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The effects of ocean acidification on habitat associations of coral reef fishes

Thesis submitted by

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in the School of Marine and Tropical Biology

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This thesis includes collaborative work with my supervisors Prof. Philip L. Munday and Geoffrey P. Jones. I was responsible for the experimental design, fieldwork and data collection, analysis and interpretation of results, and preparation of manuscripts suitable for publication. Prof. Philip Munday provided editorial assistance, statistical support, field assistance and financial contributions. Prof. Geoffrey Jones provided editorial assistance and statistical support. Funding for projects completed in this thesis was provided by Prof. Philip Munday through the ARC Centre of Excellence for Coral Reef Studies and I obtained support through the Great Barrier Reef Marine Park Authority Science for Management Award. The Lizard Island Research Station provided excellent logistical support in the form of accommodation, boating, diving, and aquarium facilities, and research equipment.

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GENERAL ABSTRACT

The amount of carbon dioxide dissolved in the oceans is steadily rising due to a 40% increase in atmospheric CO_2 since the industrial revolution, leading to significant changes in water chemistry. Recent studies show that larval reef fish exposed to near future concentrations of CO_2 experience impaired ability to discriminate between olfactory cues and exhibit changes in behaviour that increase post-settlement mortality. Reef fishes at all life history stages rely on olfactory cues for critical behaviours including feeding, reproduction, predator avoidance, and the establishment and maintenance of habitat associations. However, it is unknown if olfactory impairment due to elevated CO_2 observed in larval fish will affect critical ecological processes, such as habitat selection at settlement, and if sensory disruption will also occur in adults. This thesis examines the effects that CO_2 concentrations predicted to occur in the ocean this century (550-950ppm) have on habitat associations of coral reef fishes. Fishes tested in each study were selected to assess behavioural responses to high-CO_2 conditions at both larval and adult stages and across multiple reef fish taxa, using species with varying degrees of habitat specialisation and habitat use. The chapters comprising this thesis address the following questions: 1) does olfactory impairment alter habitat selections and settlement behaviour of larval reef fishes when all sensory cues are available?; 2) does elevated CO_2 cause sensory impairment in adult fish, and if so how might olfactory-mediated processes such as homing behaviour be affected?; and 3) does elevated CO_2 affect habitat preferences of habitat specialist species?

As larvae of many reef fish species settle to benthic substrates overnight and during new moon phases, when low light aids in predator avoidance, olfaction is potentially the key sensory system employed during the settlement process. The effect of elevated CO_2 on larval behaviour and habitat preferences was tested in three species of damselfishes with different habitat preferences. Although exposure to elevated CO_2 affected the ability of larvae to discriminate between common reef microhabitat odours (hard coral, soft coral, coral rubble), this olfactory impairment had no effect on the habitats selected at settlement when all sensory cues were available. However, the settlement behaviour of larvae following CO_2 exposure was significantly altered. While control larvae exhibited typical peak settlement around the new moon, larvae exposed to 850ppm CO_2 displayed highest settlement rates around the full moon and lowest settlement around new moon phases. Such a pronounced shift in settlement timing could have significant consequences for the success of settling larvae.

Following selection of preferred habitats at settlement, some reef fishes often depend on olfactory cues for recognition of selected habitats in addition to foraging and reproductive behaviours. Cardinalfishes nocturnally forage throughout the reef, returning each morning to the same daytime home resting site with the aid of olfactory cues associated with the home site. Adult five-lined cardinalfish, *Cheilodipterus quinquelineatus,* exposed to elevated CO_2 exhibited impaired ability to distinguish between odours of home- versus foreign-site conspecifics in pair-wise choice experiments. In a displacement experiment, fish from all CO_2 treatments exhibited a 22-31% reduction in homing success compared with control fish when released at 200m from home resting sites. While CO_2 exposed cardinalfish released directly back onto home sites exhibited similar persistence rates to control subjects, behaviour at home sites was affected, with CO_2 exposed fish exhibiting increased activity levels and venturing further from shelter. This study demonstrates that disruption of sensory mechanisms in fishes due to elevated CO_2 extends to critical adult behaviours.

Coral-dwelling gobies are some of the most habitat-specialist fish species on coral reefs, spending their entire benthic life within the branches of corals. Respiration of the coral host causes nocturnal increases in CO_2 concentrations among the coral branches, possibly enabling gobies to be more tolerant to changes in ocean chemistry than other fishes. Pair-wise olfactory tests demonstrated that exposure to elevated CO_2 impaired the ability of *Paragobiodon xanthosomus* to discriminate between odour cues of their sole host coral (*Seriatopora hystrix*) and a non-preferred coral (*Pocillopora damicornis*). A habitat choice experiment conducted in the field revealed alterations in habitat preferences following CO_2 exposure. All control *Gobiodon histrio* individuals vacated dead coral habitat and successfully located new live colonies of *Acropora nasuta* (preferred host coral) within 24 hrs, however gobies exposed to elevated CO_2 remained on dead coral habitats and associated with both preferred (*A. nasuta*) and non-preferred (*A. tenuis*) habitats in approximately equal frequency. These results suggest that rising CO_2 could affect the fitness and survival of habitat specialist fish species by impairing their ability to select favourable habitat.

This thesis demonstrates sensory disruptions due to elevated CO_2 are present in both larval and adult reef fishes, with potential alteration of habitat associations observed in multiple species groups. While fishes may be able to compensate for olfactory impairment through use of alternative sensory mechanisms, behavioural alterations observed throughout this project suggest possible interference of critical neural pathways necessary for accurate interpretation of environmental cues. This research indicates that rising CO_2 has the capacity to affect fitness of reef fishes through changes in habitat utilization, with potentially far-reaching consequences for population dynamics and community structure.

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CHAPTER 1: GENERAL INTRODUCTION

Species are distributed unevenly throughout the environment, with concentrations of individuals often occupying distinct habitat types. As habitats are heterogeneous in composition, variance in developmental rates, survival, and reproductive potential can exist between individuals inhabiting different habitats (Huey 1991; Pulliam and Danielson 1991; Pettorelli et al. 2002). Selection to persist within particular habitats can be dependent on local resources and conditions promoting optimal fitness (Pulliam and Danielson 1991; Garshelis 2000). Settlers may occupy certain areas with increased frequency if local conditions support optimal foraging, low predator abundance, or provide suitable breeding sites; however, species distributions may be influenced by both intraspecific density and co-occurrence of competitors exploiting the same habitats (Rosenzweig 1991; Orians and Wittenberger 1991). While some species have maintained use of specific habitat types (habitat specialists), others possess a wider ecological niche and have evolved to persist throughout an array of different habitats (habitat generalists) (Van Tienderen 1991; Fridley et al. 2007).

The distribution of fishes across coral reefs can be influenced by a range of factors, including depth, temperature and currents (Doherty et al. 1996; Floeter et al. 2001; Srinivasan 2003), interactions with other species (Sweatman 1985; Geange 2010), food availability (Doherty 1982; Jones 1986; Forrester 1990), and habitat composition and complexity (Williams 1982; Chabanet et al. 1997; Almany 2004). These factors are often derived from the spatial heterogeneity of coral reef environments which can influence the assemblages of marine organisms at a variety of spatial scales (Williams 1991). Within and among coral reefs, depth and exposure conditions often dictate the distribution and composition of habitats across reef zones (Montaggioni 2005).

Habitat selection appears to be particularly important in the structuring of reef fish assemblages (Shulman 1984; Wellington 1992; Tolimieri 1995; Caselle and Warner 1996; Holbrook et al. 2000), although the supply of recruits (Fowler et al. 1992) predator abundance (Caley 1993; Webster 2002) and positive or negative associations with resident conspecifics and heterospecifics (Jones 1987; Sweatman 1988; Almany 2003; Kane et al. 2009) also contribute to the distribution of fishes among reef substrates. Most reef fishes are characteristically associated with particular microhabitats throughout the reef environment (Sale 1977; Randall et al. 1997). While some reef fishes occur in a variety of reef habitats, other species exhibit specific habitat preferences, with optimal growth, reproductive success and survival dependent on associating with preferred substrates (Jones 1988; Clarke 1992; Wellington 1992; Munday 2001; Caley and Munday 2003). Approximately 10% of species are dependent on live coral habitat for most of their life (Jones et al. 2004; Pratchett et al. 2008) with some of the most specialised species associating with only a single coral species (Munday et al. 1997; Elliott and Mariscal 2001; Wong 2011).

Habitat selection by reef organisms typically occurs early in life as larvae settle to reef substrates following a pelagic larval stage (Leis 1991). Despite their naivety to the reef environment, reef fish larvae display innate affinities for particular habitats and are capable of distinguishing between various reef substrates to actively locate preferred habitat types during settlement (Williams and Sale 1981; Booth 1992; Doherty et al. 1996; Danilowicz 1996; Öhman et al. 1998; Feary et al. 2007). Locating preferred habitats during this transition to a benthic existence is critical, as habitat use has been shown to influence larval success post-settlement, with direct affects on mortality, growth rate, and age at maturation in some species (Jones 1988; Wellington

1992; Munday 2001; Lecchini et al. 2007a; Bonin et al. 2009b). For example, Jones (1988) found an approximately 50-75% reduction in survival and decreased growth rates for juvenile damselfishes 4 months post-settlement occupying *Porites* reefs compared to individuals settled to the higher complexity *Pocillopora* reefs. Thus, habitat selection can be a crucial factor determining the success of individuals following settlement to the reef environment.

Larval fish utilize a variety of sensory systems to discriminate between various reef substrates, with spatial and temporal factors likely influencing the sensory modalities used during the settlement process (Kingsford et al. 2002; Leis et al. 2011). Larvae possess highly developed sensory systems (Job and Bellwood 1996; Wright et al. 2005; Leis et al. 2011) and are able to actively swim to orientate toward specific cues while migrating to reef habitat (Fisher et al. 2000; Kingsford et al. 2002; Montgomery et al. 2001; Stobutzki and Bellwood 1997; Simpson et al. 2005; Wright et al. 2005). Post-hatching and throughout their pelagic duration larvae are capable of interpreting a range of sensory and environmental cues, enabling orientation toward favourable conditions, and eventually toward suitable reef habitat (Kingsford et al. 2002). During migration toward reefs from offshore waters larvae may use a range of far-field cues, including auditory, olfactory, and visual cues (e.g. celestial and polarization patterns) (Simpson et al. 2005; Gerlach et al. 2007; Leis et al. 2011). As larvae approach reefs, olfactory detection of chemical cues and visual interpretation of the reef environment play an important role in location of suitable settlement habitat (Elliott et al. 1995; Danilowicz 1996; Lecchini et al. 2005, 2007b; Ben-Tzvi et al. 2010). However, as larvae of most reef fishes settle overnight, often during new moon phases when low light levels aid in predator avoidance (Victor 1986;

Robertson et al. 1988; Dufour and Galzin 1993; Leis et al. 2002; Doherty et al. 2004), it is uncertain how reliable visual cues may be for discriminating between habitat types at settlement.

