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JAMES COOK UNIVERSITY
AUSTRALIA

The effects of climate change on predator-prey interactions in coral reef fish

Thesis submitted by

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In September 2015

For the degree of **Doctor of Philosophy**

In the College of Marine and Environmental Science

James Cook University

Statement of Contribution of Others

This thesis includes collaborative work with my supervisors, Professor Mark McCormick, Professor Philip Munday and Dr. Paolo Domenici as well as with Dr. Sue-Ann Watson and Dr. Gabrielle Miller. While undertaking these collaborations, I was responsible for the research concepts and designs, data collection, analysis and interpretation of results. My co-authors provided intellectual guidance, editorial assistance, financial support or technical assistance.

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Declaration of Ethics

The research presented in this thesis was conducted in accordance with the national Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition (2004) and the Queensland Animal Care and Protection Act (2001). The research received and was conducted under the animal ethics approval from the JCU Animal Ethic Committee Approval number #A2080.

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General abstract

Climate change is one of the greatest threats facing marine ecosystems. Despite a rapid increase in scientific research in this field, predicting the response and acclimatory capacity of marine animals remains difficult. Moreover, predicting the responses of interacting species is further challenging owing to different sensitivities to elevated carbon dioxide (CO_2) and increasing temperatures among species. Recent studies demonstrate that end of century predicted increases in CO_2 and temperature can affect the physiological performance and behaviour of marine fishes; however, little is known of the effect of these stressors on ecological processes, such as predator-prey interactions.

To explore this, **Chapter 2** aimed to determine whether predators and prey respond differently to modest increases in temperature (ambient 27°C and elevated temperature 30°C), and whether these responses change the outcome of predator-prey interactions by affecting the kinematics of the encounter. Exposure to elevated temperatures significantly affected the predator-prey interactions of a pair of common reef fish, the planktivorous damselfish (*Pomacentrus wardi*) and the piscivorous dottyback (*Pseudochromis fuscus*). When predators exposed to elevated temperatures interacted with prey exposed in a similar manner, maximal attack speeds increased. This effect coupled with decreasing prey escape speeds and escape distances led to increased predation rates. Prey exposed to elevated temperatures also had decreased reaction distances and increased apparent looming threshold, suggesting that their sensory performance was affected. This occurred despite the increase in maximal attack speeds, which in other species has been shown to increase reaction distances. These results suggest that the escape performance of prey is sensitive to short-term increases in ambient temperature. Predators may become more successful, leading to strong selection for the maintenance of maximal escape performance in prey.

In addition to increasing sea surface temperatures, ocean acidification is posing a problem for many marine organisms. Recent research has shown

that exposure to elevated CO_2 affects how fishes perceive their environment, affecting behavioral and cognitive processes leading to increased prey mortality. However, it is unclear if increased mortality results from changes in the dynamics of predator-prey interactions or it is due to prey increasing activity levels. **Chapter 3** examined the potential cause of increased mortality of prey fish, asking the specific question: Does exposure to elevated CO_2 change the outcome of predator-prey encounters by altering the kinematics of the predator-prey interaction? To test this question, prey (*Pomacentrus amboinensis*) were exposed to elevated CO_2 (~880 µatm) or to a present-day control (~440 µatm) and allowed to interact with similarly exposed predators (*Pseudochromis fuscus*) in a cross-factored design. This enabled me to tease apart the independent effects on the predator and prey as well as the interacting effects when both were exposed to elevated CO_2. These results show that exposure to elevated CO_2 significantly affected the interactions between *P. amboinensis* and *P. fuscus*. Specifically, predators had the lowest capture success when exposed to elevated CO_2 and interacting with prey exposed to present-day CO_2. Prey exposed to elevated CO_2 had reduced escape distances and longer reaction distances compared to prey exposed to present-day CO_2 conditions, but this was dependent on whether the prey was paired with a CO_2 exposed predator or not. This suggests that the dynamics of predator-prey interactions under future CO_2 environments will depend on the extent to which the interacting species are affected and can adapt to the adverse effects of elevated CO_2.

While there has been extensive research on the effects of elevated CO_2 and rising temperatures on marine organisms, most studies have tested the effects of these drivers in isolation. However, given that these stressors are unlikely to occur independently, there is a need to gain a more ecologically realistic understanding of how the combined effects of temperature and acidification will affect marine organisms. **Chapter 4** tested the independent and interactive effects of short-term elevated CO_2 and temperature exposure on the interactions between *P. wardi* and its predator, *P. fuscus*. Predators and prey were split into 4 different treatment groups in a 2 CO_2 (405 and 930 µatm) x 2 temperature (27 and 30°C) design. Results showed that predator success increased following predator and prey exposure to elevated CO_2 and temperature. Specifically, exposure to high temperatures resulted in the greatest capture success followed by the combined exposure to elevated CO_2 and temperature. There was a strong influence of temperature on prey escape behaviour. We observed significant changes in prey reaction distances, apparent looming thresholds and directionality, as well as declining escape speeds and distances. Contrary to expectations, there was little influence of temperature or CO_2 on the behaviour of the predator, suggesting that the attack behaviour of *P. fuscus* was robust to these environmental changes. Overall, high temperature had an overwhelming negative effect on the escape behaviour of the prey compared to the independent effect of CO_2 or the combined exposure to elevated CO_2 and temperature. These results, combining escape performance, attack behaviour and mortality rates, suggest that increasing CO_2 and warming may lead to complex changes in the predator-prey relationships of coral reef communities.

Though recent studies have shown that elevated CO_2 can increase the mortality rate of prey when exposed to predators (for example Chapter 3), the extent to which fish larvae can acclimate their escape responses across generations exposed to elevated CO_2 is unknown. **Chapter 5** tested the acute effects of elevated CO_2 exposure on the escape responses of juvenile fish and whether such effects are altered by exposure of parents to elevated CO_2 (transgenerational acclimation). To test the hypothesis that transgenerational acclimation may reduce the impact of elevated CO_2 on escape performance, adults and juveniles were reared under current-day CO_2 levels (~400 μatm) or CO_2 levels projected to occur by the end of the century (~1087 μatm). Comparisons between treatments enabled the determination of the acute (within-generation) effects of increased CO_2 on juvenile escape performance and whether such effects were mediated by exposure of parents to increased CO_2. Elevated CO_2 negatively affected the reactivity and locomotor performance of juvenile fish, but parental exposure to high CO_2 reduced the effects in some traits, indicating the potential for acclimation of behavioural impairment across generations. However, acclimation was not complete in some traits, and absent in others, suggesting that transgenerational acclimation does not completely compensate the effects of high CO_2 on escape responses. This research demonstrated that there is a potential for rapid acclimation for variables that influence escape performance, if the parents have been exposed to the same environmental history. However, parental effects did not fully restore performance in most traits. Thus, negative effects of higher CO_2 levels on behaviour remain, although at reduced levels. Whether behaviours could be fully acclimated in future generations and the potential costs of such acclimation to other processes is unknown. This highlights the need to exercise caution when making statements about whole-organism acclimation.

Examining interactions between species in response to environmental stressors can be complex due to behaviourial subtleties that may not be captured. However, only focusing on the responses of individual species is likely to provide an insufficient basis from which an understanding of long-term responses to climate change can be inferred. This is particularly true in complex ecological environments, such as coral reefs. In the present era of rapid climate change, understanding how changes to individual performance influences the relationships between predators and their prey will be increasingly important in predicting the effects of climate change within ecosystems. These are the first studies to address how the kinematics at the basis of predator-prey interactions may change in response to independent and concurrent exposure to elevated CO_2 and temperature, and whether there is any potential for acclimation to occur. Understanding how organisms and ecosystems respond to key environmental drivers remains a priority for science, management and conservation.

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Chapter 1: General introduction

A changing climate

Climate change is one of the greatest threats facing marine ecosystems yet predicting the response of ecosystems over timescales relevant to climate change predictions is challenging. The burning of fossil fuels for energy has escalated since the onset of the industrial revolution causing an unprecedented increase in anthropogenic carbon dioxide (CO_2) emissions. CO_2 emissions have risen from approximately 280ppm pre-industrial to 400ppm in 2015, the highest level in at least 600,000 years (Lüthi et al., 2008). Under the business-as-usual (RCP8.5) emission scenario from the Intergovernmental Panel on Climate Change (IPCC), atmospheric CO_2 concentration is projected to reach nearly 1000 ppm by the end of the 21st century, with the rate of increase faster than any time in the last 10,000 years (Meinshausen et al., 2011; IPCC, 2013). Greenhouse gases such as CO_2 affect the absorption, scattering and emission of radiation within the Earth's atmosphere and as a result are changing our climate in a process called global warming. Increasing greenhouse gas concentrations results in a greater retention of heat in the atmosphere and is predicted to lead to temperatures increasing by up to 4.5°C by the end of the century (IPCC, 2013). Furthermore, the world's oceans have absorbed ~93% of this excess heat created by the enhanced greenhouse effect (Hoegh-Guldberg et al., 2013) causing an increase in sea surface temperatures (0-700m). Climate change models predict that in regions with coral reefs, temperature increases of up to 3°C by the end of the century can be expected (Lough, 2012; Collins et al., 2013).

Occurring concurrently with increasing atmospheric CO_2 is increasing dissolved CO_2 in the world's oceans. The surface oceans are in approximate gas equilibrium with the atmosphere, consequently, as atmospheric CO_2 rises, oceanic CO_2 also increases (Doney et al., 2010). Since the onset of industrialization, the oceans have absorbed ~30% of the excess CO_2 produced by anthropogenic activities (Doney, 2009; Honisch et al., 2012). Increased uptake of CO_2 causes a shift in the carbonate system of seawater in a process termed ocean acidification (Caldeira and Wickett 2003). Ocean acidification occurs when dissolved CO_2 combines with seawater to form carbonic acid (H_2CO_3), which mostly dissociates into bicarbonate (HCO_3^-) and hydrogen ions. This increase in hydrogen ions reduces the pH of seawater as well as reacting with free carbonate ions ($CO3^{2-}$) to form more bicarbonate ions leading to a decline in carbonate and a subsequent decline in the saturation state of calcium carbonate minerals (aragonite and calcite) present in seawater (Gaylord et al., 2015). Ocean acidification has already reduced average ocean pH by 0.1 units since pre-industrial times (Meehl et al., 2007) and is expected to decline by a further ~0.45 units under the current RCP8.5 CO_2 emission scenario (Hoegh-Guldberg et al., 2014). This projected decline in pH will lead to the equivalent of a ~150% increase in acidity presenting a problem for organisms that precipitate calcium carbonate structures and skeletons such as corals and molluscs (Caldeira and Wickett, 2005, Meehl et al., 2007).

The effect of thermal changes on marine organisms

Climate change is occurring over decadal timeframes and potential consequences are often perceived as a future problem. However, significant shifts in species distributions in a diverse group of plants and animals and changes in the timing of life history events such as reproduction are already occurring in response to increasing global temperatures (Walther et al., 2002; Parmesan, 2006; Poloczanska et al., 2013). For ectotherms, such as fishes, performance is tightly linked to environmental temperatures. Ambient temperature is a major environmental determinate of life history processes and can influence physiologically driven functions such as swimming performance (Rome, 2007), growth (Pauly, 1980), neural control (Szabo et al., 2008), aerobic scope (Farrell, 2002; Portner and Farrel, 2008), cardiac output (Eliason et al., 2011), muscle development (Hanel and Wieser, 1996) and the contractile properties of the swimming muscles (Wakeling, 2006). These changes arise through a variety of mechanisms such as changes in the balance between ATP consumption and generation as well as direct effects on ligand binding, diffusion and the speed of chemical and enzyme catalysis (Cossins and Bowler, 1987). In addition, the energetic cost of maintaining cellular function rises, increasing the basic cost of living and, unless available energy increases to match requirements, reduces the energy available for other processes (Brett, 1971; Houde, 1989; Sokolova et al., 2012). The effects of higher temperatures on physiological processes also affect the behaviours that are directly linked to them, such as activity (Biro et al., 2010), sensory responsiveness (Webb and Zhang, 1994), anti-predator swimming behavior (Szabo et al., 2008) and the interactions between predators and their prey (Freitas et al., 2007; Grigaltchik et al., 2012).

Whilst the majority of climate change research has focused on organisms from temperate regions, it is increasingly acknowledged that it is the tropical communities closest to the equator that will be most vulnerable to the effect of global warming (Hoegh-Guldberg and Bruno, 2010). Tropical organisms have evolved in a thermally stable environment and their thermal tolerance windows tend to be narrower (more stenothermic) compared to their temperate counterparts (who tend to be eurythermic) (Fuiman and Batty, 1997; Hunt von Herbing, 2002). Even small increases in temperature may result in serious consequences for tropical organisms. Understanding the physiological and behavioral effects of thermal changes on tropical species is critically important for forecasting how communities may respond to changing temperature regimes (Tewksbury et al., 2008; Sunday et al., 2014).

Despite increasing global temperatures, most marine organisms already experience thermal variation at a range of temporal and spatial scales. This variation arises from changing seasons, currents, upwelling, tides, local topography and daily changes in solar radiation (Soon and Legates, 2013). Coral reefs occur in warm and thermally stable regions, and in part this has led to the high species diversity that characterizes these ecosystems. However, temperature fluctuations still occur both temporally and spatially. These changes are driven by the shallow, sloping and rough bottom topography (Monismith et al., 2006) typical of coral reefs that alter flow regimes, as well as predictable changes in solar radiation (Leichter et al., 2006) and cloud cover (Leahy et al., 2013). Thermal regimes on coral reefs have been observed to change by up to 4–8°C on a diurnal cycle in some places, with peaks of up to 12°C during the summer months (McCabe et al., 2010), and by up to 9°C on a seasonal basis at higher latitudes (Rummer et al., 2013), exceeding those temperatures that are predicted under current climate change scenarios (Collins et al., 2013). Assessing the effects of current day natural thermal variability can be useful for predicting future impacts of climate change on marine organisms.

The problem with acidifying oceans

In addition to increasing sea surface temperatures, ocean acidification is a threat to many marine organisms. Although ocean acidification is often perceived as a future problem, impacts on marine life and the human economies they support are already being felt in some regions. For example, failures in yields produced by oyster hatcheries in the Northwest United States have been attributed to low-pH water along the Pacific coast (Barton et al., 2012), a pattern enhanced by additions of anthropogenic CO_2 in the ocean (Feely et al., 2008). To date, most ocean acidification studies have focused on organisms that precipitate aragonite skeletons, such as calcifying plankton, mollusks and reef building corals (Guinotte and Fabry, 2008) and the changes associated with decreasing pH on the formation of their skeletons (Orr et al., 2005; Kleypass et al., 2006). Comparatively less research has been conducted on other organisms, such as fishes. Nevertheless, increasing CO_2 is predicted to affect the physiology and behaviour of many marine organisms (Portner et al., 2005; Widdicombe and Spicer, 2008; Kroeker et al., 2010; Briffa et al., 2012) with consequences for growth, development and survival

(Kroeker et al., 2010).

In fishes, acute exposure to high CO_2 causes an increase in pCO_2 and decrease in pH in the blood (Ishimatsu et al., 2004; Heuer and Grosell, 2014). Fishes experiencing acidosis caused by elevated CO_2 compensate this disturbance in acid-base balance by accumulating HCO_3^- to restore plasma pH (Ishimatsu et al., 2004; Michaelidis et al., 2006; Brauner and Baker, 2009; Heuer et al., 2012; Esbaugh et al., 2012). However, this compensatory mechanism is predicted to be costly (Ishimatsu et al., 2008; Brauner and Baker, 2009; Melzner et al., 2009) and could reduce energy available for other processes such as growth, reproduction and survival (Ishimatsu et al., 2004; Portner et al., 2004).

Some of the most dramatic effects of elevated CO_2 levels reported to date are impaired sensory ability and altered behaviours of larval and juvenile fishes (Briffa et al., 2012; Munday et al., 2012; Branch et al., 2013). Behavioural effects of elevated CO_2 include changes in anti-predator responses, olfactory and auditory preferences, activity levels, sensory performance, behavioural lateralization and visual risk assessment (Briffa et al., 2012; for review see Heuer and Grosell, 2014), which can translate into consequences for survival in natural habitats (Munday et al., 2010, Ferrari et al., 2011a). The underlying mechanism linking elevated CO_2 to these diverse changes in behaviour appears to be interference of the acid-base regulatory process with the function of a major neurotransmitter receptor ($GABA_A$) in the brain (Nilsson et al., 2012; Hamilton et al., 2014). Exposure to elevated CO_2 leads to the maintenance of intercellular acid-base balance through compensatory decreases in plasma Cl^- and increases in HCO_3^-. This can alter normal neuronal cell membrane ion gradients leading to an efflux rather than an influx of these ions leading to a reversal of $GABA_A$ receptor function i.e., from inhibition to excitation. This is proposed to be the cause of altered sensory preferences leading to subsequent changes in behaviour (Nilsson et al., 2012; Heuer and Grossel, 2014).

Concurrent exposure to elevated temperatures and CO_2

While there has already been a considerable amount of research into the effects of elevated CO_2 and rising temperatures on marine organisms (for review see Johnston and Temple, 2002; Briffa et al., 2012; Munday et al., 2012; Branch et al., 2013; Kroeker et al. 2013), most studies have tested the effects of these drivers in isolation. Given that these stressors will not occur independently, understanding how these multiple and concurrent shifts in environmental conditions will interact is critical for predicting species responses to climate change as extrapolations based on single driver responses could lead to incorrect predictions about future impacts (Harvey et al., 2013; Gaylord et al., 2015).

Ectotherms, which are predicted to be more sensitive to changing thermal conditions (Gilbert et al., 2014), can exhibit changes in metabolic performance, behaviour, growth and swimming abilities in temperatures that are close to or past their thermal optima (Portner and Farrell, 2008; Johansen and Jones, 2011). This combined with elevated CO_2, which can exert similar physiological constraints on these same traits (Kroeker et al., 2010; Briffa et al., 2012), may enhance the effects of both stressors, which can manifest in a non-linear way. For example, they can act independently (additive), or interact to either reduce (antagonistic) or enhance (synergistic) performance (Kroeker et al., 2013; Todgham and Stillman, 2013).

Despite their potential importance, relatively few studies have explored the interactive effects of these important environmental drivers (Byrne et al., 2009; Pansch et al., 2012; Byrne and Przeslawski, 2013). Recent studies have shown that concurrent exposure to elevated CO_2 and temperature can lead to a synergistic response in invertebrates. For example, Metzger et al., (2007) observed that increased CO_2 acted synergistically when coupled with temperature resulting in a narrowing of the thermal tolerance window of a marine invertebrate, the edible crab, *Cancer pagurus*. Similarly, Rosa and Seibel, (2008) showed that elevated CO_2 and temperature interacted, the metabolic rates and activity levels of the jumbo squid (*Dosidicus gigas*) were reduced. Emerging evidence also suggests that the physiology and behaviour of fishes is sensitive to changes in simultaneous exposure to CO_2 and temperature. For example, there are interactive effects of ocean acidification and temperature on aerobic scope and survivorship (Munday et al., 2009), predation rates and predator selectivity (Ferrari et al., 2015), directional changes in lateralization suggestive of neural impairment (Domenici et al., 2014), increasing food consumption and foraging behaviour (Nowicki et al., 2012) and declining offspring quality (Miller et al., 2014), all of which can determine species persistence into the future.

Is there scope for acclimation?

Despite the importance and utility of short-term temperature- or CO_2-exposure studies on fishes, an unavoidable limitation of these studies is that they are unable to describe the potential adaptive capacity of a species (Munday et al., 2013). However, despite this limitation, these studies have allowed for an interpretation of physiological and/or behaviourial effects that may be susceptible to selective pressures. Recently, Munday et al., (2015) observed that fishes living near natural highly acidic CO_2 seeps exhibited behaviourial abnormalities similar to those found in acute CO_2 exposure laboratory experiments suggesting that traits observed in short term studies will likely persist across generations. Mechanisms by which organisms might be able to maintain their performance in a high CO_2 world are acclimation or adaptation. Adaptation is driven by genetic selection that is inherited from one generation to the next involving selection on genetic traits that shift the average phenotype toward a fitness peak (Sunday et al., 2014). In contrast, acclimation through phenotypic plasticity can result in physiological, morphological and behavioural changes without genetic selection (Sunday et al., 2014). Acclimation can occur both within and between generations providing a powerful mechanism enabling individuals to cope with environmental change. Sunday et al., (2014) describes three main types of acclimation: reversible, developmental and transgenerational. Reversible acclimation occurs for species that live in highly variable environments, such as intertidal species (Somero, 2009) and occurs over days to months. Developmental acclimation occurs when exposure to a novel environment during ontogeny better prepares the individual should they encounter that environment later in life. For example, zebrafish exposed to elevated temperatures as embryos had enhanced swimming performance when encountering the same temperatures as adults (Scott and Johnston, 2012).

