 | 7.10 | 97 | 5.71 | 0.000 |
| 29.5,4 - 29.5,6 | 24.0 | 7.10 | 97 | 3.38 | 0.028 |
| 29.5,4 - 30.5,6 | 26.7 | 7.10 | 97 | 3.76 | 0.008 |
| 30.5,4 - 28.5,6 | 57.3 | 7.10 | 97 | 8.07 | <0.001 |
| 30.5,4 - 29.5,6 | 40.8 | 7.10 | 97 | 5.74 | <0.001 |
| 30.5,4 - 30.5,6 | 43.5 | 7.10 | 97 | 6.12 | <0.001 |
| 28.5,6 - 29.5,6 | -16.5 | 7.10 | 97 | -2.33 | 0.337 |
| 28.5,6 - 30.5,6 | -13.8 | 7.10 | 97 | -1.95 | 0.583 |
| 29.5,6 - 30.5,6 | 2.7 | 7.10 | 97 | 0.38 | 1.000 |

**Table A5.3** Turkey's contrasts for the capture oxygen consumption ($CMO_2$) of adult Spanish flag snapper *(Lutjanus carponotatus*). Individuals were held at either 28.5°C (28.5), 29.5°C (29.5), or 30.5°C (30.5) and were tested at 2 weeks exposure (2), 4 weeks exposure (4), and 2 weeks post-exposure (6).

| contrast | estimate | SE | df | t ratio | p.value |
|---|---|---|---|---|---|
| 28.5,2 - 29.5,2 | -38.1 | 36.24 | 97 | -1.05 | 0.979 |
| 28.5,2 - 30.5,2 | -9.4 | 36.24 | 97 | -0.26 | 1.000 |
| 28.5,2 - 28.5,4 | -33.0 | 36.24 | 97 | -0.91 | 0.992 |
| 28.5,2 - 29.5,4 | -22.8 | 36.24 | 97 | -0.63 | 0.999 |
| 28.5,2 - 30.5,4 | -31.1 | 36.24 | 97 | -0.86 | 0.995 |
| 28.5,2 - 28.5,6 | 27.3 | 36.24 | 97 | 0.75 | 0.998 |
| 28.5,2 - 29.5,6 | -142.4 | 36.24 | 97 | -3.93 | 0.005 |
| 28.5,2 - 30.5,6 | -143.0 | 36.24 | 97 | -3.95 | 0.005 |
| 29.5,2 - 30.5,2 | 28.8 | 36.24 | 97 | 0.79 | 0.997 |
| 29.5,2 - 28.5,4 | 5.1 | 36.24 | 97 | 0.14 | 1.000 |
| 29.5,2 - 29.5,4 | 15.4 | 36.24 | 97 | 0.42 | 1.000 |
| 29.5,2 - 30.5,4 | 7.0 | 36.24 | 97 | 0.19 | 1.000 |
| 29.5,2 - 28.5,6 | 65.5 | 36.24 | 97 | 1.81 | 0.678 |
| 29.5,2 - 29.5,6 | -104.2 | 36.24 | 97 | -2.88 | 0.108 |
| 29.5,2 - 30.5,6 | -104.9 | 36.24 | 97 | -2.89 | 0.103 |
| 30.5,2 - 28.5,4 | -23.6 | 36.24 | 97 | -0.65 | 0.999 |
| 30.5,2 - 29.5,4 | -13.4 | 36.24 | 97 | -0.37 | 1.000 |
| 30.5,2 - 30.5,4 | -21.7 | 36.24 | 97 | -0.60 | 1.000 |
| 30.5,2 - 28.5,6 | 36.7 | 36.24 | 97 | 1.01 | 0.984 |
| 30.5,2 - 29.5,6 | -133.0 | 36.24 | 97 | -3.67 | 0.011 |
| 30.5,2 - 30.5,6 | -133.6 | 36.24 | 97 | -3.69 | 0.011 |
| 28.5,4 - 29.5,4 | 10.2 | 36.24 | 97 | 0.28 | 1.000 |
| 28.5,4 - 30.5,4 | 1.9 | 36.24 | 97 | 0.05 | 1.000 |
| 28.5,4 - 28.5,6 | 60.3 | 36.24 | 97 | 1.66 | 0.766 |
| 28.5,4 - 29.5,6 | -109.4 | 36.24 | 97 | -3.02 | 0.076 |
| 28.5,4 - 30.5,6 | -110.0 | 36.24 | 97 | -3.04 | 0.072 |
| 29.5,4 - 30.5,4 | -8.3 | 36.24 | 97 | -0.23 | 1.000 |
| 29.5,4 - 28.5,6 | 50.1 | 36.24 | 97 | 1.38 | 0.902 |
| 29.5,4 - 29.5,6 | -119.6 | 36.24 | 97 | -3.30 | 0.035 |
| 29.5,4 - 30.5,6 | -120.2 | 36.24 | 97 | -3.32 | 0.033 |
| 30.5,4 - 28.5,6 | 58.4 | 36.24 | 97 | 1.61 | 0.796 |
| 30.5,4 - 29.5,6 | -111.3 | 36.24 | 97 | -3.07 | 0.066 |
| 30.5,4 - 30.5,6 | -111.9 | 36.24 | 97 | -3.09 | 0.063 |
| 28.5,6 - 29.5,6 | -169.7 | 36.24 | 97 | -4.68 | <0.001 |
| 28.5,6 - 30.5,6 | -170.3 | 36.24 | 97 | -4.70 | <0.001 |
| 29.5,6 - 30.5,6 | -0.6 | 36.20 | 97 | -0.02 | 1.000 |

