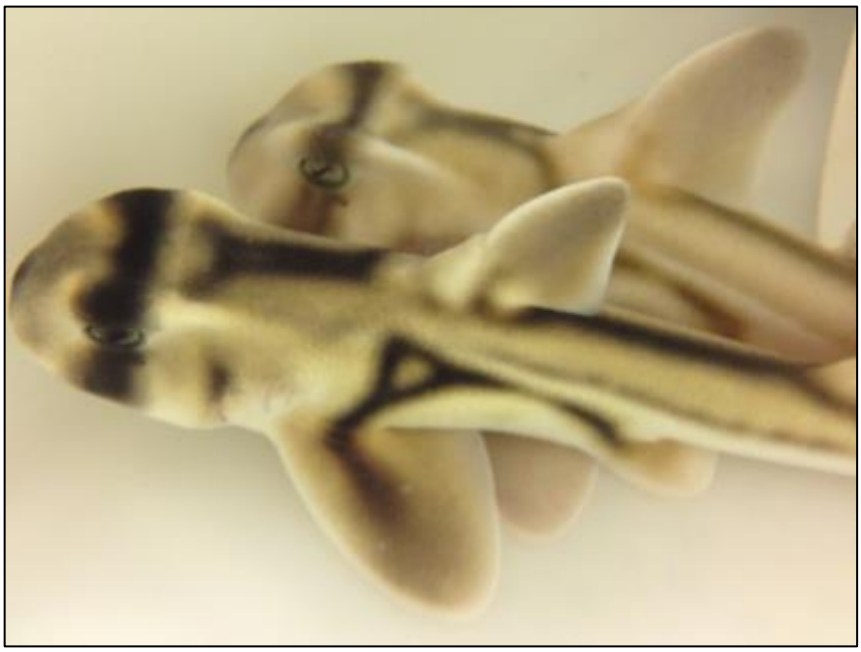
EARLY LIFE BEHAVIOUR & SENSORY ECOLOGY OF PREDATORY FISH UNDER CLIMATE CHANGE AND OCEAN ACIDIFICATION



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ABSTRACT

The early life cycle of a fish species is presumed to be the most vulnerable to abiotic change. Their successful development and growth is key to sustaining and connecting existing populations and dispersal to new habitats. Larvae and juvenile fish have to progressively develop and fine tune their behavioural and sensory capabilities in order to successfully hunt and or forage for prey, avoid larger predators and find suitable habitat to reach maturity and reproduce. Their sensory capabilities typically involve multiple senses including, vision, olfaction and audition. Ocean warming and acidification alter the physiological performance and behaviour of many small bodied fish, however, the potential interactive effects of these stressors on large predatory fish has not been explored fully and may act synergistically or antagonistically. Predatory fish can have large effects on trophically-structured systems. The potential for altered predatory function through alterations in their metabolism as a result of temperature and behaviour from ocean acidification may not only affect their hunting ability but also the communities in which their prey live. In this thesis, I show that the combination of ocean warming with acidification can alter the metabolic function and hunting behaviour of a predatory shark leading to considerable reductions in growth rates. Laboratory experiments revealed faster embryonic development under elevated temperature, however elevated temperature and CO_2 had detrimental impacts on sharks by increasing energetic demands. Subsequent mesocosm experiments showed reductions in growth rates under elevated CO_2 either alone or in combination with elevated temperatures, where their metabolic efficiency was decreased and their ability to locate food through olfaction was reduced. Additionally, while elevated temperature increased the motivational drive to locate prey, elevated CO_2 negated chemical and visual behavioural responses that enable effective hunting. I also found that ocean acidification alone altered the physicochemical sensing in a

predatory teleost fish (Barramundi) such that cues for temperature and salinity were inhibited by reduced pH. This thesis reveals a more complex reality for predators where the combination of elevated temperature and CO_2 reduces their ability to hunt effectively leading to smaller sharks, ultimately reduces their ability to exert strong top-down control over food webs. Furthermore, alterations to their perception and evaluation of environmental cues during the critical phase of dispersal have implications for ensuing recruitment and population replenishment. Alterations such as the ones brought about by ocean acidification and increased temperature far reaching consequences, not just for the individual predator population's sustainability, but also the ecosystem food webs which they inhabit.

CHAPTER 1

CHAPTER 1

GENERAL INTRODUCTION

Human activities continue to intensify and have an increasing potential to modify ecosystems and the organisms that live within them. Ocean warming and acidification, as a result of anthropogenic activities, may profoundly affect marine life by the end of the century, however, there are many gaps in understanding how their effect on marine life will manifest. Developing an understanding on how these stressors can influence marine life through alterations in their behaviour and/or physiology will allow us to better appreciate changes in the ecosystems through trophic interactions that can ultimately affect human populations, potentially leading to better management practices to mitigate the impact of climate change.

Throughout this Introduction, I discuss the impact of ocean warming and acidification as a result of human activities on marine early fish life behaviour, sensory ecology and physiology as well as the potential impact on ecosystem structure. I examine how fish exposed to higher temperatures and elevated CO_2, predicted for the end of the century, will respond through the observation and assessment of certain key behaviour processes (such as olfaction, feeding and foraging) and physiological responses (growth). I then examine how alterations to these processes affect the species studied as well as the potential implications to their ecosystems through their trophic interactions.

1.1 HUMAN INDUCED CLIMATE CHANGE AND THE SEA

For nearly 800,000 years prior to the Industrial Revolution, atmospheric carbon dioxide (CO_2) levels were relatively constant with values ranging between 172 and 300 parts per million (ppm) (Feely et al. 2004, Lüthi et al. 2008). Since the start of the Industrial Revolution (circa 1750), atmospheric CO_2 has been increasing at an unprecedented rate, driven primarily by fossil fuel combustion and deforestation (Doney et al. 2009). Within the last two centuries atmospheric CO_2 levels have risen by approximately 40%, reaching 400ppm in 2014 (NOAA, 2014). The rate of increase is predicted to rise more rapidly than previously thought and reach the range of 851 – 1370 ppm by the year 2100 (RCP8.5 high emission scenario, IPCC 2014), this rate of chance is of major concern for marine ecosystems.

Approximately one fourth of anthropogenic CO_2 emissions has already been absorbed by the oceans mitigating to some extent their effects in the atmosphere (Le Quéré et al. 2009), however, this continual uptake has led to a marked increase in CO_2 concentrations in seawater. Increased seawater pCO_2 leads to changes in the oceanic carbonate system leading to a reduction in oceanic pH, this process is termed 'ocean acidification' (Caldera & Wickett 2003). Ocean acidification has already reduced average ocean pH by 0.1 units since the industrial revolution (Meehl et al. 2007) and is one of the increasing aspects of global climate change that pose a threat to marine ecosystems (Royal Society 2005). Additionally, climate change through elevated CO_2 will also lead to an increase in global temperatures both in the atmosphere and oceanic systems (IPCC 2007). These accelerated global changes will inevitably result in biological impacts which will deleteriously affect marine organisms and ultimately the ecosystems they reside in.

1.1.1 OCEAN WARMING

As a greenhouse gas, CO_2 in its increasing concentration in the atmosphere, changes the radiative forcing by trapping solar radiation (Thomson 1997; Tuckett, 2009) increasing the earth's temperature. Any alteration in temperature impacts the whole earth's systems from the atmosphere to the sea. In the last 40 years 84% of the total heating of the earth has gone into the world's oceans (Barnett et al. 2005). Global mean sea surface temperatures have increased by 0.76°C since the industrial revolution (IPCC 2007). With the predicted rise in anthropogenic CO_2 emissions, global air temperatures could increase by up to 4.5°C by 2100 (RCP 8.5, IPCC 2007) with the mean sea surface temperatures in 2090 predicted to be 2.7°C warmer (RCP8.5, Pörtner et al. 2014).

Most fish are ectotherms whose body temperature is equal to the environment and as a result have low energetic expenditures, however, this comes at the expense of a temperature-determined metabolism and physiological performance (Kaslbeek et al. 2012). Thus, temperature has the greatest influence on fish metabolic processes such as digestion and physical performance. Increasing temperature generally increases metabolic rate (Ede & Krogh 1914; Pörtner et al. 2006).Where most biological processes are temperature sensitive they can only optimally operate within a narrow thermal window outside of which performance declines significantly (Angilletta 2009, Kearney & Porter, 2009). This could potentially lead to reduction in somatic growth and reproduction as the temperature alteration would force an organism to reallocate resources in order to maximise fitness (Pörtner et al. 2001). Temperature is, therefore, a key environmental variable in habitats as it influences over all physiological and biochemical process in marine species (Pörtner et al.

2006). As a result of climate change, temperature will substantially influence their biology and distributions depending on how close an organism is to its thermal optimum (Bosonovic et al. 2011, Pörtner et al. 2006).

1.1.2 OCEAN ACIDIFICATION

Ocean acidification results from the dissolution of carbon dioxide in water (equation 1) where it forms a weak acid that is very unstable in seawater - carbonic acid (H_2CO_3) (equation 2). This acid readily dissociates to form bicarbonate ions (HCO_3^-) and hydrogen ions [H^+] (equation 3). Subsequently bicarbonate ions dissociate further to produce carbonate ions (CO_3^{2-}) and more hydrogen ions [H^+] (Skirrow & Whitfield 1975). The increased hydrogen ions causes reaction (4) to reverse and lead to bicarbonate ions to become more stable in seawater.

$$CO_{2(gas)} \leftrightarrow CO_{2(aq)} \quad (1)$$

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad (4)$$

Ultimately this leads to increasing amounts of H_2CO_3, HCO_3^- and [H^+] ions in seawater together they form the pool of dissolved inorganic carbon (DIC) and the relative proportion of these three forms of DIC controls seawater pH which in this case, results in reduced pH (Raven et al. 2005). Ocean acidification scenarios predict a reduction in ocean pH by 0.3 to 0.5 units by the end of the century (RCP8.5 - IPCC 2007, Caldera & Wickett 2003).

Increased ocean acidification pose threats to marine habitats such as temperate reefs, as well as the organisms which live in it (Connell et al. 2013). Most research has been focused on the impact of ocean acidification on calcifying marine organisms such as corals and other invertebrates that precipitate aragonite skeletons. Due to the impact of reduced carbonate-ion saturation this has direct implications on their calcification processes affecting their growth and survival (Orr 2005, Hoegh-Guiberg 2007, Kleypass 2006). However, an increasing body of work is showing that other marine organisms such as fish will also be significantly impacted due to ocean acidification. Research on larval fish and ocean acidification has shown detrimental effects on larval survival such as growth (Baumann et al. 2011), metabolism (Franke & Clemmesen 2011, Miller et al. 2012), condition (Franke & Clemmesen 2011) and behaviour (Munday et al. 2009, Dixson et al. 2010, Devine et al. 2011, Simpson et al. 2011 Ferrari et al. 2012a 2012b, Domenici et al. 2012). Elevated CO_2 concentrations in oceans can result in hypercapnia in marine organisms due accelerated acidosis in their tissues (Guinotte & Fabry 2008). Hypercapnia occurs when increased levels of CO_2 in water enter the organism by diffusion, equilibrates between all body segments and acts predominately through its acidifying effect on the acid-base balance in body fluids. The disturbance in the acid-base balance leads to increase synthesis and activity of ATP-consuming transporters and this can have a potential metabolic cost (Pörtner et al. 2004, Deigweiher et al. 2008).

Short term impacts of increased CO_2 include alterations of the acid-base status, blood circulation, respiration and nervous system functions (Guinotte & Fabry 2008). A study by Nilsson et al. (2012) indicated that high levels of CO_2 interfere with the main neurotransmitter receptor, the GABA-A receptor. Regulatory changes in levels of HCO_3^- and Cl^- during high levels of CO_2 exposure lead to excitation of the GABA-A receptors

ultimately affecting behaviour and shifts in sensory preferences (Nilsson et al. 2012). Many of the alterations affecting fish are behavioural, where fish fail to respond appropriately to homing cues (Munday et al. 2009), predatory and alarm cues (Dixson et al. 2010, Ferrarri et al. 2011a). Additionally, fish have shown impaired response to the sight of potential predators (Ferrari et al. 2012b), reduced hearing ability (Simpson et al. 2011) and fail to respond to novel predators (Domenici et al. 2012). These alterations are particularly dangerous in the early life stages of marine fish such as the larval and juvenile phases as these are the most sensitive to environmental change and survival from predation and subsequently could lead to population declines due to increased mortality rates young.

1.2 INFLUENCE OF CLIMATE CHANGE ON FISH

Many studies assessing climate change, either temperature or ocean acidification, assess these stressors in isolation even though these two stressors will occur simultaneously. While these provide essential insights into elucidating stressor impacts they can lead to misleading conclusions about future responses as they do not account for stressor interactions. Several studies (Kroeker et al. 2013, Ferrari at al. 2015, Nowicki et al. 2012) indicate when multiple stressors are tested, the impact varies significantly depending on whether stressors were tested in isolation or in combination. Currently and in the future, increasing temperature and ocean acidification occur naturally in combination, therefore investigating them in combination is crucial to further our understanding of the impact of global stressors on marine organisms and subsequent ecosystem function and biodiversity.

The effect of the increasing partial pressure of CO_2 in surface oceans is predicted to alter marine biota as most organisms function within a narrow pH range which can interfere with their energetic and metabolic functions (Feely et al. 2004). Galloway et al. (2004) proposed that behavioural changes were a key biomarker for the presence of significant biological effects of pollution in marine environments a likely alteration on the behaviour of larval fish could therefore, dramatically decrease their survival during recruitment to adult populations. So far three main routes to altered behaviour in fish have been identified (Briffa et al. 2012) as a result of elevated CO_2 exposure:

(i) Elevated metabolic load - changes in underlying physiological conditions constraining the ability to perform key behaviours such as swimming (Dissanayake et al. 2010).

(ii) Info-disruption - the ability to gather and assess information and make decisions is impaired affecting a wide range of sensory functions and behaviours (Briffa et al. 2012).

(iii) Avoidance behaviour - altering the normal movement patterns and distribution of marine animals (Pörtner & Peck 2010).

Even though the changes in sea-water chemistry resulting from the oceanic uptake of anthropogenic CO_2, are well characterised and documented over the ocean, the understanding of biological influences from ocean acidification is still in early development (Fabry et al. 2008). A growing body of research suggests that OA with its associated drop in pH and additional rise in SST will affect the physiologies of marine organisms (Pörtner 2008) and subsequently their ecological functions and interactions with other organisms (Widdicombe & Spicer 2008).

Additionally, only a limited number of studies have investigated the synergistic effects of rising sea surface temperature and acidification on biological communities and only a few on larval fish or juvenile fish. A recent review by Wernberg et al. (2012) presents information from 110 marine climate change experiments, published between 2000 and 2009, and suggests that 65% of papers focused on a single climate change factor while the majority (73%) were using benthic invertebrates. As climate change is causing a rise in both SST and acidification, it is important to study the possible synergistic effects that may give rise to even more complex problems than one factor alone. It is important to assess the range of the likely behavioural consequences in diverse marine taxa due to stressors such as elevated CO_2 and SST rise as well as to assess the potential influences on pathways such as info disruption due to the combined effect of reduced pH and increased temperature synergies between reduced pH and elevated (Briffa et al. 2012). Finally, in order to further predict population and ecosystem level effects (Briffa et al. 2012; Simpson et al. 2011; Nagelkerken & Munday 2016), the consequences of altered behaviours and their underlying causes as a result of climate change must be understood first.

1.2.1 IMPORTANCE OF LARVAL AND JUVENILE PHASE AND BEHAVIOUR

In a fish species' life, the early life cycle is presumed to be its most vulnerable, especially when considering environmental changes (Spicer & Gaston 1999, Thorson 1950). Additionally, planktonic larvae play a crucial role in the life history of marine organisms by sustaining and connecting existing populations and connecting new habitats (Chan et al. 2011). When predicting biological and community responses to climate change therefore, it is important to consider the larval stages of certain marine fish species (Arnberg et al. 2012).

Most species of benthic marine fish have an initial planktonic larval phase and which, depending on species can last a few weeks or even a few months (Caley et al. 1996). In order to join the adult population they must transition to a benthic existence (Munday et al. 2010); first this transition period is usually associated with high mortality rates and can be a stage of strong selection (Hamilton et al. 2008). A larval fish in order to locate a suitable adult habitat and to avoid predators must use a multitude of sensory capabilities so as to detect, orient and distinguish between various settlement sites (Gerlach et al. 2007, Kingsford et al. 2002, Simpson et al. 2011).

Certain species of larval fish (clownfish, cardinalfish, damselfish among a few), however, have been observed to lose their ability to distinguish various chemical cues from their preferred settlement habitats under high CO_2 (Devine et al. 2012, Munday et al. 2009). Not only is their homing ability affected but also their ability to distinguish predators (Dixson et al. 2010) and for some predator species, their prey (Cripps et al. 2011). By conducting a variety of behavioural tests on fish larvae that have been exposed to various environmental treatments and with a combination of factors, we will be able to better assess and predict potential effects on fish species at a variety of levels of biological organisation.

Sharks lack a larval phase, having internal fertilization to produce fertile eggs or embryos. The retention time of the fertilised embryos classifies the sharks into either viviparous or oviparous forms (Carrier et al. 2004). The young of both are either born or hatched fully developed smaller versions of adults. This form of reproduction allows the shark species to reduce losses to predation and as larger fish they have a greater number of potential prey (Carrier et al. 2004). Once a shark emerges (whether born or hatched) it will rely on it senses for feeding and predator avoidance.

Climate change and ocean acidification can ultimately lead to altered habitats in various ways such as altering the performance of each life stage by either decreasing or increasing it (Podolski & Moran 2006, Kroeker et al. 2013, Rossoll et al. 2012), alteration in competition through changing organism influence within the food web (Kroeker et al. 2013), alterations in food availability (Rossoll et al. 2012).

1.2.2 ECOLOGICAL IMPLICATION OF PREDATORY FISH

Predatory fish play a role in structuring communities in marine environments that are sensitive to trophic control. They help structure prey populations and diversity (Paine 1966, Hixon & Menge 1991, Franke et al. 2005) within communities through predation, as well as indirectly by their sheer presence leading to an alteration in prey behaviour (Schmitz et al. 2004). With this influence, they can have cascading effects on ecosystems and the predator body size, metabolic function and mobility can determine the strength of such cascades (Borer et al. 2005). Thus, any changes in their size, metabolic demands, hunting strategies, density and distribution can lead to changes that affect the entire ecosystem (Estes et al. 2011, Ripple et al. 2001, 2014).

To feed effectively, predators rely on a variety of sensory adaptations to aid orientation, prey detection and location and ultimately capture of prey (Cripps et al. 2011, Guttridge et al. 2009, Gardiner et al. 2012, 2014). Predators are especially reliant on chemical and visual cues to detect prey (Cripps et al. 2011). As with many prey species, olfaction plays an important role for predators in effective prey location as well as avoiding larger predators in marine environments (Hay 2009, Yopak et al. 2014). Several studies have indicated that

teleost fish (Munday et al. 2009, Cripps et al. 2011) as well as sharks (Dixson et al. 2015) when exposed to elevated CO_2 exhibit negative alterations in olfactory ability.