Transgenerational acclimation occurs when the environment experienced by the parent's influences the performance of the offspring. Transgenerational plasticity has been observed in a diverse group of taxa such as plants (Galloway and Etterson, 2007), insects (Agrawal et al., 1999; Mondor et al., 2004), bryozoans (Burgess and Marshall, 2011), shellfish (Parker et al., 2012) and fishes (Salinas and Munch, 2012), leading to changes in morphology (Agrawal et al., 1999; Mondor et al., 2004), dispersal potential (Burgess and Marshall, 2011) and growth rates (Parker et al., 2012; Salinas and Munch, 2012). Recently, it has been observed to occur in response to environmental stressors, such as hypoxia (Ho and Burggren, 2012), elevated temperature (Donelson et al., 2012; Salinas and Munch, 2012), and elevated CO_2 (Miller et al., 2012; Parker et al., 2012), suggesting that transgenerational acclimation may be an important mechanism in helping taxa to cope with climate change (Munday et al., 2014). Parents can influence the phenotype of their offspring through a variety of non-genetic mechanisms such as variation in energy provisioning (e.g., yolk reserves) (Uller, 2008). In addition, parents can influence the quality and performance of their offspring through epigenetic mechanisms such as DNA methylation, changes in chromatic structure or RNA (Bonduriansky et al., 2012).

Does a changing world lead to changing predator-prey interactions?

Predation is the dominant source of mortality in most populations (Lima and Dill, 1990) and is an evolutionary driver for community and social structure, as well as the spatial and temporal use of habitats. Consequently, predation plays a major role in determining the life history characteristics of many species. A prey's response to predation risk represents a series of trade-offs between the perceived intensity of current threats and the importance of other fitness promoting activities such as foraging, mating and maintenance (Ferrari et al., 2010). Prey must be able to adapt and respond to predation risk as it changes in its intensity and frequency through time and space (Sih et al., 2000). Early detection and avoidance are seen as the most effective ways to avoid predation (Kelley and Magurran, 2006; Ferrari et al., 2010) as it allows prey to assess the potential risks to which they are exposed and respond accordingly without the need to directly encounter the predator (Lima and Dill, 1990; Kelley and Magurran, 2006). The effectiveness and success of a predator is dependent upon attributes of both predator and prey. A prey individual with a well-honed antipredator response will have high survival, while a predator with a reduced catch rate will be penalized by slower growth and lower reproductive output. Any factor that influences a step in the behavioral basis of the predator-prey interaction sequence may change the outcome for both parties.

For an organism to critically assess its environment, it relies on the accuracy of sensory information. Sensory cues include visual, auditory or chemical cues. Visual and chemical cues are a fundamental feature of the marine environment and they govern whether species flee, mate or consume the individual next to them (Magurr, 1989). Because of the critical importance of sensory cues, any process that disrupts these can be critical to an individual's fitness and in turn can permeate throughout the community (McCormick and Manassa, 2008) through changes in trophic links. Disruptions in the sensory system can have a profound effect on marine organisms as they rely on these cues to navigate a complex and dynamic environment.

A critical stage in the life of reef fishes is at the end of the larval phase, when they settle to the benthic environment. Mortality schedules during the first few days of settlement are high, ~60% for tropical reef fishes (Almany and Webster, 2006). Success at this life stage is predominantly a consequence of the size, growth and the fast-start performance of new recruits (Green and McCormick, 2005; Holmes and McCormick, 2010). During a predator-prey interaction, fish employ a rapid acceleration called a fast-start that is used by predators to capture prey, or by prey to avoid a predatory strike (for review see Domenici and Blake, 1997; Walker et al., 2005). Fast starts are short, high-energy swimming bursts which are driven by the rapid, anaerobically powered, contraction of large myotomal blocks of fast glycolytic muscle (Rome et al., 1988; Josephson, 1993). The fast kinematics of escape responses are usually controlled by the large Mauthner neurons which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved (Eaton et al., 2001). Successful fast starts consist of finely tuned responsiveness and locomotor performance (Domenici, 2010). However, fast-starts are sensitive to changes in ambient temperatures with responses differing between species and ontogenetic stages (Johnston and Temple, 2002; Wilson, 2010), as well as between predators and their prey due to changes in their temperature tolerance and sensitivity (Freitas et al., 2007; Grigaltchik et al., 2012). Furthermore, if elevated CO_2 alters the processing of sensory information (Nilsson et al., 2012), then it may directly influence the behavioral processes that govern the outcome of a predator-prey interaction, such as the timing of a predator strike or the distance to react to the predator by the prey. If factors that influence predator and prey success change through environmental change for instance, the impact will be first felt on the vulnerable early life stages, with repercussions for population replenishment (Munday et al., 2010).

Aims and Objectives

Although we are improving our knowledge base about the effects that increasing CO_2 and temperature can have on a number of performance-related traits in coral reef fishes, we are yet to determine how these stressors may act independently or concurrently on the kinematic responses that underpin predator-prey interactions. **Broadly, this thesis focuses on identifying the effects of climate change on the kinematics of predator-prey interactions of coral reef fishes**. In addition, there is compelling evidence that some of the negative physiological effects of climate change on marine organisms can be reduced if the parents have been exposed to the same environmental history as their offspring (Donelson et al., 2012; Miller et al., 2012; Salinas and Munch, 2012). This indicates that non-genetic parental effects may alter how marine organisms respond to elevated CO_2 and provides evidence that the response to environmental stress may depend on the history of previous life stages. However, these studies have focused on physiological and life-history acclimation across generations. Whether predator avoidance behaviour is subjected to the same rapid transgenerational acclimation is unknown. This thesis represents a significant advance in improving our understanding of how critically important behaviours of coral reef fishes may change as ocean temperatures and CO_2 levels rise.

The data chapters that comprise this thesis are self-contained publications, which address four main objectives:

Chapter 2 investigated whether acute heat stress would lead to an increase in prey vulnerability as well as an increase in attack rates from predatory fish. Because of the effects of elevated temperature exposure on predators and prey, there may be changes in predator strike success or prey escape rates. Therefore, this chapter sought to determine whether predators and prey respond differently to modest increases in temperature, and whether this changed the outcome of predator–prey interactions by affecting the kinematics of their responses to one another. **Chapter 3** examined the potential cause of increased mortality of prey fish that has been observed in previous studies (Munday et al., 2010; Ferrari et al., 2011a) by investigating whether exposure to elevated CO_2 changed the outcome of predator-prey encounters by altering the kinematics of the predator-prey interaction? Previous experiments have observed increased mortality rates of high CO_2 exposed prey when placed in the field with non-CO_2 exposed predators (Munday et al., 2010; Ferrari et al., 2011a). This research suggested that the behavior of the CO_2 exposed prey depends on whether or not the predator has also been exposed to elevated CO_2. Therefore, the responses of fish exposed to high CO_2 were compared to fish exposed to present day CO_2 levels in a fully crossed design. I sought to tease apart the independent effects on the predator and prey as well as the interacting effects when both were exposed to elevated CO_2. **Chapter 4** employed a multi-stressor approach to assess the independent and combined effects of both elevated CO_2 and high temperatures on the attack and escape performance of a common pair of coral reef fishes. By exposing predators and prey to elevated CO_2 and high temperatures, both independently and simultaneously, I sought to determine the relative effects of each stressor on the attack and escape performance that forms the basis of predator-prey interactions. **Chapter 5** investigated the extent to which fish can acclimate their escape responses across generations when exposed to elevated CO_2. Rearing of adults and juveniles under current-day CO_2 levels (400 µatm) or CO_2 levels projected to occur by the end of the century (1000 µatm Meehl et al. 2007) allowed me to test whether transgenerational acclimation reduced the impact of elevated CO_2 on the escape characteristics of a juvenile fish. Comparisons between treatments allowed us to determine the acute (within-generation) effects of increased CO_2 on juvenile escape performance and to test whether such effects were mediated by exposure of parents to increased CO_2. Furthermore, by investigating a range of behavioural and locomotor variables that are critical to a successful escape response, I was able to investigate whether the capacity for acclimation to elevated CO_2 may differ between escape processes linked to locomotor performance and those more closely linked to cognitive processes.

Chapter 2: Feeling the heat: the effect of acute temperature changes on predator-prey interactions in coral reef fish

Bridie J. M. Allan, Paolo Domenici, Phillip L. Munday and Mark I. McCormick

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2.1 Summary

Recent studies demonstrate that elevated temperatures predicted to occur by the end of the century can affect the physiological performance and behaviour of larval and juvenile fishes; however, little is known of its effect on ecological processes, such as predator-prey interactions. Here we show that exposure to elevated temperatures significantly affected the predator-prey interactions of a pair of common reef fish, the planktivorous damselfish *Pomacentrus wardi* and the piscivorous dottyback *Pseudochromis fuscus*. When predators exposed to elevated temperatures interacted with similarly exposed prey maximum attack speeds increased. This effect coupled with decreasing prey escape speeds and escape distances led to increased predation rates. Prey

exposed to elevated temperatures also had decreased reaction distances and increased apparent looming threshold suggesting that their sensory performance was affected. This occurred despite the increase in maximum attack speeds, which in other species has been shown to increase reaction distances. These results suggest that the escape performance of prey is sensitive to short term increases in ambient temperature. As marine environments become more thermally variable in future, our results demonstrate that some predators may become more successful suggesting that there will be strong selection for the maintenance of maximum escape performance in prey. In the present era of rapid climate change, understanding how changes to individual performance influence the relationships between predators and their prey will be increasingly important in predicting climate change effects within ecosystems.

2.2 Introduction

Climate change models predict that sea surface temperatures are set to increase by up to 3°C by the end of the century owing to rising atmospheric greenhouse gases (Collins et al., 2013). However, most marine organisms already experience temperature variation as a result of thermal fluctuations at a range of temporal and spatial scales resulting from changing seasons, currents, upwelling, tides, local topography and daily changes in solar radiation (Soon and Legates, 2013). For coral reefs, which are considered to be thermally stable environments, temperatures also change both temporally and spatially. These changes are driven by the shallow, sloping and rough bottom topography (Monismith et al., 2006) typical of coral reefs that alter flow regimes, as well as predictable changes in solar radiation (Leichter et al., 2006) and cloud cover (Leahy et al., 2013). Thermal regimes on coral reefs have been observed to change by up to 4-8°C on a diurnal cycle in some places with peaks of up to 12°C during the summer months (McCabe et al., 2010) and by up to 9°C on a seasonal basis at higher latitudes (Rummer et al., 2013), exceeding those temperatures that are predicted under current climate change scenarios (Collins et al., 2013). Assessing the effects of natural thermal variability now can be important for predicting future impacts of climate change on marine organisms.

Ambient temperatures strongly influence a broad range of physiological and behaviourial traits in all marine organisms. For most marine organisms, temperature is a major environmental determinate on life history processes and governs basic physiological functions such as swimming performance (Rome, 2007), growth (Pauly, 1980), neural control (Szabo et al., 2008) and behaviour (Biro et al., 2010), particularly if it increases beyond an individuals thermal optimum. Fluctuating temperatures can have an effect on swimming performance through changes in aerobic scope (Farrell, 2002; Johansen and Jones, 2011), cardiac output (Eliason et al., 2011), muscle development (Hanel and Wieser, 1996) and the contractile properties of the swimming muscles (Wakeling, 2006). However, the mechanisms underlying these changes are complex and arise from changes in the balance between ATP consumption and generation as well as direct effects on ligand binding, diffusion and enzyme catalysis (Cossins and Bowler, 1987). Elevated temperatures lead to an increase in metabolic rate with subsequent changes in energy requirements (Clarke and Johnston, 1999). This can result in reduced net food conversion, ultimately affecting muscle development (Johnston et al., 2001) through trade-offs between hypertrophy (an increase of mass of individual fibers) and hyperplasia (an increase in the number of fibers) (Hanel and Wieser, 1996). Furthermore, neural control of anti-predator swimming behavior was found to be affected by temperature (Szabo et al., 2008).

Thermal fluctuations cause changes not only in physiological processes but also in the behaviourial capacities that are directly linked to them, such as activity (Biro et al., 2010), sensory responsiveness (Webb and Zhang, 1994) and the interactions between predators and their prey (Freitas et al., 2007; Grigaltchik et al., 2012). A critical stage in the life of reef fishes is at the end of the larval phase, when they settle to the benthic environment. Mortality schedules during the first few days of settlement are high, averaging 56% for tropical reef fishes (Almany and Webster, 2006). Success at this life stage is predominantly a consequence of the size, growth and the fast-start performance of new recruits (Green and McCormick, 2005; Holmes and McCormick, 2010; Allan et al., 2013). Fast starts are short, high-energy swimming bursts which are driven by the rapid, anaerobically powered, contraction of large myotomal blocks of fast glycolytic muscle (Rome et al., 1988; Josephson, 1993). The fast kinematics of escape responses are usually controlled by the large Mauthner neurons which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved (Eaton et al., 2001). Successful fast starts consist of finely tuned responsiveness and locomotor performance (Domenici, 2010). However, fast-starts are sensitive to changes in ambient temperatures with responses differing between species and ontogenetic stages (for review see Johnston and Temple, 2002; Wilson et al., 2010), as well as between predators and their prey due to changes in their temperature tolerance and sensitivity (Freitas et al., 2007; Grigaltchik et al., 2012).

Acute heat stress, defined as physiological stress associated with short term changes in ambient temperature, can lead to an increase in prey vulnerability (Yocom and Edsall, 1974) as well as an increase in attack rates from predatory fish (Persson, 1986). However, it is unknown if modest temperature increases that an animal may naturally experience within its environment will influence predator-prey interactions in coral reef fishes. Because of the relative effects of elevated temperature exposure on predators and prey, there may be changes in predator strike success or prey escape rates. Consequently this could lead to changes in predation pressure, which could translate into changes in community structure and function. Therefore, the aim of the present study was to determine whether predators and prey respond differently to modest increases in temperature, and whether this changes the outcome of predator-prey interactions by affecting the kinematics of their responses to one another.

2.3 Methods

Study site, fish collection and maintenance

Fishes were collected during October 2012 at Lizard Island (14° 40′ S, 145°

28′ E) in the northern Great Barrier Reef (GBR), Australia. Temperature loggers (Sensus ultra) deployed around Lizard Island for the 3 years prior to the study found that water temperatures in the shallow (<10m) water where fish were collected from ranged from 20.6°C to 30.6°C, and had a diurnal range of 1.2°C during the summer months, when fish recruitment occurs. During the recruitment period of 2012, the water temperature ranged from 25.2°C and 29.2°C. The Ward's damselfish, *Pomacentrus wardi* (Pomacentridae), was used as the prey species and is a small planktivorous fish commonly found on Indo-Pacific coral reefs. The dottyback, *Pseudochromis fuscus* (Pseudochromidae), was used as the predator. *P. fuscus* is widely distributed throughout the Indo-Pacific and is an important predator of newly settled coral reef fishes (Feeney et al., 2012), including *P. wardi*. Newly metamorphosed *P. wardi* (range 11.2–15.4 mm, 13.6 ± 1.3 mm mean standard length (SL) ± standard deviation (SD)) were collected using light traps moored 100 m off the fringing reef of Lizard Island. On the morning of capture *P. wardi* individuals were transferred into tanks at ambient, control temperatures (26.7°C). These individuals were then split into control (present day temperature, 26.7°C ± 0.1°C) and treatment (elevated temperature, 29.6°C ± 0.1°C) groups. For fish in the +3°C temperature group, the temperature was raised by 1°C every 8 hours until the final temperature of ~29.6°C was reached to avoid any stress associated with rapid temperature increases. Fish were maintained in these treatments for a period of 7 days, as we were interested in behavioral changes associated with short-term increases in temperature. A week is sufficient for thermal acclimation to occur in reef fishes and previous studies have not found significant improvement of physiological processes after longer exposures to elevated temperatures (Tullis and Baillie, 2005; Nilsson et al., 2010). Tanks were heated with 300-watt bar heaters and insulated to ensure stability of the chosen temperatures of 26.7°C and 29.6°C. Fish were fed 4 times daily ad libitum with newly hatched *Artemia* sp. but were starved for the 12 hours prior to commencement of experimental trials to standardize for satiation. A 12-hour light and 12-hour dark regime was used.

Adult *P. fuscus* (range 63–96 mm, 76.9 mm mean SL, SD 9.7) were collected with a dilute solution of clove oil (Munday and Wilson, 1997) from around the shallow fringing reef off Lizard Island. Immediately after collection, fish were transported back to the Lizard Island Research Station (LIRS) where they were housed separately in mesh breeding baskets within 30 L aquaria to avoid aggressive interactions. Fish were maintained in tanks for 7 days (following the same protocol as *P. wardi*) and were fed 2 juvenile reef fish morning and night and then not fed for the last 24 hours prior to the interaction trial to standardize for satiation.

Interaction trials

Experimental trials were conducted over a period of 10 days in a temperature-controlled room at LIRS. Trials were conducted at the same water temperature as the acclimation temperature for the test fish. Predator-prey interactions were measured using a standard protocol established by (Allan et al., 2013). Briefly, this involved placing a predator and prey fish into an experimental arena (38cm x 58cm x 10cm water height) and filming the ensuing interaction at high speed (420fps) for 10 minutes or until the prey had been consumed. Trials only commenced when the predator was at the opposite end of the tank to the prey at the start of the interaction to standardize for predator position. Kinematic variables were measured based on the center of mass (COM) of the fish when stretched straight based on Webb (1976). COM was assumed to be at 35% of the body length from the tip of the snout as it is the case for generalist fish (Domenici and Blake, 1997). Stage 1 and 2 were defined by directional changes of the anterior part of the body of the fish, based on (Domenici and Blake, 1997). Predator attacks were measured only when a predator showed a fast-directed burst towards the prey (>3 body lengths s^{-1}). All variables with the exception of number of prey caught were measured using only the first attack that occurred within a trial. This was done to control for any anaerobic stress either the predator or prey may have experienced due to prolonged attacks. Both predators and prey were used once to avoid habituation to the experimental procedure. Prey suffering was minimal as prey were consumed immediately following a successful strike.

The following performance variables were measured:

Prey

1) Prey reaction distance (m): RD, i.e. the distance between the prey COM and the tip of the predator's snout at the onset of the escape response to a predator attack.

2) Apparent Looming Threshold (ALT): was defined as the apparent looming threshold for prey avoidance responses to a predatory strike and is a measure of the magnitude of the prey's response to the perceived threat of predation.

The higher the perceived threat, the higher the ALT (in radians s^{-1}) measured at the onset of the escape response and measured as the rate of change of the angle (α) subtended by the predator's frontal profile as seen by the prey. Previous work has shown that fish tend to react to an approaching stimulus (a predator) when a given threshold of dα/dt (i.e. ALT) is reached. ALT is calculated as $(4US)/(4D^2+S^2)$, based on (Dill, 1974) and (Webb, 1982). Where U= predator speed, calculated as the speed of the predator in the frame prior to the prey's response; S is calculated based on the morphological characteristics of the predator, i.e. S = (Max.Depth + Max.Width)/2, whereby both max depth and max width are at 0.25 L of the predator (pers. obs.). D = the reaction distance calculated between the prey COM and the point on the predator where its maximum width is located. Hence D = RD + 0.25Lpred. As a consequence, for any given predator speed and morphology, as RD decreases ALT increases.

3) Prey escape distance (m): the straight-line distance between the prey COM at the onset of the escape response and at the end of the escape response (i.e. when the prey came to a halt).

4) Maximum prey escape speed (m s^{-1}): the top speed achieved at any point in time during the escape response, measured using the prey COM.

5) Mean prey escape speed (m s^{-1}): was measured as the distance covered within a fixed time (24 ms). This fixed duration was based on the average duration (22.8ms), of the first two flips of the tail (the first two axial bends, i.e. stages 1 and 2 defined based on (Domenici and Blake, 1997) which is the

period considered crucial for avoiding ambush predator attacks (Webb, 1976).

Predator

6) Capture success: percentage of trials in which the predator ingested the prey within the 10 min filming period, out of the total number of trials for each treatment.