**Table A5.3** Turkey's contrasts for the recovery time of adult Spanish flag snapper *(Lutjanus carponotatus*). Individuals were held at either 28.5°C (28.5), 29.5°C (29.5), or 30.5°C (30.5) and were tested at 2 weeks exposure (2), 4 weeks exposure (4), and 2 weeks post-exposure (6).

| contrast | estimate | SE | df | t ratio | p.value |
|---|---|---|---|---|---|
| 28.5,2 - 29.5,2 | -142.50 | 24.82 | 97 | -5.74 | <0.001 |
| 28.5,2 - 30.5,2 | -172.50 | 24.82 | 97 | -6.95 | <0.001 |
| 28.5,2 - 29.5,4 | -120.83 | 24.82 | 97 | -4.87 | <0.001 |
| 28.5,2 - 30.5,4 | -115.83 | 24.82 | 97 | -4.67 | <0.001 |
| 28.5,2 - 28.5,6 | -4.17 | 24.82 | 97 | -0.17 | 1.000 |
| 28.5,2 - 29.5,6 | 7.50 | 24.82 | 97 | 0.30 | 1.000 |
| 28.5,2 - 30.5,6 | 19.17 | 24.82 | 97 | 0.77 | 0.997 |
| 29.5,2 - 30.5,2 | -30.00 | 24.82 | 97 | -1.21 | 0.953 |
| 29.5,2 - 28.5,4 | 143.33 | 24.82 | 97 | 5.77 | <0.001 |
| 29.5,2 - 29.5,4 | 21.67 | 24.82 | 97 | 0.87 | 0.994 |
| 29.5,2 - 30.5,4 | 26.67 | 24.82 | 97 | 1.07 | 0.977 |
| 29.5,2 - 28.5,6 | 138.33 | 24.82 | 97 | 5.57 | <0.001 |
| 29.5,2 - 29.5,6 | 150.00 | 24.82 | 97 | 6.04 | <0.001 |
| 29.5,2 - 30.5,6 | 161.67 | 24.82 | 97 | 6.51 | <0.001 |
| 30.5,2 - 28.5,4 | 173.33 | 24.82 | 97 | 6.98 | <0.001 |
| 30.5,2 - 29.5,4 | 51.67 | 24.82 | 97 | 2.08 | 0.492 |
| 30.5,2 - 30.5,4 | 56.67 | 24.82 | 97 | 2.28 | 0.363 |
| 30.5,2 - 28.5,6 | 168.33 | 24.82 | 97 | 6.78 | <0.001 |
| 30.5,2 - 29.5,6 | 180.00 | 24.82 | 97 | 7.25 | <0.001 |
| 30.5,2 - 30.5,6 | 191.67 | 24.82 | 97 | 7.72 | <0.001 |
| 28.5,4 - 29.5,4 | -121.67 | 24.82 | 97 | -4.90 | <0.001 |
| 28.5,4 - 30.5,4 | -116.67 | 24.82 | 97 | -4.70 | <0.001 |