Olfaction potentially plays an important role in the initial selection and subsequent recognition of habitat sites. Larval reef fishes preparing to settle are able to distinguish between habitat types using chemical cues and are known to display species-specific preferences for the odours of particular reef microhabitats (Danilowicz 1996; Elliott et al. 1995; Lecchini et al. 2005; Ben-Tzvi et al. 2010). In laboratory choice tests, Danilowicz (1996) found 93% of *Dascyllus albisella* larvae orientated toward the odour cues of their preferred coral habitat, *Pocillopora meandrina*, compared to the odours of two less preferred corals *Porites compressa* and *Montipora verrucosa*. Other species rely on the odours of conspecifics in order to locate suitable habitats at settlement (Sweatman 1988; Lecchini et al. 2005, 2007b), with preferential settlement of larvae to coral colonies with resident adult conspecifics observed in many damselfish species (Sweatman 1985, 1988; Jones 1988; Öhman et al. 1998).

Habitat selection can also be important to adult fishes. For many species, adult survival and reproduction can be critically dependent upon navigation to home sites (Colin 1996; Dittman and Quinn 1996). Possessing home sites can contribute to the overall fitness of an individual, with benefits derived from familiarity with local food resources, shelter, and the location of mates, competitors and predators (Shapiro 1986; Noda et al. 1994; Brown and Dreier 2002). Many reef fishes display strong site fidelity (Marnane 2000; Meyer et al. 2000; Willis et al. 2001) and occupy the same caves or corals for extended periods, departing daily to forage across reef habitats (Chave 1978; Marnane and Bellwood 2002). Others must regularly abandon home sites to navigate to distant breeding grounds or successfully locate sites of mass spawning (Warner 1995; Domeier and Colin 1997; Fukumori et al. 2010). Species possessing home sites are believed to rely heavily on olfactory cues associated with home sites in order to successfully navigate to return (Braithwaite 1998; Atema et al. 2002; Døving et al. 2006; Myrberg and Fuiman 2002). Therefore, interpretation of olfactory cues can be important for the maintenance of essential habitat associations for adult reef fishes.

Habitat selection is clearly an important aspect determining the distribution and abundance of reef fishes. However, coral reef habitats are predicted to change dramatically over the next few decades due to rapid climate change (Hughes et al. 2003; Hoegh-Guldberg et al. 2007). Increasing temperatures, changes in water chemistry, and increased frequency of habitat disturbances are predicted to compromise the integrity of reef ecosystems (Hoegh-Guldberg 1999; Donner et al. 2005). Mass coral bleaching in response to elevated water temperatures can significantly alter the composition of reef environments through the selective mortality of susceptible coral species (Marshall and Baird 2000; McClanahan et al. 2004). Dead coral are quickly overgrown by algae and vacant space occupied by a range of other benthic invertebrates, such as sponges and soft coral. Furthermore, dead corals erode through time, reducing the topographic complexity of reef habitats (Pratchett et al. 2008). While habitat composition will undoubtedly be transformed as a result of global climate change, relatively little is known about how such changes in the composition of benthic habitats might affect the survival and behaviours of reef-associated fishes.

Since the start of the industrial age, the global concentration of atmospheric CO_2 has risen nearly 40%, from about 280 parts per million (ppm) in preindustrial times to the present-day level of approximately 390ppm (Solomon et al. 2007). This rapid increase of CO_2 can be attributed to both direct and indirect anthropogenic effects, from fossil fuel combustion and industrial activity to alterations in land-usage through deforestation and agriculture processes (Raupach et al. 2007). Based on trajectories of future emission scenarios developed by the Intergovernmental Panel on Climate Change, at the current rate of CO_2 production atmospheric CO_2 could reach 850ppm by the end of this century. Although geological records indicate the amount of CO_2 in the atmosphere has been highly variable, the current concentration of CO_2 and rate of increase is unlike any observed in at least the past 800,000 years (Lüthi et al. 2008). While ecosystems worldwide have endured great change over time, a rapid shift in climatic conditions could severely impact the ability of organisms to acclimate and adapt to cope with a changing environment.

Oceans absorb large quantities of excess CO_2 through gas exchange at the sea surface (Revelle and Suess 1957). Recent estimates indicate approximately one-third of the fossil-fuel derived carbon released each year is incorporated into the ocean (Doney 2010). Addition of excess CO_2 into seawater has profound effects on ocean chemistry. Once dissolved in water, CO_2 is rapidly converted to carbonic acid (H_2CO_3), which then disassociates to produce a hydrogen ion and bicarbonate, leading to a decrease in oceanic pH, a process known as ocean acidification. Excess hydrogen ions bond with carbonate ions, driving a further increase in bicarbonate and a reduction in carbonate (Feely et al. 2004). The reduced saturation of carbonate ions has consequences for marine calcifying species including reef building corals and a variety of benthic invertebrates that require carbonate ions for skeletal production (Doney et al. 2009; Guinotte and Fabry 2008; Royal Society 2005). Alterations in growth, reproduction and survival due to ocean acidification have been observed in multiple calcifying taxa (Kurihara and Shirayama 2004; Kleypas et al. 2006; Gazeau et al. 2007), however how changes in ocean chemistry due to elevated ocean uptake of CO_2 might affect higher order marine organisms, such as fishes, is still poorly understood (Ishimatsu et al. 2008; Munday et al. 2008).

Gas exchange at the sea surface constantly equilibrates CO_2 concentrations between the atmosphere and ocean (Doney et al. 2009), therefore as CO_2 concentrations rise in the atmosphere they also rise in the shallow ocean (Doney 2010). The pCO_2 of body fluids of fishes is typically a few mmHg above ambient level (Evans et al. 2005). Consequently, tissue pCO_2 rises when fish are exposed to elevated CO_2 (Brauner and Baker 2009). Increased tissue pCO_2 causes acidosis, which can be detrimental to many cellular processes, including protein synthesis, enzymatic function and oxygen transport (Pörtner et al. 2004). Fish compensate for acidosis by acid-base equivalent ion transport, mostly across the gills, and to a lesser extent via the kidneys and intestine (Claiborne et al. 2002; Brauner and Baker 2009). Nevertheless, exposure to elevated CO_2 may have a range of physiological and behavioural effects in marine fishes (Ishimatsu et al. 2008; Munday et al. 2009b, 2010).

Numerous studies have investigated the physiological responses of fishes to high-CO_2 conditions, although relatively few studies have tested CO_2 levels relevant to IPCC emission scenarios. Early research found fishes display reductions in growth (Fivelstad et al. 1998), higher energetic costs (Hosfeld et al. 2008; Michaelidis et al. 2005), and increased mortality throughout ontogeny (Hayashi et al. 2004; Ishimatsu et al. 2004, 2008; Kikkawa et al. 2004) following exposure to elevated CO_2, but only at levels much higher than will occur in the atmosphere and oceans in the next 100-200 years (pCO_2 10,000-50,000µatm). More recent research has focussed on the effects of CO_2 concentrations that could occur by 2100. Although near-future CO_2 levels (500-1000ppm) appear to have little effect on physiological attributes (Munday et al. 2011), exposure to elevated CO_2 causes profound effects on the behaviour of larval reef fishes. Damselfish larvae held in CO_2 treated seawater experienced impairment of olfactory (Munday et al. 2009b) and auditory ability (Simpson et al. 2011) and were no longer able to distinguish between sensory cues that may be necessary for habitat site selection (Munday et al 2009b). Likewise, CO_2 exposed larvae were unable to recognize odours of predator and non-predator fish species, which could negatively impact survivorship of larvae following migration to the reef environment (Dixson et al. 2010; Munday et al. 2010). Larvae displayed more active, riskier behaviours, leading to 5-8 times higher predation-related mortality in natural reef habitat (Munday et al. 2010).

Disruption of sensory mechanisms and subsequent alterations in behaviour observed in larval fishes could dramatically affect successful recruitment of larvae to adult populations. Olfaction is potentially a key sense used by larvae for selection of habitat sites (Danilowicz 1996; Elliott et al. 1995; Lecchini et al. 2005; Ben-Tzvi et al. 2010). It is unknown how olfactory impairment might alter habitat selections of settling larvae, as larvae may be able to compensate for olfactory disruption through the use of alternative sensory cues. Elevated CO_2 appears to affect a range of behaviours and sensory systems, but at different concentrations. While elevated CO_2 elicits olfactory disruption in clownfish at levels of 750-850ppm (Munday et al. 2009b), auditory disruption is present at a lower concentration of 600ppm (Simpson et al. 2011), and there is no indication of visual impairment at even higher treatment levels (950-1000ppm) (Munday et al. 2009b, 2010; Devine et al. 2011). This indicates sensory systems differ in their sensitivity to changes in ocean chemistry, although how such variations in sensory impairment affects critical fish behaviours is unknown.

Fishes in early life history stages are presumably more vulnerable to changing oceanic conditions. Higher metabolic demand throughout metamorphosis coupled with increases in diffusion surface area with development of gill lamellae at juvenile stages could account for observed sensitivity to alterations in ocean chemistry (Kikkawa et al. 2003; Ishimatsu et al. 2004; Melzner et al. 2009); however, whether sensory disruptions observed in larval fishes are present in adult stages has yet to be determined. Accurate interpretation of environmental sensory cues is essential for critical adult behaviours including location of foraging (Chave 1978; Heyman et al. 2001) and breeding sites (Tesch 1967; Dittman and Quinn 1996). Therefore, it is important to test the tolerance of adults to elevated CO_2 in order to predict the dynamics of future marine fish populations.

The aim of this thesis was to investigate potential alterations in habitat associations of reef fishes following exposure to CO_2 levels predicted to occur in the ocean in the next 50-100 years. A series of experiments was conducted to assess possible effects of elevated CO_2 at various ontogenetic stages and utilising species with a range of microhabitat preferences and varying degrees of habitat specialisation. Through both laboratory and field-based experiments this study focused on the following objectives: 1) To determine if exposure to elevated CO_2 affects habitat selection and settlement behaviour of larval reef fishes, 2) To test the effects of elevated CO_2 on site fidelity and homing behaviours of adult reef fishes, and 3) explore potential alterations in habitat preferences of habitat specialist reef fishes due to increased CO_2. These aims are addressed in separate chapters, each comprised of multiple studies. Chapter 2 tests if impairment of olfactory discrimination ability in larval damselfish alters the selection of microhabitats at settlement when all sensory cues are available. Lunar-based settlement patterns were additionally examined to determine if CO_2 treated larvae could successfully interpret lunar cues necessary for coordination of settlement timing. Chapter 3 investigates potential alterations of adult habitat associations, testing the effects of elevated CO_2 on olfactory-mediated behaviours such as homing in adult cardinalfish using displacement experiments. Finally, Chapter 4 assesses the effects of a high CO_2 environment on habitat specialist fishes, using coral-dwelling gobies to determine if alterations in habitat preferences will occur due to rising CO_2.