7) Predation rate: capture success divided by the number of attacks per unit time.

8) Attack rate: number of attacks per unit time, measured for each interaction.

9) Predator attack distance (m): the straight-line distance between the predator COM at the time the attack commenced and the end of the attack (end is defined as when the predator came to a halt).

10) Maximum predator attack speed (m s^{-1}): the top speed achieved at any point in time during the attack, based on the predator COM.

Statistical analyses

The effects of elevated temperatures on performance kinematics were tested separately for prey and predators using one-factor MANOVAs. One-way ANOVAs were performed to determine the nature of any differences found by the MANOVA. Residual analysis indicated that data met the assumptions of normality and homogeneity of variance. To test the null hypothesis that predator capture success is independent of predator and prey temperature exposure, capture success was compared by 2x2 contingency table analysis. Predation rate data did not meet the assumption of homogeneity of variance;

therefore a Kruskal-Wallis test was performed to explore differences in predation rates between the two treatments.

2.4 Results

Prey

The MANOVA revealed a significant effect of temperature on prey escape performance (Pillai's trace $_{4,31}$ = 5.49, p = 0.001). One-factor ANOVAs detected significant differences in four out of the five tested behaviourial attributes: reaction distance, apparent looming threshold (ALT), escape distance and mean prey escape speed. The reaction distance of the prey to the predator at the onset of the first attack was affected by exposure to elevated temperatures (Figure 2.1a; $F_{1,34} = 5.40$, p = 0.02). Specifically, prey exposed to elevated temperatures allowed similarly exposed predators to get twice as close to them before undertaking an escape response (82 mm compared to 40 mm). Apparent looming threshold (ALT) was also significantly higher for prey following exposure to elevated temperatures (Figure 2.1b; $F_{1,34}$ = 7.30, p = 0.01). The distance travelled during an escape response **(**escape distance) was significantly shorter than when interactions occurred at the control temperatures demonstrating an acute effect of elevated temperature exposure (Figure 2.1c; $F_{1,34}$ = 6.87, p = 0.01). Exposure to elevated temperatures also significantly affected the mean response speed of the prey, with prey exposed to elevated temperatures being considerably slower compared to the control temperature group (Figure 2.1d; $F_{1,34}$ = 5.35, p = 0.02). There was no significant difference in maximum speed achieved between the two treatment groups.

Predator

Capture success was significantly associated with the elevated temperature treatment experienced by the predator and the prey (Figure 2.1e; $\chi^2 = 8.95$, df= 3, $p = 0.005$). Within this treatment, capture success was 47%; 41% greater than the control temperature exposed pairs (5.8% capture success). Exposure to elevated temperatures also significantly affected predation rates (Figure 2.1f; $H = 7.81$, df= 1, $p = 0.005$) with a markedly higher predation rate in the elevated temperature exposed predators compared to the control temperature exposed predators. A MANOVA revealed a significant effect of temperature on the attack characteristics of the predators (i.e. attack rate, attack distance and maximum attack speed; Pillai's trace $_{3,32} = 5.04$, $p = 0.002$). ANOVAs detected significant differences in only maximum speed achieved (Figure 2.1g; $F_{1,34} = 4.89$, $p = 0.03$), with predators exposed to elevated temperatures reaching greater speeds than the control temperature exposed predators. Exposure to elevated temperatures did not significantly influence distance travelled during an attack or the attack rate.

2.1

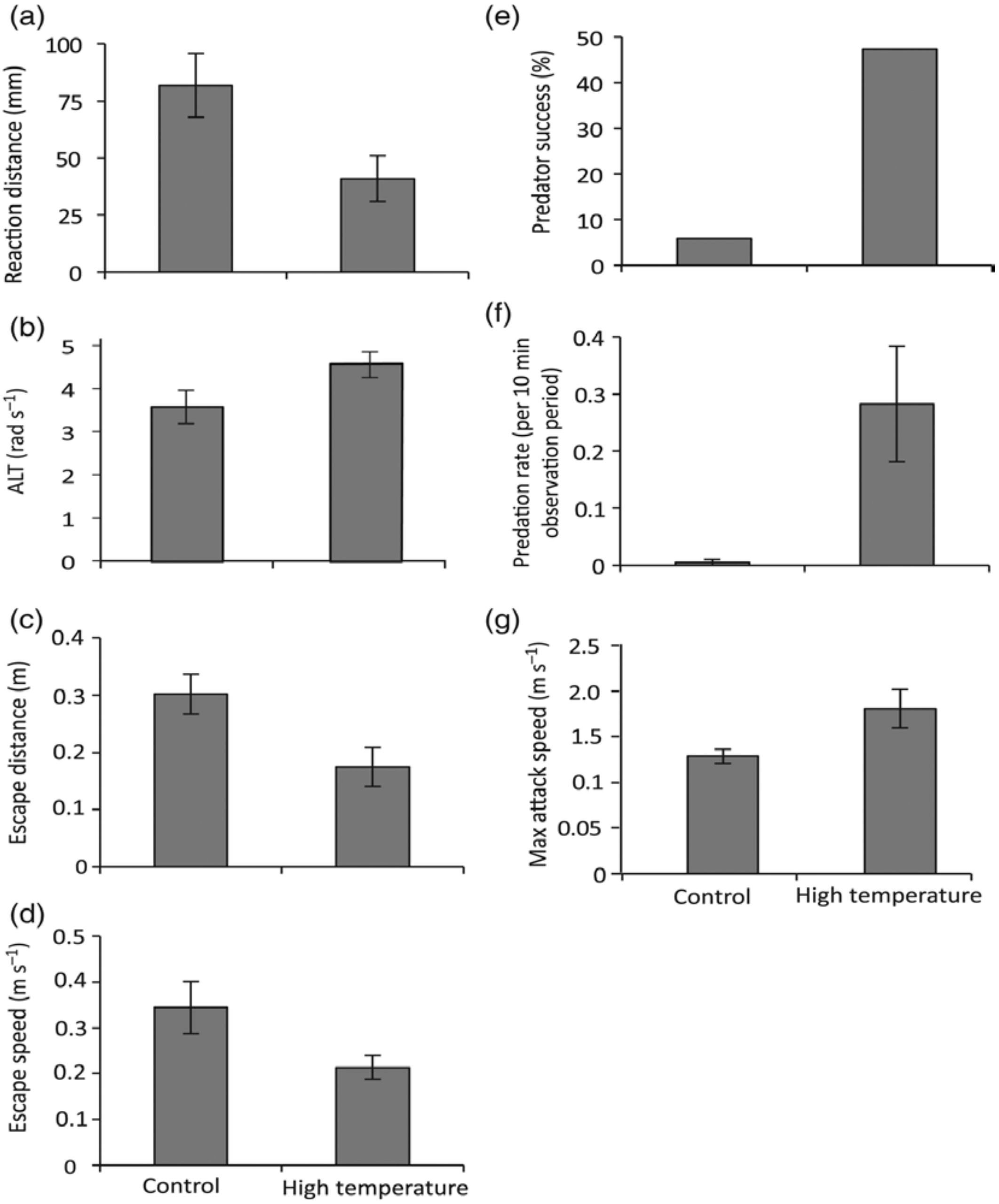

Figure 2.1. Comparison of the effects of temperature (26.7 and 29.6°C) on interactions between a predator (*Pseudochromis fuscus*) and prey (*Pomacentrus wardi*). (a) prey reaction distance, (b) prey apparent looming threshold (ALT), (c) prey escape distance, (d) prey speed, (e) predator success, (f) predation rate, and (f) maximum attack speed. N = 17 (control), N = 19 (high temperature). Error bars are standard errors.

2.5 Discussion

Fish experience temperature fluctuations at a range of temporal and spatial scales that influence their physiology (Rome, 2007) and behaviour (Biro et al., 2010). Our results demonstrate that even modest temperature increases can lead to changes in the timing, kinematics and outcome of predator-prey interactions. Specifically, prey exposed to elevated temperatures for 7 days exhibited changes in locomotor performance with decreases in swimming speeds and reduced escape distances. This reduced prey performance is likely due to a decline in muscle power at elevated temperatures (Johnson and Bennett, 1995). Many species exhibit a decline in swimming performance once a thermal threshold has been reached (for review see Johnston and Temple, 2002), with species responses differing due to the thermal sensitivity of their performance curves (Grigaltchik et al., 2012).

Alternatively, the mechanistic explanation underlying the observed responses may be declining aerobic scope caused by an increase in resting metabolic rate due to exposure to elevated temperatures (Munday et al., 2012). Although fast-starts are powered anaerobically, this energy debt has to be paid off by post-exercise oxygen consumption, which requires more energy than initially used, creating an energy deficit (Moyes et al., 1993). Moreover, reduced aerobic scope may have caused a decrease in burst swimming performance indirectly, i.e. as a result of reduced body condition.

In addition to reduced locomotor performance, there were changes in the responsiveness of the prey with reduced reaction distances and an increase in apparent looming threshold. The fast kinematics of escape responses are likely to be under the control of Mauthner neurons, which are triggered as a reaction to the fast approach of a predator (Eaton et al., 2001). Because elevated temperatures led to decreased reaction distances and an increase in apparent looming threshold, the motivational component of the motor response, which also acts upon the duration of the burst, may have been affected. Furthermore, it is also possible that the elevated temperature had a negative effect on the neural control and therefore the timing of the response. Webb and Zhang (1994) found that the responsiveness (i.e. their reaction distance) of prey (Goldfish; *Carassius auratus*) to an attacking predator (Rainbow trout; *Oncorhynchus mykiss*) was affected following acute exposure to elevated temperatures leading to increased predator success. Szabo et al., (2008) found that acclimation to high temperatures altered the escape responses of C*. auratus* through changes in the cellular physiology of the Mauthner neuron circuit, which led to differences in the balance between the excitatory and inhibitory transmission onto the Mauthner neuron. Fish acclimated to warmer water tended to show high excitability but a lower directional discrimination of the stimulation. These studies indicate that sensory responsiveness; a crucial factor for successfully escaping from predators (Domenici, 2010) can be affected by temperature changes.

Predators exposed to elevated temperatures had an increase in capture success compared to controls, which may be due to two factors. Firstly, the reactivity and locomotor performance associated with the prey's escape behaviour decreased following exposure to elevated temperatures and secondly, there was an increase in the predation rate and maximum attack speeds, which could be interpreted as an increase in predator motivation to capture prey. Hunger may have played a role in determining motivation as the energetic demands of the predator may have increased due to an increase in metabolic rate, while food availability was kept constant in the two treatment groups. In addition, Webb, (1984) showed that some predators attack at submaximal speeds. Therefore, it is possible that in the present study increased hunger (resulting from the temperature-induced increase in metabolic rate) may have caused predators to attack at a higher speed compared to the control, since they may not have exhibited maximum attack speeds in the control treatment. Furthermore, Webb, (1984) and Domenici and Blake, (1997) suggest that the strategy of striking at speeds below maximum ensures that prey do not initiate an escape response early (i.e. with a short reaction distance), which could result in displacing prey further from the predators strike trajectory. Interestingly, we found that despite the increase in predator attack speed (which usually causes an increased prey reaction distance; (Dill 1974), prey exposed to elevated temperature showed a reduction in reaction distance, as a result of their decreased sensory performance (i.e. increase in apparent looming threshold).

Although it is possible that predators increased their attack speeds at high temperature as a result of both increased hunger level and sub-maximal speeds of the control situation, additional physiological considerations may also explain the differential thermal response of predator and prey. Because only one of the locomotory variables we tested for our predator (maximum attack speed) was affected following exposure to elevated temperatures, this suggests that the attack performance of *P. fuscus* was robust to modest temperature increases. This may be because they have experienced a wider breadth of temperatures on the shallow fringing reef than the prey, which were returning to the reef from the open ocean at the end of their larval phase.

Alternatively, there may have been differential levels of thermal acclimation between the two species as a product of their recent and or evolutionary history. Both predators and prey were exposed to the elevated temperatures for a minimum of 7 days before predation trials commenced, which should have allowed acclimation to occur, but the capacity for thermal acclimation may differ between the two species. However, if fish are exposed to temperatures for longer than 7 days, then the potential for acclimation to occur may increase (Johnson and Bennett, 1995). Acclimation can lead to changes in the speed of contraction of fast muscle fibres and a corresponding change in swimming performance (Rome, 1985; Heap and Goldspink, 1986; Johnson and Bennett, 1995). Fish that can acclimate to increasing temperatures usually experience increased capture or escape success (Yocom and Edsall, 1974; Beddow et al., 1995).

Overall, we found an increase in predation rate in response to a temperature increase that was within the range naturally found at the study site (ranging from 25.2°C and 29.2°C during the 2012 recruitment period). This change in predator success resulted from temperature negatively affecting the prey's fast-start kinematics and responsiveness, while the predator's locomotor performance was enhanced. As a consequence of elevated temperatures affecting the prey and predator in opposite ways, predator strike success increased at elevated temperatures. Regardless of the possible mechanistic explanations for the changes we saw, the differing responses between *P. wardi* and its predator *P. fuscus* to small temperature increases could have consequences for ecological interactions and the relative abundance of species within coral reef fish communities. How critical these changes are, particularly when combined with other stressors, remains to be determined. In an era of rapid climate change, understanding small-scale changes in ambient temperatures and how these influence the interaction between organisms and their environment will be increasingly important in predicting climate change effects within ecosystems. We have demonstrated that the kinematics at the basis of predator-prey interactions in coral reef fish are significantly changed following exposure to modest increases in temperature within the range normally experienced. Further studies should test how predator-prey interactions change at temperatures beyond their current limits to determine the extent to which the physiological mechanisms that underpin performance will affect key ecological processes in the future.

Chapter 3: Elevated CO_2 affects predator-prey interactions through altered performance

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3.1 Summary

Recent research has shown that exposure to elevated carbon dioxide (CO_2) affects how fishes perceive their environment, affecting behavioral and cognitive processes leading to increased prey mortality. However, it is unclear if increased mortality results from changes in the dynamics of predator-prey interactions or due to prey increasing activity levels. Here we demonstrate that ocean pCO_2 projected to occur by 2100 significantly affects the interactions of a predator-prey pair of common reef fish: the planktivorous damselfish Pomacentrus amboinensis and the piscivorous dottyback Pseudochromis fuscus. Prey exposed to elevated CO_2 (880µatm) or a present-day control (440µatm) interacted with similarly exposed predators in a

cross-factored design. Predators had the lowest capture success when exposed to elevated CO_2 and interacting with prey exposed to present-day CO_2. Prey exposed to elevated CO_2 had reduced escape distances and longer reaction distances compared to prey exposed to present-day CO_2 conditions, but this was dependent on whether the prey was paired with a CO_2 exposed predator or not. This suggests that the dynamics of predator-prey interactions under future CO_2 environments will depend on the extent to which the interacting species are affected and can adapt to the adverse effects of elevated CO_2.

3.2 Introduction

Predation is one of the key processes structuring communities in ecological and evolutionary time (Lima and Dill, 1990). Prey with well-honed anti-predator responses will have high survival, while predators with low catch rates suffer slower growth and reduced reproductive output (Bengtson, 2002; Planes and Romans, 2004). Any factor that influences a part of the predator prey interaction sequence will affect the outcome for both parties. Recent research has shown that carbon dioxide (CO_2) levels projected to occur in the ocean by the end of this century (based on Representative Concentration Pathways RCPs) (Meinhausen et al. 2011) can affect the behavior of marine organisms and significantly increase predation rates in natural habitat (Bibby et al. 2007; Dixson et al. 2010; Munday et al. 2010; de la Haye et al. 2011; Ferrari et al. 2011a; Briffa et al. 2012). These findings emphasize the potential impact that elevated CO_2 in the ocean could have on marine population dynamics and ecosystem processes (Fabry et al., 2008; Harley, 2011;

Wisenden, 2012). To date however, the dynamic mechanisms of predator-prey interactions that underlie increased predation rates in high CO_2 environments are unknown.

The concentration of CO_2 in the atmosphere has increased approximately 40% since the industrial revolution to present-day levels 390 ppm. The atmosphere and surface-ocean are in approximate gas equilibrium; therefore the concentration of CO_2 in the ocean also increases as atmospheric CO_2 increases (Doney et al., 2009). If the current trajectory of emissions is maintained, atmospheric CO_2 concentrations are predicted to exceed 900 ppm by the year 2100 (Meinhausen et al. 2011). It has recently been demonstrated that these levels of dissolved CO_2 can dramatically alter the response of fish predators to prey sensory cues (Cripps et al., 2011) and of fish prey to the presence of predators (Dixson et al. 2010). Fish exposed to elevated CO_2 exhibit impaired olfactory (Dixson et al. 2010; Cripps et al. 2011) and auditory responses (Simpson et al. 2011), altered activity levels (Munday et al. 2010; Ferrari et al. 2011a) and reduced behavioral lateralization (the propensity to turn left or right) (Domenici et al., 2012). The underlying mechanism for these behavioural changes appears to be impaired neurotransmitter function in fish that are permanently exposed to elevated CO_2 (Nilsson et al., 2012). If elevated CO_2 alters the processing of sensory information, then it may directly influence the behavioral basis that governs the outcome of a predator-prey interaction, such as the timing of a predator strike or the distance to react to the predator by the prey. During a predator-prey interaction, fish employ a rapid acceleration called a fast-start that is used by predators to capture prey, or by prey to avoid a predatory strike (Domenici and Blake 1997; Walker et al. 2005; Langerhans et al. 2009). Consequently, any effect of elevated CO_2 on the timing or performance of fast-starts by either the predator or prey could lead to changes in prey mortality.

This study examined the potential cause of increased mortality of prey fish that has been observed in previous studies (Munday et al. 2010; Ferrari et al. 2011a) by asking the specific question: Does exposure to elevated CO_2 change the outcome of predator-prey encounters by altering the kinematics of the predator-prey interaction? To test this hypothesis, locomotion performance, prey reaction distance and capture success were examined in staged encounters between newly metamorphosed individuals of a prey fish, Pomacentrus amboinensis, and a common predator, Pseudochromis fuscus. Predators and prey were exposed to CO_2 levels (880 µatm) relevant to the end of the century based on the most recent representative greenhouse gas concentration pathways (Meinhausen et al. 2011). Previous experiments have demonstrated that the mortality rates of high- CO_2 exposed prey when placed in the field with non- CO_2 exposed predators are 2–3 times higher than when prey are exposed to similarly treated predators in the laboratory (Munday et al. 2010; Ferrari et al. 2011a). This suggests that the behavior of the CO_2 exposed prey depends on whether or not the predator has also been exposed to elevated CO_2. Therefore, the responses of fish exposed to high CO_2 were compared to fish exposed to present day CO_2 levels (440µatm) in a fully crossed design. This enabled us to tease apart the independent effects on the predator and prey as well as the interacting effects when both were exposed to elevated CO_2.

3.3 Methods

Study Site and Species

Fishes were collected during December 2010 at Lizard Island (14° 40′ S, 145° 28′ E), northern Great Barrier Reef (GBR) and maintained in a flow-through seawater system at the Lizard Island Research Station (LIRS). Newly metamorphosed individuals of the common damselfish, *Pomacentrus amboinensis* (Pomacentridae) were used as the prey species. The dottyback, *Pseudochromis fuscus* (Pseudochromidae) was used as the predator. *P. fuscus* is an abundant, small, widely distributed mesopredator found throughout the Indo-Pacific. It is a gape limited, highly territorial and active predator and makes up 9.5% of the piscivorous reef fish assemblage at Lizard Island (Stewart and Jones, 2001). It is considered an important predator of newly settled coral reef fishes (Munday et al., 2003; Holmes & McCormick, 2010). *P. fuscus* readily adjusts to aquarium conditions and has been observed exhibiting normal feeding and aggressive behaviors within 48 hours of collection (Messmer et al., 2005). Because of this, it has been used extensively as a model predator in predator-prey manipulation studies.

Newly metamorphosed *P. amboinensis* (range 10.3–15.1 mm, 12.6 mean standard length (SL), standard deviation (SD) 1.5) (Kerrigan, 1996) were collected using light traps (Meekan et al., 2001) moored 100 m off the fringing reef of Lizard Island. On the morning of capture they were transferred to 30 L aquaria supplied with a continuous flow of either control (present-day CO_2) or elevated- CO_2 seawater (see below) for 4 days. This period of time has been found to be sufficient to elicit the full behavioural effects of high CO_2 and larval fish do not become acclimated with longer exposure (Munday et al., 2010). Fish were fed 4 times daily ad libitum with newly hatched Artemia sp. but were starved for the 12 hours prior experimental trials to standardize for satiation.