| 28.5,4 - 28.5,6 | -5.00 | 24.82 | 97 | -0.20 | 1.000 |
| 28.5,4 - 29.5,6 | 6.67 | 24.82 | 97 | 0.27 | 1.000 |
| 28.5,4 - 30.5,6 | 18.33 | 24.82 | 97 | 0.74 | 0.998 |
| 29.5,4 - 30.5,4 | 5.00 | 24.82 | 97 | 0.20 | 1.000 |
| 29.5,4 - 28.5,6 | 116.67 | 24.82 | 97 | 4.70 | <0.001 |
| 29.5,4 - 29.5,6 | 128.33 | 24.82 | 97 | 5.17 | <0.001 |
| 29.5,4 - 30.5,6 | 140.00 | 24.82 | 97 | 5.64 | <0.001 |
| 30.5,4 - 28.5,6 | 111.67 | 24.82 | 97 | 4.50 | <0.001 |
| 30.5,4 - 29.5,6 | 123.33 | 24.82 | 97 | 4.97 | <0.001 |
| 30.5,4 - 30.5,6 | 135.00 | 24.82 | 97 | 5.44 | <0.001 |
| 28.5,6 - 29.5,6 | 11.67 | 24.82 | 97 | 0.47 | 1.000 |
| 28.5,6 - 30.5,6 | 23.33 | 24.82 | 97 | 0.94 | 0.990 |
| 29.5,6 - 30.5,6 | 11.67 | 24.82 | 97 | 0.47 | 1.000 |

**Table A5.3** Turkey's contrasts for the haematocrit levels of adult Spanish flag snapper (*Lutjanus carponotatus*). Individuals were held at either 28.5°C (28.5), 29.5°C (29.5), or 30.5°C (30.5).

| Contrast | Estimate | SE | t ratio | T ratio | p value |
|---|---|---|---|---|---|
| 28.5-29.5 | -4.67 | 0.697 | 97 | -6.693 | <0.001 |
| 28.5-30.5 | -8.19 | 0.697 | 97 | -11.752 | <0.001 |
| 29.5-30.5 | -3.53 | 0.697 | 97 | -5.059 | <0.001 |

**Table A5.3** Turkey's contrasts for the Haemoglobin levels of adult Spanish flag snapper (*Lutjanus carponotatus*). Individuals were held at either 28.5°C (28.5), 29.5°C (29.5), or 30.5°C (30.5).

| Contrast | Estimate | SE | t ratio | T ratio | p value |
|---|---|---|---|---|---|
| 28.5-29.5 | -12.27 | 2.31 | 97 | -5.319 | <0.001 |
| 28.5-30.5 | -21.41 | 2.31 | 97 | -9.279 | <0.001 |
| 29.5-30.5 | -9.14 | 2.31 | 97 | 93.961 | <0.001 |