CHAPTER 2: RISING CO_2 CONCENTRATIONS AFFECT SETTLEMENT BEHAVIOUR OF LARVAL DAMSELFISHES

Submitted to Coral Reefs July 2011

2.1 ABSTRACT

Reef fish larvae actively select preferred benthic habitat, relying on olfactory, visual and acoustic cues to discriminate between microhabitats at settlement. Recent studies show exposure to elevated carbon dioxide (CO_2) impairs olfactory cue recognition in larval reef fishes. However, whether this alters the behaviour of settling fish or disrupts habitat selection is unknown. Here, the effect of elevated CO_2 on larval behaviour and habitat selection at settlement was tested in three species of damselfishes (family Pomacentridae) that differ in their pattern of habitat use: *Pomacentrus amboinensis* (a habitat generalist), *Pomacentrus chrysurus* (a rubble specialist), and *Pomacentrus moluccensis* (a live coral specialist). Settlement-stage larvae were exposed to current-day CO_2 levels or CO_2 concentrations that could occur by 2100 (700ppm and 850ppm) based on IPCC emission scenarios. First, pair-wise choice tests were performed using a two-channel flume chamber to test olfactory discrimination between hard coral, soft coral, and coral rubble habitats. The habitat selected by settling fish was then compared among treatments using a multi-choice settlement experiment conducted overnight. Finally, settlement timing between treatments was compared across two lunar cycles for one of the species, *P. chrysurus*. Exposure to elevated CO_2 disrupted the ability of larvae to discriminate between habitat odours in olfactory trials. However, this had no effect on the habitats selected at settlement when all sensory cues were available. The timing of settlement was dramatically altered by CO_2 exposure, with control

fish exhibiting peak settlement around the new moon, whereas fish exposed to 850ppm CO_2 displaying highest settlement rates around full moons. These results suggest larvae can rely on other sensory information, such as visual cues, to compensate for impaired olfactory ability when selecting settlement habitat at small spatial scales. However, rising CO_2 could cause larvae to settle at unfavourable times, with potential consequences for larval survival and population replenishment.

2.2 INTRODUCTION

Nearly all coral reef fish have a bipartite life cycle, consisting of a pelagic larval phase lasting weeks or months followed by juvenile and adult stages spent relatively site-attached in close association with reef habitats (Leis 1991). Settlement-stage larvae possess highly developed sensory systems, and are capable of actively swimming to orientate toward specific cues while migrating to reef habitat (Stobutzki and Bellwood 1997; Fisher et al. 2000; Montgomery et al. 2001; Kingsford et al. 2002; Simpson et al. 2005; Wright et al. 2005). Many reef fish have distinct preferences amongst the benthic habitats present on coral reefs (Sale 1977) and habitat use has been shown to directly affect growth and mortality rates in new recruits of several reef fish species (Jones 1988; Wellington 1992; Munday 2001; Lecchini et al. 2007a). Consequently, larval fishes are expected to possess sensory mechanisms that enable them to select preferred habitats while transitioning from a pelagic to benthic lifestyle (a process called settlement).

Larvae are capable of utilizing a suite of sensory cues to discriminate between various reef attributes, with spatial and temporal factors likely influencing the sensory modalities used during the settlement process (Kingsford et al. 2002; Lecchini et al. 2005; 2007b, Leis et al. 2011). Settlement to reef habitats in many species occurs overnight, when low light levels likely enhance predator avoidance by settling larvae (Dufour and Galzin 1993; Leis and McCormick 2002; Doherty et al. 2004). To further reduce mortality, peak settlement overnight on many reefs has been shown to occur during the new moon (Victor 1986; Robertson et al. 1988), when lunar illumination is minimal. Settlement during low light conditions renders visual cues less reliable, therefore chemical and auditory cues (Atema et al. 2002; Simpson et al. 2004) undoubtedly play an important role in successful orientation to preferred habitat substrata.

Many coral reef fish are associated with particular habitats as adults, with individuals selecting a narrow range of habitat types or even a single coral species (Chave and Eckert 1974; Sale 1977; Gardiner and Jones 2005). Habitat use has been shown to directly affect the growth and mortality of recent settlers in many reef fish species (Jones 1988; Wellington 1992; Munday 2001; Lecchini et al. 2007a; Bonin et al. 2009). Despite having no prior experience of the reef environment, reef fish larvae are known to display affinities for specific habitats at settlement (Williams and Sale 1981; Booth 1992; Öhman et al. 1998; Feary et al. 2007) and possess the ability to distinguish between various microhabitat structures using olfactory (Elliott et al. 1995; Danilowicz 1996; Lecchini et al. 2005; Ben-Tzvi et al. 2010), visual cues (Lecchini et al. 2005, McCormick et al. 2010) and auditory cues (Radford et al. 2011). Likewise, settling larvae are able to determine the presence or absence of conspecifics near settlement habitats, with larvae of some aggregating species preferring odours of conspecific adults when selecting settlement sites (Sweatman 1988; Öhman et al. 1998; Lecchini et al. 2005).

The rapid rise of atmospheric CO_2 since the industrial age has resulted in a corresponding increase in the partial pressure of CO_2 (pCO_2) at the ocean surface (Doney 2010). Shallow water ecosystems such as coral reefs are potentially at great risk from rising CO_2, as increased ocean uptake of CO_2 changes water chemistry, leading to a decrease in oceanic pH (ocean acidification) and a reduction in the concentration of carbonate ions necessary for calcification (Orr et al. 2005; Fabry et al. 2008). Furthermore, recent studies have shown larval fish exposed to elevated CO_2 are unable to discriminate between various olfactory cues that may be necessary for site selection at settlement (Munday et al. 2009; Dixson et al. 2010). Larvae held in elevated CO_2 conditions were no longer able to distinguish between chemical cues of preferred and non-preferred habitat, as well as between chemical cues associated with kin versus non-kin adult conspecifics (Munday et al. 2009) and predator versus non-predator species (Dixson et al. 2010). Behavioural changes have also been detected in larvae exposed to elevated CO_2, such as increased activity and bolder behaviour after settling onto reef habitat (Munday et al. 2010). Levels of CO_2 eliciting behavioural abnormalities and olfactory impairment in larval reef fishes (700-850ppm) could occur in the oceans by the end of this century according to predictions based on current CO_2 emission trajectories (Meehl et al. 2007; Raupach et al. 2007).

In this study we experimentally tested: 1) if exposure to elevated CO_2 affects the ability of settlement-stage reef fish larvae to select preferred settlement habitat using chemical cues, 2) if impairment of olfactory ability affects habitat selection at settlement when all sensory cues are available, and 3) if lunar settlement patterns are affected by exposure to near-future CO_2 concentrations. Settlement-stage larvae of three species of damselfish with different habitat preferences (a habitat-generalist, a rubble-associate and a live branching-coral specialist) were tested for discrimination among three common reef microhabitats: hard coral, soft coral and coral rubble. Wild-caught damselfish larvae with no experience of the reef were exposed for 4 days to CO_2 concentrations of 440ppm (current-day control), 700ppm or 850ppm CO_2; representing levels that will likely occur this century based on current CO_2 emission scenarios (Raupach et al. 2007). Olfactory trials were performed to assess the ability of treated fish to discriminate between habitat types using chemical cues alone and if this ability was affected by exposure to elevated CO_2. Habitat selection at settlement for each species was then determined using a multi-choice settlement experiment to test if olfactory impairment altered species-specific preferences for settlement substrata. Finally, nightly settlement experiments were conducted in outdoor aquaria for two complete moon cycles to determine if elevated CO_2 alters settlement timing in relation to lunar phases. We predicted that if elevated CO_2 affects larval behaviour and sensory perception, CO_2 exposed larvae would display alterations in habitat selection and settlement patterns following arrival to the reef environment.

2.3 MATERIAL AND METHODS

2.3.1 Study species and location

This study was conducted at Lizard Island in the Great Barrier Reef, Australia (145°27’E, 14°41’S) during November and December 2009-2010. Settlement-stage damselfish larvae with no prior contact with reef substrata were collected overnight in light-traps deployed on the leeward side of the island in the evening and retrieved between 0600-0630 hrs the following morning. Larvae were sorted according to species and placed in treatment aquaria (see below). *Pomacentrus amboinensis* (Bleeker), *P. chrysurus* (Cuvier), and *P. moluccensis* (Bleeker) were selected for this study based on the variety of habitat preferences exhibited among these species (Figure 2.1). *Pomacentrus moluccensis* is a live-branching coral specialist, while *P. amboinensis* is often found associated with both live and dead hard coral and soft coral habitats (Allen 1991; Syms and Jones 2001). *Pomacentrus chrysurus* inhabits areas high in coral rubble, especially on shallow reef flats (Allen 1991). Using damselfish with different habitat preferences provided a test of how elevated CO_2 affects habitat selection. Microhabitats used in experiments included the hard coral *Pocillopora damicornis* (Linnaeus), soft coral *Sarcophyton spp*, and coral rubble collected from the reef flat. These microhabitats were selected based on their use by the focal fish species and their generally high abundance on reefs at Lizard Island.

Figure 2.1 Study species: a) *Pomacentrus moluccensis* (Photograph by JE Randall), b) *Pomacentrus amboinensis* (Photograph by Dr. Line Bay), and c) *Pomacentrus chrysurus* (Photograph by Kashiwajima)

2.3.2 Seawater manipulation

Seawater was pumped directly from the ocean into 3 x 60 L sumps and diffused with either ambient air (control ~440ppm) or CO_2-enriched air to achieve concentrations of ~700ppm or 850ppm. Equilibrated seawater from each sump was supplied to four replicate aquaria at a rate of ~200-250ml sec^{-1}. Aquaria were individually aerated with the same CO_2 concentrations as sump water. The concentration of CO_2-enriched air was controlled by scientific-grade pressure regulators and needle valves, and measured continuously with an infrared CO_2 probe (Vaisala GMP222). Water temperatures within aquaria averaged 29.9 ± 1.18 (±SE) in 2009 and 27.6 ± 0.03 in 2010. Dissolved CO_2 in the aquaria was measured at regular intervals using a submerged CO_2-permeable membrane connected to an infrared CO_2 probe (Vaisala GMP222) in a closed loop (Hari et al. 2008). Average pCO_2 measurements for treatments in 2009 (olfactory trials and habitat choice experiments) were: 449.76 ± 6.84 (±SE) ppm (control), 678.00 ± 15.68 ppm, and 875.00 ± 32.79 ppm, and in 2010 (lunar experiment): 421.47 ± 4.87 ppm (control) and 873.76 ± 5.51 ppm. In-water measurements of pCO_2 were verified in 2010 by calculations of pCO_2 from measured pH, temperature, salinity and total alkalinity. Temperature and pH_{NBS} were measured twice daily using a Hach 40d pH meter calibrated with fresh buffers. Total alkalinity was measured by Gran titration from water samples taken twice weekly from each CO_2 treatment. The program CO_2SYS (Lewis and Wallace 1995) was used to estimate pCO_2 using the constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987). Average calculated pCO_2 was: 440.53 ± 1.49 µatm (±SE) and 879.90 ± 4.71 µatm, consistent with direct in-water estimates.

Each morning light-trap caught larvae of each test species were divided into equal-sized groups and transferred to one of the four replicate 35 L aquaria in each CO_2 treatment. Previous studies indicate the behavioural effects of elevated CO_2 are fully manifest within 4 consecutive days in treatment (Munday et al. 2010; Ferrari et al. 2011), consequently all larvae were held in CO_2 treated seawater for 4-5 days prior to testing. Aquaria were devoid of natural habitat structure and were opaque to limit external visual stimuli. Fish had not completed pigmentation metamorphosis (McCormick et al. 2002) by the end of treatment and thus their behaviour was considered to be a reasonable representation of settlement-stage larvae. Larvae were fed newly hatched *Artemia* nauplii three times daily. As previous studies show no difference in behavioural responses of CO_2 treated individuals when tested in either control or elevated CO_2 test water (Munday et al. 2009), all tests were performed in ordinary seawater.