Adult *P. fuscus* (range 64–83 mm, 72.3 mm mean SL, SD 0.6) were collected with a dilute solution of clove oil (Munday and Wilson, 1997) from of the shallow fringing reef around Lizard Island. Immediately after collection, fish were transported back to LIRS where they were housed separately in mesh breeding baskets within 30 L aquaria to avoid aggressive interactions. Fish were maintained in tanks for 4 days and were fed 2 juvenile reef fish for the first 2 days and then not fed for the last 2 days to standardize for satiation. Food deprivation in the wild is not unusual and previous work has demonstrated a high prevalence of gut emptiness for piscivorous fish (Sweatman,1984).

CO_2 Treatment

The pCO_2 of treatment seawater was manipulated by CO_2 dosing to a set pH_{NBS}. Seawater was pumped from the ocean into 2x60 L sumps where it was diffused with ambient air (control) or CO_2 to achieve the desired pH (CO_2 treatment). A pH of 7.89 was selected to achieve the approximate pCO_2 required, based on preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH-controller (Tunze

Aquarientechnik, Germany) was attached to the CO_2 treatment sump to maintain pH at the desired level. A solenoid injected a slow stream of CO_2 into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. Equilibrated seawater from each sump was then supplied at a rate of ~500 $ml.min^{-1}$ to eight replicate 35 L aquariums, four housing small groups of *P. amboinensis* and four housing *P. fuscus*. Temperature and pH_{NBS} of each aquarium was measured each morning and afternoon using an HQ40d pH meter (Hach, Colorado, USA) calibrated with fresh buffers. Total alkalinity (TA) of seawater was estimated by Gran titration from water samples taken twice weekly from control and treatment tanks. Alkalinity standardizations achieved accuracy within 1% of certified reference material from Dr. A. Dickson (Scripps Institution of Oceanography). Average seawater pCO_2 was calculated using measured values of pH, TA, temperature and salinity in the program CO2SYS (Pierrot et al., 2006) and using the constants of Mehrbach et al., (1973) refit by Dickson and Millero, (1987). Seawater parameters are shown in Table 1.

Table 3.1. Mean (±SD) seawater parameters in the experimental system. Temperature, pH salinity, and total alkalinity (TA) were measured directly. pCO_2 was estimated from these parameters using CO2SYS.

pH_{NBS}	**Temp (°C)**	**Salinity (ppt)**	**TA (µmol.kg^{-1}SW)**	**pCO_2 (µatm)**
8.15 (0.04)	27.66 (0.98)	35	2269.66 (15.01)	440.53 (44.46)
7.89 (0.06)	27.74 (0.99)	35	2261.23 (14.92)	879.95 (140.64)

Laboratory Assays

Trials were conducted over a period of 10 days in a temperature-controlled room at LIRS, ensuring the water temperature remained between 26 and 28°C. One predatory *P. fuscus* was placed into the experimental arena (38 cm x 58cm x 10cm water height) and one *P. amboinensis* was then released into a length of PVC tube (11cm diameter, 15cm high) placed upright in the middle of the experimental arena. Both fish were allowed to acclimate for 30 min. The PVC tube was then carefully raised and removed from the tank using a wire connected to the top of the tube. This allowed the predator and the prey to start the interaction. A soundproof polystyrene lid was placed on the experimental arena to minimize disturbance and to eliminate observer effect. A high-speed video camera (Casio ex-fh20; 420 fps) recorded fish behavior through a hole in the lid and trials were filmed until the prey was consumed or 10 min had elapsed. The water in the experimental arena was changed following each trial to maintain oxygen saturation. SL (defined as the length of a fish measured from the tip of the snout to the posterior end of the last vertebra) of the predator and prey and the water temperature were recorded for each trial.

In order to partition the relative effects of CO_2 on predators, prey and the full interaction, four combinations of CO_2-treated and control predators and prey were undertaken in a crossed design: control predator vs. control prey (n= 21); treated predator vs. treated prey (n= 21); control predator vs. treated prey (n =16); treated predator vs. control prey (n= 16). Predator and prey fish were used only once in each trial. This ensured both were naive to the experimental procedure. All combinations of treatments were undertaken daily to control for any potential daily variation. Video-analysis could not be performed on all interaction trials, due to fish leaving the screen at the time of the response and/or technical problems with the video. As a result, the sample size varied slightly among the performance traits measured. Trials were only used when the predator was at the opposite end of the tank to the prey at the start of the interaction. This was done to standardize for predator position. Maximum predator attack speed and maximum prey escape speed ($UMAX_{pred}$ and $UMAX_{prey}$) were measured based on the center of mass (COM) of the fish when stretched straight based on Webb (1976). COM was assumed to be at 35% of the body length from the tip of the snout as it is the case for generalist fish. Speed was smoothed using a 5-point differentiation-based moving polynomial regression (Lanczos 1956). Stage 1 and 2 where defined based upon directional changes of the anterior part of the body of the fish, based on Domenici and Blake (1997).

Prey escape variables were measured only when prey performed a C-start. Predator attacks were measured only when a predator showed a fast-directed burst towards the prey (>3 body lengths s^{-1}). All variables with the exception of number of prey caught were measured using only the first attack that occurred within a trial. This was done to control for any anaerobic stress either the predator or prey may have experienced due to prolonged attacks.

Kinematic Analysis

Video recordings were analyzed using WinAnalyze motion analysis software (v. 1.9 2D; Mikromak Service Brinkmann, Berlin, Germany). In each frame the snout and the COM (center of mass) were located on each fish. These points were chosen to standardize each frame. The following performance variables were measured:

Predator

1) Capture success: percentage of trials in which the predator ingested the prey within the 10 min filming period, out of the total number of trials for each treatment.

2) Attack rate: number of attacks per unit time, measured for each interaction.

3) Predation rate: capture success divided by the number of attacks per unit time.

4) Predator attack distance (D_{pred} m): the straight-line distance between the predator centre of mass (COM) at the time the attack commenced and the end of the attack. The end of the attack is defined as when the predator came to a halt.

5) Maximum predator attack speed ($UMAX_{pred}^{m\ s^{-1}}$): the top speed achieved at any point in time during the attack, based on the predator COM.

Prey

6) Prey reaction distance (R_D m): the distance between the prey COM and the tip of the predator's snout at the onset of the escape response to a predator attack.

7) Apparent Looming Threshold (ALT) is defined as the apparent looming threshold for prey avoidance responses to a predatory strike and is a measure of the magnitude of the preys response to the perceived threat of predation. The higher the perceived threat, the higher the angle, (ALT; radians s^{-1}): measured at the onset of the escape response and measured as the rate of change of the angle (α) subtended by the predator's frontal profile as seen by the prey. Previous work has shown that fish tend to react to an approaching stimulus (a predator) when a given threshold of dα/dt (i.e. ALT) is reached. ALT is calculated as $(4US)/(4D^2+S^2)$, based on Dill, (1974) and Webb, (1982). Where U= predator speed, calculated as the speed of the predator in the frame prior to the prey's response; S = (Max. Depth+Max. Width)/2, where Max. Depth (D_{MAX}) was estimated to be positioned at one-quarter of the body length of the predator (L_{pred}) (pers. obs.) and maximum width (W_{MAX}) at $0.25L_{pred}$; $D=R_D+0.25L_{pred}$.

8) Prey escape distance ($D_{prey}{}^{m\ s-1}$): the straight-line distance between the prey COM at the onset of the escape response and at the end of the escape response (i.e. when the prey comes to a halt).

9) Maximum prey escape speed ($UMAX_{prey}{}^{m\ s-1}$): the top speed achieved at

any point in time during the escape response, measured using the prey COM.

10) Mean prey escape distance during stage 1 & 2 (Ds1s2; m): the distance between the COM of the fish at frame 0 and 24 ms later. This fixed duration was based on the average duration (22.8±3.2 ms), of the first two tail flips of the tail (the first two axial bends, i.e. stages 1 and 2 defined based on (Domenici and Blake, 1997), which is the period considered crucial for avoiding ambush predator attacks (Domenici and Blake, 1997; Webb, 1976).

Statistical Analyses

To test the null hypothesis that feeding success is independent of predator and prey CO_2 exposure, predator success was compared by 2x4 contingency table analysis. The effects of CO_2 elevation on predator-prey interactions were examined using a 2-factor MANOVA with the fixed factors: Prey treatment (Control and CO_2 elevated) and Predator treatment (Control and CO_2 elevated). Univariate 2-factor ANOVAs with Tukey's HSD post hoc tests were performed to determine the nature of any differences found by MANOVA. Predation rate data was arcsine transformed to meet the assumption of homogeneity of variance. Residual analysis indicated that data met the assumptions of normality and homogeneity of variance.

3.4 Results

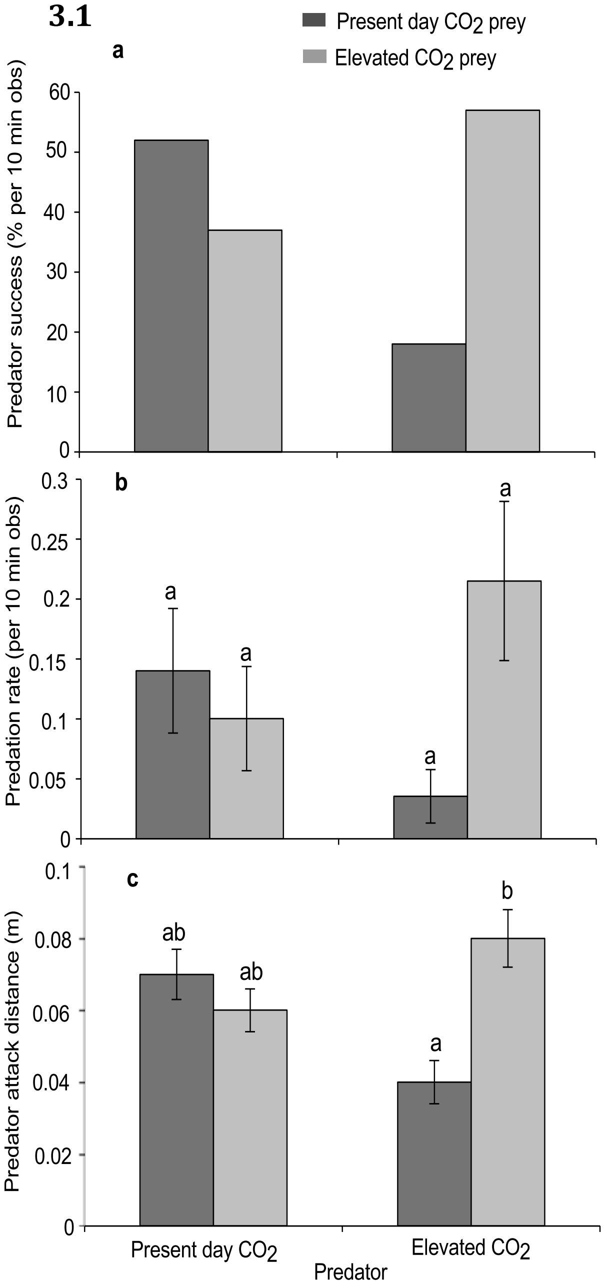
Capture success was significantly associated with the CO_2 exposure treatment experienced by the predator and prey ($\chi^2 = 8.95$, df = 3, p = 0.03; Fig. 1a). When both the predator and prey had been exposed to elevated CO_2 the capture rate (52%) was similar to that of the control group where both predators and prey were exposed to present-day CO_2 (51%). In contrast, the capture rate of these two treatment groups was markedly higher than the CO_2 predator-Control prey group. When predators exposed to elevated CO_2 were given prey exposed to present-day CO_2, capture success of the predator was 33% less than the treatment groups where both predators and prey had been exposed to the same levels of CO_2 (either elevated or present day CO_2). Capture success was also 14% less for predators exposed to present-day CO_2 with elevated CO_2 exposed prey compared with treatment groups where both predators and prey had been exposed to the same levels of CO_2. The 2-factor MANOVA revealed a significant interaction between the effects of elevated CO_2 on the performance characteristics of predators and prey (Pillai's trace (3,52) = 7.50, $p<0.0001$). ANOVA detected a significant interaction in the CO_2 levels of predators and prey for six of the tested behavioral attributes: attack rate, predation rate, predator attack distance, reaction distance, apparent looming threshold and prey escape distance (see Table 2).

Elevated CO_2 significantly affected predator attack rates, however, this was influenced by prey CO_2 exposure (Table 3.2). There was no difference in attack rate when predators and prey were exposed to the same CO_2

treatments. However, when predators were exposed to elevated CO_2, they displayed a significantly decreased attack rate against prey that had not been exposed to elevated CO_2. Similarly, predators exposed to present day CO_2 levels had a reduced attack rate when paired with prey that had been exposed to elevated CO_2 levels. Exposure to elevated CO_2 significantly affected predation rates (Table 3.2; Figure 3.1b). For predators, exposure to elevated CO_2 resulted in a significant reduction in the predation rates of prey that had not been exposed to elevated CO_2. Post-hoc tests yielded a border line p-value ($p = 0.05$) for the comparison between the elevated CO_2 exposed predators that interacted with present-day CO_2 exposed prey and the elevated CO_2 exposed predators that interacted with similarly exposed prey. Furthermore, when predators exposed to elevated CO_2 were paired with similarly exposed prey, there was a significant increase in predation rates. Elevated CO_2 significantly affected predator attack distance, but the nature of the effect differed according to whether prey had been exposed to elevated CO_2 or not (Table 3.2; Figure 3.1c). There was no effect of CO_2 on attack distance when prey encountered control predators, however, when predators had been exposed to elevated CO_2 they displayed significantly increased attack distances against prey that had also been exposed to elevated CO_2 compared to control prey. The reaction distance of the prey to the predator at the onset of the first attack was affected by the CO_2 treatment of the prey and predator (Table 3.2; Figure 3.2a).

Prey exposed to elevated CO_2 allowed CO_2 exposed predators to get closer to them before undertaking an escape response. Max predator speed was found not to differ between treatments. Apparent looming threshold (ALT) was significantly affected following exposure to elevated CO_2. When prey that had been exposed to elevated CO_2 were paired with similarly exposed predators, ALT increased significantly. In contrast, when control prey were paired with CO_2 exposed predators, ALT decreased substantially. There was no significant difference in ALT between the crossed trials (Table 3.2; Figure 3.2b). Prey escape distance was also found to be significantly affected following exposure to elevated CO_2 (Table 3.2; Figure 3.2c) with prey exposed to present-day CO_2 having the highest escape distance compared to the other three treatment combinations. Prey escape speed during stage 1 and 2 and maximum prey escape speed were not influenced by the exposure of either prey or predator to elevated CO_2 and no interactions between predator and prey treatments were found.

3.1



3.2

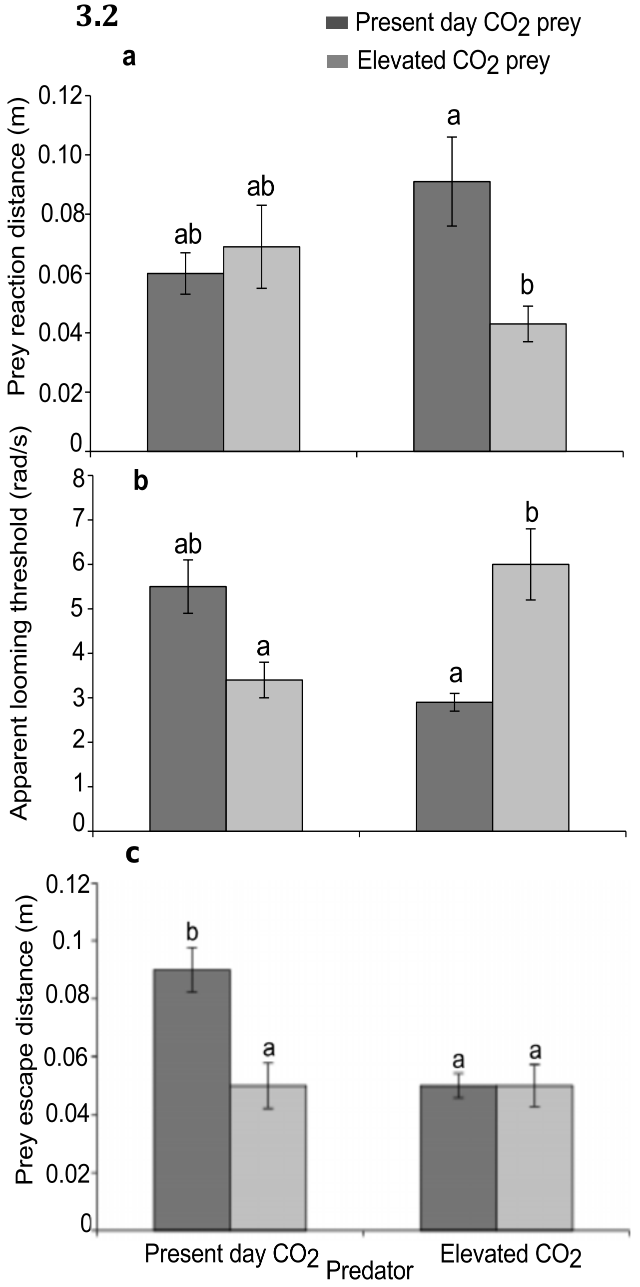


Figure 3.1. Effects of elevated CO_2 on predator attack performance. Comparison of the effects of elevated CO_2 (440, 880 µatm) on interactions between a predator (*Pseudochromis fuscus*) and prey (*Pomacentrus amboinensis*) on 3 performance variables of the predator: (a) predator success (b) predation rate and (c) predator attack distance. (a) N = 21, 16, 16, 21, (b) N = 21, 16, 16, 21 and (c) N = 17, 15, 12, 16 (left to right). Errors are standard errors. Letters above bars represent Tukey's HSD groupings of means.

Figure 3.2. Effects of elevated CO_2 on prey escape performance. Comparison of the effects of elevated CO_2 (440, 880 µatm) on interactions between a predator (*Pseudochromis fuscus*) and prey (*Pomacentrus amboinensis*) on 3 performance variables of the prey: (a) prey reaction distance (b) apparent looming threshold (ALT) and (c) prey escape distance. (a) n = 19, 14, 11, 19 (b) N = 17, 14, 11, 16 and (c) N = 17, 14, 11, 18 (left to right). Errors are standard errors. Letters above bars represent Tukey's HSD groupings of means.

Table 3.2 Comparison of the effects of elevated CO_2 (440, 880 µatm) on interactions between a predator (*Pseudochromis fuscus*) and prey (*Pomacentrus amboinensis*) on 6 performance variables: (a) attack rate (b) predation rate (c) predator attack distance (d) prey reaction distance (e) ALT and (f) prey escape distance.

Behavior	**Source of variation**	***df***	**MS**	***F***	***P***
(a) Attack rate	predator	1	0.0042	0.143	0.705
	prey	1	0.0070	0.238	0.626
	predator*prey	1	0.1427	4.845	**0.030**
	error	70	0.0294		
(b) Predation rate	predator	1	0.0049	0.464	0.829
	prey	1	0.1459	1.358	0.247
	predator*prey	1	0.4387	4.083	**0.020**
	error	67	0.1074		
(c) Predator attack speed	predator	1	2017	0.213	0.646
	prey	1	38512	4.070	**0.048**
	predator*prey	1	61256	6.474	**0.013**
	error	56	9462		
(d) Prey reaction distance	predator	1	685.5	0.422	0.519
	prey	1	8898.2	5.471	**0.022**
	predator*prey	1	16555.2	10.179	**0.002**
	error	56	1626.4		
(e) ALT	predator	1	228	0.011	0.916
	prey	1	12548	0.616	0.435
	predator*prey	1	313294	15.396	**<0.001**
	error	54	20349		
(f) Prey escape distance	predator	1	57241	6.365	**0.014**
	prey	1	40388	4.491	**0.038**
	predator*prey	1	69008	7.674	**0.007**
	error	56	0.4445		

3.5 Discussion

Although recent studies have demonstrated that exposure to elevated CO_2 significantly increases prey mortality rates (Munday et al., 2010; Ferrari et al., 2011a; Ferrari et al., 2011b), the effects of elevated CO_2 on the kinematics at the basis of predator-prey interactions has not been investigated. Here, we demonstrated that CO_2 levels that may occur in the surface oceans by the end of the century impact on both the kinematics and the timing of predator-prey interactions. More specifically, prey treated with elevated CO_2 showed changes in escape performance, such as shorter reaction distances, reduced escape distances and changes in apparent looming threshold (ALT). Because CO_2 exposed prey were closer to the predator at the onset of their escape reaction and they swam shorter distances, elevated CO_2 had a clear negative effect on the reactivity and locomotion performance of the prey. The fast kinematics of the escape responses are likely to be under the control of Mauthner neurons (although control by other reticulospinal cells cannot be ruled out (Kohashi and Oda, 2008), which are triggered as a reaction to the fast approach of a predator. It is therefore possible that increased CO_2 levels may have an intrinsic effect on the sensory performance and neural control by the Mauthner neurons or other reticulospinal cells at the basis of the escape response, leading to increased prey vulnerability and ultimately increased mortality. Because escape distance is affected by CO_2, it is possible that CO_2 affects mainly the motivational component of the motor response, which acts upon the duration of the burst. Nevertheless, the effects of CO_2 on the onset of the response (i.e. reaction distance), suggest that the sensori-motor performance and the timing of the Mauthner neurons firing are also affected.