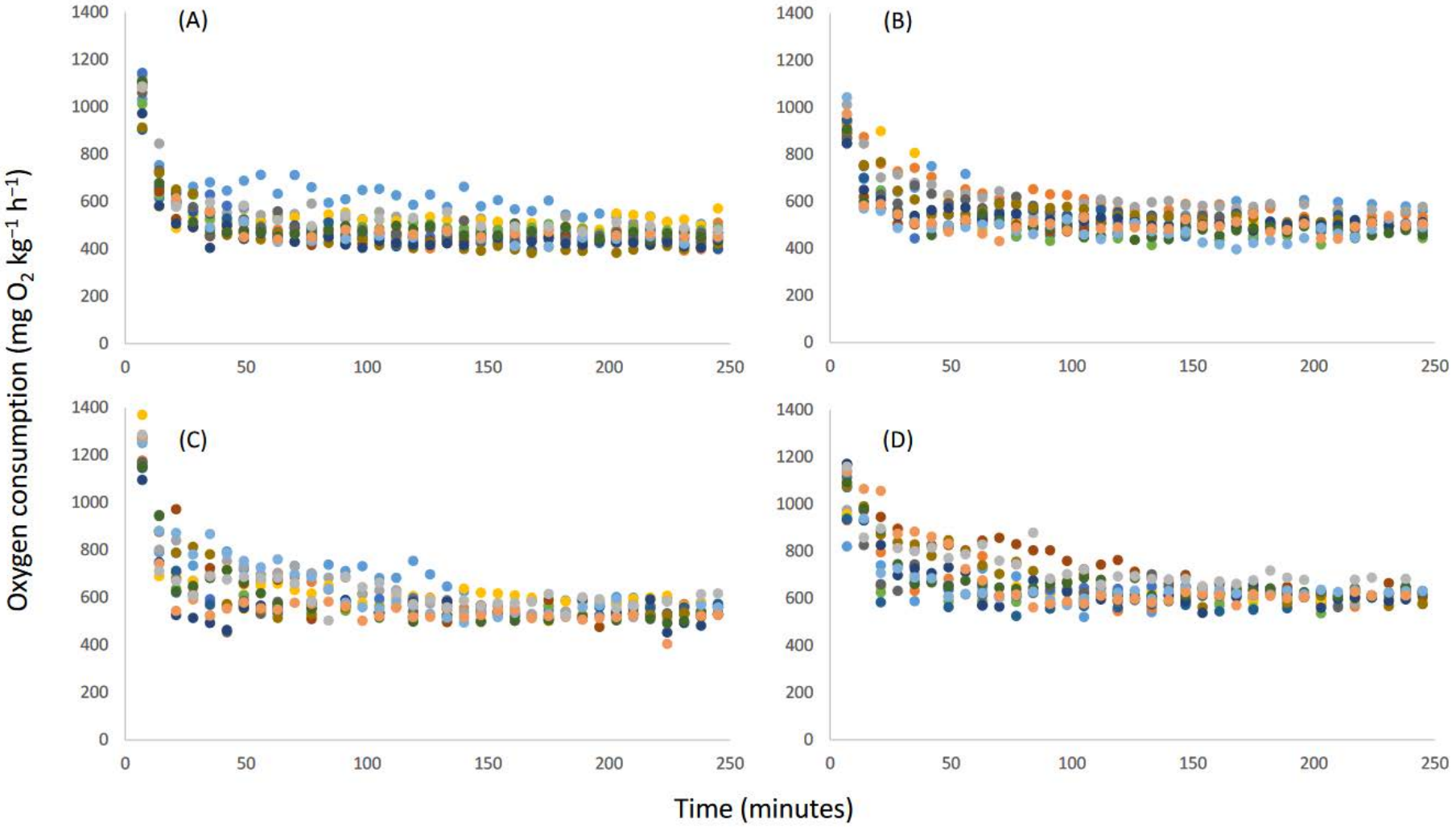


Figure A1.1 MO2 measurements over time for juvenile *Chrysophrys auratus* held for 21 days in treatments of 18°C & 400 $pCO_2$ (a), 18°C & 1000 $pCO_2$ (b), 22°C & 400 $pCO_2$ (c), and 22°C & 1000 $pCO_2$ (d). Colours in each plot represent an individual fish.