As predatory fish are so influential in the structuring of marine ecosystems it is vital to understand how they will respond to future changes of global warming and ocean acidification. With models suggesting a reduction in body size and a collapse in populations (Sheridan & Bickford 2011) there is little evidence to show the underlying mechanisms of such a response as well as how predators are likely to respond to future global stressors. Additionally, predators (such as sharks) tend to be slower growing with late sexual maturity, low fecundity with longer gestation periods (Dulvy et al. 2010). Thus, it is important to identify any potential alterations due to global warming and ocean acidification on the functioning of the predatory fish within ecosystems to enable us to predict any potential changes to the food web and marine community functioning (Estes et al. 2011, Ripple et al. 2014).

1.3 THESIS SCOPE AND OUTLINE

Due to anthropogenic climate change and ocean acidification, coastal systems are undergoing rapid changes with potentially severe impacts for marine ecosystems. Predatory fish are a key component to any marine ecosystem through the control they impose in their respective trophic systems (Ripple et al. 2014; Heithaus et al. 2008). Assessing their resulting response to future stressors would further our understanding on their respective populations as well as their impacts on the trophic systems they inhabit. Few studies have assessed the impact of climate change on predatory fish species and even fewer have looked

at their early life behaviour and even fewer still have looked at the combined effect of elevated temperature and ocean acidification on predatory fish. Using fish from different latitudes, permits an understanding of a more holistic picture on the effects of climate change that is not limited by location or species (Weinberg et al. 2012). This approach will demonstrate the potential far-reaching implications of elevated pCO_2 and rising temperature on marine diversity and more specifically on predatory fish species. For this purpose, I used two predatory fish, *Heterodontus portusjacksoni* (Port Jackson shark) found in temperate marine ecosystems and *Lates calcarifer* (Barramundi) found in tropical marine and freshwater ecosystems.

By combining long-term laboratory and habitat scale mesocosm experiment for a juvenile temperate predator, as well as laboratory experiments covering the most sensitive larval phase of a tropical predator fish species, this study aims to provide a comprehensive overview on the sensitivity of two important predator species covering two different ecosystems to the combined effects of climate change and ocean acidification.

Manipulative and observational experiments assessed the specific aims:

1 to investigate how ocean warming and acidification affected a predatory shark species' behaviour and growth and how this could might alter their population sustainability (chapter 2)

2 to examine whether ocean warming and acidification create synergisms or antagonisms between physiological and behavioural processes in a shark species (chapter 3)

3 to examine how ocean acidification can alter physicochemical sensing used for dispersal between ocean and estuarine systems by a larvae of a tropical predator species (chapter 4)

By furthering our understanding in the fields of fish behaviour and physiology and how this will impact the fish's condition and potential trophic interactions, this thesis provides insights to inform policy makers and environmental managers about the potential impact of anthropogenic stressors on the marine environment and the need to mitigate increasing levels of atmospheric carbon dioxide.

1.3.1 THESIS SUMMARY

Each thesis chapter is outlined below.

Chapter 2

Alterations in predation pressure can vastly influence trophically-structured ecosystems, however, the extent of which ocean acidification with ocean warming will influence these alterations remains unexplored. In chapter 2, I investigate how the direct and indirect effects of ocean warming and ocean acidification impacted a predatory shark species behaviour and growth in laboratory and mesocosm experiments.

Chapter 3

Ocean warming and acidification alter the physiological performance and behaviour of many small bodied fish, however, the potential interactive effects of these stressors on larger predatory species remains poorly understood. In chapter 3, I examine whether ocean warming and ocean acidification create synergisms or antagonisms between physiological and behavioural processes.

Chapter 4

Ocean acidification alters the way animals perceive and respond to their word by altering a variety of senses such as audition, olfaction, pH sensing and vision. Marine organisms rely on additional senses but little is known how these will be impacted by ocean acidification. In chapter 4, I examine how ocean acidification can alter physicochemical sensing by a larval fish used for dispersal between ocean and estuarine systems.

Chapter 5

Chapter 5 provides a general discussion on the key findings of the previous chapters and outlines possible directions for future research.

Thesis

Each data chapter (2 - 4) has been written in the form of an individual scientific paper and therefore uses the journal formatting. A list of co-authors and their contributions to the paper

has been highlighted in the statement of authorship for each data chapter. A comprehensive reference list is included at the end of each chapter. Chapters 2 and 3 are published journal articles; the remaining chapter is in review with a journal.

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CHAPTER 2

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CHAPTER 2

OCEAN ACIDIFICATION AND GLOBAL WARMING IMPAIR SHARK HUNTING BEHAVIOUR AND GROWTH

Short title: Climate stressors impair hunting and growth in a large predator

2.1 ABSTRACT

Alterations in predation pressure can have large effects on trophically-structured systems. Modification of predator behaviour via ocean warming has been assessed by laboratory experimentation and metabolic theory. However, the influence of ocean acidification with ocean warming remains largely unexplored for mesopredators, including experimental assessments that incorporate key components of the assemblages in which animals naturally live. We employ a combination of long-term laboratory and mesocosm experiments containing natural prey and habitat to assess how warming and acidification affect the development, growth, and hunting behaviour in sharks. Although embryonic development was faster due to temperature, elevated temperature and CO_2 had detrimental effects on sharks by not only increasing energetic demands, but also by decreasing metabolic efficiency and reducing their ability to locate food through olfaction. The combination of these effects led to considerable reductions in growth rates of sharks held in natural mesocosms with elevated CO_2, either alone or in combination with higher temperature. Our results suggest a more complex reality for predators, where ocean acidification reduces their ability to effectively hunt and exert strong top-down control over food webs.

Key words: animal behaviour, foraging, trophic cascades, sharks, olfaction, growth

2.2 INTRODUCTION

Apex and mesopredators shape ecosystem structure and function through their control of prey populations[1–3]. Their influence on ecological communities is driven by consumptive effects (i.e. by predation) as well as non-consumptive effects (e.g. the presence of a predator that leads to an alteration in prey behaviour interactions[4]). Predators often have cascading effects on ecosystems. A well-documented example is that of killer whale predation on sea otters and the consequences for kelp forests[5], where killer whales mediate otter numbers whose predation on herbivorous sea urchins mediates the presence of kelp forests. The body size, metabolism and mobility of predators are strong determinants of the strength of such trophic cascades[6]. Alterations in the body size, metabolic demands, hunting tactics, density, and distribution of predators can therefore lead to changes that cascade through entire ecosystems[2,3,7]. Because of this important function, there has been a long-standing interest in understanding the impact of predators in both terrestrial and marine ecosystems[8–10]. However, we have entered an era where rapid environmental changes are affecting the functioning and persistence of many species. Changing climatic conditions are likely to lead to altered community compositions, population dynamics and ecosystem functioning[11]. The mechanisms by which apex and mesopredators are vulnerable to global change and the consequences for the ecosystems in which they live is a relatively new area of enquiry. While models have suggested decreases in body size and collapse of their populations[12], there is a tremendous gap in empirical studies that have studied the underlying mechanisms and have tested how such predators may respond to multiple global stressors (but see[13–15]).

Global average sea surface temperatures are predicted to rapidly rise due to the greenhouse effect by 1°- 3°C in 2100 and this is in addition to an increase of ~0.76°C in the last 150 years[16]. Increased temperature can have both negative and positive effects on a multitude of

biological responses, including vertical and latitudinal range shifts, species interactions, and feeding, growth, survival and development rates[17–19] . However, warming will not occur in isolation, but in combination with ocean acidification which is predicted to decrease ocean pH by 0.3-04 units by the end of the century[16,20]. Most studies have focused on the effects of ocean acidification and climate change on marine invertebrates, with the few studies on fish largely restricted to small-bodied species[21–23]. Furthermore, many studies evaluate the effects of increased CO_2 and temperature in isolation rather than in combination with factors that have a strong probability of altering the outcome of single factor effects. Indeed, studies on the interactive effects of warming and ocean acidification on the performance of larger predators such as sharks are very limited[14], preventing us from better understanding their fate due to future change and how this might affect a change in the intensity of predation. Another concern is that studies regularly use unrealistic elevations of temperature or CO_2 and that most studies are done over short time periods and under simple laboratory conditions requiring cautious interpretation when applied to natural conditions.

Several studies have shown effects of elevated temperature on fish metabolism and growth, with tropical species suggested to be more sensitive than temperate species (as tropical species have evolved in a more stable environment) due their narrower thermal reaction norm and as such reducing their ability to cope with temperatures above their thermal optimum[24] .While elevated temperatures enhance basal metabolic rates they can also raise respiratory demand leading to a reduced aerobic scope for activity such as feeding, digestion and predator avoidance and as such reducing available energy for growth and reproduction[25].

Recent short-term studies (up to 2 months) on elasmobranch species have discovered a range of changes to shark physiology and behaviour as a function of elevated CO_2. The epaulette

shark (*Hemiscyllium ocellatum*), a species that exhibits exceptionally high tolerance to severe hypoxia, showed no effects of elevated CO_2 on their metabolic performance [13], although metabolic rates of embryonic bamboo shark (*Chiloscyllium punctatum*) were negatively affected[14]. A study on small-spotted cat sharks (*Scyliorhinus canicula*) indicated no changes in growth; however, alterations in blood chemistry and a shift in swimming patterns and increased lateralization were detected, suggesting some effects on elasmobranch physiology leading to altered behaviour[13]. Elevated CO_2 also reduced survival in early juvenile bamboo sharks[14], and reduced odour tracking behaviour in smooth dogfish (*Mustelus canis*) by avoiding food odour cues as well as displaying reduced attack behaviour[26]. However, long-term studies that provide an understanding of the interactive effects of elevated CO_2 and temperature on the behaviour and physiology of large, long-lived predators such as shark are clearly lacking.

Many predators rely on a variety of cues such as odour to locate their prey as part of their hunting and foraging strategy. This is especially important in nocturnal feeders that almost solely rely on this function[26–28]. Olfaction plays an important role in many predators' ability to locate prey at a distance as odour cues disperse further than most other cues and it is often the first cue of many encountered[26]. Olfaction is also important for avoiding predators and chemosensory communication with conspecifics[29]. Recent studies have shown negative impacts of CO_2 on olfaction in several fish species[21,30–32]. Since olfaction is an essential mechanism of the foraging strategy of many species, any disruption to this mechanism due to increased CO_2 could leave animals vulnerable to malnutrition and predation and ultimately reduced growth and survival.

Here we test the potential effects of near-future ocean warming and acidification on a temperate shark species, the Port Jackson shark (*Heterodontus portusjacksoni*). This study aims to determine: (i) the extent to which temperature and/or ocean acidification modify somatic growth through altered foraging rates, when food supply is unlimited, (ii) the effects of CO_2 on hunting behaviour through olfaction, and (iii) the interactive effects of elevated temperature and CO_2 on shark growth in mesocosms containing natural habitats and prey, where sharks need to hunt for their food. The direct effects of ocean warming on physiological performance was assessed in the laboratory, while in large mesocosms we studied the longer-term effects on shark performance by integrating metabolic effects and potential CO_2 effects on hunting behaviour under more natural conditions.

2.3 RESULTS

Elevated temperature increased the rate of embryonic development of sharks (Fig. 1, ANOVA; temperature, $F_{1,12} = 49.565$; $P = 0.0001$) but CO_2 had neither an independent nor an interactive effect on hatch rates (Table S1) and 100% of the eggs hatched successfully with no mortality across any treatments. The forecasted end-of-century increase in temperature reduced the embryonic period by approximately 40 days out of 10–12 months on average. No significant differences in hatching size or weight were detected between treatments ($P > 0.2$).

Newly hatched sharks were held under controlled laboratory conditions and fed *ad libidum* for 33-81days to determine the metabolic effects of temperature and CO_2 on feeding and growth. Sharks tripled their food consumption rates under elevated temperatures compared

to the control treatments, irrespective of normal or elevated CO_2 (Fig. 2a, ANOVA; temperature, $F_{1,12} = 49.566$; $P = 0.0001$). The increased food intake in both temperature treatments resulted in significantly increased growth rates compared to the control (Fig. 2b, ANOVA; temperature, $F_{1,76} = 62.733$; $P = 0.0001$; temperature x CO_2: $F_{1,76} = 4.001$; $P = 0.0460$). Whilst elevated temperature yielded the highest F-value for any term in the analysis accounting for most of the variation in the treatments, elevated CO_2 had an antagonistic effect on growth when combined with the elevated temperature (Table S2). Nevertheless, growth under combined elevations of temperature and CO_2 was still significantly higher than under control conditions (Fig. 2b).

To understand how hunting behaviour may be affected by the treatments, experiments were repeated in mesocosms mimicking a natural mini-ecosystem in which sharks had to locate familiar, but hidden prey. We observed that sharks reared under elevated CO_2 (66-68 days in mesocosms) took almost 4 times longer than those in controls to locate their prey (Fig. 2c). However, in combination with an elevated temperature the time it took to locate prey was reduced by a third (although with a larger variance), which was still significantly higher than that for the control and elevated temperature only treatment groups (Table S3). All sharks in the control mesocosms approached the sand trays with hidden prey as soon as they were placed into the mesocosms and started shifting through the sand to find the food. However, under elevated CO_2 and its combination with temperature not all sharks responded immediately, with 2 out of 9 sharks in the high CO_2 treatment not responding to the introduction of prey at all. Additionally, for the sharks that responded there was a significantly higher failure rate (chi square test; $\chi^2 = 27.88_{219.9}$) in terms of number of sharks that successfully located their prey in the elevated CO_2 treatment and the elevated CO_2 and

temperature treatments (50% failure across sharks from both elevated CO_2 treatments vs. 27% failure across both non-elevated CO_2 treatments).

The reduced effectiveness of sharks to locate their prey through olfaction due to increased CO_2 was reflected in their growth. Sharks reared for over 2 months in mesocosms with either elevated CO_2 or elevated temperature and CO_2 showed significantly lower growth rates (Fig. 2d, Table S3, ANOVA; CO_2: $F_{1,29} = 25.33$; $P = 0.0002$) compared to the other treatments at ambient CO_2 levels, where their growth was reduced by 70% in the elevated CO_2 treatment and by 75% in the combined elevated temperature and CO_2 treatment (Fig. 2d).

2.4 DISCUSSION

Our results show that ocean acidification and ocean warming can strongly govern embryonic duration, hunting behaviour, food consumption rates, and growth of a mesopredator. Impairment of effective foraging and growth may reduce the resilience and sustainability of predator populations. Under temperature forecasted for the end of the century, sharks increased their food consumption when fed *ad libidum*. However, when combined with the concurrent predicted elevation in levels of ocean CO_2 there was a failure to allocate these resources towards maximal somatic growth. This indicates the presence of an antagonistic effect of CO_2 on temperature reflecting a direct metabolic cost of increased CO_2 in conjuncture with higher temperatures. With temperature-driven increases in metabolism, the likelihood of predator starvation increases when it is not matched by elevated ingestion rates; in some cases (such as the juvenile hammerhead) sharks are at provisioning limits and these stresses could push them into starvation[33]. As these temperature treatments were based on

winter values, we would expect that the summer temperatures to be even higher, further exacerbating the stress response we see here, potentially leading to a tipping point for many sharks. A mismatch between food demands and food availability has for example been shown in low-productivity ecosystems[17,18]and low-fertility systems[34]. Possible pathways of negative CO_2 effects on animal physiology are a reduction in protein synthesis, and the costs of acid-base regulation or cardiorespiratory control[35]. Predator-prey relationships across marine ecosystems are strongly dependent on the body mass of the predator and prey and size-based predation is responsible for the transfer of energy across the food chain[36]. With increasing temperature, different sensitivities of species to rising CO_2 might therefore lead to alterations in the body sizes of some predator species, which may have cascading effects on other species through altered predator-prey relationships.

Embryonic development time in Port Jackson sharks was reduced by temperature, but unaffected by elevated CO_2, and with 100% survival in all cases. Faster development would result in reduced exposure times to egg predation which would increase their early life stage survival; like many fishes, sharks optimise energetics to favour early growth to reduce neonate and juvenile vulnerability. Port Jackson sharks usually suffer from very high embryonic mortality (89%) with 98% of the loss due to predation[37]. In contrast, elevated temperature and CO_2 reduced hatching success in a temperate skate species (*Leucoraja erinacea*)[15] and elevated temperature reduced juvenile condition and survival in a tropical shark (*C.punctatum)* as well as reduced embryonic survival (with no effect of pH on embryonic survival)[14].

Organisms typically have some capacity to acclimate to potential stressors either by altering aspects of their physiological, behavioural or morphological characteristics to enable them

to cope with changes[38]. Some are more permanent alterations (developmental acclimation) whereas others are reversible. Many studies use juveniles or adults and expose them to high temperature and or elevated CO_2 for a short period of time and cannot realistically account for within-generation acclimation, including developmental acclimation[38]. We provide the first insight into within-generational acclimation potential by exposing sharks from their embryo stage through to their juvenile stage to two major global stressors both in a laboratory and in a mesocosm setting. Importantly, after seven months of experimental exposure we find no clear signs of acclimation over this critical period of growth and survival. Recent studies have shown only partial acclimation to elevated temperature and CO_2 for metabolic rates and growth in fish when parents experience the same stressors as the offspring[38–40], but this was not the cause for behaviour[41]. It is therefore highly unlikely that our sharks, which are slow growing, long-lived animals, would show any significant acclimation at a later developmental stage. It is important to note that the temperature within our mesocosms was 1°C higher and the pCO_2 was approximately 300 ppm lower than in our laboratory experiment and this is important because negative behavioural effects were still detected at these levels (~700 ppm) which will be reached before the end of the century based on the current CO_2 emission trajectory[16]. Moreover, shallow coastal habitats that naturally experience naturally high CO_2 levels from upwelling and/or eutrophication[42] will reach predicted levels sooner than open oceans[43].

Impacts of global change stressors could alter survival (through altered anti-predator behaviour) as well as foraging success in mesopredators and thus directly affect upper and lower trophic levels[44,45]. Detecting sufficient prey in a large aquatic environment is difficult and sharks and other aquatic predators have evolved a variety of senses to aid prey detection[27], with odour taking a primary role in the sensory hierarchy[26]. This is especially

true for predators that hunt at night to avoid predation pressure. Odour is an important cue in aquatic environments as it can disperse further and be detected sooner than any other cue, especially as a directional cue (vision: <100 m, sound: 25–150 m, odour: up to 10 km[46]). Here we show that sharks exposed to elevated CO_2 levels were slower and less successful in finding prey through olfaction (as prey was dead and thus electroreception can be excluded) and that this resulted in significantly reduced growth in mesocosms that mimicked natural environments. Failure in detection of olfactory cues due to elevated CO_2 has been observed in smooth dogfish (*M. canis)*, but this study did not include tests of the effect of ocean warming[30]. Sensory failure due to ocean acidification could affect predators in several ways. Reduced olfactory capacity would leave some prey items undetected, while predators might spend more time actively searching to compensate for reduced prey capture success. It would also make them vulnerable to higher-order predators, for example towards ambush predators such as the wobbegong, pinnipeds, and even other fish during their juvenile phase[47]. If mesopredators altered their nocturnal hunting to daytime hunting strategies to rely more on visual cues than just olfactory cues they would be more susceptible to predation as well. Elevated CO_2 has been shown to alter the nocturnal swimming pattern of small-spotted cat sharks as well as significantly increase lateralization[13] providing further support to potentially altered hunting strategies. At the same time predators need to cope with the increased energetic demands due to elevated temperature, as well as with the increased metabolic costs of CO_2. Predators may adapt to olfactory disruption by relying more on other senses to detect prey (e.g. vision, electroreception, mechanoreception), but these may be affected by ocean acidification as well[48] and because these typically detect cues at shorter distances, search times may increase and successful prey capture may decrease leading to lower food intake with consequences for their fitness. Reduced predator detection and

recognition by mesopredators likely increases mortality, and any alteration of their anti-predator behaviour comes at a cost of other behaviours such as foraging.