Previous work has demonstrated that the regulation of plasma and cellular HCO_3^- and Cl^- following exposure to high CO_2 may lead to the excitation of $GABA_A$ receptors (Nilsson et al., 2012). Given that $GABA_A$ receptors are found throughout the Mauthner neuron (Diamond and Huxley, 1968), these results suggest that high CO_2 interferes with $GABA_A$ receptor function resulting in the misfiring of action potentials. Therefore, it is possible that this interference with brain neurotransmitters may be responsible for the changes observed. These results are consistent with the increased mortality rates observed in CO_2-exposed prey (Munday et al., 2010; Ferrari et al., 2011a) and suggest that higher vulnerability to predation may be caused by a combination of changes in escape performance and other behavioural traits, such as increased activity levels and distance from shelter.

The results for the predators are complex to interpret, because the overall predation rate is the result of both predator performance during the attack and its motivation to attack. Attack rates and attack distances were affected by elevated CO_2, with the lowest attack rates and distances occurring when predators and prey had experienced different CO_2 histories. This demonstrates that high CO_2 has an effect on predator performance during a predator-prey encounter, however the extent of this effect appears to be dependent upon the extent to which prey are affected by high CO_2. Predatory attacks require not only high speed but also precision to aim at the prey. The neural mechanisms that control predatory attacks are poorly understood, although it has been suggested that in some cases they may also be controlled by Mauthner neurons (Wohl and Schuster, 2007). It is possible that future levels of high CO_2 will impact on the complex circuitry needed to carry out attack processes. This may explain why we see behavioural changes that result in either decreased or increased capture success when predators and prey are exposed to different CO_2 histories, but when both are treated similarly, these changes appear to ‘level out’ resulting in no change in overall capture success compared with controls. Similar results have been suggested in estuarine systems involving crustaceans and molluscs, where for individual species there was a negative effect following CO_2 exposure, but these affects were not manifest at the community level (i.e. within the predator-prey interaction) (Landes and Zimmer, 2012). This suggests that elevated CO_2 will have a marked impact on the dynamics and outcome of predator prey encounters, but the population and community level effects are likely to be dependent on the sensitivity of species to elevated CO_2.

This is the first study to examine performance-based attributes of both predators and prey under a high CO_2 environment within the context of a predator-prey interaction. While our findings are specific to the species used, the fact that the behavior of both predator and prey were affected is strongly suggestive that elevated CO_2 will affect the behavior of other predator and prey fish. Whether in other cases the increase in CO_2 will provide an advantage for the prey, the predator or neither, will depend on the extent to which the specific predators and prey individuals are tolerant to CO_2 changes. Recent studies have shown that effects of elevated CO_2 can differ markedly between species (Ferrari et al., 2011a). Moreover, the effects of elevated CO_2 are not necessarily restricted to single fish-fish interactions, but might be manifest through other trophic pathways. Changes in seawater pCO_2 has been shown to impact fishes at all stages of development (Briffa et al., 2012; Munday et al., 2012), with significant impacts on individual fitness occurring at the vulnerable juvenile stage. Alterations to the dynamics of regulating processes at this early life stage could have significant affects on the replenishment and sustainability of marine populations (Munday et al., 2010). In addition to affecting the physiology and behavior of given species, elevated CO_2 is likely to influence interactions between species, including predator-prey encounters. This may have important ecological effects, such as changing the balance of an interaction in favor of the predator or the prey. This study highlights the importance of considering species interactions when making predictions concerning the response of communities to climate change (Gilman et al., 2010; Cripps et al., 2011).

Chapter 4: Differential effects of CO_2 and warming on predator-prey interactions in coral reef fish

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4.1 Summary

Ocean acidification and warming, driven by anthropogenic CO_2 emissions, are considered to be among the greatest threats facing marine organisms affecting physiology and behaviour in fishes. While each stressor in isolation has been studied extensively, there has been less focus on their combined effects, which could impact key ecological processes. We tested the independent and interactive effects of short-term elevated CO_2 and temperature exposure on the predator-prey interactions of a common pair of coral reef fishes (*Pomacentrus wardi* and its predator, *Pseudochromis fuscus*). We found predator success increased following predator and prey

exposure to elevated CO_2 and temperature. Overall, high temperature had an overwhelming negative effect on the escape behaviour of the prey compared to the independent effect of CO_2 or the combined exposure to elevated CO_2 and temperature. We observed little influence of temperature or CO_2 on the behaviour of the predator, suggesting that the attack behaviour of *P. fuscus* was robust to these environmental changes. This is the first study to address how the kinematics at the basis of predator-prey interactions may change in response to concurrent exposure to elevated CO_2 and temperature and represents an important step to forecasting the responses of interacting species to climate change.

4.2 Introduction

Atmospheric CO_2 has risen from approximately 280ppm pre-industrial to 400ppm in 2015, the highest level in at least 600,000 years (Lüthi et al., 2008). Ocean pCO_2 is rising at the same rate as the atmosphere, leading to a decline in seawater pH (Doney et al., 2009). If the current rate of anthropogenic CO_2 emissions is maintained, climate models project that CO_2 concentrations in the atmosphere and surface ocean will exceed 900 ppm by the end of this century and ocean pH will decline by 0.14-0.43 units compared with current day values (Collins et al., 2013). These changes in ocean chemistry are set against a background of warming, with average ocean temperatures projected to increase by up to 3°C (Collins et al., 2013). Consequently, marine organisms face complex environmental changes throughout this century. While there has been extensive research into the effects of ocean acidification or rising temperature on marine organisms, most studies have tested the effects of these drivers in isolation. Relatively few studies have explored the interactive effects of these important environmental drivers. The combined effects of elevated CO_2 and warming could either be additive, antagonistic or synergistic (Munday et al., 2010; Kroeker et al., 2013). Consequently, understanding how multiple drivers affect key physiological or behaviourial traits in marine organisms is critical, as extrapolations based on single driver responses could lead to incorrect predictions about future impacts (Gaylord et al., 2015).

Some of the most dramatic effects of elevated CO_2 and warming reported to date are impaired physiology and altered behaviours of marine fishes. Behavioural effects of elevated CO_2 include changes in anti-predator responses, olfactory and auditory preferences, activity levels, behavioural lateralization and visual risk assessment (Briffa et al 2012; Heuer and Grosell, 2014). These behavioural changes translate into consequences for survival in natural habitats (Munday et al., 2010). Similarly, temperature can influence the physiological performance of fishes leading to changes in aerobic scope (Farrell, 2002; Johansen and Jones, 2011), cardiac output (Eliason et al., 2011), muscle development (Hanel and Wieser, 1996) and the contractile properties of the swimming muscles (Wakeling, 2006), and sensory performance (Szabo et al., 2008). Similar to exposure to elevated CO_2, thermal fluctuations lead to changes in behaviours such as activity rates (Biro et al., 2010), sensory responsiveness (Webb and Zhang, 1994) and the interactions between predators and their prey (Freitas et al., 2007; Grigaltchik et al., 2012; Allan et al., 2015).

Emerging evidence suggests that the behaviour and physiology of coral reef fishes is particularly sensitive to changes in simultaneous exposure to CO_2 and temperature. For example, there are effects on predation rates and predator selectivity (Ferrari et al., 2015), directional changes in lateralization suggestive of neural impairment (Domenici et al., 2014), increasing food consumption and foraging behaviour (Nowicki et al., 2012), reduced activity rates (Faleiro et al., 2015), reduced offspring quality (Miller et al., 2014) and lower aerobic scope (Munday et al., 2009), all of which can influence persistence of species into the future. However, the direction of reactions to multiple stressors is not always clear. For example, if multiple stressors affect similar pathways, an additive effect may be observed (Crain et al., 2008). In contrast, any stress-induced tolerances could lead to antagonisms (Blanck, 2002), resulting an attenuation of the observed stress response, while those stressors that affect different pathways may act synergistically (Kneitel and Chase, 2004).

A critical stage in the life of reef fishes is at the end of the larval phase, when they settle to the benthic environment. Mortality rates during the first few days of settlement are high and it is at this stage that fishes undergo a severe predation-induced bottleneck (Almany and Webster, 2006). Success at this life stage is dependent upon the size, growth and fast-start performance of new recruits (Green and McCormick, 2005; Holmes and McCormick, 2010; Allan et al., 2013). Fast starts are short, high-energy swimming bursts that are driven by the rapid contraction of the white (anaerobic) muscle fibres (Rome et al., 1988; Domenici and Blake, 1997). The fast kinematics of escape responses are usually controlled by the large Mauthner neurons, which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved (Eaton et al., 2001). Successful fast starts consist of finely tuned responsiveness and locomotor performance (Domenici, 2010). However, fast starts are sensitive to changes in ambient temperatures and elevated CO_2 due the interplay between temperature and muscle contraction rates (Wakeling, 2006), and CO_2 exposure and sensory performance (Szabo et al., 2008). Fast-start performance can also differ between predators and their prey due to differences in their temperature tolerance and sensitivity (Freitas et al., 2007; Grigaltchik et al., 2012; Allan et al., 2015).

To date, only one study has investigated the combined effects of climate change on predator-prey dynamics in coral reef fishes (Ferrari et al., 2015). Therefore, the aim of the present study was to employ a multi-stressor approach to assess the independent or combined effects of both elevated CO_2 and high temperatures on the attack and escape performance of a common pair of coral reef fishes: the piscivore, the dusky dottyback (*Pseudochromis fuscus*), and its prey, the juvenile of a common damselfish (*Pomacentrus wardi*). Previous work has shown that both the predator (*Pseudochromis fuscus*) and prey (*Pomacentrus wardi*) are sensitive to changes in elevated CO_2 and ambient temperature (Munday et al., 2010; Cripps et al., 2011; Allan et al., 2015). *Pomacentrus wardi* also displayed behavioural impairment following simultaneous exposure to both stressors (Domenici et al., 2014), making them ideal model organisms for studying the combined effects of environmental changes on predator-prey interactions. By exposing predators and prey to elevated CO_2 and high temperatures, either independently or simultaneously, we were able to determine the relative effects of each stressor on the attack and escape performance that forms the basis of predator-prey interactions. To date, this is the first study examining how the swimming kinematics of an attacking predator and its escaping prey may change following concurrent exposure to elevated CO_2 and high temperatures.

4.3 Methods

Study species

The Ward's damselfish, *Pomacentrus wardi* (Pomacentridae), was used as the prey. *Pomacentrus wardi* is a small planktivorous fish commonly found on Indo-Pacific coral reefs. Newly metamorphosed *Pomacentrus wardi* larvae (range 11.2–15.5 mm, average 13.2 ± 1.2mm standard length (SL) ± standard deviation (SD)) were collected using light traps moored 100 m off the fringing reef off Lizard Island. On the morning of capture, larvae were transferred into 30 L aquaria at either ambient (~405 µatm) or elevated CO_2 (~930µatm) and cross-factored with ambient (~27°C) or elevated temperature (~30°C). Control conditions were ambient for the study site at the time of the study (October 2012). Larvae were split into 4 different treatment groups in a 2 CO_2 x 2 temperature design: Ambient CO_2 - Ambient Temperature (ambient; n = 17); Ambient CO_2 - Elevated Temperature (high temperature; n = 19); Elevated CO_2 - Ambient Temperature (elevated CO_2; n = 18); Elevated CO_2 - Elevated Temperature (high temperature and elevated CO_2; n = 18). For fish in the +3°C temperature treatments, the temperature was raised by 1°C every 8 hours until the final temperature of ~30 °C was reached (Allan et al., 2015). Once individuals were acclimated to the increase in temperature, they were placed into their final experimental tanks where they remained for 7 days. Fish were fed 4 times daily ad libitum with newly hatched *Artemia* sp. but were starved for the 12 hours prior to commencement of experimental trials to standardize for satiation.

The dottyback, *Pseudochromis fuscus* (Pseudochromidae), was used as the predator. *Pseudochromis fuscus* is widely distributed throughout the Indo-Pacific and has been observed to prey upon newly settled coral reef fishes (Feeney et al., 2012), including *Pomacentrus wardi*. Adult *Pseudochromis fuscus* (range 63–99mm, mean 78.2 ± 9.4mm) were collected on SCUBA using a dilute solution of clove oil from around the shallow fringing reef off Lizard Island. Immediately after collection, fish were housed separately in mesh breeding baskets within 30 L aquaria to avoid aggressive interactions. Predators were maintained in treatment tanks for 7 days (following the same treatment exposure as *Pomacentrus wardi*) and were fed 2 juvenile reef fish morning and night and then not fed for the last 24 hours prior to the interaction trial to standardize for satiation. A 12-hour light and 12-hour dark regime was used.

CO_2 and temperature treatments

Tanks were heated with 300-watt bar heaters and insulated to ensure stability of the experimental temperatures (27 and 30°C). CO_2 treatments were maintained by CO_2 dosing to a set pH. Seawater was pumped from the ocean into 60 L sumps where it was diffused with ambient air (control) or CO_2 to achieve a pH_{NBS} of 7.87. The pH value was selected to achieve the approximate CO_2 conditions required, based on preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH-controller (Aqua Medic, Germany) was attached to the CO_2 treatment sump to maintain pH at the desired level. A solenoid injected a slow stream of CO_2 into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. Equilibrated seawater from each sump was supplied at a rate of ~720 $ml.min^{-1}$ to each of the aquaria. Temperature and pH_{NBS} of each aquarium was measured daily with a temperature probe (C22, Comark, Norwich, UK) and a pH meter (HQ40d, Hach, Colorado, USA) calibrated with fresh buffers. Seawater CO_2 was confirmed with a portable CO_2 equilibrator and nondispersive infrared (NDIR) sensor (GMP343, Vaisala, Helsinki, Finland) (Munday et al. 2014). Total alkalinity of seawater was estimated by Gran titration (888 Titrando, Metrohm, Switzerland) from water samples taken twice weekly from control and treatment tanks. Alkalinity standardizations achieved accuracy within 1% of certified reference material from Dr. A. Dickson (Scripps Institution of Oceanography). Seawater pCO_2 was calculated from seawater parameters in the program CO2SYS (Pierrot et al., 2006) using the constants of (Mehrbach et al., 1973), refit by (Dickson and Millero, 1987). Seawater parameters are shown in Table 1.

Table 4.1: Mean (+SE) seawater parameters in the experimental system. Temperature, pH salinity, and total alkalinity (TA) were measured directly. pCO_2 was estimated from these parameters using CO2SYS.

CO_2 treatment	Temp treatment	Temp (°C)	Salinity	pH_{NBS}	Total alkalinity ($\mu mol.kg^{-1}$ SW)	pCO_2 (µatm)
Ambient	Ambient	26.7 (+0.1)	35.2	8.18 (+0.01)	2274 (+6)	396 (+8)
Ambient	Elevated	29.6 (+0.1)	35.2	8.17 (+0.01)	2274 (+6)	415 (+9)
Elevated	Ambient	26.8 (+0.1)	35.2	7.87 (+0.01)	2257 (+6)	921 (+19)
Elevated	Elevated	29.8 (+0.1)	35.2	7.87 (+0.01)	2257 (+6)	935 (+19)

Laboratory assays

Predator-prey interactions were evaluated using a standard protocol established by Allan et al., (2013). Briefly, this involved placing a predator and prey fish into an experimental arena (38 cm x 58 cm x 10 cm water height) and filming the ensuing interaction at high speed (420 fps) for 10 minutes or until the prey had been consumed. Trials were only used when the predator was at the opposite end of the tank to the prey at the start of the interaction to standardize for predator position. Maximum predator attack speed and maximum prey escape speed were measured based on the center of mass (COM) of the fish when stretched straight based on (Webb, 1976). COM was assumed to be at 35% of the body length from the tip of the snout as it is the case for generalist fish (Domenici and Blake, 1997). Predator attacks were measured only when a predator showed a fast-directed burst towards the prey (>3 body lengths s^{-1}). All variables with the exception of number of prey caught were measured using only the first attack that occurred within a trial. This was done to control for any stress either the predator or prey may have experienced due to prolonged attacks. Both predators and prey were used only once regardless of the trial outcome. Prey suffering was minimal because prey were consumed immediately after a successful strike. Trials were conducted over the course of 5 days in a temperature-controlled room at the same water temperature as the relevant experimental treatment. Trials were randomized to account for any potential day effect.

The following performance variables were measured:

Predator

1) Capture success: percentage of trials in which the predator ingested the prey within the 10 minute filming period, out of the total number of trials for each treatment.

2) Predation rate: Number of captures divided by the number of attacks within the 10 minute filming period.

3) Attack rate: number of attacks occurring within the 10 minute filming period or until the prey was consumed.

4) Predator attack distance (m): the straight-line distance between the predator COM at the time the attack commenced and the end of the attack (end is defined as when the predator came to a halt).

5) Predator attack speed ($m\ s^{-1}$): was measured as the average speed occurring over the attack.

Prey

6) Prey reaction distance (RD, measured in m): the distance between the prey COM and the tip of the predator’s snout at the onset of the escape response to a predator attack.

7) Apparent looming threshold (ALT) was defined as the apparent looming threshold for prey avoidance responses to a predatory strike and is a measure of the reactivity of the prey to the perceived threat of predation. The higher the

perceived threat, the lower the ALT (in radians per second) measured at the onset of the escape response and measured as the rate of change of the angle (α) subtended by the predator's frontal profile as seen by the prey. Previous work has shown that fish tend to react to an approaching stimulus (a predator) when a given threshold of $d\alpha/dt$ (i.e. ALT) is reached. The ALT is calculated as $(4US)/(4D^2 + S^2)$, based on (Dill, 1974) and (Webb, 1982); where U is the predator speed, calculated as the speed of the predator in the frame prior to the prey's response; S is calculated based on the morphological characteristics of the predator, i.e. S = (maximal depth + maximal width)/2, whereby both maximal depth and maximal width are at 0.25 lengths of the predator (personal observation); and D is the distance measured at the onset of the escape response, between the prey COM and the point on the predator where its maximal width is located. Hence, D = RD + 0.25 lengths of the predator. For any given speed, a large reaction distance RD will result in a low ALT.

8) Directionality was measured by dividing escape responses into 'away' and 'towards' responses based on the first detectable movement of the head relative to the approaching predator (Domenici et al., 2011a).

9) Prey escape distance (m): the straight-line distance between the prey COM at the onset of the escape response and at the end of the escape response (i.e. when the prey came to a halt).

10) Mean prey escape speed ($m\ s^{-1}$): was measured as the distance covered within a fixed time (24 ms). This fixed duration was based on the average

duration of the first two flips of the tail (22.8ms; the first two axial bends, i.e. stages 1 and 2) defined based on Domenici and Blake, (1997), which is the period considered crucial for avoiding ambush predator attacks (Webb, 1976) and is typical for fish within the size range used.

Statistical analyses

To test the null hypothesis that capture success is independent of predator and prey temperature and elevated CO_2 exposure, capture success was compared by 4x4 contingency table analysis. Predation rate data did not meet the assumption of homogeneity of variance; so we rank transformed the data and performed a non-parametric 2-way ANOVA to explore differences in predation rates between the four treatments. The effects of temperature and CO_2 exposure on predator-prey interactions were examined using a 2-factor MANOVA with temperature and CO_2 as the fixed factors by including the variables: attack rate, attack distance, attack speed, reaction distance, ALT, escape distance and escape speed. Univariate 2-factor ANOVAs with Tukey's HSD post-hoc tests were then performed to determine the nature of any differences found by the MANOVA. Assumptions of normality and homogeneity of variance were examined using residual analysis. Two-tailed binomial tests were performed to determine whether CO_2 or temperature exposure had an effect on the directionality of the escape response, comparing the proportion of individuals that moved away from or towards the stimulus and testing the null hypothesis of a 0.5 probability of turning either way. Where assumptions were violated, data was transformed.