2.3.3 Olfactory trials

A two-channel flow chamber (Atema et al. 2002) was used to test olfactory preferences of the settlement-stage damselfishes when presented with habitat odour from hard coral, soft coral, and coral rubble (Figure 2.2). Subjects were presented with pair-wise choice tests of: 1) hard coral vs. soft coral, 2) hard coral vs. coral rubble, 3) soft coral vs. coral rubble, and a blank seawater vs. seawater as a control. To attain cues, habitat structures approximately 10cm in diameter were placed in 8L containers of untreated seawater for 2h. Habitat structures were removed and the conditioned water was gravity fed into the chamber at 100ml-min^{-1}. For each trial, a single fish was introduced in the downstream end of the chamber and allowed to acclimate for 2 minutes. The position of the fish within the chamber was then recorded at 5 second intervals for 2 minutes. The water sources were switched and the acclimation and test period repeated. At least 15 individuals per species were tested per habitat comparison for each CO_2 treatment. All olfactory trials were performed blind, without knowledge of odour cues administered during each trial. Chi-square goodness-of-fit tests were used to compare responses to odour in the CO_2 treatment groups with the responses in the control group.

2.3.4 Habitat choice experiment

To test if habitat selection was affected by elevated CO_2 when all sensory cues are available, settlement-stage larvae were released overnight in outdoor aquaria designed to establish the preferred settlement habitat for each species. Six replicate open-air 300L cylindrical outdoor aquaria (Figure 2.2) contained the same three habitats used in olfactory trials; hard coral, soft coral, and coral rubble. Habitat patches were 15-20cm in diameter and placed 30 cm apart over 2-3cm of sand covering the aquarium base. At the start of each trial, a single fish was allowed to acclimate for 1 hour in an opaque PVC holding tube (diameter 20cm) placed in the centre of the testing aquaria. Fish were introduced to the holding tube in the evening between 1730 and 1830 hrs and released into the test arena after dark. The position of each fish relative to habitats was recorded at first light (0530-0545 hrs) and every 30 minutes afterwards until 1130 hrs. Twenty settlement-stage larvae were tested per treatment level for each species. Log-linear models were used to examine relationships between observation time, CO_2 treatment level and settlement rates for each species. Model terms were removed from the saturated model until removal of a term resulted in a significant deviance from the previous model. Only terms relating to settlement rates were removed in order to test the effects of time and CO_2 treatment levels on settlement rates for each species. Chi-square tests of independence were then used to compare habitat selection among species and to test for any differences in habitat preferences between control and elevated CO_2 treatments for each species. In this analysis, the habitat occupied at the final observation period was considered to be the preferred settlement habitat. Any fish that had not settled by the final observation period were not included in the analysis of habitat preference.

Fig 2.2 Two-channel flow chamber used for olfactory trials (left); Outdoor test aquaria for overnight habitat choice experiments each containing portions of hard coral (*Pocillopora damicornis*), soft coral (*Sarcophyton spp*), and coral rubble.

2.3.5 Lunar settlement experiment

Settlement-stage *P. chrysurus* larvae were tested in outdoor aquaria to assess settlement rates overnight across two lunar phases for control and 850ppm CO_2 treated fish to determine if alterations in settlement patterns occur following exposure to elevated CO_2. Fish were placed individually in open-air 300L cylindrical outdoor aquaria containing settlement habitat in the centre of each arena. Habitat structures were ~15cm diameter clusters of coral rubble pieces, the preferred microhabitat for *P. chrysurus*. Fish were allowed to acclimate in an opaque PVC (polyvinyl chloride) holding tube (diameter ~20cm) for 1 hour prior to testing. Each fish was introduced to the acclimation tube in the evening at 1830 hrs, with release into the test arena occurring after dark. The following morning each aquarium was surveyed at first light (0530-0540 hrs) to record if overnight settlement had occurred. Ten aquaria were used throughout the study, providing approximately 5 replicates per treatment daily across two lunar cycles (n=265-

268 for each treatment). Log-linear analysis was used to examine relationships between moon phase (first quarter, full moon, last quarter, new moon) and CO_2 exposure on settlement rates for *P. chrysurus*. Model terms were removed from the saturated model until a significant deviance from the previous model was observed, with only terms relating to settlement rates removed to determine the effects of moon phase and CO_2 treatment levels on nightly settlement rates.

2.4 RESULTS

2.4.1 Olfactory preferences

Larvae exposed to elevated CO_2 exhibited altered responses to olfactory stimuli compared with controls. *Pomacentrus moluccensis* larvae from the current-day control group spent >60% of the test period associated with hard coral odours in all trials containing hard coral cues (Figure 2.3). Larvae from 700ppm and 850ppm treatments displayed a general decrease in attraction to hard coral odour, with significant differences observed in hard coral versus soft coral trials (700ppm: χ^2=6.83 df = 2, $p<0.04$; 850ppm: χ^2=9.33, df = 2, $p<0.01$). In soft coral versus coral rubble trials, control larvae displayed no preference for either cue, whereas larvae in 700ppm and 850ppm treatments swam in the soft coral cue for the majority of the test period, with a significant attraction to soft coral exhibited by 850ppm larvae (χ^2=6.97, df = 2, $p<0.03$).

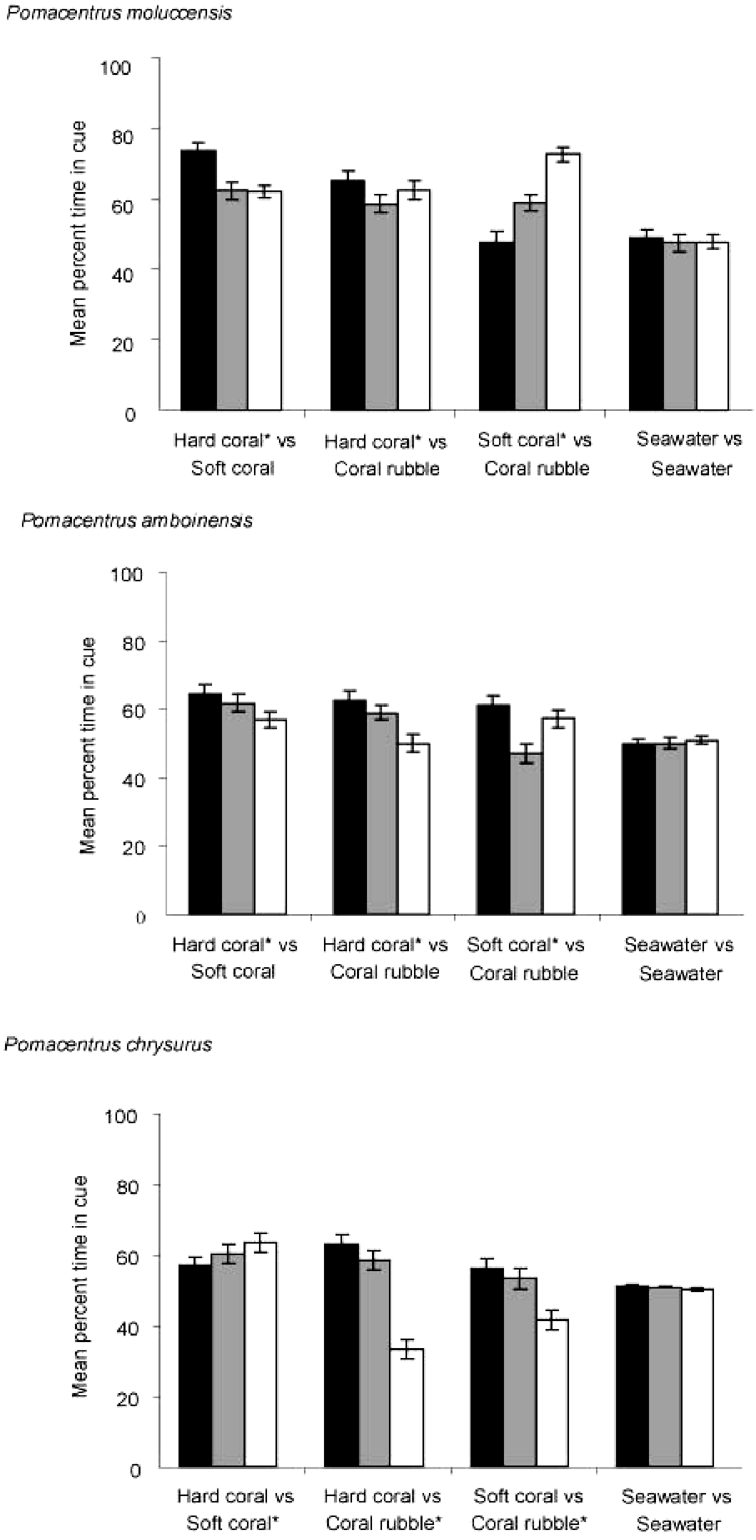

Figure 2.3 Mean percent time larvae spent in a given cue (*) for each odour comparison test for *Pomacentrus moluccensis*, *P. amboinensis,* and *P. chrysurus*. Black bar: control; gray bar: 700ppm; white bar: 850ppm.

Pomacentrus amboinensis control larvae exhibited an attraction (>60% of the test period) to chemical cues of hard coral versus soft coral and coral rubble, and preference for soft coral habitat over the coral rubble cue (Figure 2.3). Although not significant, *P. amboinensis* larvae in 700ppm and 850ppm CO_2 treatments displayed a trend of general decrease in attraction to odours preferred by control larvae in all comparison trials, with a significant decline in attraction to soft coral observed in 700ppm CO_2 treated individuals in soft coral versus coral rubble trials (χ^2=9.64, df = 2, p<0.01).

Pomacentrus chrysurus control larvae spent a majority of the test period in coral rubble habitat cues, and favoured odour of soft coral when coral rubble cues were unavailable (Figure 2.3). Larvae exposed to elevated CO_2 treatments exhibited a general trend of increased attraction to hard and soft coral habitats in all trials, with 850ppm CO_2 treated fish significantly avoiding their preferred habitat odour (χ^2=10.14, df = 2, p<0.01), spending less than 40% of the test period within the coral rubble cue in hard coral versus coral rubble trials.

2.4.2 Habitat choices at settlement

Log-linear analysis revealed that the proportion of larvae settling overnight varied significantly for all species, and that exposure to elevated CO_2 affected settlement rates of *P. moluccensis* and *P. amboinensis*, but not *P. chrysurus.* For *P. moluccensis* and *P. amboinensis* the best-fit log linear model contained all the two-way interactions, including the terms time * settlement rate and CO_2 treatment * settlement, indicating that time had a significant effect on settlement rate, and CO_2 treatment had a significant effect on settlement rate, but there was no interacting effect between time and CO_2 treatment on settlement rate (Tables 2.1, 2.2). For *P. chrysurus* the best-fit log linear model included the term time * settlement rate, indicating that time had a significant effect on settlement rate, but not the level of CO_2 larvae were exposed to, or an interaction between time and CO_2 treatment (Table 2.3). The greatest variation in settlement occurred at the first observation time (0530 hrs), with a greater proportion of fish having failed to settle by this time (Figure 2.4). For *P. moluccensis* fewer fish had settled at the first observation period in the 850 ppm CO_2 treatment compared with controls and the 700 ppm CO_2 treatment. For *P. amboinensis* fewer fish generally had settled at the first observation period in the 700 ppm CO_2 treatment compared with controls and the 850 ppm CO_2 treatment.