Future ocean warming and acidification will not be uniform across the globe due to the interaction of multiple climatic and non-climatic factors at local spatio-temporal scales[49], and it is at these scales that organisms are most affected. In regions where temperatures rise at relatively higher rates than CO_2, predators such as sharks may grow faster due to higher food intake rates, but the outcome will be highly dependent on food availability. With the predicted reductions in abundances of many species at intermediate and lower trophic levels[50], the energetic demands of large predators may not be met. In areas with relatively more rapid increases of CO_2 rather than temperature, predators might not meet their energetic requirements either, but through alternative mechanisms, i.e. reduced effectiveness in locating prey. Additionally, the potential for sharks to migrate would also influence the type of ecological impacts these stressors impose as sharks are highly mobile species that are able to move vast distances[51]. Sharks may be able to relocate to a more suitable habitat or higher latitudes thus affecting the strength of their interaction within the systems they leave behind and introduce new pressures to the new habitats they occupy[44]. Our parallel laboratory and mesocosm approach it is not able to evaluate such adaptations, but range shifts could mitigate the negative effects on some shark populations.

One third of shark and ray species are threatened worldwide[52]. Their life histories of late sexual maturation and slow reproduction rates followed with long gestation periods result in very low population growth rates making them highly sensitive to elevated fishing mortality[53]. While overfishing remains the greatest direct threat on shark populations, the additive effects of increasing ocean acidification and warming is likely to further exacerbate

their demise[44,52,54]. Considering that both stressors will increase concurrently, the implications for populations of high-order carnivores are likely to be more considerable than estimates derived from single-factor studies on sharks. This has important management implications for their populations. Since it is not possible to reverse the effects of climate change and ocean acidification in the short term, the importance of reducing fishing mortality of large-bodied predators are even greater on the short-term.

With elevated temperatures leading to higher metabolic rates and the need for higher food intake, predators may exert a stronger control on their prey populations due to climate change[10]**.** We challenge this model because CO_2 may negate these temperature effects by reducing the effectiveness of hunters to successfully capture prey and exert such top-down control (Fig. 3). A reduction under future climate conditions in the growth rates of mesopredators, as demonstrated in our mesocosm experiment, could therefore potentially lead to modified predator-prey interactions[55] and have cascading effects on food web structure. Depending on the species and their role in the ecosystem, reduced predator influence could lead to weakened top-down control over prey allowing lower-order consumers to increase in abundances and affecting their prey species. This would be primarily true for predators that rely on olfaction as a sense to find prey, particularly sharks. Rather than an increase in top-down control as is currently predicted, our results suggest a more complex reality for predators, where ocean acidification reduces their ability to effectively hunt and exert strong top-down control over food webs. Nevertheless, it is also important to consider how ocean acidification affects the behaviour of prey species and the predator-prey interactions [63] and how results from our meso-predatory shark scale up to that of apex predator sharks.

2.5 MATERIALS AND METHODS

2.5.1 ETHICS STATEMENT

Research was carried out under approval of the University of Adelaide animal ethics committee (permit: S-2013-095) and according to the University's animal ethics guidelines. Egg collections around the Gulf St. Vincent were carried out with permission of the South Australian Government Department of Primary Industry and Regions SA (permit: 990295).

2.5.2 STUDY SPECIES AND SAMPLE COLLECTION

The study species *Heterodontus portusjacksoni* (Meyer, 1793) is an ideal model species because it is robust to handling stress that could affect their physiology[56] . It is a medium-sized benthic oviparous shark endemic throughout the southern half of Australia[57,58]. It is known to aggregate in groups as juveniles, however, this is also influenced by habitat[59]. It breeds annually, between the months of September and November, laying a pair of eggs every 10-12 days over 2-3 month period[28] and the incubation can last up to a year. *H. portusjacksoni* lays large eggs containing a single embryo with an average weight of 155.5 g[58]. A total of 98 eggs were collected from Gulf St. Vincent, South Australia, over two collection dates (7th and 28th June 2013) via snorkelling.

2.5.3 EGG AND SHARK REARING

The collected eggs were held in a temperature-controlled laboratory until hatching. Egg capsules were observed for changes in developmental stages[58] so that eggs were acquired of similar stage (stage 14 - at least 7.5 months). The eggs were placed in 40 L tanks containing natural filtered seawater which was partially exchanged every 2-3 days. The tanks were

placed in water baths with temperatures maintained using heater chiller units (TR15 Aquarium chillers, TECO refrigeration technologies, Ravenna, Italy), and 300 W glass heaters. Pumps were connected to the chiller units which ensured an even temperature distribution throughout the water baths. The eggs were left to acclimatize over a period of seven days where temperature was steadily increased by 1°C to the elevated temperature treatment. The eggs were kept in either control (~400 µatm) or elevated CO_2 (~1000 µatm) [16,60] crossed with control (~16°C) or elevated temperature (~19°C) (Table S4). Target pH was reached over two days. Eggs were evenly distributed over 4 tanks per treatment with a max density of 9 eggs per tank. Exposure time of the embryos varied from an average of 108 days for the elevated temperature treatment to 143 days for the lower temperature treatments (hatching rate was affected by temperature which affected embryonic exposure time).

Upon hatching the juvenile sharks were relocated to new tanks with the exact same treatment set-up as described above, again with 4 tanks per treatment. The sharks were placed into large tubs of 100 L or 150 L in volume. The number of sharks in each of the tanks ranged from 1-8 for the 150 L tanks and 1-4 for the 100 L tanks (differing numbers due to differences in hatching time and because at some point 33 sharks were removed for the subsequent mesocosm experiment). Sharks were kept in their respective treatments for at least 2 months. Water parameters (Table S4) were measured daily. Tanks received water changes every other day (minimum 40% of total volume). Sharks were fed *ad libidum* with thawed frozen prawns daily.

A thermal mass flow meter/controller (PEGAS 4000 MF Gas Mixer, Columbus Instruments, Columbus, Ohio) was used to achieve different CO_2 concentrations in the seawater by bubbling the CO_2 enriched air directly into the tanks. The gas mixer was connected to a CO_2

tank and an air compressor. Temperature and pH_{NBS} of each tank was measured daily using a pH and temperature meter (Mettler Toledo SevenGo™ SG2) calibrated with fresh buffers each day. Additionally, oxygen and salinity were also measured daily within the tanks. Total alkalinity of seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken weekly from each of the treatment tanks. Alkalinity standards were accurate within 1% of certified reference material from Dr A. Dickson (Scripps Institution of Oceanography; Langdon et al. 2000). Average seawater pCO_2 (Table S4) was calculated using CO_2SYS with the constants of Mehbrach *et al.* [61] refit by Dickson and Milero[62]. The variability in pCO_2 is higher than for pH because it was calculated using weekly measurements of total alkalinity, whereas pH was measured on a daily basis.

2.5.5 HATCHING RATE, FEEDING AND GROWTH MEASUREMENTS IN THE LABORATORY

The tanks holding the eggs were checked daily for new hatchlings. As soon as new hatchlings were observed, their weight and sex was recorded as well as a photo taken of each individual for future identification. The newly hatched shark was then placed into a new tank with the same CO_2 and temperature treatment as it experienced while still in the egg. The sharks were measured each week for changes in weight (± 1 g) and also photographed to aid identification of individuals to track their growth for the duration of the experiment. Sharks were fed *ad libidum* with mussels and prawn meat during the first month after hatching, and afterwards with prawn meat alone. Food consumption was recorded daily by comparing the difference in weight of food offered and food remaining after 30 minutes of feeding. Thirty minutes was selected as the end period because this was well beyond the time it took sharks to feed to satiation (usually ~10 min). Because multiple sharks were kept in a tank, food consumption was calculated at the level of tanks and divided by the number of sharks in the respective tank. This was deemed as a fair representation of individual shark consumption

rates because leftover food in the tanks indicated they were all fully fed and competition for food resources was unlikely to take place because food was not limiting. Although Port Jackson sharks usually feed at night, our sharks were conditioned to feed during the day directly upon hatching and therefore we expect this to represent true demand of food intake.

2.5.5 GROWTH IN MESOCOSM EXPERIMENTS

After the laboratory experiment, a subset of the sharks was relocated to a mesocosm setup in South Australia. Three sharks were placed in each of the 12 mesocosm tanks (2,000 L volume each) which were manipulated to mimic a shallow temperate reef habitat. The mesocosms had the same crossed design of elevated CO_2 and temperature as the laboratory experiments with 3 replicate mesocosm per treatment (Table S4). Each mesocosm had the same biological set up which included 5 kelp plants (*Ecklonia radiata*) with an average weight of 250 g per plant, a single spiny rock lobster (*Jasus edwardsii*) of ~2 kg in weight, 1 crab (*Ozius truncatus*), 15 snails (*Turbo undulatus)*, 6 urchins (*Heliocidarcis erythrogramma*) and amphipods (>1,000). The kelp, snails and urchins were replenished 3 times over the duration of the experiment (68 days) as needed. The snails, crab, lobster and urchins were too large for the sharks to consume, and their primary food source was the amphipods that successfully populated and reproduced within the tanks. Turf algae started growing naturally and covered the major part of the substratum in the mesocosms. The mesocosms had a flow-through system using natural seawater filtered through a sand filter. Temperatures were manipulated using external heater/chiller units (TC60 Aquarium chillers, TECO refrigeration technologies, Ravenna, Italy). The same thermal mass flow meter/controller as in the laboratory experiments was used to achieve an elevated CO_2 concentration in the seawater of the mesocosm via bubbling of enriched air directly into the tanks, and both temperature and pH were measured daily.

The sharks were measured individually for total weight and photographed (to aid with the identification and tracking of individual growth for the duration of the experiment) prior to placement in the mesocosms. Sharks were re-measured after 61 days and after 68 days at the end of the experiment. During the first two weeks of the experiment, the sharks in both high temperature treatments were fed 2 g of fresh prawn meat, whereas sharks in both ambient temperature treatments were fed 1 g of meat each. These were similar to the food intake quantities as measured in the laboratory prior to placement into the mesocosms. This served as an acclimation period during which the sharks could familiarize themselves with the natural prey items in the mesocosms. After 2 weeks the feeding was standardized to 1 g per shark for all treatments. Due to the lowered food provisioning and due to their continuing increase in growth, the sharks increased their reliance on foraging on natural prey in the mesocosms such as amphipods. Observations showed shark foraging in-between the turf algae (which occupied most of the substratum and vertical tank walls of the mesocosms). Biomass of amphipods was not enhanced in the control treatments compared to the elevated CO_2/temperature treatments (single sampling event of total weight and numbers of all amphipods found on the kelp: Control = 0.06 g, n= 124, Temperature = 0.06 g, *n*= 56, CO_2=0.09 g, *n* = 86 and T x CO_2= 0.05 g, *n*= 114) and could therefore not have been responsible for the observed reductions in growth rates in the latter treatments. There was no strong differences in growth between the three sharks in one tank (this was the same for all tanks and no shark was observed to compete while feeding individually.

2.5.6 HUNTING BEHAVIOUR IN MESOCOSM EXPERIMENTS

After an average 36-day exposure (range: 35–38 days because sharks were introduced into the mesocosms over a 4 day interval) to the experimental treatments in the mesocosms, the

effect of elevated CO_2 on shark prey hunting behaviour through olfaction was tested. Prior to the day of testing the sharks were not fed, although they were still able to obtain prey (amphipods) from the tanks. Nevertheless, the sharks showed high degree of motivation towards the food offered in the olfactory trial the next day. The olfaction tests consisted of placing two equally sized (33 x 23 x 5 cm) sand-filled trays within each of the mesocosms. One tray (i.e. the food tray) had a combination of prawn meat (4 equally sized pieces of approx. 1 g each) and 5 fresh cockles of equal size (still in their shell but opened), buried into the sand. The control tray contained no food but had 5 empty and cleaned out cockle shells buried in the sand to reduce any visual bias of the slightly exposed top ends of the shell in the food tray. Both trays were placed near each other (average distance of 5 cm between the trays) and the shark responses were recorded using a GoPro HD HERO3 video camera (white edition) for a period of 40 min. The recordings were then analysed to determine the length of time it took for each shark to locate the hidden food and to determine the number of sharks that responded to the introduction of the prey. The timer started counting from the moment the trays were lowered onto the bottom of the mesocosm until the time each shark found the hidden prey items in the tray and started retrieving them from the sand or until the end of the experiment (after 40 minutes). Although Port Jackson sharks are nocturnal feeders these sharks were accustomed since birth to being fed during the day and responded actively when food was offered. It was possible to distinguish individual sharks within each mesocosm due to the markings on their upper bodies between the eyes, first dorsal and pectoral fins. These areas showed the most variation in patterning between sharks and remained consistent from hatching (photos were taken weekly after hatching).

2.5.6 STATISTICAL ANALYSIS

Separate linear regressions estimated individual growth of sharks over time. The slopes of each regression per shark was used for statistical analyses of factorial treatments; PERMANOVA version 1.0.3 (Anderson, 2005) that tested the effects of elevated CO_2, temperature, and their interactive effects on growth, food consumption and hunting behaviour of the 2 x 2 factorial experiments. Subsequent pair-wise tests were used to determine the specific significances of each separate treatment combination. A significant tank effect was found for the hatching (Table S1) and consumption data (Table S2) only, this was not significant in any subsequent behaviour trials and on growth. For behaviour: tank did not have a significant effect when nested in factors temperature and CO_2 and the statistical test was thus rerun without tank nested as a factor.

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2.7 FIGURES

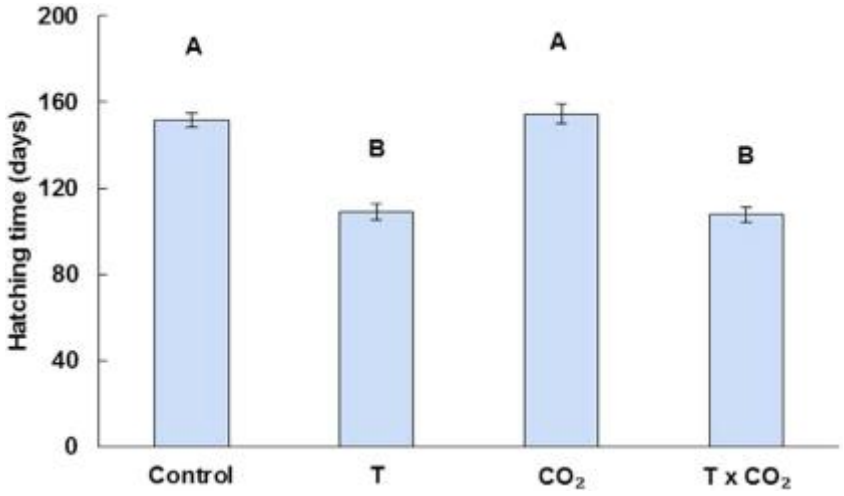

Figure 1. Mean duration until hatching for sharks eggs incubated in a factorial experiment of increased temperature (T) and CO_2. Error bars represent $\pm$ 1 standard error of the mean. Bars with different letters (A, B) differ significantly ($P < 0.05$).

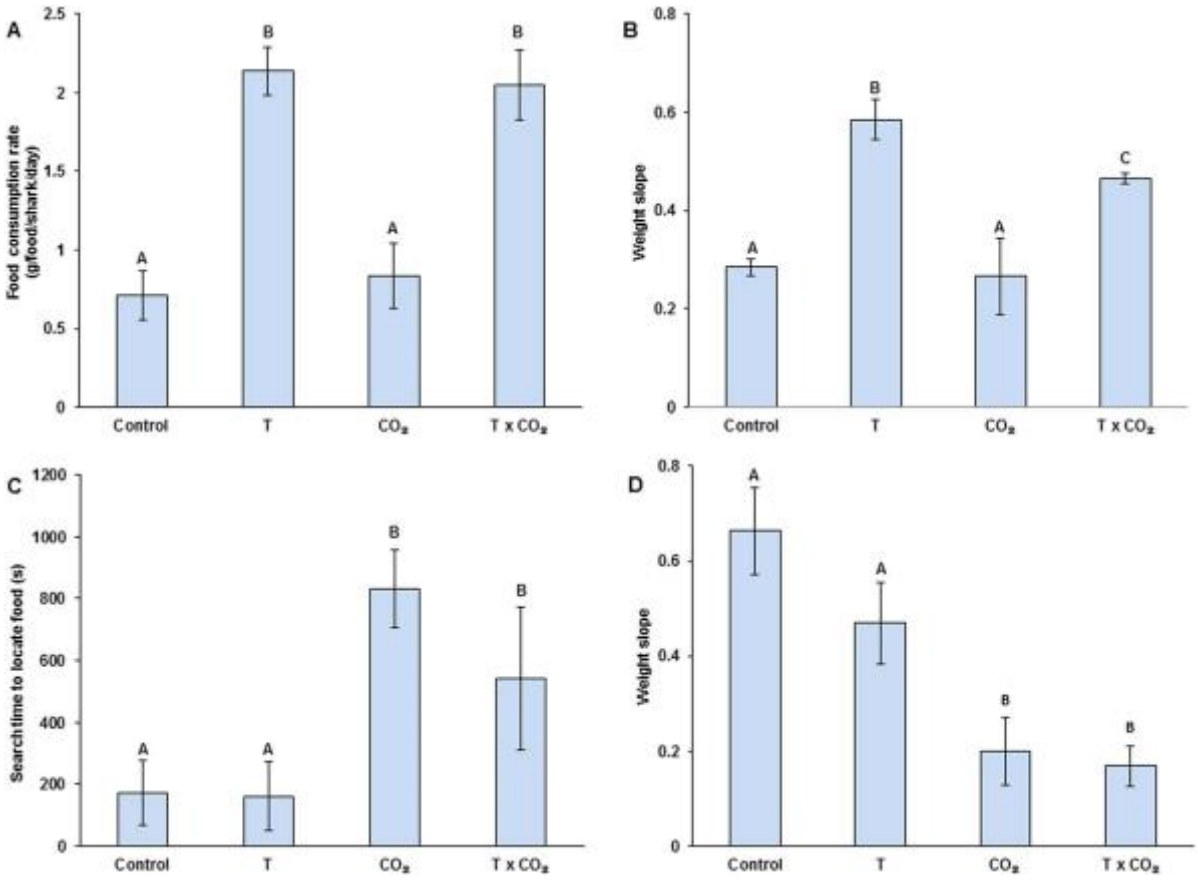

Figure 2. Shark food consumption rates, somatic growth rates, and hunting behaviour tested in a factorial design of elevated temperature (T) and CO_2 as predicted for the end of this century. (A) Net food consumption rates in the laboratory where sharks were fed *ad libidum*. (B) Mean growth rates (slope of biomass increase over time) of sharks reared in the laboratory for 56 days on average and fed *ad libidum*. (C) Total time to successfully locate prey hidden in sand trays at the bottom of the mesocosms. (D) Mean growth rates (slope of biomass increase over time) for sharks reared in mesocosms with natural habitat and prey over a period of 68 days. For (c) and (d) the representative means are per tank/treatment. Error bars represent standard error of the mean, different letters represent significant differences ($P < 0.05$).