4.4 Results

Capture success was significantly affected by the treatment experienced by the predator and prey (χ^2 = 7.82, d.f. = 3, p = 0.04) and highest under elevated temperature (Figure 4.1a). Capture success was greatest in the high temperature-ambient CO_2 treatment, with 47.3% of prey caught, and next highest in the combined elevated CO_2 and temperature treatment (33.3%). Capture success was moderate (22%) in the elevated CO_2-ambient temperature treatment combination, and lowest under ambient conditions (5.8%).

There was a significant trend for predators exposed to high temperature to have a higher attack rate, with more than double the attacks compared to the ambient temperature treatment ($F_{1,67}$ = 4.38, p = 0.04) (Figure 4.1b). There was no effect of elevated CO_2 or the combination of CO_2 and temperature exposure on attack rates (p >0.05). Total distance travelled by the predator was unaffected by the CO_2 or temperature treatments. Predation rate changed in response to the treatments with the temperature-exposed group displaying a marked increase in predation rate compared to the ambient treatment ($F_{1,67}$ = 5.99, p = 0.01) (Figure 4.1c). The overall pattern of predation rate mirrored that of capture success.

The performance characteristics of prey were affected by the combined exposure of elevated temperature and CO_2 (MANOVA, Pillai's trace $_{8,61}$ = 2.233, p = 0.03), and there was a significant main effect of temperature (MANOVA, Pillai's trace $_{8,61}$ = 3.271, p = 0.003). There was no multivariate effect of elevated CO_2 independent of temperature (p >0.05). Univariate 2-

factor ANOVAs detected significant differences between treatments in 5 of the tested performance attributes: attack rate, reaction distance (RD), apparent looming threshold (ALT), prey escape distance, and prey escape speed (Table 4.2).

There was a strong effect of temperature on the RD of the prey ($F_{1,67}$ = 15.7, p = 0.0001) (Figure 4.1d). Specifically, prey exposed to high temperatures allowed the predator to get twice as close to them before undertaking an escape response (0.08m compared to 0.04m). There was little effect of elevated CO_2 exposure on the RD of the prey, with values nearly identical to the ambient treatment (0.08m compared to 0.075m). When prey were exposed to the combined CO_2 and temperature treatment, RD decreased to 0.034m, however, this was not significantly different to the other treatments.

ALT increased from 1.39 rad s^{-1} in the ambient treatment to 14.52 rad s^{-1} in the high temperature treatment ($F_{1,67}$ = 16.4, p = 0.0001) (Figure 4.1e; Table 4.2).

Directionality did not differ among treatments (χ^2 = 6.25, d.f. = 3, P = 0.1) (Fig. 1f). However, when treatments were individually compared to the ambient treatment, there were significant differences (the combined elevated CO_2 and temperature treatment: χ^2 = 9.21, d.f. = 2, p = 0.01; high temperature, χ^2 = 7.37, d.f. = 2, p = 0.02 and the elevated CO_2, χ^2 = 5.99, d.f. = 2, p = 0.03). Specifically, in the ambient treatment, only 6% of the prey turned towards the predator. By contrast, 42% of prey turned towards the predator following combined exposure to CO_2 and temperature. The direction turned by the high temperature treatment and the elevated CO_2 treatment was intermediate to the ambient treatment and combined CO_2 and temperature treatment group. The proportion of responses directed away and towards the predator differed significantly from random (50:50) for the ambient treatment (n_{norm} = 17, p = 0.001), but not for the other three treatments (high temperature: n_{norm} = 19, p = 0.09; elevated CO_2; n_{norm} = 16, p = 0.06 or the combined CO_2 and temperature treatment: n_{norm} = 17, p = 0.1).

There was a strong effect of temperature on the total prey escape distance ($F_{1,67}$ = 14.8, p = 0.0002) (Figure 4.1g), with prey exposed to high temperatures having 50% lower escape distances compared to prey from ambient temperatures (0.30m vs. 0.17m). In contrast, CO_2 exposure had no significant effect on escape distances (0.31m to 0.30m; p >0.05) and there was no significant interaction between the two treatments (p >0.05) (Table 4.2).

There was a significant interaction between temperature and CO_2 treatment for mean prey escape speed ($F_{1,67}$ = 9.54, p = 0.002) (Figure 4.1h) Prey from the ambient treatment escaped significantly faster than those from the combined elevated CO_2 and temperature treatments (0.4 vs. 0.26 m s^{-1}).

4.1

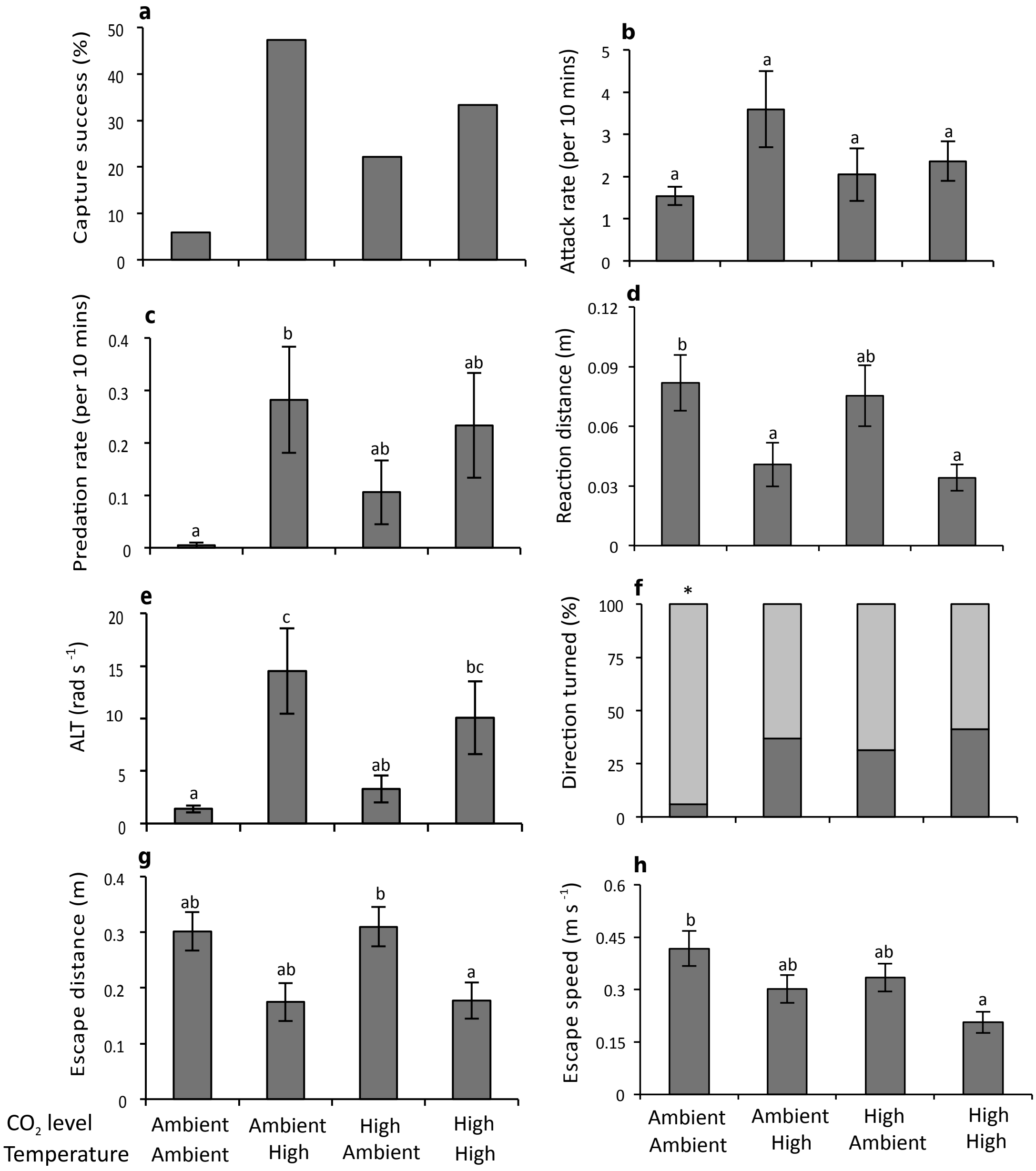

Figure 4.1: Comparison of the effects of elevated CO_2 (~405 µatm and 930 µatm) and temperature (ambient - 26.7 and elevated - 29.6°C) on interactions between a predator (*Pseudochromis fuscus*) and prey (*Pomacentrus wardi*), on eight performance characteristics: capture success (**a**); attack rate (per 10 minute observation) (**b**); predation rate (per 10 minute observation) (**c); prey** reaction distance (**d**); prey apparent looming threshold (ALT); **e**); directionality =(**f**); prey escape distance (**g**); and prey escape speed (**h**). *n* = 17 for ambient, *n* = 19 for H temperature, *n* = 18 for elevated CO_2 and *n* = 18 for H temp/E CO_2. Error bars are SEM. Letters above bars represent Tukey's HSD groupings of means. Asterisk above panel **(f)** represents the proportion of responses directed away (light grey portion) and towards (dark grey portion) the stimulus that differed significantly from random.

Table 4.2 Effects of water temperature (~27, 30 °C) and CO_2 (~405, 930 µatm) on the behavioural interaction between a predator (*Pseudochromis fuscus)* and its prey (*Pomacentrus wardi*) on 6 performance variables: (a) predation rate (b) attack rate (c) prey reaction distance (d) ALT (e) prey escape distance and (f) prey escape speed (n = ambient; n = 17; high temperature; n = 19; elevated CO_2; n = 18; high temperature and elevated CO_2; n = 18).

Behaviour	**Source of variation**	***df***	**MS**	***F***	***P***
(a) Predation rate	Temp	1	18.282	5.992	**0.0169**
	CO_2	1	0.7952	0.2606	0.6113
	Temp*CO_2	1	5.7200	1.8747	0.1754
	Error	67	3.0511		
(b) Attack rate	Temp	1	1.9700	4.3823	**0.0401**
	CO_2	1	0.2235	0.4971	0.4832
	Temp*CO_2	1	0.5780	1.2858	0.2608
	Error	67	0.4495		
(c) Reaction distance	Temp	1	0.1436	14.785	**0.0002**
	CO_2	1	0.0026	0.2741	0.6023
	Temp*CO_2	1	0.0003	0.0338	0.8547
	Error	67	0.0009		
(d) ALT	Temp	1	8.7191	16.402	**0.0001**
	CO_2	1	0.0193	0.0363	0.8494
	Temp*CO_2	1	0.4005	0.7535	0.3884
	Error	67	0.5315		
(e) Escape distance	Temp	1	0.3145	14.829	**0.0002**
	CO_2	1	0.0001	0.0050	0.9437
	Temp*CO_2	1	0.0005	0.0267	0.8707
	Error	67	0.0212		
(f) Escape speed	Temp	1	0.0034	0.0877	0.7680
	CO_2	1	0.1897	4.8022	**0.0318**
	Temp*CO_2	1	0.3769	9.5418	**0.0029**
	Error	67	2.6862		

4.5 Discussion

Increasing CO_2 and changing temperatures are occurring concurrently as a result of global climate change and these changes can act antagonistically, additively or synergistically leading to the physiological and behaviourial impairment of marine animals (Kroeker et al., 2013; Heuer and Grosell, 2014). We show that short-term exposure to elevated CO_2 and high temperature led to pronounced changes in the attack and escape performance of reef fish. Overall, high temperature had the strongest effect on responsiveness and locomotory performance. However, the interaction between CO_2 and temperature was complex resulting in a mildly additive response with CO_2 exposure slightly increasing the effect of temperature for some performance traits.

Prey exposed to high temperatures displayed a decrease in reactivity with an attenuation of reaction distance (RD) and an increase in apparent looming threshold. The fast kinematics of escape responses are likely to be under the control of Mauthner neurons, which are triggered as a reaction to the fast approach of a predator (Eaton et al., 2001). Reactivity is a crucial factor for predator evasion (Domenici, 2010). These changes suggest there was impairment of the motor response, which also acts upon the duration of the burst. Our findings are similar to Webb and Zhang, (1994) who found that the RD of prey (goldfish, *Carassius auratus*) to an attacking predator (rainbow trout, *Oncorhynchus mykiss*) deteriorated following acute exposure to high temperatures, contributing to increased predator success. Our results also suggest that high temperatures affect the neural control of the reaction leading to changes in the timing of the response. Exposure to high temperatures can lead to changes in the physiology of the Mauthner neuron circuit, resulting in differences in the balance between the excitatory and inhibitory transmission onto the Mauthner neuron (Szabo et al., 2008). Given this, it is not surprising that directionality was also affected in the fish exposed to high temperatures. Directionality is an important trait and an early mistake, such as turning towards a predator, may increase the risk of capture (Domenici, 2010). Prey exposed to higher temperatures tended to show lower directional discrimination of the predator. In contrast, individuals that were exposed to ambient conditions exhibited directionality that was non-random. We also observed both an independent effect of elevated $CO_{2,}$ as well as an effect due to the combined exposure of CO_2 and temperature on directionality, with both of these treatment groups turning away and towards the predator randomly. Directionality was one of the only escape variables affected by CO_2 exposure. Because capture success increased from 5% in the ambient treatment to 22% in the CO_2 treatment, we suggest that directionality is a fundamental factor facilitating successful escapes.

Interestingly, we saw little influence of elevated CO_2 on prey responsiveness with both RD and ALT at similar levels to fish under ambient CO_2. This is a surprising result as earlier studies have demonstrated numerous behaviourial effects of elevated CO_2 on the behaviour of coral reef fishes, including *P. wardi* (Munday et al., 2010; Nilsson et al., 2012; Munday et al., 2014) demonstrating that the neural mechanisms causing behavioural abnormalities involved altered function of the $GABA_A$ neurotransmitter, a malfunction restored following treatment with the $GABA_A$ receptor antagonist gabazine. $GABA_A$ receptors are found throughout the Mauthner neuron (Diamond and Huxley, 1968). Previous work with a closely related species (*Pomacentrus amboinensis*) suggested that impairment of these receptors may be the cause of decreased prey responsiveness and escape performance during a predator-prey interaction (Allan et al., 2013). Alternatively, these conflicting responses may be due to species-specific differences despite their close phylogenetic history; behaviour of *P. amboinensis* is highly sensitive to increasing CO_2 (Ferrari et al., 2011a; McCormick et al., 2013).

Several studies have shown little influence of CO_2 exposure on the physiological performance of most juveniles reef fishes (Couturier et al., 2013; Munday et al., 2014). Similarly, Couturier et al., (2013) found no effect of high CO_2 on metabolic rates of *P. fuscus*. The physiological tolerance of these species to high CO_2 may at least partly explain why there were limited effects of high CO_2 on kinematics of predator-prey interactions in this study. Furthermore, recent work has shown that a change in behaviour in response to CO_2 exposure is not ubiquitous, with some temperate species showing little influence of CO_2 exposure on behaviour (Jutfelt and Hedgärde, 2013). There could also be behaviourial subtleties that we did not measure resulting in increased capture success. For example, previous work examining the influence of CO_2 on visual responses in damselfish found the maximal flicker frequency of the retina was disrupted, potentially reducing the capacity of fish to react to fast events such as predatory attacks (Chung et al., 2014).

We observed significant interactive effects of temperature and CO_2 on prey escape speed. These changes may be due to reduced muscle power resulting from changes in the contractile properties of the swimming muscles (Wakeling, 2006) associated with increased temperature exposure, as well as changes in aerobic scope. Although not directly measured in the present study, declining aerobic scope at higher temperatures may explain the reduction in locomotory performance. Despite fast starts being powered anaerobically, an energy debt has to be paid off by post-exercise oxygen consumption, requiring more energy than used initially, creating an energy deficit (Moyes et al., 1993). Fishes with reduced aerobic scope in high temperature conditions may therefore show low performance escapes as an energy-saving strategy (Johansen and Jones, 2011). Similarly, previous work on fish exposed to hypoxia has shown that their burst (anaerobic) swimming performance is impaired (Domenici et al., 2007).

The results for the predators are more complex to interpret, because capture success is determined by predator performance and motivation as well as the interplay between the predator and the prey. Predators exposed to high temperatures had a significant increase in capture success compared to the other three groups, which may be due to two factors. Firstly, prey responsiveness decreased and secondly, there was an increase in attack rates and predation rates. Increasing temperatures increase metabolic rate, which in turn increases energetic demand. This may manifest as an increase in predator motivation to capture prey. As food availability was kept constant between the four treatment groups, this seems likely to be the cause of the increase in attack motivation. In contrast, Ferrari et al., (2015) found predation rates increased after the combined exposure of elevated CO_2 (995µatm) and high temperatures (31°C), yet failed to find an increase in predation rates in response to high temperature exposure alone. Despite the increase in capture success, we saw little influence of temperature on the locomotory performance of the predator suggesting that *P. fuscus* may be immune to temperature increases such as those used here, potentially as a result of its recent thermal experience or evolutionary history. Alternatively, there may have been differential acclimation occurring between the predator and the prey despite the consistent time spent in treatment between the two species. In contrast to earlier work by Allan et al., (2013), there was little influence of elevated CO_2 on the locomotory performance on the predator despite observing a 4-fold increase in capture success compared to controls. This is consistent with the physiological tolerance to high CO_2 in *P. fuscus* reported by Couturier et al., (2013).

The absence of an effect of temperature on the behavior of the predator suggests that changes in the prey's behavior may have led to an increase in capture success. We observed a strong effect of short-term exposure to high temperature on predator-prey interactions, which may have a substantial effect on the replenishment of recruits to coral reefs. The temperature used in our experiment was 3°C higher than the ambient October temperature of 27°C, but *Pomacentrus wardi* and *Pseudochromis fuscus* do experience temperatures around 30°C in the middle of summer. However, October is the start of the breeding season and settling recruits experience a severe predation-induced bottleneck at this vulnerable time. Therefore, any changes in the numbers of fish that successfully settle could lead to changes in community structure. Examining interactions between species in response to environmental stressors can be complex due to behaviourial subtleties that may not be captured. However, only focusing on the responses of individual species is likely to provide an insufficient basis from which an understanding of long-term responses to climate change in complex ecological environments, such as coral reefs (Harley, 2011) due to differing tolerances to environmental perturbations (Grigaltchik et al., 2012; Allan et al., 2015;).

Whilst this study examined short-term exposure to elevated temperature, the timeframe is relevant to exposure of new recruits on the reef. Furthermore, there is little evidence to suggest that reef fishes acclimate to elevated temperatures over longer durations (7-22 days) (Nilsson et al., 2010). However, there is evidence for transgenerational thermal acclimation in reef fishes (Donelson et al., 2012). Multigenerational experiments are needed to test whether we still see these effects on predator-prey interactions. Our results, combining escape performance, attack behaviour and mortality rates, suggest that increasing CO_2 and warming may lead to complex changes in the predator-prey relationships of coral reef communities. Understanding how organisms and ecosystems respond to key environmental drivers concurrently remains a priority for science, management, and conservation.

Chapter 5: Parental effects improve escape performance of juvenile reef fish in a high CO_2 world

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5.1 Summary

Rising CO_2 levels in the oceans are predicted to have serious consequences for many marine taxa. Recent studies suggest that non-genetic parental effects may reduce the impact of high CO_2 on the growth, survival and routine metabolic rate of marine fishes, but whether the parental environment mitigates behavioural and sensory impairment associated with high CO_2 remains unknown. Here we tested the acute effects of elevated CO_2 on the escape responses of juvenile fish and whether such effects were altered by

exposure of parents to increased CO_2 (transgenerational acclimation). Elevated CO_2 negatively affected the reactivity and locomotor performance of juvenile fish, but parental exposure to high CO_2 reduced the effects in some traits, indicating the potential for acclimation of behaviourial impairment across generations. However, acclimation was not complete in some traits, and absent in others, suggesting that transgenerational acclimation does not completely compensate the effects of high CO_2 on escape responses.