There was no observable difference in settlement habitat preferences between control individuals and those exposed to elevated CO_2; however, there were significant differences in habitat preferences at settlement between species (Figure 2.4; Table 2.4). *P. moluccensis* were found most often on hard coral habitat, with 70-85% of individuals associating with this habitat throughout the day in all CO_2 treatment levels. *P. amboinensis* associated mostly with live coral habitats, with 75-90% of individuals selecting either hard or soft coral. *P. chrysurus* were found to be associated with all habitats throughout the day, although 35-60% of individuals were associated with coral rubble habitat throughout the day.

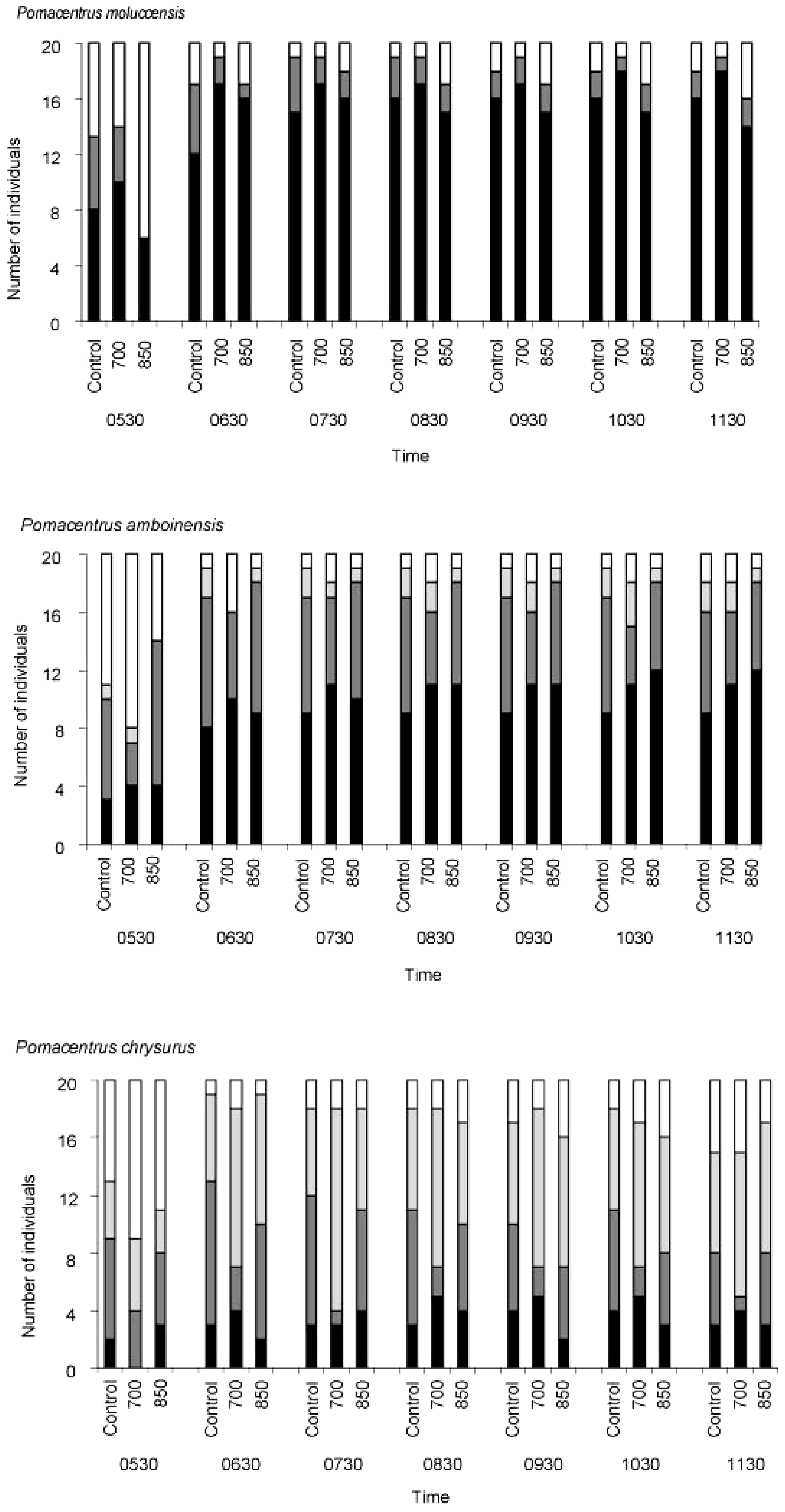

Figure 2.4 Number of larvae associated with each microhabitat in overnight habitat choice experiments for control and CO_2 treatments at each observation time. Colours within each bar represent hard coral (black), soft coral (dark gray), coral rubble (light gray), and a no choice (white) category for larvae not associated with habitat structure at each time.

Table 2.1 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C), and settlement rates (S) by larval *Pomacentrus moluccensis.* NS=not significant ($p>0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

Model	Likelihood ratio χ^2	df	Difference between models	df
1) T*C*S	0.00	0		
2) T*C+T*S+C*S	2.57 NS	12	1 and 2, 2.57 NS	12
3) T*C+T*S	14.04 NS	14	2 and 3, 11.47 **	2
4) T*C+C*S	40.35**	18	2 and 4, 37.78***	6
5) T*C+S	50.59***	20	3 and 5, 36.55***	6

Table 2.2 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C), and settlement rates (S) by larval *Pomacentrus amboinensis.* NS=not significant ($p>0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

Model	Likelihood ratio χ^2	df	Difference between models	df
1) T*C*S	0.00	0		
2) T*C+T*S+C*S	1.58 NS	12	1 and 2, 1.58 NS	12
3) T*C+T*S	7.96 NS	14	2 and 3, 6.38*	2
4) T*C+C*S	45.57***	18	2 and 4, 43.99***	6
5) T*C+S	57.11***	20	3 and 5, 49.15***	6

Table 2.3 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C), and settlement rates (S) by larval *Pomacentrus chrysurus.* NS=not significant ($p>0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

Model	Likelihood ratio χ^2	df	Difference between models	df
1) T*C*S	0.00	0		
2) T*C+T*S+C*S	3.66 NS	12	1 and 2, 3.66 NS	12
3) T*C+T*S	4.33 NS	14	2 and 3, 0.67 NS	2
4) T*C+C*S	36.03**	18	2 and 4, 32.37***	6
5) T*C+S	36.64*	20	3 and 5, 32.31***	6

Table 2.4 A summary of Chi-square statistics for habitat choice comparisons between treatments for each species. Between species comparisons based on control preferences for each species.

	Chi-square (χ^2)	df	p-value
P. moluccensis	0.488	4	$p>0.90$
P. amboinensis	1.134	4	$p>0.80$
P. chrysurus	3.581	4	$p>0.40$
Between species	20.762	4	$p<0.0001$

2.4.3 Lunar settlement patterns

Exposure to elevated CO_2 caused a dramatic shift in settlement timing in relation to moon phases in *P. chrysurus* (Figure 2.5). The saturated model containing the terms moon phase, CO_2 treatment, and settlement rate best described the relationship between lunar phase and CO_2 exposure on settlement rates. Removal of any higher-level term caused a significant increase in deviance from the saturated model (χ^2 21.78, $p<0.001$), indicating that settlement rates in overnight experiments were dependent on moon phase and CO_2 treatment level, with a significant interaction between the two. Peak settlement of control fish coincided with new moon phases and lowest settlement rates occurred during full moon phases (Figure 2.5). Fish exposed to elevated CO_2 exhibited a complete reversal in lunar settlement patterns. Peak settlement for CO_2 treated individuals occurred during the full moon period and lowest settlement occurring throughout new moon phases (Figure 2.5).

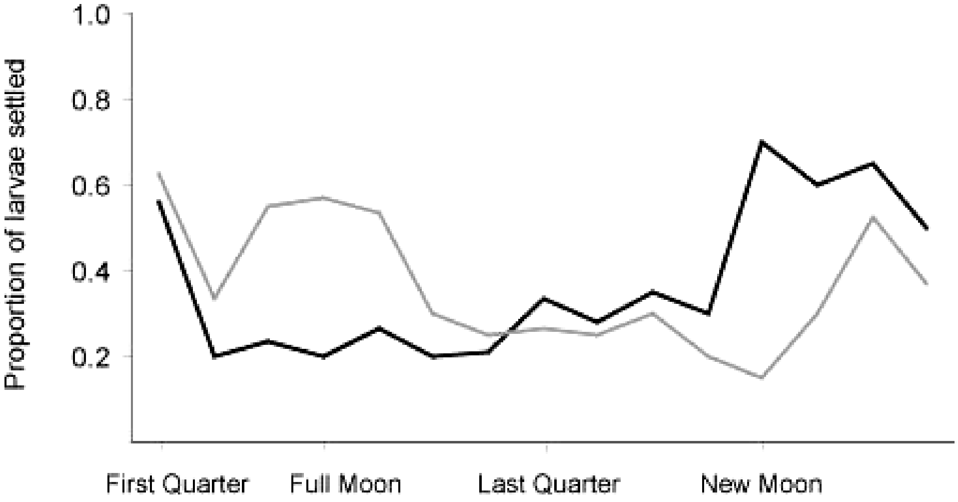

Figure 2.5 Proportion of larvae settling overnight in each CO_2 treatment for each lunar phase. Proportions are based on combined data from two complete lunar cycles at two day intervals. Each data point represents approximately 15-20 individuals. Black line represents control treatment; grey line, 850ppm CO_2.

2.5 DISCUSSION

The transition from pelagic to benthic environment is a critical period for reef fish larvae, as settling larvae must successfully locate appropriate habitat amid a reef full of potential predators. The ability to correctly select an appropriate benthic habitat is essential as an unfavourable choice can have an immediate impact on growth and survival (Jones 1987, 1988). While reef fish larvae have been shown to use chemical cues when locating settlement habitats, recent studies show exposure to elevated CO_2 impairs their olfactory ability (Munday et al. 2009, 2010; Dixson et al. 2010). Our results also show that exposure to elevated CO_2 affects olfactory discrimination between habitat chemical cues. Olfactory responses to preferred substrata declined, and in some cases, larvae developed an attraction to stimuli normally ignored. Notably, however, the ability of larval fishes to select specific preferred habitats at settlement was unaffected when all sensory cues were available. Although auditory cues were not excluded in the test aquaria, olfactory and visual cues are presumably the primary cues utilised by larvae for discriminating habitats in our experimental arena. Despite the ability of alternative senses to compensate for impaired olfactory ability during habitat selection, exposure to elevated CO_2 caused a dramatic shift in the timing of settlement, with CO_2 exposed fish settling predominantly near the full moon, whereas more control fish settled during the new moon.

Olfactory cues are thought to be important during settlement site selection by reef fish larvae because migration to reef habitat for most species occurs primarily at night (Victor 1991). The ability of larvae to distinguish between chemical cues of specific microhabitats at settlement would enable larvae to select preferred habitats immediately upon arrival to the reef and avoid the unnecessary risk of movement post-settlement (Frederick 1997). Consistent with previous studies (Munday et al. 2009; Dixson et al. 2010), we found larvae displayed olfactory impairment following exposure to elevated CO_2. Although not all behavioural responses were statistically significant, trends indicated larvae from CO_2 treatments were often attracted to odours emitted from non-preferred habitats that were avoided by control individuals, or displayed a general decrease in attraction to preferred habitat odours. The strength of responses here were typical for duel odour comparisons (Gerlach et al. 2007) and further replication would potentially elicit significant values for observed trends.