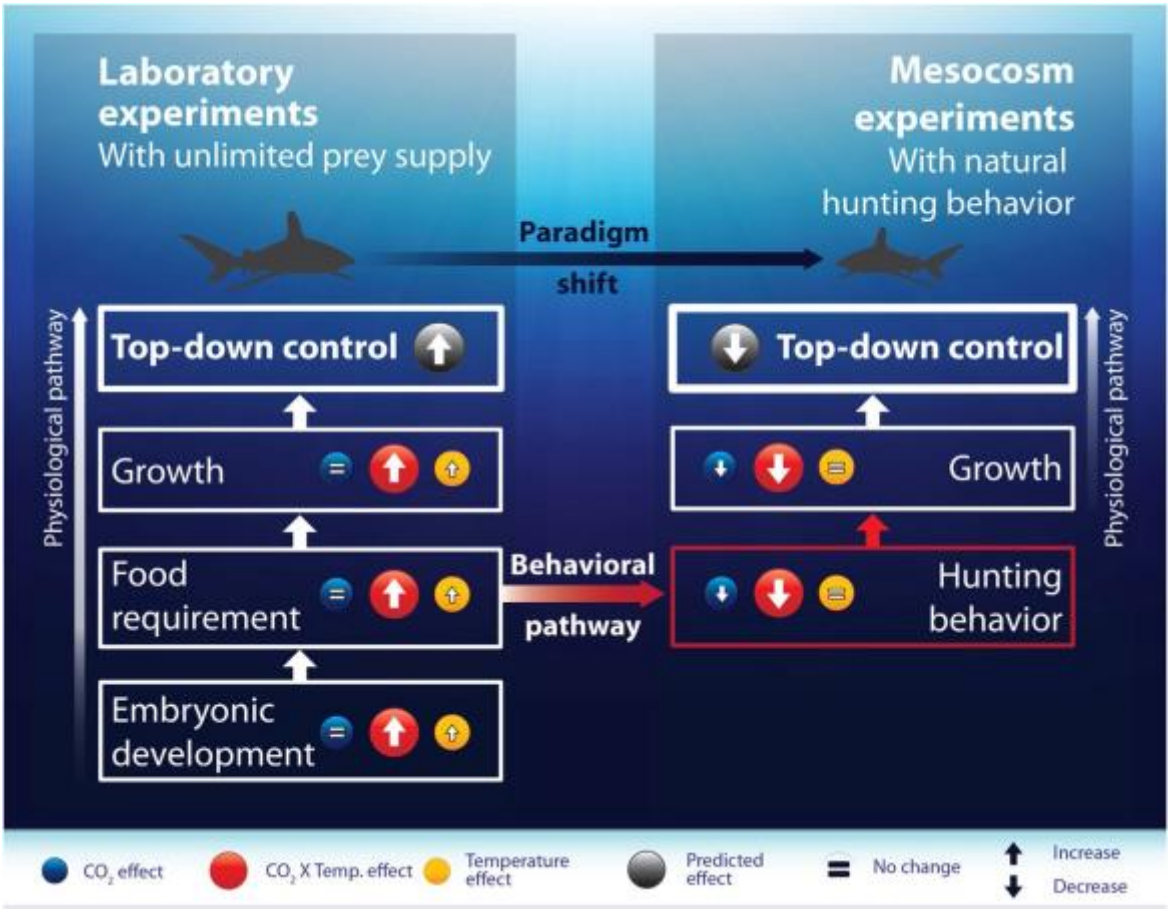

Figure 3. Conceptual diagram showing the individual and interactive effects of elevated temperature and CO_2 on the physiology (development rate, food consumption rate, and growth rate) and behaviour (hunting for prey through olfaction) of sharks, based on the results of our long-term laboratory and mesocosm experiments. Arrows within circles show whether the respective factors increase, decrease, or remain the same. Left-hand panel shows results that support the current predicted increase in energetic demands by predators leading to a potential increase of top-down control on food-webs. Right-hand panel shows our suggested paradigm shift linked to a negative effect of CO_2 on olfaction-driven predation. CO_2 leads to a reduced effectiveness in finding prey, leading to reduced growth, and therefore negates the predicted increase in top-down control based on elevated temperature alone. Figure designed by T.Rossi

2.8 SUPPLEMENTARY INFORMATION

Table S1: Analyses of variance of the effects of temperature (target: 16 °C and 19 °C) and CO_2 (target: 400 and 1000 ppm) on shark egg hatching rates.

Source	DF	MS	F	P
T	1	30535	49.565	**0.0001**
CO_2	1	5.00	8.12×10^3	0.9330
T x CO_2	1	14.364	2.33×10^2	0.8758
TK (T x CO_2)	12	8003.4	2.154	**0.0206**
Residual	75	23227		
Total	90			

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T x CO_2 = combined elevated temperature and elevated CO_2 treatment, TK = tank nested within T x CO_2 interaction term. Degrees of freedom (DF), mean squares (MS), the *F*-ratio (F), *P*-value (P). Bold values indicate significance at $p < 0.05$.

Table S2: Analyses of variance of the effects of temperature (target: 16 °C and 19 °C) and CO_2 (target: 400 and 1000 ppm) on (a) food consumption rates and (b) growth rates for sharks reared over 56 days, on average, in the laboratory.

Source	DF	MS	F	P
(a) Consumption				
T	1	318.25	49.566	**0.0001**
CO_2	1	3.80×10^{-2}	5.92×10^{3}	0.9415
T x CO_2	1	2.028	0.316	0.5801
TK (T x CO_2)	12	6.563	9.568	**0.0001**
Residual	766	0.686		
Total	781			
(b) Growth				
T	1	1.259	62.733	**0.0001**
CO_2	1	8.89×10^{-2}	4.427	**0.0365**
T x CO_2	1	8.03×10^{-2}	4.001	**0.0460**
Residual	76	2.01×10^{-2}		
Total	79			

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T x CO_2 = combined elevated temperature and elevated CO_2 treatment, TK = tank nested within T x CO_2 interaction term. Degrees of freedom (DF), mean squares (MS), the F-ratio (F), *P*-value (P). Bold values indicate significance at $p < 0.05$.

Table S3: Analyses of variance of the effects of temperature (target: 16 °C and 19 °C) and CO_2 (target: 400 and 1000 ppm) for sharks reared in the mesocosms on (a) search time to locate prey, and (b) growth rates over 68 days.

Source	DF	MS	F	P
(a) Search time				
T	1	2.82×10^{-5}	2.5195	0.1292
CO_2	1	8.44×10^{-5}	7.5297	**0.0120**
T x CO_2	1	4.22×10^{-5}	3.7654	0.0660
Residual	19	1.12×10^{-5}		
Total	22			
(b) Growth				
T	1	0.101	2.20	0.1446
CO_2	1	1.165	25.33	**0.0002**
T x CO_2	1	5.31×10^{-2}	1.16	0.2982
Residual	29	4.60×10^{-2}		
Total	32			

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T x CO_2 = combined elevated temperature and elevated CO_2 treatment. Degrees of freedom (DF), mean squares (MS), the F-ratio (F), *P*-value (P). Bold values indicate significance at $p < 0.05$.

Table S4: Mean (± SE) seawater parameters in the experimental systems with two crossed factors of elevated temperature and CO_2 for the (a) shark eggs (b) sharks in the laboratory and (c) sharks in the mesocosms. Numbers in brackets following the treatment names represent the number of sharks in each treatment. N= no of replicates for pH readings, n = no of replicates for alkalinity readings The SE in S4 represents the variability of both replicates and measurements.

	Salinity	pH_{NBS}	Temp (ºC)	N	TA ($\mu mol.kg^{-1}SW$)	pCO_2 (ppmv)	SE	n
(a) Egg stage								
Control (24)	40 (±0.0)	8.02 (±0.01)	16.3 (±0.0)	143	2377.9 (±49.4)	517.6 (±19.9)	19.9	11
T (20)	40 (±0.0)	8.06 (±0.00)	19.2 (±0.1)	98	2528.9 (±32.2)	531.8 (±22.4)	22.4	8
CO_2 (23)	40 (±0.0)	7.82 (±0.01)	16.2 (±0.1)	136	2400.2 (±67.1)	946.7 (±39.7)	39.7	11
T x CO_2 (27)	40 (±0.0)	7.81 (±0.01)	19.1 (±0.1)	118	2442.2 (±42.0)	1048.5 (±45.9)	45.9	10
(b) Laboratory								
Control (24)	40 (±0.0)	7.96 (±0.01)	16.4 (±0.1)	73	2346.1 (±102.5)	589.4 (±50.9)	50.9	3
T (20)	40 (±0.0)	7.87 (±0.02)	18.8 (±0.3)	55	2179.0 (±61.9)	661.0 (±15.4)	15.4	3
CO_2 (25)	40 (±0.0)	7.69 (±0.01)	15.9 (±0.1)	73	2075.0 (±29.8)	1003.6 (±69.9)	69.9	3
T x CO_2 (27)	40 (±0.0)	7.68 (±0.01)	18.7 (±0.1)	56	1944.4 (±98.1)	1014.3 (±115.0)	115.0	3
(c) Mesocosm								
Control (9)	40 (±0.0)	8.11 (±0.01)	17.7 (±0.2)	54	2444.2 (±6.4)	470.2 (±39.8)	39.8	9

T (9)	40 (±0.0)	8.10 (±0.01)	19.5 (±0.1)	54	2465.2 (±16.8)	517.9 (±43.5)	43.5	9
CO_2 (9)	40 (±0.0)	8.02 (±0.01)	17.7 (±0.2)	54	2437.8 (±6.8)	680.0 (±91.9)	91.9	9
T x CO_2 (9)	40 (±0.0)	7.98 (±0.01)	19.5 (±0.1)	54	2445.0 (±4.9)	734.3 (±64.4)	64.4	9

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T x CO_2 = combined elevated temperature and elevated CO_2 treatment, TA = total alkalinity.

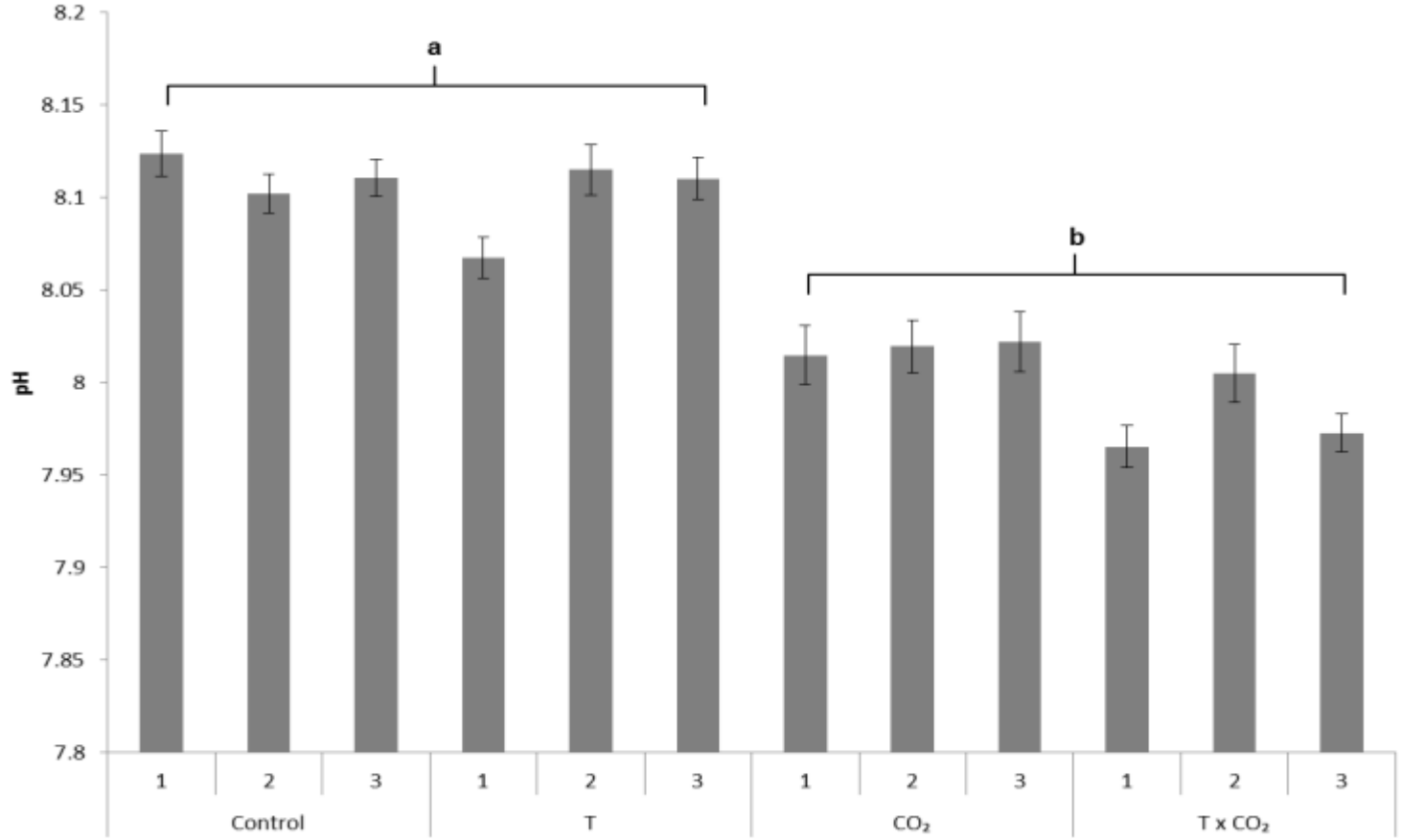

Figure S1: Mean pH in each mesocosm. No significant differences were found between replicate tanks within treatments as tested with a nested ANOVA ($p > 0.05$) A 2-way ANOVA showed a significant effect of pH in the CO_2 treatments (ANOVA; CO_2 $F_{1,635} = 105.5$, $P=0.0003$). Letters in figure denote significant differences. Error bars represent standard error of the mean.

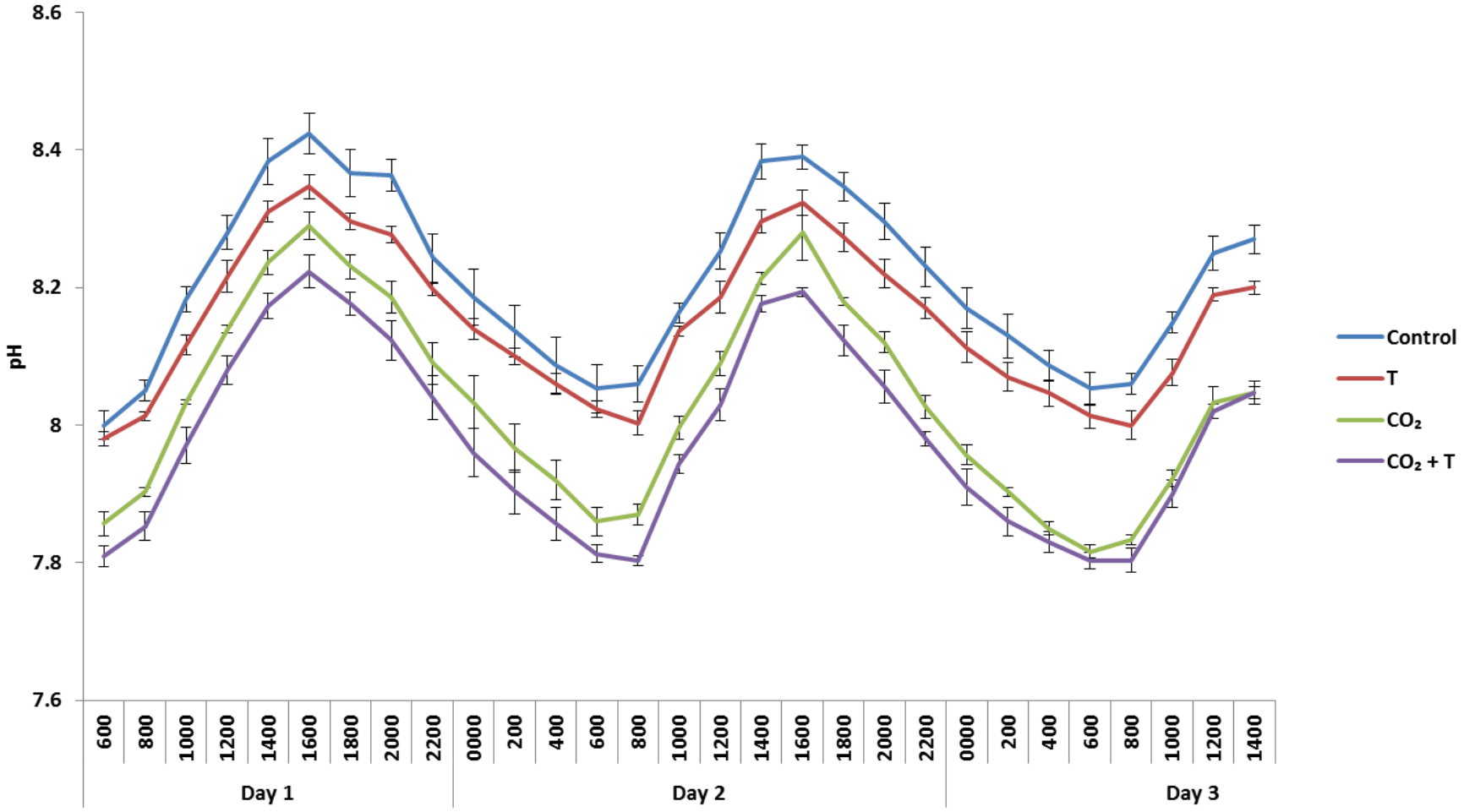

Figure S2: Diurnal variation of mean pH in each treatment over 3 days in the mesocosm experiment accounting for the variability in pH as seen in Table S2c. Error bars represent standard error of the mean. The difference in pH between the elevated temperature treatments compared to the

control could be attributed to higher respiration rates (biological activity) as well as dissolution of the added rocky substrate within the tanks contributing to increased CO_2 levels within those tanks (Falkenberg et al. 2016).