5.2 Introduction

Carbon dioxide (CO_2) levels in the world's oceans are increasing due to rising atmospheric CO_2 concentrations (Doney et al., 2009). Higher CO_2 levels are predicted to affect the physiology and behaviour of many marine species (Kroeker et al., 2010; Briffa et al., 2012). Exposure to elevated CO_2 can lead to extra- or intra-cellular acidosis in marine animals, with effects on growth, development and survival (Pörtner et al., 2004; Widdicombe and Spicer, 2008). This may be particularly relevant for early life stages as the physiological costs of maintaining acid-base balance could be greater due to the relatively large surface area to volume ratio of early life stages and less developed acid-base regulatory mechanisms (Melzner et al., 2009). Some of the most dramatic effects of elevated CO_2 levels reported to date are impaired sensory ability and altered behaviours of larval and juvenile fishes (Briffa et al., 2012; Munday et al., 2012; Branch et al., 2013). Behavioural effects of high CO_2 include changes in anti-predator responses (Allan et al., 2013), olfactory (Munday et al., 2009; Dixson et al., 2010) and auditory preferences (Simpson et al., 2011), activity levels (Munday et al., 2010; Ferrari et al., 2011a), behavioural lateralization (Domenici et al., 2011; Jutfelt et al., 2013) and visual risk assessment (Ferrari et al., 2012). These behavioural changes affect key ecological processes such as habitat selection (Devine and Munday, 2012), the timing of settlement (Devine et al., 2011) and predator-prey interactions (Ferrari et al., 2011a; Allan et al., 2013). The underlying mechanism linking elevated CO_2 to these diverse changes in behaviour is difficult to assess, but appears to be interference of the acid-base regulatory process with the function of major neurotransmitter receptors in the brain (Nilsson et al., 2012).

While our understanding of how elevated CO_2 can constrain individual performance is increasing, the potential for adaptation or acclimation over climate change relevant timescales remains largely unresolved (Kelly and Hofmann, 2013). Importantly, the environmental conditions experienced by parents may affect their physiological condition and provide the opportunity for non-genetic effects to be transferred to their offspring (Marshall and Morgan, 2011; Bonduriansky et al., 2012;). Indeed, recent work has demonstrated that some of the negative physiological effects of climate change on marine organisms can be reduced if the parents have been exposed to the same environmental history as their offspring (Donelson et al., 2012; Miller et al., 2012; Salinas and Munch, 2012). This indicates that non-genetic parental effects may alter how marine organisms respond to elevated CO_2 and provides evidence that the response to environmental stress may depend on the history of previous life stages. However, these studies have focused on physiological acclimation across generations and it is not known if behaviour is subjected to the same rapid transgenerational acclimation.

A critical stage in the life of reef fishes is the end of the larval phase, when they settle to the benthic environment. Mortality within the first few days of settlement is variable, but uniformly high, averaging 56% for tropical reef fishes (Almany and Webster, 2006). Success at this life stage is predominantly a consequence of the size, growth and escape performance of new recruits (Green and McCormick, 2005; Holmes and McCormick, 2010). Escape responses consist of sudden accelerations in response to a startling stimulus, such as a predator strike, and are observed in many fish species from various taxa (Domenici and Blake, 1997). A successful escape response consists of finely tuned responsiveness and locomotor performance. The speed of escape and duration of response may be related to locomotor performance (Domenici, 2011). In contrast, the perception of a threat, response latency and directionality of escape are related to neural and sensory capacity (Eaton et al., 2001; Domenici et al., 2007; Domenici, 2010). The fast kinematics of the escape responses are usually controlled by the Mauthner neurons which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved (Eaton et al., 2001).

While recent studies have shown that elevated CO_2 can increase the mortality rate of prey when exposed to predators (Munday et al., 2010; Ferrari et al., 2011a), the extent to which fish larvae can acclimate their escape responses across generations exposed to elevated CO_2 is unknown. Rearing of adults and juveniles under current-day CO_2 levels (400µatm) or CO_2 levels projected to occur by the end of the century (1087µatm) (Meehl et al., 2007) allowed us to test the hypothesis that transgenerational acclimation may reduce the impact of elevated CO_2 on the outcome of predator-prey interactions. Comparisons between treatments allowed us to determine the acute (within-generation) effects of increased CO_2 on juvenile escape performance and to test if such effects were mediated by exposure of parents to increased CO_2. Furthermore, by investigating a range of behavioural and locomotor variables that are critical to a successful escape response (Walker et al., 2005) we were able to test if the capacity for acclimation to elevated CO_2 may differ between escape processes linked to locomotor performance and those more closely linked to cognitive processes.

5.3 Methods

Study species and brood-stock maintenance

To examine the effect of elevated CO_2 on the escape responses of juvenile reef fish, adult breeding pairs of the cinnamon anemone fish, *Amphiprion melanopus,* were collected from the Palm Island Region of the central Great Barrier Reef, Australia (18°37’ S, 146°30’ E). Breeding pairs were housed in individual 45-litre aquaria and maintained at long-term average temperatures for the collection location (22.5°C winter and 28.5°C summer). Pairs were randomly assigned to the control (400µatm) and high (1087µatm) CO_2 treatments in June 2011 and CO_2 was slowly adjusted to the desired level over a two-week period. This allowed pairs to be conditioned in their CO_2 treatments for five months before the start of the breeding season in November 2011. Temperatures were increased from winter temperatures of 22.5°C at a rate of 0.5°C per week until the summer breeding temperature of

28.5°C was reached in the first week of November 2011. Breeding pairs were maintained in their CO_2 treatments until May 2012. Breeding pairs were provided with half of a terracotta pot as a shelter and a spawning site. Pairs were allowed to spawn naturally during the breeding season (November 2011–May 2012) and spawning sites were checked daily for the presence of a new egg clutch.

Juvenile rearing

To examine the difference between acute exposure to CO_2 and parental effects of CO_2 on juvenile reef fish, three juvenile treatment groups were used: 1) juveniles from control parents were hatched into control CO_2 levels (control-control); 2) juveniles from high CO_2 parents were hatched into high CO_2 (high-high); and 3) juveniles from control parents were hatched into high CO_2 (control-high). On the night of hatching, terracotta pots with clutches were moved to 60-litre larval rearing tanks and juveniles were reared in their designated CO_2 level from hatching. Tanks were filled with treated system water and aerated with premixed air to the desired CO_2 level. Juveniles were held under 13h:11h light:dark regime. Aquaria were slowly flushed for 11 h overnight with fresh treatment water at the relevant CO_2 level to remove any excess food and to maintain water quality. Larvae were fed live rotifers (10 indv/ml of tank water) and then weaned to max of 5 indv/ml of *artemia* spp. Fish were allowed 13 hours to feed as food was administered in the morning. Fish were reared in clutch groups until 11 d post-hatching at which point the juveniles are considered competent to settle. Clutches from 3-4 different parents per CO_2 treatment were used in the experiment to ensure genetic diversity. Offspring used in the experiments were approximately equally distributed among the 3-4 breeding pairs in each treatment: 29-40% per pair from 3 pairs in Control-control, 29-37% per pair from 3 pairs in High-high and 21-30% per pair from 4 pairs in Control-high. At the end of their pelagic larval phase (10-11 days post hatching), juveniles (mean SL 13.2mm ± 0.06 SD) were used for escape performance analysis. We found no significant difference among the 3 groups in respect to standard length, body depth, caudal peduncle length and tail width.

Experimental system and seawater chemistry

Adult and juvenile anemone fish were reared in an environmentally controlled aquarium facility at James Cook University, Townsville, Australia. Two x 8,000 l recirculating seawater systems, one maintained at control CO_2 (400 µatm) and the other at elevated CO_2 (1087µatm), supplied seawater to individual aquariums. CO_2 treatments were achieved by CO_2 dosing to a set pH following standard techniques. A pH computer (Aquamedic AT-Control, Germany) regulated CO_2 dosing in a 3,000 l temperature-controlled sump within each system. Temperature (Comark C22) and pH_{NBS} (Hach HQ40d) were recorded daily in the rearing tanks. Salinity and total alkalinity were measured weekly. Total alkalinity was measured by Gran titration from water samples of replicate tanks in each system. Accuracy of titrations was within 1% of certified reference material (Prof. A. Dickson, Scripps Oceanographic Institute). Average seawater pCO_2 was calculated in the program CO2SYS using the Mehrbach constants refit by (Dickson and Millero, 1987) Seawater parameters are shown in Table 1.

Table 5.1. Seawater parameters for parents and juveniles reared at control and high CO_2. Values are means ± s.d.

treatment	temp. (°C)	salinity (ppt)	TA µmol kg^{-1} SW)	pH_{NBS}	pCO_2 (µatm)
control	28.4 ± 0.01	33.3 ± 0.12	2058.32 ± 15.81	8.15 ± 0.005	400 ± 6.33
high	28.5 ± 0.01	33.6 ± 0.09	2168.24 ± 6.70	7.81 ± 0.008	1087 ± 24.67

Experimental protocol

Juvenile fish were individually introduced into a grey circular experimental arena (diameter 250mm; water level 100mm) filled with water at the same CO_2 treatment as they were reared in. Shallow water depth (100 mm) was used in the experimental tank in order to minimize displacement in the vertical dimension. Fish were introduced to the arena via a water filled sample jar. This minimized any stress associated with the movement from the tank to arena. Water temperature in the experimental arena was 28.5°C. The arena was illuminated with four 150 W spotlights, placed above the water surface, by the side of the tank. One minute after being released into the experimental tank an escape response was elicited by the release of a clear plastic cylinder with a tapered end (38.3g, 25mm diameter, 120mm length) from 350mm above the water surface. This was accomplished by turning off an electromagnet to which the cylinder was attached via a metal disc (15mm in diameter) applied to the top end of the cylinder. In order to provide a sudden stimulation and allow calculation of the escape latency (Dadda et al., 2010; Lefrancois et al., 2005), the stimulus was released through a white PVC tube (diameter 6cm, length 30cm) suspended above the experimental tank, with the bottom edge at a distance of 10mm above the water level. In order to record the frame during which the stimulus hit the water surface (i.e. stimulus onset), a small mirror (5cm x 5cm) was placed at 45° near the cylinder, above the water surface. Fish were startled >2 body lengths from the arena's edge to minimize edge effects on escape responses (Eaton and Emberley, 1991). The area above and around the tank was screened off with polystyrene to avoid laboratory disturbances. Responses to the stimulus were filmed from above with a high-speed camera (Casio ex-fh20; 420 fps) and video recordings were analysed using Image J software. Videos were calibrated with a 5cm line inside the tank. Fish that displayed no reaction to the stimulus were omitted from the directionality analysis leading to a slight variation between sample sizes. Sample sizes are stated in figure 1.

Kinematic analysis

Escape response variables were defined as follows:

Locomotor variables

1. Response distance (in mm) is a measure of the total distance covered by the fish from the onset to the end of the escape response (i.e. when the prey comes to a halt).

2. Mean response speed (U_{mean} in mm s^{-1}) was measured as the distance covered within a fixed time (24 ms) which corresponds to the average duration of the first two tail flips of the tail (the first two axial bends, i.e. stages 1 and 2 defined based on Domenici and Blake (1997) which is the period considered crucial for avoiding ambush predator attacks (Domenici and Blake, 1997; Walker et al., 2005).

3. Maximum speed (U_{max} in mm s^{-1}) was measured as the maximum speed reached at any time during the response.

4. Response duration (in sec) was measured as the elapsed time from start of response to end of response (i.e. when the fish came to a halt).

Non-locomotor variables

5. Responsiveness was defined for each treatment as the proportion of animals that responded with a sudden acceleration after being startled, out of the total number of fish.

6.Directionality: escape responses were divided into ‘away’ and ‘towards’ responses when the first detectable movement of the head was oriented away and towards the stimulus, respectively (Domenici et al., 2011).

7. Response latency (in ms) was measured as the time interval between the stimulus onset and the first detectable movement leading to the escape of the animal.

Statistical analyses

The effects of CO_2 exposure on the escape responses of juvenile fish were examined using a one-way multivariate analysis of variance (MANOVA). The effect of CO_2 exposure on each locomotor variable (response distance, mean response speed, maximum response speed, response duration and latency) was further explored using an Analysis of Variance (ANOVA). Residual analysis was used to determine whether the data met the assumptions of normality and homogeneity of variance. Tukey's HSD post-hoc tests were used to examine the differences detected by the ANOVA. The effect of CO_2 exposure on responsiveness was analysed with a χ^2 test on the count data (number of responding and non-responding fish at each treatment). Two-tailed binomial tests were performed to determine if CO_2 exposure had an effect on directionality of response, comparing the proportion of individuals that moved away from or towards the stimulus and assuming a 0.5 probability of turning either way.

5.4 Results

The MANOVA revealed that escape responses were influenced by the CO_2 treatment (Pillai's trace $_{(12,212)}$ =2.93, $p < 0.0001$). ANOVA detected significant differences in four out of the six variables tested (distance travelled, mean response speed, maximum response speed and response duration).

Locomotor variables:

The response distance following stimulation was significantly shorter in the control-high group compared to the control-control group demonstrating an acute effect of high CO_2 on distance travelled (Figure 5.1a; $F_{2, 122}$= 4.135, $p = 0.018$). In contrast, there was no significant difference between the high-high group and the control-control group (Tukey's test, $P = 0.6$), demonstrating a parental effect on distance travelled in the high-high group. However, the distance travelled by the high-high group was intermediate to the control-control group and control-high group (Figure 5.1a), indicating that exposing parents to high CO_2 did not completely reduce the effect of high CO_2 on response distance.

The mean response speed during an escape response differed significantly among the groups (Figure 5.1b; $F_{2, 122}$= 4.13, p = 0.01). Response speed was significantly less in the control-high group when compared to the control-control group demonstrating an acute effect of elevated CO_2 on escape speed (Tukey's test, p = 0.03). There was no significant difference in response speed between the high-high group and the control-control group (Tukey's test p = 0.3). Nevertheless, the response speed exhibited by the high-high group was intermediate to the control-control group and control-high group (Figure 5.1b), indicating that parental exposure to high CO_2 was not sufficient enough to fully reduce the negative effects of CO_2 exposure on response speed in juveniles.

The maximum response speed was significantly less in the control-high group compared to the control-control and high-high groups (Figure 5.1c; $F_{2, 122}$= 6.995, p = 0.001) demonstrating an acute effect of CO_2 treatment on the maximum speed achieved. However, there was no difference in maximum response speed between the control-control group and the high-high group (Tukey's test p = 0.7, Figure 5.1c) indicating that parental effects had completely reduced the negative effects of high CO_2 on maximum response speed in juveniles.

The duration of the burst response following stimulation (response duration) was significantly shorter in the control-high group compared to the control-control group demonstrating an acute effect of high CO_2 on response duration (Figure 5.1d; $F_{2, 122}$= 7,78, p <0.001). In contrast, there was no significant difference between the high-high group and the control-control group, suggesting a parental influence on response duration in the high-high group (Tukey's test p = 0.09). However, the response duration exhibited by the high-high group was intermediate to the control-control group and control-high group (Figure 5.1d), indicating that parental effects did not completely reduce the acute effect of high CO_2 on distance travelled.

Non-locomotor variables:

The proportion of juveniles that displayed no reaction to the stimulus was significantly greater in the control-high group compared to the control-control group (χ^2 = 5.16, df = 1, p = 0.023) and the high-high group (χ^2 = 4.09, df = 1, p = 0.043), demonstrating a clear negative effect of acute CO_2 exposure on reactivity to the stimulus (Figure 5.1e). The high-high group was not significantly different to the control-control group (χ^2 = 0.27, df = 1, p = 0.6), suggesting that parental exposure to elevated CO_2 completely restored reactivity in juveniles exposed elevated CO_2.

Exposure to high CO_2 had a detrimental effect on the directionality of the escape response. In the control-control group, 72% of the escape responses were away from the stimulus. By contrast, only 45% of responders turned away from the stimulus in the control-high group. The direction turned by the high-high group was intermediate to the control-control group and control-high group (Figure 5.1f). The proportion of responses directed away and towards the stimulus differed significantly from random (50:50) for the control-control group (Fig. 1f; n_{norm}=46, p = 0.001), but not for the other two treatments (high-high, Fig. 1f: n_{norm}=34, p = 0.1; control-high, Fig. 1f; n_{norm}=34, p = 0.1). Directionality differed among treatments (χ^2 =6.32, df = 2, p = 0.042). Specifically, directionality differed between the control-control and the control-high groups (χ^2 = 6.63, df = 1, p = 0.01). However, the control-control and the high-high groups (χ^2 = 2.70, df = 1, p = 0.1) and the control-high and the high-high groups were not significantly different (χ^2 = 0.9, df = 1, p = 0.3). Overall, these results suggest that CO_2 affected directionality and that parental exposure to a high CO_2 environment partially reduces this effect. However, individuals whose parents were exposed to elevated CO_2, exhibit a directionality that does not differ from random. Latency was not affected by any of the CO_2 treatments (Fig. 1g; $F_{2, 112}$= 1.63, p = 0.19).

5.1

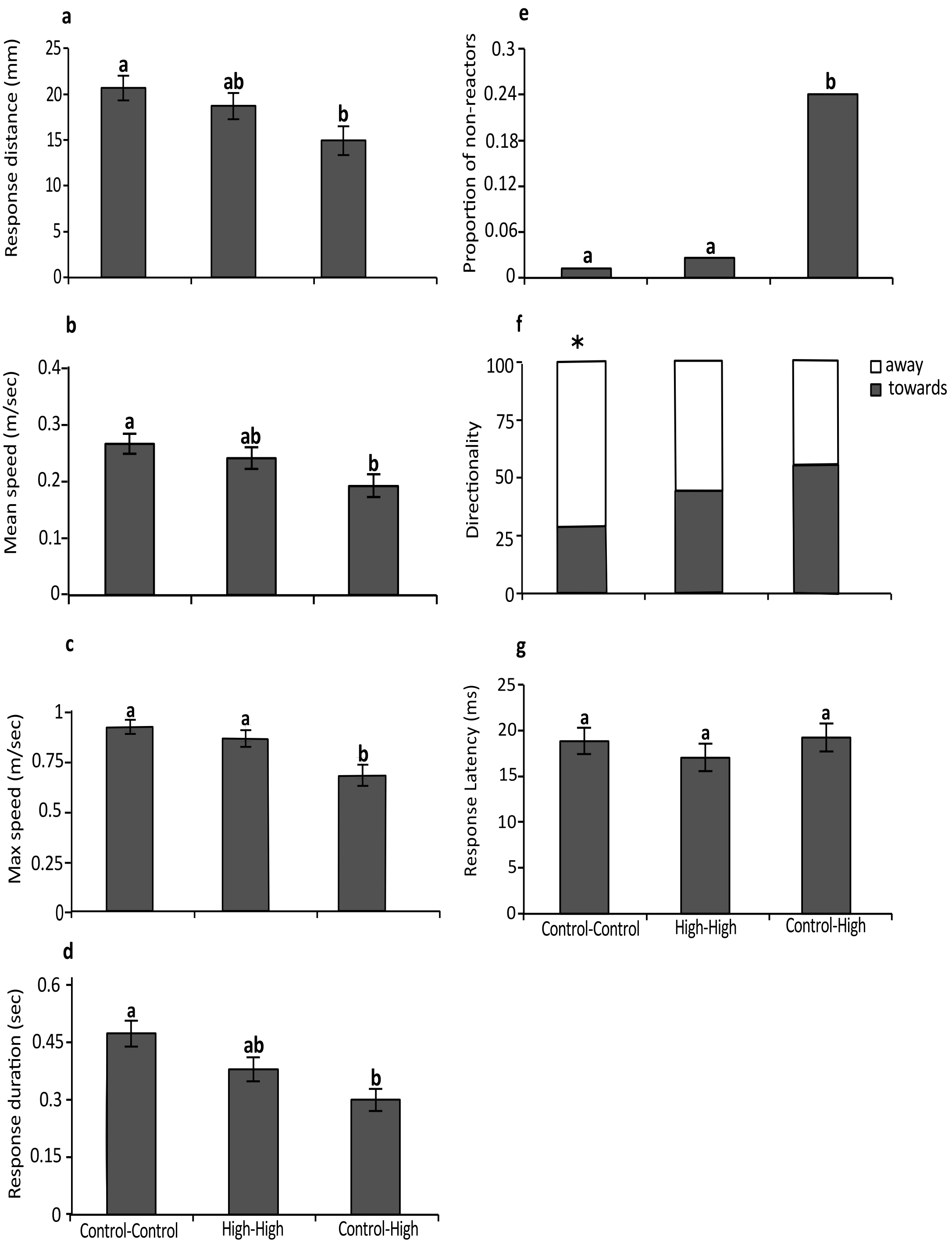

Figure 5.1 The effect of elevated CO_2 on the escape performance of juvenile *A. melanopus* in three different parent–offspring CO_2 treatments (control–control, high–high and control–high). The comparison between control–control and control–high assesses acute effects of elevated CO_2 on juveniles, while the comparison of the high–high group with the other two groups assesses the effects of parental exposure to high CO_2 on juvenile performance. Variables displayed are: (*a*) response distance, (*b*) mean speed, (*c*) max. speed, (*d*) response duration, (*e*) proportion of non-reactors, (*f*) directionality and (*g*) response latency. Errors are standard errors. Letters above bars represent Tukey's HSD groupings of means. Asterisk above panel (*f*) represents the proportion of responses directed away (unfilled portion) and towards (filled portion) the stimulus that differed significantly from random. *n* = control–control 45, high–high 37, control–high 43 (*a*–*e*); *n* = control–control 46, high–high 34, control–high 34 (*f*,*g*).