Despite altered olfactory performance of CO_2 treated larvae, overnight settlement tests demonstrated that fish from all treatments successfully settled to preferred habitats when other sensory cues were available. The ability of CO_2 treatment fish to select the same habitats as control individuals likely occurs through the use of visual cues, which appear to be unaffected by elevated CO_2 levels tested here. However, if larvae exposed to elevated CO_2 are forced to rely primarily on visual cues, in addition to spatially limiting their sensory field, settling larvae might be forced to delay overnight settlement until light levels are high enough to allow visual cues to be interpreted. Consistent with this hypothesis, lunar settlement experiments indicted preference for settlement during the full moon by larvae exposed to 850ppm CO_2, possibly relying on elevated moonlight in order to utilize visual cues. This disparity in lunar settlement patterns between CO_2 treatments likely explains variations observed at the first observation time in habitat choice experiments where species and CO_2 treatment groups were randomly tested across several months without consideration of lunar phases.

Lunar periodicity in activity levels and behaviour of predators and prey has been observed in both terrestrial and aquatic ecosystems. Nocturnal predators hunting by moonlight are significantly more efficient at prey capture during full moon phases when lunar illumination is highest (Clarke 1983; Gliwicz 1994), and successful prey capture by predators decreases as light levels are lowered (Chesney 1989; Fraser and Metcalfe 1997). Consequently, prey have adapted their nocturnal behaviours to minimize predation risk, reducing foraging ventures and remaining closer to shelters during the full moon (Trillmich and Mohren 1981; Daly et al. 1992; Mitchell and Hazlett 1996; Metcalfe et al. 1997). Prey species have modified nocturnal behaviours in response to the reduced capacity of predators in poor light conditions, maximizing foraging ventures under darkness of new moon phases and restricting movement during the full moon (Lueke and Wurtsbaugh 1993; Horning and Trillmich 1999). Although the effects of lunar periodicity on predation rates have not been specifically tested for larval settlers on coral reefs, predation efficiency on zooplankton by planktivorous fishes in freshwater systems has been shown to increase during the full moon period (Gliwicz 1986). Therefore, it is likely pelagic larvae choosing to settle at the full moon would be equally vulnerable during this time. Mortality rates of settling larvae could be significantly increased if elevated CO_2 disrupts these behavioural adaptations and causes an increased proportion of larvae to settle around the full moon compared with new moon phases.

Settlement occurring under darkness of new moon phases is already a high risk event, with >50% mortality of larvae believed to occur by the morning following settlement (Almany and Webster 2006; Doherty et al. 2004). Alteration of settlement timing to coincide with full moon phases would undoubtedly exacerbate risks incurred by settlers. Even if suitable habitat is located and larvae successfully settle to the reef, multiple factors that are potentially dependent on the sensory aptitude and competence of larvae in their new benthic environment can influence survivorship post-settlement (Doherty and Sale 1985; Jones 1991; Bonin et al. 2009). Munday et al. (2010) found that newly-settled larvae exposed to elevated CO_2 were more active and ventured further from shelter resulting in increased mortality. Any additional pressure on survival rates during settlement to reef habitats could ultimately affect population abundance (Caley et al. 1996).

Visual cue reception appears to be unaffected at near-future CO_2 concentrations; however, if elevated CO_2 disrupts brain function related to sensory behaviours (Munday et al. 2010; Domenici et al. 2011; Simpson et al. 2011), fishes may have difficulty with interpretation of perceived sensory cues. Such cognitive disruption could account for the reduced ability of larvae to differentiate between chemical cues and respond appropriately to lunar cues following exposure to elevated CO_2. Limitation of accurate sensory information due to impairment of brain functions across critical sensory pathways could have dramatic effects on larval survival during transition to reef habitats.

In conclusion, this study shows larvae exposed to elevated CO_2 maintain species-specific habitat preferences at settlement despite impairment of olfactory discrimination, likely through reliance on visual cues to locate and differentiate reef habitats. Although habitat selection by settling larvae was unaffected, elevated CO_2 caused a dramatic shift in the timing of settlement, possibly because visual cues become the dominant sensory mechanism for habitat selection. Based on current IPCC emission scenarios, CO_2 levels causing altered olfactory preferences in larval reef fishes will occur in oceans by 2100 (Meehl et al. 2007). Further research is needed in order to discover the physiological impacts elevated CO_2 imposes on brain processes, and assess how interference with these processes might impact vital fish behaviours.

CHAPTER 3: HOMING ABILITY OF ADULT CARDINALFISH IS AFFECTED BY ELEVATED CARBON DIOXIDE

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3.1 ABSTRACT

The levels of carbon dioxide (CO_2) predicted for the oceans by the end of this century have recently been shown to impair olfactory discrimination in larval fishes. However, whether this disruption extends to olfactory-mediated behaviour in adult fishes is unknown. In many fishes, adult survival and reproduction can be critically dependent upon navigation to home sites. Here we tested the effects that near-future levels of pCO_2 (550ppm, 700ppm or 950ppm) have on the ability of adult five-lined cardinalfish, *Cheilodipterus quinquelineatus*, to home to their diurnal resting sites after nocturnal feeding. Cardinalfish exposed to elevated CO_2 exhibited impaired ability to distinguish between odours of home- versus foreign-site conspecifics in pair-wise choice experiments. A displacement experiment demonstrated that fish from all CO_2 treatments displayed a 22-31% reduction in homing success compared with control fish when released at 200m from home sites. While CO_2 exposed cardinalfish released directly back onto home sites exhibited similar site fidelity to control subjects, behaviour at home sites was affected, with CO_2 exposed fish exhibiting increased activity levels and venturing further from shelter. This study demonstrates that the potential disruption of chemosensory mechanisms in fishes due to rising CO_2 levels in the ocean extend to critical adult behaviours.

3.2 INTRODUCTION

Many aquatic organisms display remarkable homing behaviour, capable of transversing vast distances while navigating to return to specific sites (Tesch 1967; Dittman and Quinn 1996; Luschi et al. 1996). Possessing home sites can contribute to the overall fitness of an individual, with benefits derived from familiarity with local food resources, shelter, and the location of mates, competitors and predators (Shapiro 1986; Noda et al. 1994; Brown and Dreier 2002). While homing is often contingent upon imprinting of sensory cues associated with natal sites during early development (Scholz et al. 1976; Lohmann et al. 2008), adults may also regularly home to non-natal sites used for feeding (Dalpadado et al. 2000; Heyman et al. 2001), breeding migrations (Dawbin 1966; Broderick et al. 2007), and shelter (O'Gower 1995; Karnofsky et al. 1989). Homing in fishes is likely achieved with the aid of a range of sensory cues depending on the required navigational distance. While migratory species transversing great distances are thought to rely on celestial orientation and detection of geomagnetic fields (Quinn 1980; Klimley 1993), navigation through local environments likely occurs primarily through use of visual mapping and chemoreception (Braithwaite 1998; Atema et al. 2002; Myrberg and Fuiman 2002).

Ocean acidification, caused by the uptake of additional CO_2 at the ocean surface, is recognised as a significant threat to marine species (Orr et al. 2005; Royal Society 2005; Hoegh-Guldberg et al. 2007; Fabry et al. 2008). The extent to which it disrupts the chemical environment and therefore critical behavioural and physiological processes is still being evaluated. Recent studies simulating ocean acidification have revealed profound effects of elevated CO_2 on the chemosensory behaviour of larval reef fishes. Larvae exposed to elevated CO_2 are incapable of distinguishing between chemical cues likely used for navigation and settlement site selection at the end of their larval phase (Munday et al. 2009b; Dixson et al. 2010). Furthermore, CO_2 treated larvae are more active and exhibit riskier behaviour after settlement than fish exposed to present-day conditions, leading to higher mortality rates from predation (Munday et al. 2010). Based on current emission trajectories, CO_2 concentrations causing alterations in larval behaviour and survival rates (~700 ppm CO_2) will be observed in the oceans before 2100 (Meehl et al. 2007; Raupach et al. 2007). However, whether elevated CO_2 could have similar effects on olfactory-mediated behaviours and homing in adult fish is unknown.

Testing the potential ecological effects of ocean acidification on adult homing is challenging as homing often takes place in the open ocean and can involve migrations of thousands of kilometres. However, the localized homing behaviour of coral reef fishes (Sale 1971; Meyer et al. 2000; Willis et al. 2001) provides a manageable arena for testing the effects of elevated CO_2 on olfactory-mediated behaviours in a natural setting. Some of the best examples of homing are from the family Apogonidae (cardinalfishes) (Marnane 2000; Døving et al. 2006; Gardiner and Jones 2010), a diverse and abundant group of fishes found in both temperate and tropical regions (Randall et al. 1996). Cardinalfish maintain daytime resting sites, often forming large, multispecific aggregations primarily within branching corals (Greenfield and Johnson 1990; Gardiner and Jones 2010). By night, cardinalfishes depart home resting sites to forage over sand and reef habitat (Chave 1978; Marnane and Bellwood 2002). Apogonids display strong site fidelity, returning to the same resting sites each day, with some species maintaining site persistence for over 12 months (Marnane 2000). Previous studies have demonstrated successful homing of individuals following displacement 2km away from home sites (Marnane 2000) and spatial memory in migratory cardinalfish allowing return to within 30cm of previously occupied areas after 6 months away from breeding territories (Fukumori et al. 2010). Homing likely occurs through navigation based on visual landmarks and chemical cues, with preference for odours of home site conspecifics and substrate marked by home conspecifics at resting sites having been experimentally demonstrated (Døving et al. 2006). Cardinalfishes therefore provide an ideal group in which to test the potential impacts of ocean acidification on olfactory-mediated behaviour and homing ability in marine fishes.

We used a combination of laboratory and field-based experiments to investigate how near-future levels of CO_2 (550ppm, 700ppm, and 950ppm) could affect olfactory discrimination and homing ability in adult cardinalfish. First, we used pair-wise choice experiments in the laboratory to test if elevated CO_2 affected the ability of cardinalfish to distinguish between olfactory cues of home site conspecifics versus conspecifics from foreign reef sites. Tagged cardinalfish exposed to control or CO_2 acidification levels were then released back on the reef, either 0m or 200m from their home resting site, with presence of released individuals at home sites monitored over a 3 day survey period. Finally, observations were performed to determine if normal daytime behaviour at home sites was affected by CO_2 exposure.

3.3 MATERIALS AND METHODS

3.3.1 Study site and fish collection

This study was conducted at Lizard Island in the Great Barrier Reef, Australia (145°27’E, 14°41’S) during March and October 2010. Adult five-lined cardinalfish, *Cheilodipterus quinquelineatus*, were collected from various sites throughout the lagoon and returned to the research station where they were placed in one of four CO_2 treatments (control, 550ppm, 700ppm or 950ppm). Previous experiments have demonstrated that the behavioural effects of elevated CO_2 manifest within 4 days of CO_2 exposure (Munday et al. 2010), therefore cardinalfish were maintained in CO_2 treatments for 4 consecutive days prior to testing. Approximately 30 individuals (mean standard length 8.5cm) were removed from each of 18 sites with aggregations of >50 individuals per aggregation. Cardinalfish were fed commercial fish pellets (Spectrum Aquaculture) twice daily.