Supplementary reference:

Falkenberg, LJ, Russel BD & Connell SD (2016) Design and performance evaluation of a mesocosm facility and techniques to simulate ocean acidification and warming, *Limnology and Oceanography: Methods* doi:10.1002/lom3.10088

CHAPTER 3

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Principal Author

Name of Principal Author	Jennifer .C.A. Pistevos		
Contribution to the Paper	Contributed to the conception and design of the project. Conducted the study, collected and analysed the data and drafted and revised the manuscript.		
Overall percentage (%)	85		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Contributed to the conception and design of the project and assisted with the development and revision of the manuscript and acted as Corresponding author. Provided funding.		
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Contribution to the Paper	Contributed to the conception and design of the project, assisted with the development and revision of the manuscript.		
Signature		Date	04/07/2016

CHAPTER 3

ANTAGONISTIC EFFECTS OF OCEAN ACIDIFICATION AND WARMING ON HUNTING SHARKS

3.1 ABSTRACT

Ocean warming and acidification alter the physiological performance and behaviour of many small-bodied fishes, yet the potential interactive effects of these stressors on larger predators remains poorly understood. In particular, the combined effects of elevated temperature on metabolism and of elevated CO_2 on the behaviour of large predators may not only affect their foraging behaviour, but also the communities in which their prey live. We used a factorial design to assess how projected warming and acidification create synergies or antagonisms between physiological and behavioural processes, such as swimming activity and feeding behaviour through odour tracking and vision. Temperature increased swimming activity during feeding, independent of CO_2. Although temperature also increased motivational drive to locate and accept prey, elevated CO_2 negated chemical and visual behavioural responses that enable effective hunting. Fundamental to these effects was the negligible effect of high CO_2 in isolation, but its power to negate the positive effects of temperature when brought in conjunction. The reduced potential to locate prey due to the interactive effects of ocean acidification and warming, in combination with increases in energetic demand, suggests that energetic trade-offs will be needed for sharks to sustain themselves at an individual and population level in a future ocean.

3.2 INTRODUCTION

The capacity for large predators to trophically influence the structure and function of marine ecosystems may be altered by the intensification of fishing (Myers et al. 2007, Ripple et al. 2014) and CO_2 absorption in a warming ocean (Nagelkerken and Connell 2015). Whilst predators face an intensifying set of human-induced stressors (Estes et al. 2011, Heithaus et al. 2012) little is known about these effects on their hunting behaviour and sensory perception of prey. Sharks possess multiple sensory systems that provide them with visual, acoustic, mechano-sensory, chemical and electric stimuli about their physical environment which are essential for orientation, prey location, and prey sourcing (Guttridge et al. 2009, Gardiner et al. 2012, 2014). Ocean warming and acidification can alter the foraging activity and various senses of sharks, causing behavioural switches that may cascade throughout ecosystems in which they forage (Nagelkerken and Munday 2016). By recognising how an increasingly warming and acidifying ocean (Caldeira and Wickett 2003, Portner et al. 2014) alters predator behaviour and prey reception, we may understand how change in predatory behaviour could weaken or even strengthen its influence over marine ecosystems (Myers et al. 2007, Estes et al. 2011, Ripple et al. 2014).

The behaviour of marine animals is sensitive to change in acidification (Nagelkerken and Munday 2016), including impaired decision-making (Domenici et al. 2012), reduced olfactory preferences (Munday et al. 2009, Cripps et al. 2011) and auditory responses (Rossi et al. 2016). In elasmobranchs, acidification causes a spectrum of behavioural and physiological responses that range from no effects (Heinrich et al. 2015) to reduced odour tracking and hunting (Dixson et al. 2015, Pistevos et al 2015). Whilst elasmobranchs physiologically compensate for acid-base disturbances in similar ways to teleosts (Claiborne

et al. 2002, Brauner and Baker 2009), they have mechanisms that involve stronger buffering capacity (Berenbrink et al. 2005, Heinrich et al. 2014) and acid excretion processes (Wood et al. 1995).

Temperature is fundamental to the biology of organisms that do not strongly regulate their body temperature (Portner et al. 2014). In fish, temperature regulates feeding, growth, survival and development (Pörtner and Farrell 2008, Rall et al. 2009, Twomey et al. 2012). Temperatures above optimum lead to a reduction in available energy for growth and reproduction due to increased basal metabolic rates as well as increased respiratory demand leading to reduced aerobic scope for other activities such as feeding, digestion as well as predator avoidance (Portner and Knust 2007). How elevated temperature combines with ocean acidification to alter foraging in sharks is largely unknown. In the few studies to observe the combined effects of elevated temperature and CO_2 on sharks, Rosa et al. (2014) detected decreased survival and development time of embryonic and hatched sharks and increased metabolic rates, while Pistevos et al. (2015) found reduced growth. These demographic effects suggest that foraging behaviour is unlikely to go uninfluenced by future ocean conditions.

We here test whether ocean warming and acidification alter behavioural traits that mediate foraging success in a temperate shark; the Port Jackson shark (*Heterodontus portusjacksoni*). We experimentally investigated the independent and interactive effects of projected future temperature and CO_2 levels on odour tracking behaviour and swimming activity to acquire insight on potential change in population persistence and influence on trophic control. We focuses on the early life-history because of its renown sensitivity to environmental change (Rombough and McDonald 1997, Baumann et al. 2011) for which behavioural alterations

could lead to disproportionate change in adults (Przeslawski et al. 2015). Using recently-hatched Port Jackson sharks, we tested the effects of near-future ocean warming and elevated CO_2 on their (i) speed of recognition of novel food items through odour tracking/vision, (ii) rate of swimming during hunting and (iii) time spent searching for prey via odour tracking. Whilst the effect of high CO_2 was negligible in isolation, it powerfully negated the positive effects of temperature; a particularly relevant finding because both these factors are inescapably interlinked in our future ocean.

3.3 MATERIALS AND METHODS

3.3.1 ETHICS STATEMENT

This research was approved and adhered to the animal ethics guidelines of the University of Adelaide animal ethics committee (permit: S.2013-095). Collections of eggs from the Gulf of St Vincent were performed under Ministerial exemption # 9902595 (South Australian Government Department of Primary Industry and Regions SA).

3.3.2 STUDY SPECIES AND SAMPLE COLLECTION

Heterodontus portusjacksoni (Meyer, 1793) is endemic throughout the southern half of Australia (Rodda and Seymour 2008, Last and Stevens 2009). It is a medium-sized benthic shark that breeds annually during the months of September to November. The eggs of oviparous elasmobranchs are among the largest in marine animals and Port Jackson sharks contain a single embryo with an average weight of 155 g (Rodda and Seymour 2008). A pair of eggs is laid every 10-12 months (McLaughlin and O'Gower 1971) with the incubation

period lasting up to a year. Initially the egg capsules are plugged with a mucous plug, but after 4 months the plug dissolves and the embryo and yolk are surrounded by sea water and the egg casing provides a safe environment as the embryo develops and feeds off the yolk (Rodda and Seymour 2008). Hatching is triggered once the internalised yolk sac is depleted and hatchlings must feed soon after hatching (Rodda 2000). *H. portusjacksoni* is an opportunistic carnivore (Powter et al. 2010, Sommerville et al. 2011) feeding mostly on benthic invertebrates dominated by echinoderms, molluscs, and teleost fish (Last and Stevens 2009, Sommerville et al. 2011).

A total of 95 eggs were collected from the Gulf St. Vincent, South Australia, over two collection dates (7th and 28th June 2013) via snorkelling. It was possible to estimate their development stage at time of collection by opening the capsules slightly. All egg capsules were acquired at a similar development stage (stage 14 – at least 7.5 months old (Rodda and Seymour 2008). Temperature at time of collection was 16°C and this was used as the control temperature throughout the duration of the experiment with an addition of 3 °C for the elevated temperature treatment.

Eggs were maintained in a temperature controlled laboratory at the University of Adelaide. Eggs lacked the mucus plug so that the embryo was in full contact with the intended treatment of water. Embryo development was observed by opening the egg casing slightly. The eggs were incubated in 40 L tanks (max 8 eggs per tank – 4 replicate tanks per treatment) containing an internal biological filter and filled with natural sand-filtered seawater that was partially exchanged every 2–3 days (min 40% volume). The total number of individuals per treatment is indicated in Figs. 1 and 2. The eggs were kept in either control (~400 µatm) or elevated CO_2 (~1000 µatm) crossed with control (16 °C – temperature at time of collection)

or elevated temperature (19 °C), representing the end of century forecasts of sea surface temperature and pCO_2 increases based on the representative concentration pathway (RCP) 8.5 emission scenario (business-as-usual) (Meinshausen et al. 2011, Pörtner et al. 2014). Target pH was reached over two days. The longer-term (2010–2014) mean seawater temperature at 5 m depth in the Gulf St. Vincent (egg collection area) for the month June was ~ 15.3 ± 1.4 ℃ (SARDI Aquatic Sciences unpubl data). In the elevated temperature treatments, water temperature was steadily increased from 16 ℃ to reach the target temperature of 19 ℃ after 7 days (~ 0.5 °C per day). Exposure time of the embryos varied across treatments due to the positive temperature effects on hatching rates (mean ± SE): 147 ± 3.94 (control), 138 ± 7.87 (high CO2), 109 ± 3.78 (elevated temperature), and 108 ± 3.37 days (T × CO2), but did this not have a significant effect on the results (see Statistical analyses). No significant differences in hatching size (range in length across treatments: 19.8–20.2 cm) or weight (range in weight: 50–53 g) were detected between treatments (1-way ANOVA, weight: p = 0.604; length: p = 0.570; Pistevos et al. 2015). We observed 100% hatching success and survival.

3.3.3 SEAWATER MANIPULATION

Each treatment tank was placed in temperature maintained baths using heater chiller units (TR15 Aquarium chillers, TECO refrigeration technologies, Ravenna, Italy) and/or 300 W glass heaters with pumps connected to each chiller unit to maintain even temperature distribution. Eggs were kept in either control (~400 µatm) or elevated CO_2 (~1000 µatm) crossed with control (16°C) or elevated temperature (19°C). Elevated CO_2 concentrations in the seawater were maintained using a single gas mixer (PEGAS 4000 MF Gas Mixer, Columbus Instruments, Columbus, Ohio) that bubbled enriched air into each tank separately and thus creating independent treatment tanks. Target pH was reached over two days. The

pH_{NBS} and temperature of each tank was measured daily using a pH meter (Mettler Toledo SevenGo™ SG2) which was calibrated daily with fresh buffers. Total alkalinity of seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken weekly from each of the treatment tanks. Oxygen levels were maintained above 90% saturation and were measured daily within the tanks with an oxygen probe (OxyGuard® Handy Polaris 2). Total alkalinity of the seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken weekly from each of the treatment tanks. Alkalinity standards were accurate within 1% of certified reference material from Dr. A. Dickson (Scripps Institution of Oceanography; Langdon et al. 2000). Average seawater pCO_2 (Table S1) was calculated using CO_2SYS using already established constants (Mehrbach et al. 1973, Dickson and Millero 1987).

3.3.4 EXPERIMENTAL PROTOCOLS

3.3.4.1 ODOUR TRACKING BEHAVIOUR

Within 24 hrs of hatching each shark was placed into an olfactory test arena, which consisted of a white plastic tank (150 L, 100 × 50 × 50 cm) filled with seawater to the height of the tip of the dorsal fin (on average 5 cm; to limit vertical movement). No stress response was observed by the shark as a result of the shallow water. Based on preliminary trials, sharks appeared to quickly habituate to their environment and displayed normal swimming behaviour within 2 min of placing them in the trial tank. The olfactory test arena was nearly identical to their rearing tank with the exception of the presence of the urchin containers. Trials were limited to 15 min as the sharks appeared to lose interest in the urchin holding containers after this time. Sharks were tested within 24 hrs of hatching and those that did not move during the trial were considered unresponsive and not tested. Testing arenas were of

the same temperature and salinity as the original incubation treatments, but were not acidified as fish are known to retain the behavioural effects of elevated CO_2 for over 24–48 hrs when placed in control conditions (Munday et al. 2010). Although, sharks differ from teleost in their pH buffering capacity, it is unlikely that the high CO_2 effects had disappeared during the choice experiments as we placed the sharks from their long-term high CO_2 treatment bins directly into the choice arena for 15 min of filming. Moreover, the reduced behavioural response observed here (i.e. time to accept food) is in accordance with a compromised behavioural response (i.e. odour tracking behaviour) for the same sharks as tested in treatment water (Dixson et al. 2015, Pistevos et al. 2015).

Within the test arena (Fig. S1) there were two metal containers (12 cm in diameter, 13 cm high) with 108 (∅ 7 mm) holes, placed in equidistant locations at either end of the tank, one containing the chemical cue (urchin) and the other a dummy made with rolled up cotton fabric (which was rinsed between each trial) similar in size to the urchin containing no chemical cue. Before the start of the behavioural experiments, dye tests were performed which showed limited dispersal of the cue. During a time frame of 5 min the dye was mostly restricted to the container and within a few mm around the container, even when disturbing the water around the tank (i.e. mimicking the effect of a swimming shark). We only performed a 5-min dye test to ensure that the chemical cue spread out of the metal container within 5 min. In each trial, one of four urchins (*Heliocidaris erythrogramma* and *Holopneustes pycnotilus*) was used that were rotated between replicate trials. For each treatment half of the sharks were exposed to one species of urchin and the other half to the other species. All urchins were collected at the same site as the shark eggs. The shark was at first restricted in the centre of the tank using a black 15 L bin (with holes to allow for chemical cues to penetrate without the shark being able to perceive any visual cues from

outside) and left to acclimatise for 2 min after which it was released to swim freely in the tank for 15 min. Two minutes was more than sufficient for the sharks to show exploratory behaviour and swim throughout the entire arena. Each experimental trial was recorded on camera (Canon Legria HFM52) and analysed using the software EthoVision (EthoVision XT10, Noldus Information Technology, Wageningen, The Netherlands), which was able to track the fish without the need of any markers on the individual shark. This allowed an objective measurement of their behaviour without observer bias. The tank was divided into three equally sized zones: control zone (blank), central zone (equal area between the cue and control side) and cue zone (urchin) (Fig. S1 dashed lines). Data was obtained for the cumulative amount of time that was spent in each zone and the total distance each shark moved during the duration of the trial. The water in the choice arena was replaced with fresh seawater before the next individual was tested.

3.3.4.2 MOTIVATIONAL DRIVE TO ACCEPT PREY

At the end of the 15 min olfactory choice experiment the urchin and metal container were removed and immediately two pieces of 1 gram each of fresh prawn meat and/or mussel meat were placed at the opposite side of the tank in relation to the shark's position. In preliminary trials prawn meat elicited the strongest response when feeding. Nevertheless, we offered two food types to increase chances of observing a feeding response. If the shark ate any of the introduced food within 2 min ('independent feeding') the experiment was ended. The time it took from the release of the food into the tank until the shark accepted the food and swallowed it was recorded as the time to independent feeding. However, because most sharks did not feed independently we adjusted the approach by trying to hand feed the animal for 2 min using a stainless steel thumb forceps containing food ('facilitated feeding'). The time it took from the moment the food was introduced into the tank with the

forceps until the shark accepted the food was recorded as the time to facilitated feeding. All sharks that did accept the food also consumed it. If the shark did not accept the food in the latter trial, it was categorised as 'did not feed'.

The behavioural response tested in this trial was performed as a second test for motivational drive to forage or accept prey, but in presence of chemical as well as visual food cues (as opposed to the odour tracking experiment where vision was excluded). We did not consider the residual urchin chemical cues to be interfering with this second experiment as the sharks did not show any interest in the urchin cues after 15 min, but did show interest to the new food cues that were added to the chamber. When the food was added there was minimal disturbance and no additional stress observed by the sharks. The sharks were not restricted in any way and could reject the food if they lacked motivation.

3.3.4 STATISTICAL ANALYSIS

To statistically test the effects of elevated CO_2, temperature and their interactive effects on swimming distance, time to accept prey under facilitated feeding, and cumulative duration of time spent in the prey zone during the odour tracking experiment for the 2 × 2 factorial experiments, a two-way ANOVA was used with temperature and CO_2 treatments as factors. No significant tank effect was detected (3-way ANOVA with CO_2 treatment, temperature, and tank nested in treatment as factors) and therefore tank was pooled as a term ($p > 0.25$; Winer et al. 1991). There was no significant effect of exposure time to treatment conditions on our response variables tested; we used simple linear regression to test for correlations between treatment exposure time and % time spent in prey zone (see Fig. 1a), total distance moved (see Fig. 1b), and time to accept food (see Fig. 2b). Of these 12 regressions, no significant effect of exposure time was found for 11 of the regression analyses (range in p

values: 0.166–0.968; range in R^2 values: 0–0.124). One exception was % time spent in prey zone for the elevated temperature alone treatment (p value: 0.027; R^2 value: 0.212). However, for the latter analysis, the effect of exposure time was opposite (decrease in % time) that of the elevated temperature treatment effect (increase) and therefore did not affect our results. Subsequent pair-wise tests were used to determine the specific significances of each separate treatment combination for the interaction terms that were significant (Tables S2, S3). No statistical analysis was performed for total time to feed independently as only 7 out of 83 sharks showed this behaviour.

3.4 RESULTS

Elevated temperature and CO_2 as main factors did not increase the sharks' attraction to potential prey based on chemical cues, but there was an interactive stressor effect (Fig. 1a; Table S3, ANOVA: T x CO_2, $F_{1,85} = 13.334$; $P = 0.001$). In the absence of elevated CO_2, temperature significantly increased the time (55%) the sharks spent near chemical cues of prey, but this attraction was neutralised when combined with elevated CO_2 (29%) (pairwise tests for interaction: $p < 0.01$) leading to a similar low-responsiveness to prey cues as observed in the CO_2-alone (42%) and control treatments (29%).

Elevated temperature caused an increase in swimming activity while searching for food through olfaction (Fig. 1b, Table S3, ANOVA: temperature, $F_{1,85} = 7.5094$; $P = 0.007$). In contrast, CO_2 did not have any effect on swimming behaviour (Table S3).

Of the 83 sharks tested for motivational drive to locate prey/forage (tested directly after the olfactory trials), only seven sharks fed independently (Fig. 2a; four from the control group

and one from each of the other three treatments). However, the majority of sharks did respond positively to facilitated feeding. While elevated temperature and CO_2 did not have direct independent effects on time to initiate feeding, their combination was antagonistic compared to the temperature alone treatment (Fig. 2b, Table S2, ANOVA: T × CO_2 interaction, $F_{1,42} = 4.172$; $P = 0.049$). Pairwise tests revealed that elevated temperature significantly reduced the time for sharks to accept novel food compared to control sharks, but CO_2 increased the time it took for the sharks to accept food at elevated temperatures.

3.5 DISCUSSION

Ocean warming and acidification had antagonistic effects on shark hunting behaviour. While ocean acidification is renown for its negative effects on olfaction (Munday et al. 2009b, Nagelkerken and Munday 2016), we demonstrate negligible effects of acidification as an isolated stressor, but it acted to negate the otherwise enhanced effects of elevated temperature. Elevated temperature increased activity levels, time spent next to food as well as latency to feed and together these could be interpreted as a response to increased energetic demands due to warming. Temperature-driven enhancement of hunting was neutered by and interactive effect of acidification on chemical and visually mediated responses. This finding highlights the value of multi-stressors experiments on multiple traits that provide the capacity for identifying antagonisms and synergisms that can negate or exacerbate predictions based on single factor experiments and single behavioural responses (Nagelkerken and Munday 2016). Typically synergisms, where the interaction produces a greater enhanced response than simply two stressors combined, prevail in marine ecosystems (Crain et al. 2008, Przeslawski et al. 2015). For instance, elevated CO_2 or temperature in

isolation reversed prey selectivity of a predatory fish but with similar total prey consumption, whereas the interaction between the two stressors cancelled selectivity but led to increased overall prey consumption (Nowicki et al. 2012, Burnell et al. 2013) compared to when stressors were tested in isolation and elevated temperature had no effect while high CO_2 reduced feeding rates (Nowicki et al. 2012). However, antagonistic responses in interaction studies are very uncommon with only 17% of the studies reviewed showing such a response (Przeslawski et al. 2015). Therefore, studies that have for example argued the potential existence of naturally-adapted populations based on lack of a negative CO_2 effects in single-stressor experiments (Munday et al. 2011, Heinrich et al. 2015) may offer necessary, but insufficiently tested insights on the existence of resilient populations that might replenish more sensitive populations in a future warmer as well as acidified ocean.