5.5 Discussion

Elevated CO_2 can lead to changes in growth, survival, routine metabolic rate (Munday et al., 2009; Miller et al., 2012), behaviour (Munday et al., 2009; Dixson et al., 2010; Domenici et al., 2011; Ferrari et al., 2011a; Simpson et al., 2011), and predator-prey interactions (Ferrari et al., 2011b; Allan et al., 2013) of reef fish. Here we demonstrate that CO_2 concentrations that are predicted for the end of this century adversely affected the escape-response performance of juvenile fish. In the control-high treatment there was a significant increase in the number of fish that did not initiate an escape response compared with control-control group, and negative effects on distance travelled, mean response speed, maximum response speed and response duration. This demonstrates that exposure of juvenile fish to elevated CO_2 has a highly significant effect on the kinematics of the escape response that is likely to impact on the ability of prey to avoid predators (Walker et al., 2005). However, negative effects of high CO_2 exposure on escape responses were greatly reduced or absent in the high-high group, with fish showing similar performance levels found in the control-control group for some traits. This result is important because it indicates that transgenerational acclimation can help overcome behavioural impairment observed in fishes exposed to high CO_2. As CO_2 levels rise over coming decades, both parental and offspring generations will experience similar elevated CO_2 levels, thus our results indicate that this parental exposure will help reduce some of the negative effects of high CO_2 on behaviour.

While parental effects moderated the impacts of high CO_2 on most of the escape-response traits measured, performance was intermediate between control-control and control-high treatment groups for over half the traits examined. This indicates that although parental effects may be beneficial, they may not fully restore performance in many instances and some negative effects of high CO_2 on escape performance will remain. Furthermore, while some responses by juveniles, such as proportion of non-reactors, was completely reduced by exposing parents to high CO_2, other responses, such as the direction of response, were not improved. Consequently, our results demonstrate that transgenerational acclimation of behaviour to high CO_2 can be complete, partial, or absent and that the magnitude of acclimation can vary markedly among behavioural responses.

Recent research suggests that cognitive impairment in fishes exposed to elevated CO_2 is due to the interference of acid-base regulatory process with the functioning of brain neuroreceptors (Nilsson et al., 2012). Given this, it is not surprising that directionality was affected in the fish exposed to elevated CO_2. Directionality is an important trait and an early mistake, such as turning towards a predator, may increase the risk of capture (Catania, 2009; Domenici, 2010). Although transgenerational acclimation improved performance in this particular trait, individuals whose parents were exposed to elevated CO_2 exhibit a directionality that does not differ from random, likely due to an impairment of the sensory-motor system. Consequently, exposure to elevated CO_2 could have a significant effect on the outcome of the predator-prey interactions, even if parents have been exposed to similar CO_2 levels. This contrasts to the behaviourial acclimation seen with responsiveness, which highlights the specific nature of behaviourial acclimation. Interestingly, elevated CO_2 had no effect on escape latency, which has also been observed in fish exposed to hypoxia suggesting that decreased oxygen may impair the sensory mechanisms involved in the detection of the mechano-acoustic signal. However, as long as the response threshold is reached, the time course to initiate the response does not appear to be affected (Domenici et al., 2007).

We found that almost all locomotory variables were affected following acute CO_2 exposure. More specifically, there were reductions in the distance travelled, mean response speed, maximum response speed and response durations. The indirect mechanism underlying this response could be a decline in aerobic scope caused by an increase in resting metabolic rate after short-term exposure to elevated CO_2 (Munday et al., 2009). This effect may be similar to the observed reduction in escape swimming performance observed in hypoxia, which has been attributed to a trade-off between physiological exhaustion requiring energy saving, and the need to escape from a predatory attack (Domenici et al., 2007). Because the anaerobic burst is eventually paid off by excess post-exercise oxygen consumption, fish with reduced metabolic scope under elevated CO_2 conditions may therefore show low performance escapes as an energy-saving strategy. In addition, reduced aerobic scope may have caused a decrease in burst swimming performance indirectly, i.e. as a result of reduced condition (Miller et al., 2012). Notably, the negative effects of CO_2 exposure on all locomotory variables were less apparent if the parents had been exposed similarly. This suggests that transgenerational acclimation may play a major role not only in juvenile development and metabolism (Miller et al., 2012), but also in important locomotory behaviour, such as escape performance. Previous work has demonstrated that various physiological parameters (i.e. routine metabolic rate, growth and survival) are improved in juvenile fish that have been exposed to elevated CO_2, providing the parents have been exposed to a similar environment (Miller et al., 2012). The restoration of routine metabolic rate seen in Miller et al. (2012) could explain why we see an improvement in locomotory capabilities. Energy saved through improved cellular processes simply means more energy is available to escape predation.

Transgenerational plasticity (acclimation) occurs in a diverse group of taxa such as plants (Galloway and Etterson, 2007), insects (Agrawal et al., 1999; Mondor et al., 2004), bryozoans (Burgess and Marshall, 2011), shellfish (Parker et al., 2012) and fish (Salinas and Munch, 2012) leading to changes in morphology (Agrawal et al., 1999; Mondor et al., 2004), dispersal potential (Burgess and Marshall, 2011) and growth rates (Parker et al., 2012; Salinas and Munch, 2012). Recently, it has been observed to occur in response to environmental stressors such as hypoxia (Ho and Burggren, 2012), elevated temperature (Donelson et al., 2012; Salinas and Munch, 2012) and elevated CO_2 (Miller et al., 2012; Parker et al., 2012) suggesting that transgenerational acclimation may be an important mechanism in helping taxa cope with climate change. For example, Salinas and Munch (2012) found that Sheepshead minnows (*Cyprinodon variegatus*) reared under high or low temperatures had optimal growth providing their parents had been exposed to similar temperatures. While our result are consistent with growing evidence for the potential importance of transgenerational acclimation in enabling organisms to cope with rapid climate change, they also demonstrate that such acclimation has limits and that not all traits will be beneficially influenced by parents being exposed to the same environmental conditions as their offspring.

We have demonstrated that there is a potential for rapid acclimation for many variables that influence escape performance, if the parents have been exposed to the same environmental history. However, parental effects did not fully restore performance in most traits. Thus negative effects of higher CO_2 levels on behaviour remain, although at reduced levels. Whether behaviours could be fully acclimated in future generations, and the potential costs of such acclimation to other processes is not yet known. This highlights the need to exercise caution when making statements about whole-organism acclimation. It remains to be seen if these non-genetically inherited parental effects will be extended into future generations, thus potentially reducing the effects of rising CO_2 levels on populations, communities and ecosystems.

Chapter 6: General Discussion

Anthropogenic driven increases in CO_2 and temperature are one of the greatest threats facing marine ecosystems today. Yet despite a rapid increase in scientific research, predicting the ecological effects of these climatic changes remains difficult owing to the different sensitivities of individual species and the consequences this could have on interactions among species (Chapter 2-4).

For ectotherms, such as fishes, physiological and behaviourial performance is tightly linked to the external environment. Exposure to elevated CO_2 and temperature can influence predator and prey interactions through changes in locomotory (Chapter 2-4; Webb and Zhang, 1994) and sensory performance (Chapter 2; Szabo et al., 2008) leading to increased prey mortality (Chapter 2-4; Ferrari et al., 2015). Physiologically, many fishes exhibit deteriorating performance once a thermal threshold has been reached (Johnston and Temple, 2002; Wilson, 2010) with interacting species often differing in their responses owing to the thermal sensitivity of their performance curves (Grigaltchik et al., 2012). This can manifest in declining swim performance (Chapter 2; Chapter 3), likely driven by reduced muscle power (Johnson and Bennet, 1995) and/or reduced metabolic performance (Munday et al., 2012). Fishes with reduced aerobic scope in elevated temperature conditions may therefore show low-performance escapes as an energy-saving strategy (Johansen and Jones, 2011). Surprisingly, the thermal optima for escape performance are unknown for any coral reef fish. Understanding where optimal performance lies is important as fish are exposed to temperatures that fluctuate both temporarily and spatially owing to changing seasons, currents, upwelling and tides (Soon and Legates, 2013). Fishes often show phenotypic plasticity, which is thought to buffer the effects of varying environmental temperature on physiological and behaviourial processes (Johnston and Temple, 2002). However, because fast starts are a consequence of both physiological and behavioural performance, each may have different thermal reaction norms, and therefore, may respond differently to changes in temperature.

Sensory system impairment also occurs after exposure to elevated temperature (Chapter 2). Webb and Zhang (1994) found that the reaction distance of prey (goldfish, *Carassius auratus*) to an attacking predator (rainbow trout, *Oncorhynchus mykiss*) deteriorated following acute exposure to high temperatures, contributing to increased predator success. Exposure to high temperatures can lead to changes in the cellular physiology of the Mauthner neuron circuit, resulting in differences in the balance between the excitatory and inhibitory transmission onto the Mauthner neuron (Szabo et al., 2007). Specifically, high temperatures led to synaptic inputs that were weighted on the side of excitation with fish demonstrating impaired sensory performance during rapid escapes (Szabo et al., 2007). These studies indicate that sensory responsiveness, a crucial factor for successfully escaping from predators (Domenici, 2010), can be affected by temperature changes. This may be why capture success increases in fish exposed to elevated temperatures (Chapter 2-4), and may be due to two factors. Firstly, prey sensory performance decreases and secondly attack rates/speeds and predation rates increase (Chapter 2; Chapter 4). Under ambient conditions, predatory attacks usually occur at submaximal speeds (Webb, 1984). Domenici and Blake, (1997) suggest that the strategy of striking at speeds below maximum ensures that prey do not initiate an escape response early (i.e., with a long reaction distance), which could result in displacing prey further from the predators strike trajectory. However, elevated temperatures can lead to increasing attack speeds (Chapter 2; Chapter 4), suggesting temperature may influence predator motivation. This may be due to a temperature-induced increase in metabolic rate, although links between predator attack motivation and metabolic rate under conditions of elevated temperatures have never been examined.

Exposure to elevated CO_2 can further lead to impaired escape performance (Chapter 2-4; Munday et al., 2009), suggesting that sensorimotor performance and the timing of the Mauthner neuron's firing is affected. However, this occurs through a very different mechanism than when organisms are exposed to elevated temperatures. Research suggests that the regulation of plasma HCO_3^- and Cl^- following exposure to elevated CO_2 may lead to the altered function of $GABA_A$ receptors (Nilsson et al., 2012). Given that $GABA_A$ receptors are found throughout the Mauthner neuron (Diamond and Huxley, 1968), it is suggested that elevated CO_2 interferes with $GABA_A$ receptor function causing behaviourial impairment of the escape response (Chapter 3). If it were a malfunction of the $GABA_A$ neurotransmitter causing impairment of escape responses, then escape behaviour should be restored following treatment with a $GABA_A$ neurotransmitter antagonist, such as gabazine (Nilsson et al., 2012; Chivers et al., 2014). This could be an informative way to understand the mechanisms that drive escape performance and how they are impaired at high $CO_{2.}$

However there is variability in CO_2 tolerance even among fish that share a close phylogenetic history (Munday et al., 2012). For example, the escape performance of *Pomacentrus amboinensis* is significantly impaired following exposure to end of century projected CO_2 levels (Chapter 3), yet the escape performance of *Pomacentrus wardi* shows little impairment when exposed to similar CO_2 levels (Chapter 4). This “differential tolerance” could lead to shifts in community structure through changes in competition as the more tolerant species become more dominant (McCormick et al., 2013). Recent work has shown that a change in physiology and/or behaviour in response to CO_2 exposure is not ubiquitous, with some temperate and tropical species of fish showing little impact of CO_2 exposure (Chapter 3; Chapter 4; Jutfelt and Hedgärde, 2013; Heinrich et al., 2014). For example, the physiological tolerance of *Pseudochromis fuscus* to high CO_2 may at least partly explain why there are limited effects of elevated CO_2 on locomotory behaviour (Chapter 3; Chapter 4; Couturier et al., 2013). In contrast, behaviours associated with sensory performance such as olfactory ability and strike success are impaired in *P. fuscus* following CO_2 exposure (Chapter 3; Cripps et al., 2011). The mechanisms that drive these responses are unknown for predatory fishes, but it probably involves an alteration in the $GABA_A$ neurotransmitter function.

Elevated CO_2 and increasing temperatures are not going to occur in isolation. Therefore more studies examining the influence of multiple concurrent stressors are required as their effects can act antagonistically, additively or synergistically leading to the physiological and behaviourial impairment of marine animals (Chapter 4; Kroeker et al., 2013; Heuer and Grosell, 2014). The combined exposure to elevated CO_2 and temperature can lead to pronounced changes in the attack and escape performance of coral reef fishes (Chapter 4), leading to increased mortality (Chapter 4; Ferrari et al., 2015), although the influence of each stressor can be species specific (Chapter 2-4). Examining interactions between species in response to environmental stressors can be complex due to behaviourial subtleties that may not be captured. On the other hand, only focusing on the responses of individual species is unlikely to facilitate a realistic prediction of the future dynamics of complex ecological environments, such as coral reefs (Harley, 2011).

Most ocean acidification studies conducted to date have focused on relatively short-term CO_2 exposure periods and as such are limited in their capacity to predict the long-term responses of species. Despite this limitation, acute exposure to CO_2 facilitates examination of the mechanisms that may be affected, and therefore, may be subject to selection. One criticism of short-term CO_2 exposure experiments is that they could lead to an overestimation of the impact of elevated CO_2 (Sunday et al., 2014). Recent studies at natural CO_2 seeps indicate that behavioural impairment persists even when fish are consistently exposed to elevated CO_2 for weeks (Munday et al., 2014), which suggests that there is limited potential for within-generation acclimation of impaired behavioural responses. Although the possibility for evolutionary adaptation to ocean acidification is increasingly recognized, the field is at a nascent stage. Multigenerational studies allow for the scope of adaptation to be assessed, yet despite this importance, there have been few studies, with the majority focusing on physiological acclimation (growth, metabolic rate) (Donelson et al., 2012; Miller et al., 2012; Salinas and Munch et al., 2012; Donelson et al., 2014; Murray et al., 2014; Schade et al., 2014). Studies that examine the potential for behavioral acclimation to occur are rare (Chapter 5; Welch et al., 2014).

Experiments investigating the escape performance of juvenile reef fishes show that elevated CO_2 negatively affects the reactivity and locomotor performance of juvenile fishes (Chapter 3; Chapter 5). However, parental exposure to high CO_2 reduces these effects, indicating the potential for acclimation of escape performance across generations (Chapter 5). Interestingly, not all performance traits are improved, suggesting that transgenerational acclimation does not completely compensate the effects of high CO_2 on escape responses (Chapter 5). Similarly, Welch et al., (2014) found minimal potential for olfactory or neurological acclimation across generations in both mid- and high-CO_2 levels. Possibly traits that are less plastic than somatic growth (i.e., sensory performance vs. metabolic performance) may not possess the essential phenotypic flexibility to enable acclimation, at least over the course of one generation. Recently, Veilleux et al., (2015) identified the molecular pathways associated with transgenerational thermal acclimation of metabolic traits in a common coral reef fish and found that 53 key genes involved in metabolic performance, immunity and stress were upregulated leading to a shift in energy production and energy utilization enabling fish to cope in higher temperatures. To date, the molecular pathways associated with transgenerational acclimation involving CO_2 exposure are unknown, but a similar approach to that of Veilleux et al., (2015) could be informative in understanding both the mechanisms of impaired behaviours at high CO_2 and the potential for transgenerational acclimation. Combining experimental and molecular approaches represents an exciting advance in testing evolutionary potential in response to climate change.

This thesis represents the first series of studies to examine performance-based attributes of both predators and prey under elevated CO_2 and temperature conditions within the context of a predator-prey interaction (Chapter 2-4) as well as the potential for behavioral acclimation. While these findings are specific to the species used, the fact that the behavior of both predator and prey are affected is strongly suggestive that the influence of climate change will affect the kinematic behavior of other predator and prey fish. Furthermore, acclimation may cause one species to be more or less sensitive than another species (Chapter 2-4), which could cause a shift in community structure either through changes in prey availability and selection (Ferrari et al., 2015) or competitive interactions (McCormick et al., 2013); highlighting the importance of considering species interactions when predicting the response of ecosystems to climate change. How much these results can be generalized remains to be seen but future research using other predators and their prey should focus on this. To date experiments have kept CO_2 and temperature levels at constant levels, whereas in nature they fluctuate seasonally, diurnally and hourly. Future research should consider how behaviours might change in response to frequent fluctuations in these and other interacting physiological drivers. The results from this thesis, combining escape performance, attack behaviour and mortality rates, suggest that elevated CO_2 and temperature may lead to complex changes in the predator-prey relationships of coral reef communities. Understanding how organisms and ecosystems respond and acclimate to key environmental drivers remains a priority for science, management, and conservation.

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Appendix List

Publications arising from this thesis

Allan B.J.M., Domenici P., McCormick M.I., Watson S-A., Munday P.L., 2013. Elevated CO_2 affects predator–prey interactions through altered performance. *PloS ONE* 8, e58520

Allan B.J.M., Miller G.M., McCormick M.I., Domenici P., Munday P.L., 2014. Parental effects improve escape performance of juvenile reef fish in a high-CO_2 world. *Proceedings of the Royal Society B 281*: 20132179

Allan B.J.M., Domenici P., Munday P.L., McCormick M.I., 2015. Feeling the heat: the effect of acute temperature changes on predator-prey interactions in coral reef fish. *Conservation Physiology* 3.

Allan B.J.M., Domenici P., Munday P.L., McCormick M.I., (In review). Differential effects of climate change on predator prey interactions in coral reef fish *Scientific Reports*

Publications not arising from this thesis

Domenici P., **Allan B.J.M.**, McCormick M.I., Munday P.L., 2012 Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biology Letters 8*, 78–81

Domenici P., **Allan B.J.M.**, Watson S-A., McCormick M.I., Munday P.L., 2014 Shifting from right to left: the combined effect of elevated CO_2 and temperature on behavioural lateralization in a coral reef fish. *PLoS ONE* 8, e87969

Ferrari M.C.O., McCormick M.I., **Allan B.J.M.**, Choi R., Ramasamy R., Chivers D.P., 2015. The effects of background risk on behavioural lateralization in a coral reef fish. *Functional Ecology.* DOI: 10.1111/1365-2435.12483

Ferrari M.C.O., Munday P.L., Rummer J.L., McCormick M.I., Corkill K., Watson S-A., **Allan B.J.M.**, Meekan M.G., Chivers D.P., **2015**. Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. *Global Change Biology* 21 (5), 1848-1855

Ramasamy R.A., **Allan B.J.M.**, McCormick M.I., 2015. Plasticity of escape responses: prior predator experience enhances escape performance in a coral reef *PloS ONE* 10 (8), e0132790

Munday P.L., Watson S-A., Parsons D., King A., Barr N., McLeod I., **Allan B.J.M.**, Pether S., (In Press) Effects of ocean acidification on early life history development of the yellowtail kingfish, Seriola lalandi, a large pelagic fish *ICES Journal of Marine Science*

Ferrari M.C.O., McCormick M.I., **Allan B.J.M.**, Choi R., Ramasamy R.A., Johansen J.L., Mitchell M.D., Chivers D.P., (2015) Living in a risky world: the onset and ontogeny of an integrated antipredator phenotype in a coral reef fish *Scientific reports*