3.3.2 Seawater manipulation

Cardinalfish from each site were divided across CO_2 treatment levels and placed in replicate 35 L aquaria with approximately 6-8 individuals per group. All elevated CO_2 treatments were maintained with a pH-stat system. Seawater was pumped directly from the ocean into 4 x 60 L sumps and diffused with ambient air (control) or CO_2 to achieve a pH_{NBS} of approximately 8.14 (control), 8.06, 8.00 or 7.86. A pH-controller (Tunze Aquarientechnik, Germany) was attached to each CO_2 treated sump to maintain pH at the desired level, with a solenoid injecting a slow stream of CO_2 into a powerhead in each sump whenever seawater pH rose above the set point. Equilibrated seawater from each sump was supplied at a rate of ~500ml min^{-1} to four replicate 35 L aquariums, each housing a group of fish. To maintain oxygen levels and the required pCO_2 levels, aquariums were individually aerated with air (control ~390 ppm) or CO_2-enriched air (~550ppm, 700ppm, or 950ppm). The concentration of CO_2-enriched air was measured continuously with an infrared CO_2 probe (Vaisala GMP222). Temperature and pH_{NBS} of each aquarium was measured each morning and afternoon using a HQ40d pH meter (Hach, Colorado, USA). Dissolved CO_2 in the aquariums was measured at regular intervals using a submerged

CO_2-permeable membrane connected to an infrared CO_2 probe (Vaisala GMP222) in a closed loop (Hari et al. 2008). Average pCO_2 for the treatments were: 450 ± 6.84 (SE) ppm (control), 563 ± 15.69 ppm, 678 ± 46.65 ppm, and 961 ± 7.14 ppm.

3.3.3 Olfactory trials

The effect of CO_2 on the ability of adult cardinalfish to distinguish non-visual cues of their home resting sites were tested using a pair-wise choice experiment. Individuals were tested in outdoor, cylindrical 300L aquaria containing different stimuli on opposite sides of the arena. Each stimulus consisted of a porous opaque 3.5L container housing either a native conspecific, collected at the same home site as the test individual, or a foreign conspecific collected at a reef site approximately 400m away. We assumed that the stimulus was primarily olfactory, as previous studies have demonstrated that cardinalfish respond to odours of home site conspecifics and substrate marked by home conspecifics (Døving et al. 2006), although we cannot exclude the possibility that auditory cues might have been present. Test subjects were released in the centre of the aquarium and allowed to acclimate to the arena for 30 minutes. Following this acclimation period the position of the test individual in relation to the two containers was recorded at 5 minute intervals for 1 hour, with preference for a stimulus source indicated by the presence of the test subject in an aquarium half for >50% of the test period. Test water was changed between trials, with no water flow during the test period. Prior to each trial, test water was maintained still for 5 minutes to allow concentration gradients to form before introduction of the test subject. Previous studies show no difference in behavioural responses of CO_2 treated individuals when tested in either control or elevated CO_2 test water (Munday et al. 2009), therefore all tests were performed in ordinary seawater. Binomial distribution tests were used to analyse preferences based on the probability (0.5) of test subjects spending >50% of the test period within the home site conspecific cue for each trial.

3.3.4 Homing ability

A displacement experiment was used to determine if exposure to elevated CO_2 impairs the ability of adult cardinalfish to navigate to their home resting sites. To resight individuals after release all fish were uniquely tagged with coloured elastomer (Northwest Technologies) injected into the dorsal musculature. Following 4 days in CO_2 treatments fish were measured, transported to the reef and released at 0m or 200m from their respective home site. Fish were released in the late afternoon between 1500 and 1700. For individuals released away from home resting sites, the release point in relation to the home site was determined through random rotation of cardinal compass direction. Each home resting site was surveyed between 0800-1000 the following three mornings for the presence or absence of tagged individuals. Additional releases (n=15-16 individuals per treatment group) followed by night dives were conducted at three sites to determine if normal nocturnal feeding migration occurred following release of fish at home sites. Previous research with damselfishes has found that the behavioural effects of elevated CO_2 are retained for at least 24 hrs after fish are returned to current-day conditions, but dissipate over a period of 48 hrs, and full sensory ability is restored within 72 hrs (Munday et al. 2010). Therefore the 3 day survey period is sufficient to capture any effects caused by exposure to elevated CO_2. Chi-square tests for independence were used to compare return success (for fish displaced 200m) and site persistence (for fish released back on home sites) among treatments based on the number of individuals observed at home sites throughout the 3 day test period.

3.3.5 Behavioural observations

Behavioural observations of tagged individuals from each CO_2 treatment at their home sites were conducted the morning after release. The maximum distance (cm) ventured from habitat structure, maximum cumulative distance (cm) moved throughout the habitat, and activity level was recorded during 3 minute observation periods. Activity level was scored on a scale from 1 to 3 where 1 is remaining predominately stationary with movement no more than 5cm, 2 consisting of periodic movements not remaining still for longer than 30 seconds with frequent advances out of habitat structure and possible conspecific interactions, and 3 is nearly constant movement throughout home site with frequent interactions with conspecifics initiated by the test individual. ANOVAs were used to compare maximum distance ventured and cumulative distance moved among treatments, with means from each CO_2 treatment compared with control fish in post-hoc tests. Categorical data from activity level assessments were analyzed using chi-square tests. In this analysis the number of fish scored in each activity level in CO_2 treatment groups was compared with the observed distribution of the controls.

3.4 RESULTS

3.4.1 Olfactory preference

Pair-wise choice tests demonstrated impairment of non-visual discrimination at CO_2 concentrations as low as 550ppm. Control subjects displayed a strong preference for home site conspecifics vs. foreign conspecifics ($p<0.03$), spending 67% of the test period associated with the home site conspecific source. In contrast, cardinalfish in all CO_2 treatments exhibited no preference for either home or foreign conspecifics ($p>0.11$), associating with both stimuli throughout the test period (Figure 3.1).

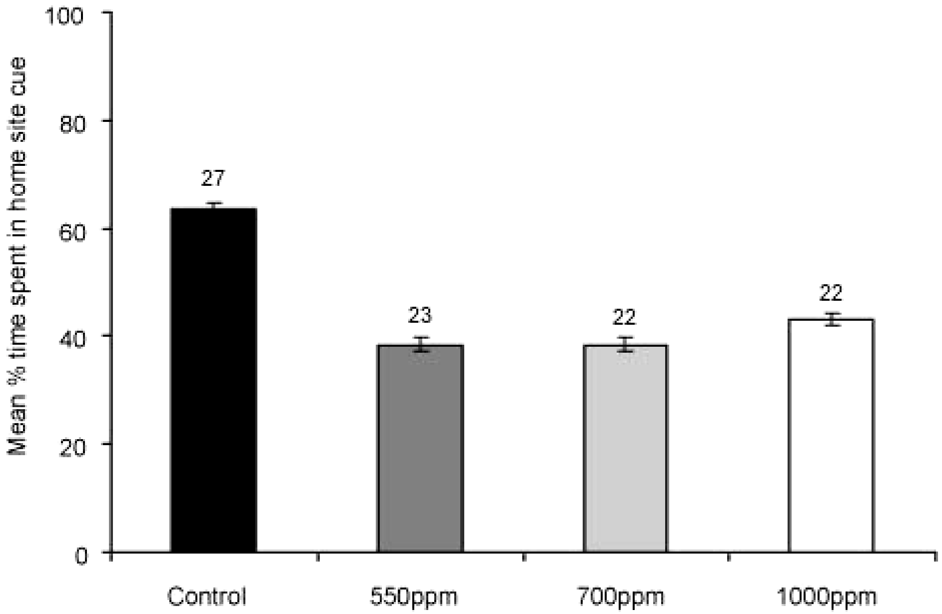

Figure 3.1 Percent time (mean ± SE) cardinalfish, *Cheilodipterus quinquelineatus*, spent associated with cues emitted from home reef site conspecifics versus foreign reef site conspecifics. Sample sizes shown above bars.

3.4.2 Homing ability

Adult cardinalfish exposed to elevated CO_2 exhibited impaired homing ability with a significant decline in successful return rate following displacement 200m from home resting sites ($p<0.02$). Sixty-two percent of control fish returned to their home resting sites within 3 days (Figure 3.2). In contrast, only 40% of fish exposed to 550ppm and 700ppm CO_2, and 32% of individuals exposed to 950ppm CO_2 returned to their home site within 3 days (Figure 3.3). The majority of successful returns observed in all CO_2 treatments occurred within 24h, with 79-89% of returned tagged fish sighted the morning following release. No tagged fish were ever found at release sites the morning following displacement. Neither site location nor size of tagged individuals had an effect on return success ($p>0.10$ using chi-square tests).

Figure 3.2 Tagged individuals of study species, *Cheilodipterus quinquelineatus*, associating with conspecifics at home resting site.

There was no significant difference among CO_2 treatments with respect to the ability of tagged individuals to persist at home sites when released 0m from their home resting habitat ($p>0.21$). Site persistence was 64% for control fish and 51%, 73%, and 66% for 550ppm, 700ppm, and 950ppm CO_2 treatments, respectively (Fig. 2). Of cardinalfish present at home sites after release, 64-84% persisted for 2 or more days throughout the survey period in both control and CO_2 treatments. Night dives indicated that fewer CO_2 exposed fish might be departing each night to feed compared with control fish, with 2 of 15 tagged individuals from each elevated CO_2 treatment sighted at home sites whereas all control fish departed at night to feed, although this trend was not statistically significant.

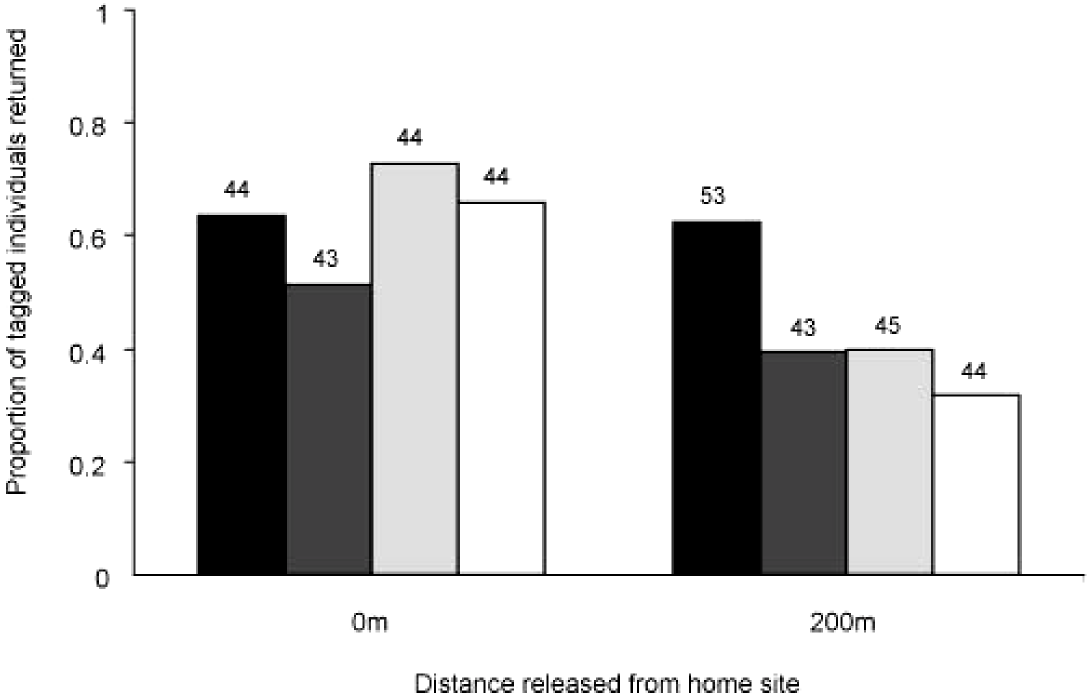

Figure 3.3 Proportion of tagged fish from each CO_2 treatment resighted at home sites over 3 days following release at distances of 0m or 200m from home sites. Sample size for each group shown above bars. CO_2 treatments were control (black bars), 550ppm (gray bars), 700ppm (hatched bars), and 950ppm (white bars).