Elevated temperature caused sharks to increase their swimming activity during hunting and their attraction to olfactory/visual prey cues, whereas CO_2 in combination with temperature negated the attraction to prey cues. Elevated temperature has a fundamental effect on fish metabolism, performance and activity, including increased motivational drive to locate food to compensate for increased energetic needs (Nilsson et al. 2009). Elevated CO_2 can interfere with their ability to meet the elevated metabolic demand through diminishing their ability to locate prey. Marine fish regulate their acid-base balance in order to avoid acidosis though alterations in levels of HCO^-_3 and Cl^- under elevated CO_2 (Ishimatsu and Hayashi 2008, Baruner and Baker 2009) and these alterations under high CO_2 are thought to affect some $GABA_A$ receptors causing sensory and behavioural impairment in fish (Nilsson et al. 2012). Increased energetic costs and concomitant demands of food could put predators at risk if increasing energetic demands are not met by successful prey encounters (Rall et al. 2009, Vucic-Pestic et al. 2011, Nagelkerken and Connell 2015). Hence, partitioning of energy

expenditure may trade off against other physiological processes (Pörtner 2008), and the allocation of energy (food intake) is prioritised towards somatic maintenance over growth and maturation and reproduction (Sarà et al. 2013). Indeed, Pistevos *et al.* (2015) observed that when forced to actively hunt for food in large mesocosms, there was a reduction in growth of sharks exposed to elevated CO_2 and temperature.

Previous single stressor studies on sharks have shown a range of effects on their behaviour. Elevated CO_2 caused a shift in swimming patterns and increased lateralization in the small-spotted cat sharks *Scyliorhinus canicula* (Green and Jutfelt 2014), reduced food odour tracking and attack behaviour in the smooth dogfish *Mustelus canis* (Dixson et al. 2015), and reduced the ability of Port Jackson shark *Heterodontus portusjacksoni* to locate food through odour tracking (Pistevos et al. 2015). However, no effect of high CO_2 was found on the foraging and sheltering behaviour of the epaulette shark *Hemiscyllium ocellatum* which also showed a high tolerance to hypoxia (Heinrich et al. 2015). Furthermore, elevated temperature increased food consumption rates in the Port Jackson shark (Pistevos et al. 2015). Together, these studies indicate that a range of shark species are negatively affected in behaviours associated with finding food, suggesting sharks may have an uncertain future because changing ocean environments. Top predators play and important role in marine ecosystems by directly enforcing selection pressures on and regulating prey populations (Myers et al 2007, Paine 1966, Shepherd and Myers 2005) as well as indirectly through buffering against trophic cascades (Ripple et al. 2014, Estes et al. 2011, Rizzarri et al. 2014). A reduction under future climate conditions in the hunting ability of top predators such as sharks, as demonstrated in our experiments, could therefore potentially lead to modified predator-prey interactions (Cheung et al. 2013) and have cascading effects on food web structure. Depending on the species and their role in the ecossytem, reduced predator

influence could lead to weakened top-down control over prey allowing lower-order consumers to increase in abundances and affecting their prey species (Nagelkerken et al. 2016). Reduced predator influence over other species due to changes in metabolism and behaviour as shown in our study could therefore lead to altered species interactions and affect the structure of food webs. As one-third of oceanic shark species are threatened with extinction under contemporary climate (Camhi et al. 2009), then quantifying situations in which large predators fail to meet growth and survival requirements in a future ocean will provide important insight into their future persistence.

3.6 REFERENCES

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3.7 FIGURES

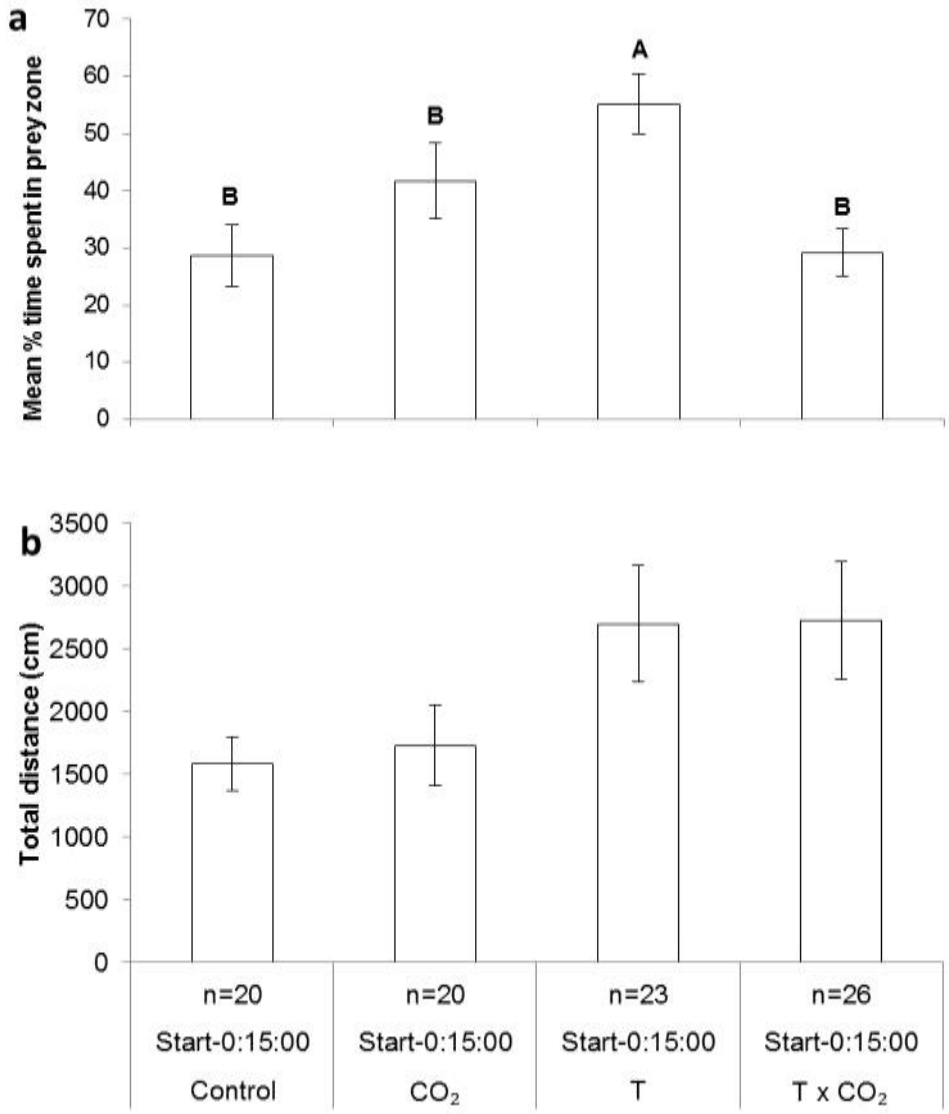

Figure 1 (a) Percent cumulative time (± SE) in minutes spent in the prey zone by sharks in the test arena, over a 15 min olfactory choice test. Different letters above columns denote significant differences ($p < 0.05$) and (b) Total swimming distance (±SE) during the 15 min olfaction trials (temperature had a significant effect as a main factor, Table S3).

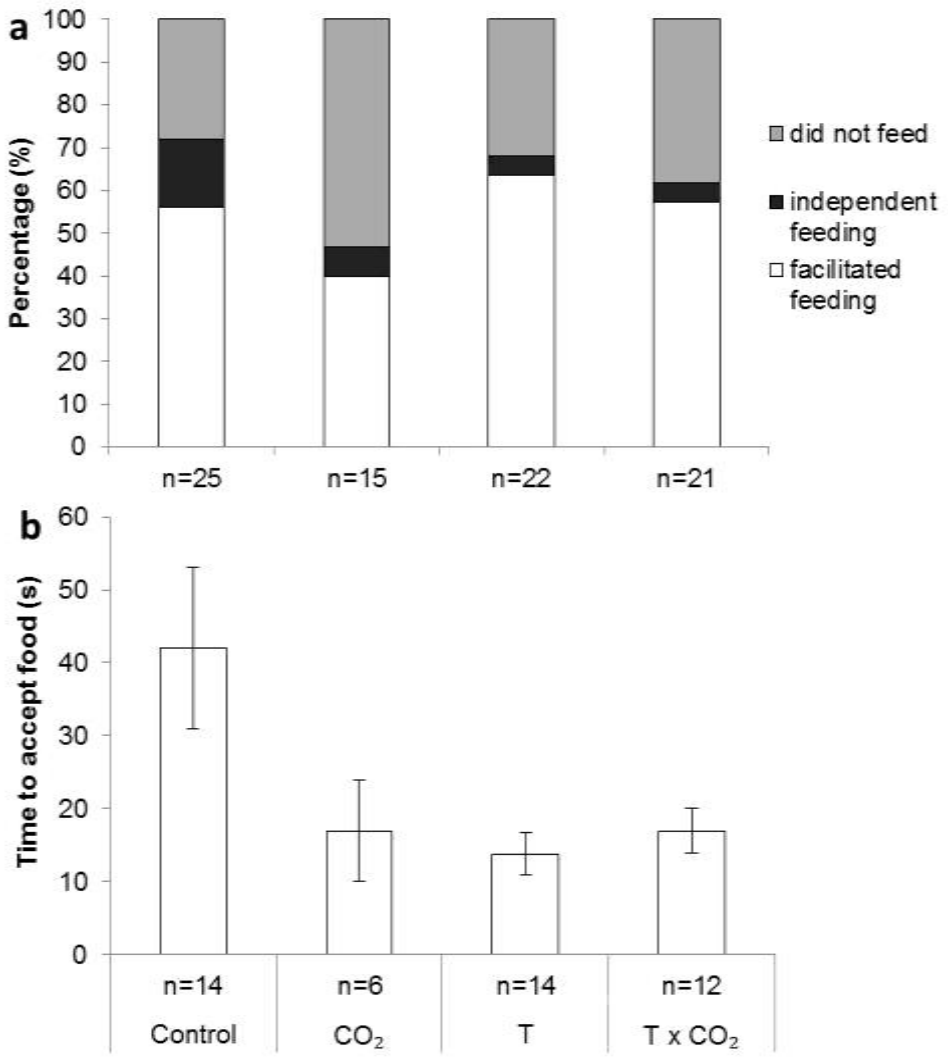

Figure 2 (a) Shark feeding response towards novel visual prey items directly after hatching. (b) Average amount of time (± SE) it took sharks to accept visual prey when fed under facilitation as shown in Fig. 1a. n = number of sharks tested.

3.8 SUPPLEMENTARY INFORMATION

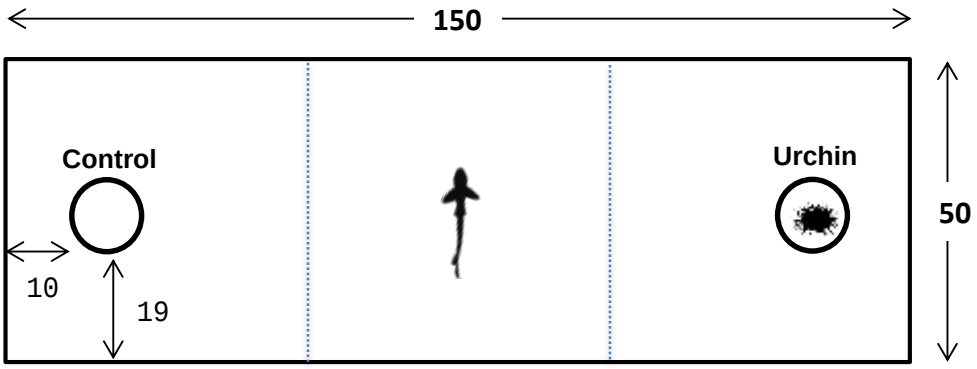

Figure S1. Diagram (top view) of the choice arena used for the olfaction test. Measurements are in cm. The position of the prey (sea urchin) in the chamber was alternated from left to right with each subsequent trial. Control indicates a dummy prey lacking any relevant chemical cues. Dashed lines indicate the equal arena division settings in the tracking program EthoVision XT (Noldus) used to determine each zone.

Table S1: Mean (± SE) seawater parameters in the experimental systems with two crossed factors of elevated temperature and CO_2 for the sharks during their embryonic life stage before hatching.

	pH_{NBS}	SE	Temp (ºC)	SE	*n*	TA (μmol.kg^{-1}SW)	SE	pCO_2 (μatm)	SE	*n*
Control	8.02	0.01	16.3	0.0	143	2377.9	49.4	517.6	19.9	11
T	8.06	0.00	19.2	0.1	98	2528.9	32.2	531.8	22.4	8
CO_2	7.82	0.01	16.2	0.1	136	2400.2	67.1	946.7	39.7	11
T × CO_2	7.81	0.01	19.1	0.1	118	2442.2	42.0	1048.5	45.9	10

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T × CO_2 = combined elevated temperature and elevated CO_2 treatment, TA = total alkalinity, *n* = number of measurements for each variable.

Table S2: Analyses of variance of the effects of temperature (target: 16 °C and 19 °C) and CO_2 (target: 400 and 1000 μatm) on time to accept food under facilitated feeding (see Fig. 2b); data was log transformed to adjust for positive skew in distribution of the data.

Source	DF	MS	F	P
Feeding				
T	1	0.2251	1.3962	0.244
CO_2	1	0.1267	0.7858	0.378
T × CO_2	1	0.6726	4.172	**0.049**
Residual	42	0.1612		
Total	45			

Pairwise test on T × CO_2		
Within levels:		P
Ambient CO_2:	Elevated temperature < ambient temperature	**0.021**
Elevated CO_2:	Elevated temperature = ambient temperature	0.538
Ambient Temp:	Elevated CO_2 = ambient CO_2	0.131
Elevated Temp:	Elevated CO_2 = ambient CO_2	0.276

See legend of Table S1 for abbreviations. Degrees of freedom (DF), mean squares (MS), the F-ratio (F), *p*-value (P). Bold values indicate significance at $p < 0.05$.

Table S3: Analyses of variance of the effects of temperature (target: 16 °C and 19 °C) and CO_2 (target: 400 and 1000 μatm) on (a) total swimming distance during the olfactory trial (see Fig. 1b), and (b) on the cumulative duration of time spent in the prey zone based on chemical cues (see Fig. 1a).

Source	DF	MS	F	P
(a) Swimming distance				
T	1	2405.2	7.5094	**0.007**
CO_2	1	8.05×10^2	2.51×10^{-4}	0.987
T × CO_2	1	2.54×10^2	7.94×10^{-5}	0.992
Residual	85	320.29		
Total	88			
(b) duration in prey zone				
T	1	95595	1.942	0.166
CO_2	1	1.17×10^5	2.3741	0.127
T × CO_2	1	6.56×10^5	13.334	**0.001**
Residual	85	49224		
Total	88			

Pairwise test on T × CO_2		
Within levels:		P
Ambient CO_2:	Elevated temperature > ambient temperature	**0.001**
Elevated CO_2:	Elevated temperature = ambient temperature	0.111
Ambient Temp:	Elevated CO_2 = ambient CO_2	0.200
Elevated Temp:	Elevated CO_2 < ambient CO_2	**0.0002**

T = elevated temperature treatment, CO_2 = elevated CO_2 treatment, T × CO_2 = combined elevated temperature and elevated CO_2 treatment, TK = tank nested within T × CO_2 interaction term. Degrees of freedom (DF), mean squares (MS), the F-ratio (F), *p*-value (P). Bold values indicate significance at $p < 0.05$.

CHAPTER 4

STATEMENT OF AUTHORSHIP

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Principal Author

Name of Principal Author	Jennifer .C.A. Pistevos		
Contribution to the Paper	Contributed to the conception and design of the project. Conducted the study, collected and analysed the data and drafted and revised the manuscript.		
Overall percentage (%)	85		
Signature		Date	04/07/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	04/07/2016

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Contribution to the Paper	Contributed to the conception and design of the project and assisted with the development and revision of the manuscript.		
Signature		Date	04/07/2016

Name of Co-Author	Sean D Connell		
Contribution to the Paper	Contributed to the conception and design of the project, assisted with the development and revision of the manuscript.		
Signature		Date	04/07/2016

CHAPTER 4

OCEAN ACIDIFICATION ALTERS SENSING OF TEMPERATURE AND SALINITY IN LARVAL FISH

Short Title: Altered temperature and salinity sensing

4.1 ABSTRACT

Ocean acidification alters the way in which animals perceive and respond to their world by affecting a variety of senses such as audition, olfaction, vision and pH sensing. Marine species rely on other senses as well, but we know little of how these might be affected by ocean acidification. We tested whether ocean acidification can alter the preference for physicochemical cues used for dispersal between ocean and estuarine environments. We experimentally assessed the behavioural response of a larval fish (*Lates calcarifer*) to elevated temperature and reduced salinity, including estuarine water of multiple cues for detecting settlement habitat. Larval fish raised under elevated CO_2 concentrations were attracted by warmer water, but temperature had no effect on fish raised in contemporary CO_2 concentrations. In contrast, contemporary larvae were deterred by lower-salinity water, where CO_2-treated fish showed no such response. Natural estuarine water – of higher temperature, lower salinity, and containing estuarine olfactory cues – was only preferred by fish treated under forecasted high CO_2 conditions. We show for the first time that attraction

by larval fish towards physicochemical cues can be altered by ocean acidification. Such alterations to perception and evaluation of environmental cues during the critical process of dispersal can potentially have implications for ensuing recruitment and population replenishment. Our study not only shows that freshwater species that spend part of their life cycle in the ocean might also be affected by ocean acidification, but that behavioural responses towards key physicochemical cues can also be negated through elevated CO_2 from human emissions.

4.2 INTRODUCTION

The sensory sensitivity (the ability to correctly identify a cue) and relevant responses of an organism to its physicochemical environment are often critical to their survival and therefore can also have population-level consequences (Réale et al. 2007; Hay 2009). This sensory capacity is particularly important for species that need to transition from one contrasting environment to another in order to complete their life cycle. Indeed, life-history transitions across marine, estuarine and freshwater environments typically involves the use of sensory cues to these vastly different systems (Kingsford et al. 2002; Huijbers et al. 2012; Igulu et al. 2013). The range of physiological adaptations and life histories have allowed certain species, like those that utilise estuaries, to become quite robust to dramatic environmental changes to allow them to exploit a wider range of habitats for maturation and reproduction (Allen et al. 2006; Feyrer et al. 2015). Ocean acidification is predicted to have a significant impact on marine organisms (Doney et al. 2009; Nagelkerken & Connell 2015) and several studies have shown negative effects on their metabolism and calcification (Fabry et al. 2008) through to behaviour (Briffa et al. 2012, Nagelkerken & Munday 2016). However, few studies have looked at the impact of ocean acidification on estuarine species (Gillanders et

al. 2011). Potential impacts on early life history of estuarine species can affect dispersal and connectivity, and as such population size and ultimately food-web interactions.

A critical phase in the demography of fish is the dispersal phase of oceanic larvae. This does not only determine the structure of metapopulations, but also their population replenishment (Cowen 2002; Kinlan & Gaines 2003; Sale 2004; Cowen et al. 2006) . Dispersal in oceanic larvae is mediated by their sensory abilities (such as detecting relevant cues through olfaction or audition) and associated behaviours in response to the presence of informative environmental cues that provide information about the location of suitable habitat onto which to settle and complete their adult life (Leis 2006). Larvae are highly sensitive to environmental cues and often selective over where they settle via sensory organs which are well developed by the time of metamorphosis and settlement (Myrberg and Fuiman 2002). The chemical environment in particular, offers key cues for sensing settlement habitat by larvae. Recent studies, however, have indicated that ocean acidification reduces the ability of some coral reef fishes to distinguish relevant odour cues of habitats and predators (Munday et al. 2009; Dixson et al. 2010; Briffa et al. 2012; Leduc et al. 2013; Chivers et al. 2014). What is less well understood is whether such responses to ocean acidification also apply to environmental cues (e.g. temperature), particularly for species that spend only a small proportion of their life in the marine environment, such as catadromous species.

Catadromous fish spend most of their adult lives in freshwater, and then move downstream through estuaries and to coastal marine areas to breed. The capacity of their larvae to sense and respond to habitat-associated cues is critical for their transition from seawater to their freshwater adult habitats. Compared to ocean environments, the intermediary environment of estuaries experiences elevated and fluctuating CO_2 levels due to alterations in freshwater inputs, tidal exchange and eutrophication (Feely et al. 2010; Gillanders et al. 2011; Hofmann

et al. 2011). It is anticipated that organisms that routinely experience such contrasting and often fluctuating environments, may be more resistant to anthropogenic ocean acidification (Smith 1983). Indeed, the catadromous cobia, *Rachycentron canadium*, are resistant to forecasted rises in CO_2 in traits such as growth, development, swimming ability and swimming activity (Bignami et al. 2013). Because of their developmental shifts between marine and freshwater environment, such species are responsive to changes in water temperature and salinity (Edeline et al. 2006; Serrano et al. 2010), but we do not know whether the preferences for these cues can be affected by ocean acidification.

Using the commercial barramundi (*Lates calcarifer*) as a model species we assessed the effect of increasing carbon dioxide concentration on the preference of their marine larvae towards habitat-related cues (chemical, temperature and salinity cues) critical to their transition from a coastal-marine to a benthic-estuarine life stage. Short-term fluctuations in CO_2 concentrations in estuaries can be greater than the long-term and more gradual changes anticipated via ocean acidification models (Feely et al. 2010; Gillanders et al. 2011; Hofmann et al. 2011). Hence, we assess the hypothesis that ocean acidification has little effect on physicochemical and olfactory preference in barramundi that use their senses for successful dispersal between ocean and estuarine environments, because they are adapted to these high levels of CO_2 in their natural environment. This hypothesis was assessed by testing the prediction that larvae retain the same ability to sense and respond to their physicochemical environment under future levels of elevated CO_2 as their counterparts would under today's concentrations.

4.3 MATERIALS AND METHODS

4.3.1 ETHICS STATEMENT

Research was carried out under approval of the University of Adelaide animal ethics committee (permit: S-2012-171) and according to the University's animal ethics guidelines.

4.3.2 STUDY SPECIES

Barramundi, *Lates calcarifer* (Boch), is a fish species widely distributed throughout coastal areas and rivers of the tropics (eastern Africa through Asia to eastern Australia and southern Japan), supporting commercial, indigenous and recreational fishers (Keenan 1994; Balston 2009). It is a large catadromous, euryhaline species whose eggs and larvae develop in coastal waters and requires high salinity water to reproduce (Russell et al. 2004). Juveniles settle into mangroves and wetland habitats (Moore 1982) before moving into estuaries and progressing into the freshwater reaches of rivers and creeks (Russell et al. 2004).

Fish larvae were obtained at 11 days post-hatching from a commercial hatchery (Robarra Pty Ltd - Broodstock Sanctuary and Hatchery) and were of a 7th generation broodstock from a single spawning event. The broodstock consisted of ~120 adult individuals, and larvae obtained from the spawning event consisted of a random mix from the entire spawning event. Ideally wild caught larvae would have been used however, this wasn't feasible, the use of 7th generation commercial larvae would mean they are not exposed to natural selection processes giving rise to more robust genetic mix. However, the number of adult individuals used provides a large pool of gene mixing allowing for variation in responses, additionally the study is testing for the mechanisms rather than their adaptation potential. Larvae were

reared at The University of Adelaide in a duplicated recirculating larval rearing system using seawater of 27 °C and a salinity of 35 ppt.

For each treatment (control vs. elevated CO_2) there were two replicate food-safe 80 l tanks. Multiple larvae were held within the same tank and were treated as replicates given the wide variation among individuals as a function of differences in personality (Norin et al. 2016), which is likely to decrease rather than increase Type I error rates. Tanks were placed in temperature-controlled water baths that were heated using standard glass aquarium heaters. Fish larvae were exposed to either control (~400 µatm) or elevated CO_2 (~1400 µatm), both at a temperature of 27 °C and a salinity of 35 ppt (Table 1). This temperature and salinity was chosen as it reflects the conditions under which barramundi eggs typically develop in nature (Katersky and Carter 2007). CO_2 concentrations in the seawater were maintained by means of a pH controlling solenoid valve (ceramic pH controller, Sera® precision). The pH_{NBS} and temperature of each tank was measured daily using a pH meter which was calibrated daily with fresh buffers (pHep®, HANNA). Oxygen levels were maintained above 90% saturation and were measured daily within the tanks with an O_2 oxygen probe (Handy Polaris, 2 OxyGuard ®). Total alkalinity of the seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken weekly from each of the four tanks. Alkalinity standards were accurate to within 1% of certified reference material from Dr. A. Dickson (Scripps Institution of Oceanography; Langdon et al. 2000). Average seawater pCO_2 (Table 1) was calculated using CO2SYS using already established constants (Mehrbach et al. 1973; Dickson and Millero 1987).

Larval fish were fed with rotifers on the first day and then with *Artemia nauplii* until they reached settlement stage at the end of the experiment (22 days post-hatching). Behavioural trials were done on larvae of 18–21 days old, meaning they had been exposed to elevated

CO_2 for at least 7 days. Previous studies have shown that 3-4 days of high CO_2 exposure already leads to behavioural changes in fishes (Munday et al. 2009, 2010; Simpson et al. 2011; Briffa et al. 2012; Dixson et al. 2014). Although larvae were not exposed to high CO_2 from the egg stage onwards we believe this makes our conclusions more conservative given the small window of exposure. Indeed, our experience shows that when barramundi larvae are raised in high CO_2 water from day 0, the sensory effects are similar (Rossi et al. 2015) to what we report here. Furthermore, the additional exposure (i.e. during the first 7 days post-hatching) is more likely to exaggerate rather than ameliorate the detected effects as the egg-stage is more sensitive to ocean acidification than the post-hatch stages (Baumann et al. 2012). There is also currently no evidence for fish to exhibit any genetic, epigenetic, or physiological adaptation to acidification over such short time spans.

4.3.3 Behavioural Choice Tests

To assess the effect of elevated CO_2 on larval ability to discriminate between water plumes of different temperature, salinity and olfactory cues we used a two-channel choice flume (13 × 4 cm) developed by Gerlach *et al*. (2007). Inside the flume larval fish can freely swim and choose between two different water sources that continuously flow from one end to the other end of the flume. We used a gravity-driven flow that was controlled by flow meters (100 ml/min per channel) with dye tests performed at each water change ensuring distinct and parallel water flow with no disturbance from either channel (Munday et al. 2009). As indicated by Dixson *et al.* (2010) using either acidified or control water in the flow meters did not affect behaviour choice when tested without any cues and thus control water was used throughout all choice trials. Trials were performed following Munday et al. (2009) where an individual larval fish was placed at the downstream end of the flume and left to acclimate for 2 minutes while exposed to both water flows with different cues and able to

explore both sides of the flume. After acclimation, the position of the fish was visually recorded as either at the left or the right-hand side of the flume for 2 minutes at 5 second intervals. This was following by a 1-minute rest period during which the water sources were switched sides (controlling for any side preferences) after which the entire process including acclimation was repeated (Munday et al. 2009). Unresponsive fish that did not actively swim during the acclimation time were not used (N = 1 from the control treatment) and each fish was used once. To avoid the observer interfering with the choice behaviour of the fish, the observer sat motionless at the back of the flume; i.e. the opposite direction to the flowing water in which fish orientated.

Three experiments tested the response of fish larvae towards 1) elevated water temperature, 2) reduced salinity, and 3) estuarine water of elevated temperature and reduced salinity. We first tested the individual effects of two key and novel cues (temperature and salinity) with the third treatment mimicking estuarine water with all of its relevant abiotic and biotic cues vs. control oceanic water as fish would experience in nature. This latter combination of temperature and salinity provided an ecologically relevant context for interpretation (i.e. to test prediction) of the first two tests that isolated these factors (i.e. to test mechanisms). Larval barramundi are able to sense multiple cues of estuarine waters for locating estuaries for settlement beyond their~ 3-week pelagic larval stage (Mukai et al. 2007). Chemical cues, such as lower salinity, provide a continental signal due to mixing with river outflow, estuary and run off (Sabatés 1990). Compared with coastal waters, estuarine water is typically warmer (due to their shallow depth) and of lower salinity (due to river inflow) but can also be hypersaline (Smith 1983, Melzner et al. 2009). Therefore, freshly-collected estuarine water containing biological as well as physico-chemical cues was used to test the difference in response of larval fish from control vs. elevated CO_2 treatments. In each of the above three experiments, the control constituted of seawater of 27 °C with a salinity of 35. Tests of

temperature alone involved larval fish choosing between seawater at ambient (27 °C) vs. elevated (30 °C) temperature (both at ambient salinity of 35). Tests of salinity alone involved larval fish choosing between seawater at ambient (35) vs. reduced (25) salinity (both at ambient temperature of 27 °C). Tests that combined both factors involved larval fish choosing among control seawater at ambient temperature (27 °C) and ambient salinity (35) vs. fresh estuary water at elevated temperature (30 °C) and reduced salinity (25). The latter reflected the *in situ* conditions of the estuary water at the time of collection in December 2012.

The environmental conditions for rearing and testing were set at current day (~400 µatm) and elevated CO_2 concentrations (~1400 µatm) as forecast for estuarine environments (Feely et al. 2010; Gillanders et al. 2011; Hofmann et al. 2011). Natural sand-filtered seawater was used for the rearing as well as the experiments and was collected several kilometres offshore (at 10 m depth) from West Beach, Adelaide; the same source of seawater used by the broodstock facility. Salinity was reduced in the experiments using purified tap water. Use of tap water could have changed aspects of water chemistry other than salinity, but we do not believe that this has affected our results because the procedure was performed on both treatments; hence the only interpretable difference between treatments was salinity. Water temperature was regulated using standard glass aquarium heaters. Estuarine water was collected from Barker Inlet, South Australia and used in the flume experiment on the same day of collection.

Subsequent to the behavioural tests, individual fish were euthanized using clove oil and frozen for use in identifying development stages under a dissecting microscope.

Table 1. Summary of the seawater chemistry parameters (mean (± SE) in the control and elevated CO_2 treatments of the larval fish rearing tanks.

CO_2 treatment	Temperature (°C)	pH_{NBS}	N	TA (µmol.kg^{-1}SW)	pCO_2 (µatm)*	N	Salinity	N
Control	27.2 (± 0.1)	8.13 (± 0.02)	31	2259 (± 20)	465 (± 32)	4	35.2 (± 0.1)	22
Elevated	27.2 (± 0.2)	7.70 (± 0.01)	31	2265 (± 20)	1477 (± 12)	4	35.0 (± 0.1)	22

TA = total alkalinity; * = pCO_2 calculated using CO_2SYS

4.3.4 STATISTICAL ANALYSIS

We used a log-linear model to test the relationship between the number of individuals in each of the developmental stage, day post hatching (dph) and CO_2 treatment. Initiating from a saturated model (containing all main effects and their interactions), higher order terms were removed from the model until there was a significant increase in deviance from one model to the next.

Side preferences of the fish within the flume chamber was controlled by switching the flows half way through the trial and each subsequent fish tested with the cue flow starting at the opposite side to the fish before. Attraction or deterrence towards the relevant cues was then determined by testing each distribution of percentages of time spent in the half of the flume chamber close to the relevant cue against the threshold for random response set at 50%. Percentage data were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$); therefore a non-parametric One-Sample Wilcoxon Signed Rank Test was used. Power analysis for a Wilcoxon signed-rank test was conducted in G*Power to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, a large effect size ($dz = 0.8$), and one tail. Based on the aforementioned assumptions, the desired sample size is 12. Thus, the

chances of a Type II error are very small. To compare the responses between untreated and CO_2-treated fish, a two-way ANOVA was performed for the estuary cue using CO_2 and DPH as factors and with Tank nested in CO_2 initially, but because there was no effect of DPH ($p=0.2981$), nor Tank ($p = 0.2353$) the test was rerun as a single factor ANOVA. For tests of temperature, we initially tested for Tank effects nested in CO_2, but no effect was found ($p = 0.7057$) and the analysis was pooled to a single factor ANOVA. For tests of salinity cues, one-way ANOVA tested the effect of CO_2 as a single factor.

4.4 RESULTS

Elevated CO_2 did not affect the timing of metamorphosis in larval barramundi (16–22 days old) used for the choice experiments. The log-linear model showed that developmental stage was dependent on days post hatching and not CO_2 treatment. The best fitting model contained the interaction between developmental phase and DPH (Likelihood chi-square = 5.246, d.f. = 14, $p = 0.982$). Removal of the three way interaction between developmental phase, DPH and CO_2 treatment did not lead to a significant increase in deviance. However, removal of the two way interactions involving developmental phase caused a significant increase in deviance. Removal of the interaction between developmental phase and DPH had a significant effect on the model deviance (Chi-square = 60.15, d.f. = 14, $p < 0.001$) while removing the interaction between developmental phase and CO_2 treatment did not. At 22 days post-hatching, the remaining control ($n = 6$) and elevated CO_2-treated ($n = 5$) larval barramundi that had not been used for the experiments had all undergone complete metamorphosis, indicating the initiation of the settlement stage where they start associating with benthic habitats.

Pre-settlement fish larvae exposed to elevated CO_2 showed a significant attraction towards warmer water (Fig. 1a; $p = 0.001$, Wilcoxon signed rank test), whereas control fish showed neither an attraction nor a deterrence to this physical cue ($p = 0.393$). Also the response between control and CO_2-treated fish was significantly different (Table S1; ANOVA: $F_{1,23} = 6.249$, $p = 0.020$).

The response towards water of lower salinity differed significantly between control and CO_2-treated larvae (Table S1; ANOVA: $F_{1,22} = 11.21$, $p = 0.004$), but in this case the control fish showed a significant deterrence (Fig. 1b; $p = 0.011$, Wilcoxon signed rank test) and the CO_2-treated fish showed no response ($p = 0.155$) towards water of lower salinity.

Finally, whereas control fish were not attracted ($p = 0.120$) during their pre-settlement stage towards estuarine water of higher temperature, lower salinity and containing biological olfactory cues, the CO_2-treated fish showed positive attraction (Fig. 1c; $p < 0.001$, Wilcoxon signed rank test). The attraction towards estuarine water also differed between control and CO_2-treated larvae (Table S1; ANOVA: $F_{1,71} = 13.07$, $p < 0.001$).

4.5 DISCUSSION

We show that elevated CO_2 can alter the preference of larval fish towards physicochemical cues such as water temperature and salinity. Ocean acidification has previously been shown to alter a variety of animal behaviours that are related to cognition and physiology, such as lateralization, activity levels, swimming behaviour, learning, boldness, schooling,

reproduction, and foraging (Nagelkerken & Munday 2016; Leduc et al. 2013). The main inhibitory receptor in the vertebrate brain is the $GABA_A$ neuroreceptor which has been linked as a driver of modified behaviours due to its altered functioning under elevated CO_2 (Smith 1983; Nilsson et al. 2012; Hamilton et al. 2014). This also affects multiple sensory modalities in larval as well as post-settlement stage fishes, such as audition (Simpson et al. 2011; Rossi et al 2015), vision (Ferrari et al. 2012; Chung et al. 2014), olfaction (Munday et al. 2009; Pistevos et al. 2015), and pH sensing (Caprio et al. 2014). We show that preference for temperature and salinity cues can also be modified by ocean acidification, although it remains unclear whether specific senses themselves are affected or behavioural preferences are modified. Temperature sensing occurs peripherally and centrally in fish, likely performed by highly responsive and rate sensitive free nerve endings (Crawshaw and Podrabsky 2011). Changes in salinity can be perceived through the process of osmosensing which is coordinated via neuronal, endocrine, paracrine and autocrine signals emanating from specialised cell types on the skin (osmoreceptor cells) that are highly sensitive towards osmolarity change (Kültz 2012). Additionally, there are indications that calcium polyvalent cation-sensing receptors (CaRs) act as salinity sensors in teleost fish (Nearing et al. 2002). Calcium is involved in olfactory signalling (itself being an odourant for fish (Bodznick 1978; Hubbard et al. 2000). Both water temperature and salinity cues are sensed by fishes and associated neurological information is processed and coordinated through the neural system (i.e. via $GABA_A$) and therefore has the potential to be affected by high CO_2 leading to altered sensory responses or cue preferences.

If the sensory sensitivity and responses of an organism to its physicochemical environment is critical to the successful completion of its lifecycle, then disturbances that interfere with such decision making could potentially have negative implications for population replenishment. Temperature and salinity cues create environmental gradients that can be

sensed over long distances from their source and are therefore potentially important directional cues for shoreward-navigating oceanic larvae (Arvedlund and Kavanagh 2009). Temperature is a predominant factor determining habitat selection, also guiding daily and seasonal movements in many fish species (Smith 1983; Crawshaw and Podrabsky 2011), whereas salinity influences the assembly of euryhaline fish assemblages across entire estuarine systems. Temperature and salinity can have strong effects on somatic growth (Ong et al. 2015) and therefore being able to select habitats of appropriate temperature and salinity is central to future growth, especially with a changing climate.

During their pre-settlement pelagic stage, larvae from the control treatment were not attracted to environmental cues related to their consecutive post-settlement environment (i.e. warmer water, lower-salinity water, and estuarine water). Ocean acidification reversed this response and this effect was not related to a different rate of metamorphosis. Similarly, Simpson *et al.* (2011), established that pre-settlement coral reef fish larvae avoid reef sound cues during their early planktonic stage, but ocean acidification reverses avoidance to attraction. Non-responsiveness of pelagic larvae to cues related to benthic habitats relevant to consecutive life stages is a strategy to avoid early entry into a habitat with potentially high predation rates (Leis 1991). It is only during the settlement stage when larvae change their oceanic life phase to a benthic life phase that they become sensitive to benthic habitat cues (Kingsford et al. 2002) for which this sensitivity may only last a few days (Rossi et al. 2015). Although evidence is lacking to confirm the mechanisms by which estuaries act as cues for settlement (i.e. temperature, salinity and other estuary cues), the mere fact that larvae raised under high CO_2 were attracted to these cues suggests a maladaptive response to environmental cues due to ocean acidification. Hence, an avoidance behaviour towards post-settlement habitats during the pre-settlement stage might have consequences for the survival of fish larvae and potentially alter connectivity patterns and their population dynamics

(Milton 2009). In their model of larval dispersal and recruitment, Codling et al. (2004) showed that survival of larvae was highly sensitive to both the sensing and orientating abilities of the larvae. We here show that physicochemical cues that would normally help guide pelagic larvae towards their benthic habitats during settlement become attractants due to ocean acidification during a life phase at which they do not yet respond to such cues.

Estuarine fish such as barramundi would be expected to be more tolerant to rapid environmental fluctuations because of their adaption to the variable environments in which they spend their lives (Smith 1983; Able 2005; Shaw et al. 2013) and their occupation of a range of freshwater and marine habitats. Nevertheless, we show that pre-settlement stages of euryhaline catadromous species are also detrimentally affected in key behaviours by elevated CO_2, just like more sensitive marine and oceanic fish species. Although the timing of responsiveness towards habitat cues for settlement is poorly known in most fish (Leis et al. 2011), some studies have shown that there is a narrow window of competency for settlement Thresher et al. 1989; Wellington & Victor 1989) that can be affected by elevated CO_2 (Rossi et al. 2015). Because barramundi have adult populations that live in freshwater as well as marine habitats (Pender & Griffin 1996) failure in the ability of their larvae to properly respond to estuarine habitat cues might potentially affect the biogeography of adult populations in marine vs. freshwater environments. This could have consequences for fisheries productivity and food web dynamics considering their trophic position as high-trophic-order carnivores (Glencross 2006).

Our study reveals that ocean acidification may not only alter physicochemical preferences in fish larvae, but can also alter potential connectivity between freshwater and marine environments for catadromous or anadromous species. Furthermore, the hypothesis that ocean acidification has little effect on physicochemical preference in a species robust to

fluctuating environmental conditions, including high CO_2, is not supported. This result is particularly profound because these catadromous fish rely on their senses for dispersal between ocean and estuarine environments. A disruption of the sensory sensitivity due to altered preferences and responses has profound implications for the completion of life-cycles, particularly those species that must navigate across contrasting environments to replenish their populations.

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4.6 FIGURES

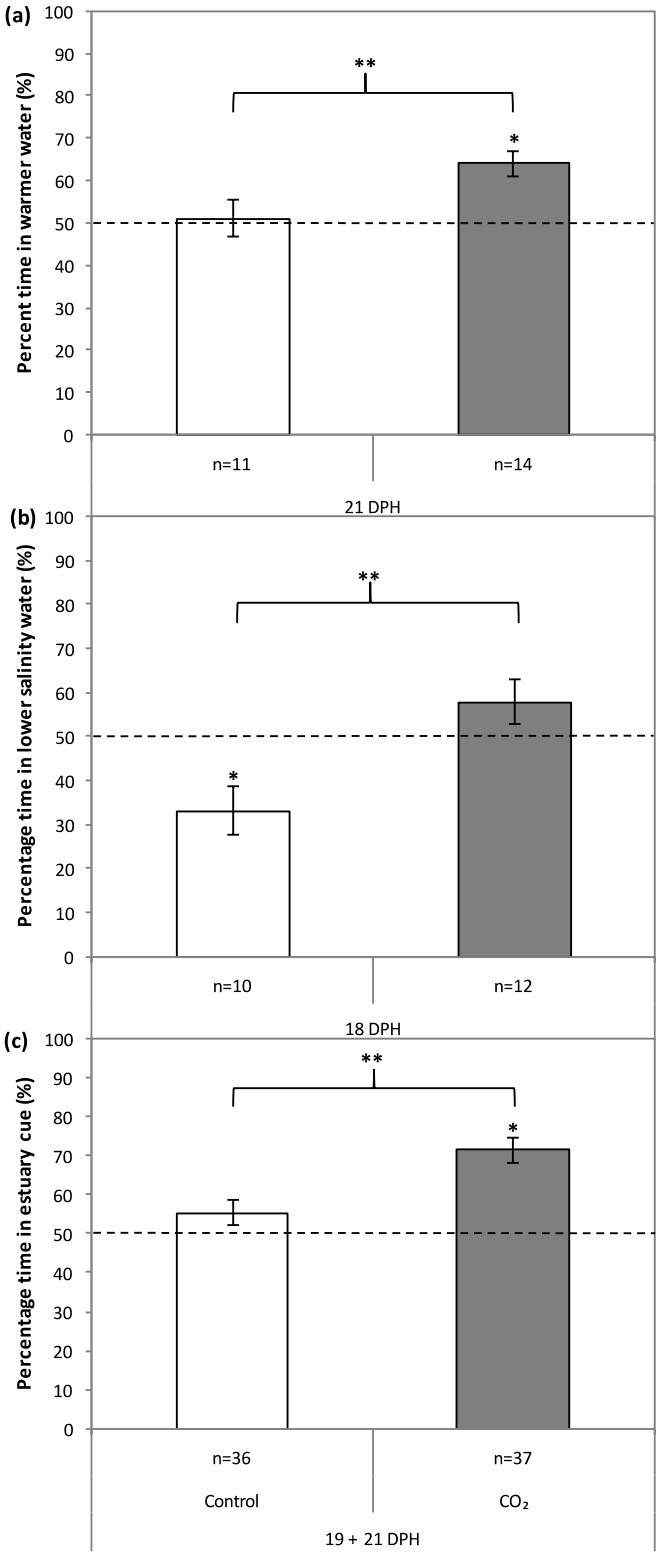

Fig. 1 Effect of ocean acidification on larval fish choice preferences between cue and control water. Mean (± SE) percentage of time spent in **(a)** seawater with elevated

temperature (+3 °C), (Control n: 11, CO_2 n: 14) **(b)** seawater with reduced salinity (–10 units) (Control n: 10, CO_2 n: 12), and (**c)** estuarine water with both elevated temperature (+3 °C) and reduced salinity (–10 units) (Control n: 36, CO_2 n: 37). Dashed line indicates 50% choice. * indicates significant differences to a random response of 50% (Wilcoxon signed rank test). ** indicates significant differences between treatments ($p < 0.05$, ANOVA; Table S1).

4.7 SUPPLEMENTARY INFORMATION

Table S1. Analysis of variance for the effects of elevated CO_2 on the choice preference of larval fish

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
(a) estuary water					
Between Groups	4713.8	1	4713.80	13.07	**0.0005**
Within Groups	25606.0	71	360.64		
Total	30320	72			
(b) salinity					
Between Groups	3646.6	1	3646.6	11.21	**0.004**
Within Groups	7157.8	22	325.36		
Total	10804	23			
(c) temperature					
Between Groups	948.43	1	948.43	6.249	**0.0201**
Within Groups	3339	22	151.77		
Total	4287.5	23			

CHAPTER 5

CHAPTER 5

GENERAL DISCUSSION

5.1 GENERAL DISCUSSION

The aim of this thesis was to provide a deeper mechanistic understanding into how ocean warming and acidification alone or in combination, will affect vital behavioural processes, sensory ecology and metabolic functioning in the early life history of predatory fish and ultimately fish populations, by the end of the century (Nagelkerken & Munday 2016). While we know that elevated temperature and ocean acidification affect fish in different ways in isolation (Perry et al. 2005, Pörtner & Knust 2007, Munday et al. 2009, Dixson et al. 2010) less is known on the combined effects of these two stressors and less so on how it would affect predatory species' performance and subsequently their influence over their respective ecosystems. This thesis provides new knowledge by filling some of these gaps through the provision of evidence of altered predatory behaviours such as reduced or altered hunting and foraging performance, reduced environmental assessment and reduced growth due to global warming and ocean acidification. A reduced performance and persistence of larval or juvenile predatory fish could undermine the resilience and sustainability of fish populations through reduced growth and reduced population replenishment (via altered sensory functioning of recruiting larvae). This thesis has also advanced insights into the potential impact of climate change on trophic cascades by indicating a reduced predatory performance that could weaken a top-down control over prey communities.

5.2 THE EFFECT OF CLIMATE CHANGE AND OCEAN ACIDIFICATION ON EARLY LIFE FISH BEHAVIOUR

Studies have indicated that ocean acidification can alter numerous behavioural traits (Munday et al. 2009, Simpson et al. 2011, Ferrari et al. 2012). These effects are likely to be driven by disruption of the neurological functioning of the $GABA_A$ neuroreceptor (Nilsson et al. 2012) as a result of elevated CO_2. While the effects of ocean acidification on tropical prey fish behaviour are well-studied (Munday et al. 2009, Simpson et al. 2011, Ferrari et al. 2012) less is known on predatory species and less so on temperate species. While more is known on the effect of elevated temperature on fish there are few studies looking in combination with elevated CO_2. To date only one previous study looked at the combined effect of elevated temperature and ocean acidification on tropical sharks (Rosa et al. 2014). Our study on the Port Jackson shark was the first study (Chapter 2) to assess at the combined effects of ocean warming and acidification on temperate shark behaviour and development. Additionally, it was the only study that combined both long-term laboratory and mesocosm experiments containing natural prey and habitat. Our study provided the first insight into within-generational acclimation potential by using sharks from their embryo stage right through to their juvenile stage while exposing them to two global stressors. While laboratory experimentation with *ad libitum* feeding indicated that temperature increased food consumption and higher growth rates, when placed in mesocosm and left to hunt on their own, there was a significant growth reduction in the elevated CO_2 treatments, suggesting interference with hunting behaviour. The mesocosm studies indicate that under future climatic conditions sharks face the potential of starvation, which is already a natural stressor

influencing survival in early life fish (Houde 1989) This early life-history alteration indicates the importance of studying animal behaviour in a natural setting as small laboratory settings can vastly contrast these results. We showed that ocean acidification affected hunting behaviour in sharks, and this alteration led to considerable reductions in growth rates of sharks. Ultimately, this could lead to cascading effects on trophically-structured systems through a reduced top-down control.

We also studied the interactive effects of ocean acidification and temperature on shark hunting performance (Chapter 3). While several studies indicate a negative effect of ocean acidification on behaviour (Munday et al. 2009, Nagelkerken and Munday 2016), we show that acidification had a negligible effect as an isolated stressor but when in combination with temperature, negated the positive effects of elevated temperature. While in our first study (chapter 2) we found an effect of CO_2 in isolation with regards to foraging (reduced olfactory response) and thus reduced growth, in contrast our second study (chapter 3) we showed no effect of CO_2 in isolation with only elevated temperature having an isolated effect by increasing activity and motivational drive. The main difference in the two studies was the age of the sharks, which could potentially account for the difference in the results of the two studies – highlighting the need to explore various life stages when conducting predictive studies as results may differ. While our first shark study was focused on sharks that were a few months old, in our second study sharks were tested when they had just hatched. Hatching rate was dependent on temperature with sharks exposed to elevated temperature hatching sooner. Hatching occurs once the shark has depleted the yolk thus the need to find food is the motivating factor to exit the egg (Rodda & Seymour 2008). With temperature accelerating hatching the main motivation is to feed and feed sooner due to the increased energetic demand, hence the increased activity and time spent in the prey zone. CO_2 had no

effect on shark hatching and thus sharks under elevated CO_2 alone responded similarly to control sharks. This non-independence highlights the need of multi-stressor studies on multiple traits that provide the ability to identify antagonisms and synergisms that can negate or exacerbate predictions based on single factor experiments and single behavioural responses (Nagelkerken and Munday 2016).

Additionally, we show that barramundi (chapter 4), a highly commercial predatory species, will be affected by ocean acidification, by alteration of key physicochemical sensory perception used for dispersal between ocean and estuarine environments. This finding is novel as no studies to date have shown any alteration in physicochemical sensing in fishes in any stage as a result of ocean acidification. We discovered a new suite of senses affected by elevated CO_2. This finding was unexpected as barramundi were expected to be more tolerant due to their adaptation to the fluctuating environments where they spend their adult lives and their high ability to rapidly shift between waters of different salinities (Able, 2005; Melzner et al. 2009; Shaw et al. 2013; Hofmann et al. 2011). This alteration is important as alterations to perception and evaluation of environmental cues during the critical process of dispersal have implications for ensuing recruitment and population replenishment. Comparable to the sharks, barramundi grew faster under unlimited food supply (Rossi et al. 2015). However, it is likely than when they need to hunt for their food, their growth rates will be slower due to negative effects on their olfactory (this study) and auditory (Rossi et al. (2016). This could potentially lead to alterations in local food webs due to their position as higher order predators, and ultimately affect their fisheries

5.3 ECOLOGICAL IMPLICATIONS

The studies in the present thesis illustrate how predatory fish in general might respond to rising ocean warming and combined acidification. The main implication is a likely reduced predator influence over structuring of prey communities. Although prey species are also negatively affected by CO_2 (see Dixson et al 2010, Ferrari et al 2012, Munday et al. 2009) it remains to be seen how predator-prey interactions will influence communities under the influence of combined future climate change when they will both be affected and needs further study. Within an ecosystem, predation governs the energy flow as it regulates both growth and mortality rates of species (Benoit & Rochet 2004). Sharks and barramundi as higher order predators exert significant influence over their ecosystem by preying upon other organisms and the same is true for barramundi. With a smaller body size, as found in our shark study (chapter 2), there is less scope for exerting this top-down influence as size determines the size of prey taken (in gape-limited predators), how much food can be gathered, how much energy is required to meet basic metabolic demands and importantly, its own vulnerability to predation (Cohen et al. 1993). As predators naturally consume prey smaller than themselves, and larger prey will consume a wider range of prey sizes than smaller predators (Wilson 1975) body size can be used for ordering in a cascade model (Cohen et al. 1993) of a trophic ecosystem. As a consequence of ocean acidification and global warming, smaller predators can alter this balance within the trophic system resulting in a shift in species dominance. After species range shifts to higher altitudes and latitudes and seasonal shifts in life cycle events, the third ecological response in aquatic ecosystems to global warming is said to be reduced body size (Daufresne et al. 2009, Gardner et al. 2011). A model simulation by Lefort et al. (2015) using the RCP8.5 scenario found that at low and mid-latitude areas, biomass and maximum body size will strongly decrease. This

was attributed to larger organisms being unable to maintain their high metabolic needs because of limited and declining food availability (Lefort et al. 2015). Several studies have also indicated that global warming will result in reduced fish sizes (Thresher et al. 2007, Todd et al. 2007, Daufresne et al. 2009, Sheridan & Bickford 2011, Cheung et al. 2012) with some predicting a reduction by 14-24% in average body weight globally from 2000 to 2050 under a high emission scenario (Cheung et al. 2012). Indeed, our studies reflect a similar reduction, however through an additional altered response to climate change such as reduced predator performance (reduced foraging and hunting ability) attributed to elevated CO_2 rather than just warming alone, resulting in smaller sharks.

Further study of predatory teleost fish needed to explore the potential for acclimation and/or adaptation due to their faster growth strategies (especially when considering longer lived species such as sharks) as well as added stressors such as eutrophication. Longer term studies spanning not only a few months, but years is required to determine true acclimation potential for both teleost and elasmobranch fish. Although the thesis tries to cover the mechanisms that are affected that could easily be extrapolated to other taxa it certainly would be advantageous to use different predatory species, especially to explore alternative strategies used by other species to compensate for reduced functioning due to climate change.

5.4 FUTURE RESEARCH

I have shown how predator hunting and foraging can be affected by ocean warming and acidification in simple laboratory experiments as well as larger mesocosms involving realistic habitats and prey. However, even these have limitations in predictions. I can only infer a reduced predatory influence on the ecosystem based solely on poor performance of

the predator as a result of testing specific behavioural responses to artificially placed cues. To get a more realistic insight into the impact of these behavioural alterations on the food webs and any subsequent trophic cascades we would need to include several predatory and prey species and assess their interactions. Indeed, a recent study by Nagelkerken et al. (2015) which focused on ocean acidification effects only, used several vent sites to show drastic alterations in abundance of some fish species with associated changes in resources such as habitat and prey availability as well as predator abundances. Future research would need to focus beyond single species studies but take into account communities to get a better estimation on the impact of climate change and ocean acidification on community responses as a result of species interactions (Nagelkerken & Munday 2016). However, we showed that despite long acclimation potential in our first study (chapter 2) the sharks still showed significant effects of climate change and ocean acidification which according to a meta-analysis by Nagelkerken & Connell (2015) would reflect a high probability of community change, especially for longer lived species.

Having more than one predator present would also allow for potential shifts in dominance over community structure where one predator might exhibit increased tolerance to future changes over another and the same could be true for multiple prey species. This would be a powerful predictor for community structure in the future under climate change. However, studies of this kind with increasing complexity are costly and difficult to maintain and coordinate artificially. An alternative would be to perform these in the field in areas such as vent sites with naturally elevated CO_2 (Munday et al. 2014, Nagekerken et al. 2015), and ocean warming hot spots (Verges et al. 2014). These would allow for habitat to be considered as well as species interactions (Nagelkerken & Munday 2016, Nagelkerken et al. 2015). However, these would be restricted to a single future stressor influence.

Lengthening experiments to include multigenerational experiments would be an advantage but this is not feasible for some species such as slow growing long lived pelagic species that would require significantly extensive aquaculture facilities. Thus, there is a need to use more sensitive and informative metrics that can detect less conspicuous effects in the short term. Metrics from genetic techniques such as utilizing genomic, transcriptomic and proteomic processes (Somero 2012) could detect effects which could help explain the reasoning behind higher level effects, for example Nilsson et al. (2012) accounting for the cellular process driving behavioural responses of acidification in fishes. Recent studies in epigenetics showed a potential for rapid acclimation to warming conditions (Veilleux et al. 2015). Studying metabolic genes and lipid metabolism, results suggested shifts in energy production for maintaining performance at elevated temperatures (Veilleux et al. 2015). A study by Shao et al. (2016) found that acidified seawater suppressed insulin like growth factor I mRNA expression and reduced the growth rated in a teleost fish. Interpreting how these conspicuous effects could influence processes on a population and or ecosystem level might be difficult to interpret, this certainly would enhance the information we receive from them.

The study of global warming and ocean acidification has room for advancement and expansion. Extending the duration of experiments with complex community structured mesocosms as well as incorporating multi-stressor scenarios, and using the rapidly evolving field of genomics will allow for the better understanding of future climate changes on individual fish, their population and the ecosystems they inhabit.

5.5 CONCLUSION

This thesis has shown that with the future climatic changes of elevated temperature and CO_2 by the end of this century may negatively affect the behavioural and sensory functioning of important predatory fishes. This alteration could disrupt settlement to new habitats for larval fish as well as the hunting and foraging techniques of juveniles and adults leading to elevated risk of predation and starvation, and settlement in unsuitable habitats. The probable consequences of this would be a reduction in their populations leading to trophic cascades within the ecosystems that they inhabit. Additionally, this might lead to economic impacts with the potential reduction of a commercial fish species. These insights are key to inform and instigate effective adaptive management to help mitigate the negative impacts of accelerated anthropogenic CO_2 release in the atmosphere, and lessen the deleterious effects on the marine environment.

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