3.4.3 Behavioural observations

Observations conducted the morning after release indicate changes in daytime behaviour at resting sites following CO_2 treatment. Individuals exposed to elevated CO_2 ventured further from habitat structure (Table 3.1; $p<0.03$) and displayed increased activity in the 950ppm CO_2 group ($p<0.001$) compared with individuals in the control group. Although not statistically significant ($p=0.054$), fish held in 700ppm and 950ppm CO_2 levels appeared to have increased cumulative movement throughout the habitat (Table 3.1).

Table 3.1 Behavioural traits of fish from control, 550ppm, 700ppm and 950ppm CO_2 treatments released at home resting sites. Data are means ± SE for each behaviour category

Treatment	N	Max distance ventured (cm)	Cumulative distance moved (cm)	Activity level (1 to 3)
Control	19	3.16 ± 0.87	14.72 ± 3.54	1.42 ± 0.14
550ppm	20	4.10 ± 0.83	13.89 ± 3.35	1.55 ± 0.17
700ppm	20	7.15 ± 1.87	26.00 ± 4.55	1.55 ± 0.15
950ppm	19	7.95 ± 1.44	26.84 ± 5.62	1.89 ± 0.17

3.5 DISCUSSION

Since navigation and homing in fishes likely involves a suite of sensory cues, including detection of chemical cues, ocean acidification has the potential to interfere with critical migratory behaviours. Recent studies have shown that larval behaviour is severely disrupted by increasing CO_2 (Munday et al. 2009b, 2010; Dixson et al. 2010). Given that olfaction is also a critical sense used by adult fishes, there is a strong potential for acidification to impact fishes at multiple life history stages. Our results demonstrate that continued uptake of CO_2 by the ocean could affect navigational capabilities and homing behaviour of adult cardinalfishes. Exposure to elevated CO_2 appeared to impair olfactory discrimination in adult cardinalfishes and significantly affected their ability to home to resting sites after displacement. However, CO_2 treated fish returned to their home sites were still able to navigate from nocturnal excursions, likely following unaffected individuals or using other sensory cues, demonstrating the importance of testing the impacts of sensory impairment in an appropriate ecological setting.

Cardinalfish exposed to elevated CO_2 exhibited impaired sensory and homing ability at concentrations as low as 550ppm. Current IPCC emission scenarios predict that atmospheric CO_2 will exceed this level during the second half of the century, with present-day CO_2 emissions already surpassing the stabilization trajectory for 650ppm, and could exceed 800ppm by the end of the century (Meehl et al. 2007; Raupach et al. 2007). While Munday et al. (2010) found no significant difference in larval damselfish behaviour following exposure to low CO_2 concentrations (550ppm), it appears members of the family Apogonidae may be more sensitive to alterations in ocean chemistry and could therefore be one of the first groups of reef fishes to be affected by ocean acidification. Apogonids form an important dietary component for many reef dwelling piscivores (Chystal et al. 1985; Beukers-Stewart and Jones 2004) and likely play an important role in energy transfer and provisioning for reef ecosystems (Marnane and Bellwood 2002), hence factors affecting their fitness and survival could have important implications for other reef species.

Chemical cues are important for navigation and site selection at a variety of life history stages in aquatic animals (Hara 1993; Døving and Stabell 2003). Although the potential for auditory disruption was present in this study (Simpson et al 2011), olfaction is believed to be the dominant sensory mechanism involved in cardinalfish homing. Cardinalfishes are known to be attracted to the chemical cues from shelters previously marked by home site conspecifics (Døving et al. 2006), suggesting they use olfactory cues to help locate home territories after nocturnal foraging. Adult cardinalfish exposed to elevated CO_2 were no longer able to distinguish between conspecifics at their home site versus conspecifics from a foreign social group, and consequently would have impaired ability to locate their home resting site.

Consistent with this hypothesis, CO_2 treated individuals were less able to locate their home site when displaced 200m for their resting site. Furthermore, tagged individuals from 700ppm and 1000ppm treatments released both at 0m and 200m were sighted on multiple occasions associating with foreign conspecific groups at least 400m from the nearest collection site, likely due to their inability to recognize home site odours. The decrease in homing success following displacement, which coincided with impaired olfactory discrimination behaviour at all elevated CO_2 levels tested, suggests that adult cardinalfish use chemical cues associated with home sites to aid in navigation to and identification of their diurnal resting sites. If ocean acidification interferes with homing and home site recognition, individuals may be forced to seek refuge in foreign territories with unfamiliar social groups following nocturnal excursions to foraging sites. It is unknown how easily individuals may be incorporated into foreign social groups as resident adults often defend established territories from foreign intruders (Turner 1994; Chellappa et al. 1999).

Despite clear disruption of homing ability in fish displaced from their home resting sites, there was no effect of elevated CO_2 on site fidelity for fish returned directly to their resting sites. Night observations suggest that a smaller proportion of CO_2 treated individuals might be departing home sites each night to feed, whereas all control fish appeared to resume nocturnal foraging behaviour. While cardinalfish maintain a high degree of spatial separation between species groups by night (Marnane and Bellwood 2002), many species form small intraspecific aggregations while foraging, suggesting that CO_2 treated fish may also be able to return to their daytime resting sites by simply following other individuals. Likewise, interpretation of visual cues appears to be unaffected by exposure to the CO_2 levels used in this study, therefore feeding nearby within visual reference to home sites may help CO_2 treated individuals return. Thus, failure to vacate resting sites, or reliance on small feeding aggregations and visual cues for direction, could account for the high persistence rates we observed in the elevated CO_2 treatments.

Variation in behaviour observed in CO_2 treated fish at daytime resting sites could potentially decrease survival rates by increasing susceptibility to predation. Individuals from 700ppm and 950ppm CO_2 treatments appeared less cautious, spending more time further from shelter and displaying an increase in activity throughout the habitat. Behavioural changes such as these have been found to cause higher mortality rates from predation in newly settled damselfish larvae (Munday et al. 2010), and would likely increase mortality of adult cardinalfishes at home sites.

The prospects for adaptation of sensory systems to maintain performance in high CO_2 environments remain unknown. Although the acute exposure to elevated CO_2 used in this study does not allow observation of long-term effects, the consistency of behavioural responses observed at CO_2 concentrations likely to occur within the next 30 years (550ppm) indicates there is limited time for adaptation to occur before impacts are present in wild populations. While Munday et al. (2010) observed substantial variation among damselfish larvae exposed to 700ppm CO_2, variation between treatment levels was not observed in this study. The short generation time observed in most apogonid species might contribute to development of adaptations over time, however the lack of variability in behavioural responses observed here suggests there may be limited opportunity for selection to occur. The plasticity of the physiological mechanisms responsible for impairment of sensory systems will be important in predicting the potential for adaptation; however, these mechanisms are currently unknown and are an important area for further research. Homing itself may be critical for local adaptation and any impairment of homing ability may significantly impact the ability of species to evolve in rapidly changing environments.

Alterations in homing ability, olfactory discrimination, and behaviour caused by elevated CO_2 suggest possible impairment of general cognitive function. Cardinalfish held in elevated CO_2 were no longer able to distinguish cues associated with home sites, and consequently displayed an inability to successfully return to home sites following displacement, suggesting failure both to recognize familiar home site chemical cues and to retain spatial memory of the surrounding reef environment. Attaining the benefits provided by maintenance of home ranges is dependent on mental retention of familiar aspects, such as locality of food, shelter and conspecifics throughout the territory. Without the capacity to identify previously learned resources, many of the benefits gained from maintaining home territories may be lost. Therefore, interruption of pathways necessary for sensory cue recognition and subsequent elicitation of appropriate behavioural responses could have significant implications for individual fitness.

This study is the first to demonstrate effects of ocean acidification on ecologically important behaviour of adult fishes in the field and at CO_2 concentrations that could occur in the oceans within 40 years (Meehl et al. 2007). While the apogonids tested here appear to be more sensitive to rising CO_2 than the damselfish species tested previously (Munday et al. 2009b, 2010; Dixson et al. 2010), potential effects on other species are unknown. If other species are affected at similar CO_2 levels, there could be significant implications for homing and site attachment in fishes from a range of different ecosystems. Further research is needed to explore the sensitivity of other fish species to elevated CO_2 and predict possible impairment of other sensory systems in order to understand the mechanisms responsible for impairment of sensory cue recognition and the ecological impacts likely to be experienced in natural ecosystems.

CHAPTER 4: HABITAT PREFERENCES OF REEF FISH MAY CHANGE IN FUTURE SEAS

Submitted to Marine Biology

4.1 ABSTRACT

Rising atmospheric carbon dioxide (CO_2) concentrations are causing additional CO_2 to be absorbed by the oceans, leading to alterations in water chemistry such as ocean acidification. Recent studies show that exposure to elevated CO_2 causes impairment of olfactory discrimination in reef fishes; however the ecological consequences of this impairment are largely unknown. Here we tested the effects of elevated CO_2 on habitat preferences of coral-dwelling gobies, some of the most habitat-specialist fishes on coral reefs. Gobies were exposed to ambient CO_2 (440μatm) or an elevated CO_2 level (880μatm) that could occur in oceans by the end of the century. Pair-wise olfactory tests indicated that exposure to elevated CO_2 impaired the ability of *Paragobiodon xanthosomus* to discriminate between odour cues of their sole host coral (*Seriatopora hystrix*) and a non-preferred coral (*Pocillopora damicornis*). To test if olfactory impairment affected habitat utilization, habitat choice experiments were then conducted in the field, simulating a future scenario in which the preferred host coral dies and gobies must search for a new coral colony to occupy. Adult *Gobiodon histrio* placed individually on dead *Acropora nasuta* colonies were given the choice of live colonies of *Acropora nasuta* (preferred habitat) or *Acropora tenuis* (non-preferred habitat). All control individuals moved to live *A. nasuta* habitat within 24 hrs, however gobies exposed to 880μatm CO_2 associated with both preferred (*A. nasuta*) and non-preferred (*A. tenuis* and dead *A. nasuta*) habitats in approximately

equal frequency. These results suggest that rising CO_2 could affect the fitness of habitat specialist fish species by reducing their ability to select favourable habitat.

4.2 INTRODUCTION

Reef fish are not distributed evenly among coral reef habitats, but rather exhibit habitat partitioning at multiple spatial scales (Waldner and Robertson 1980; Williams 1991; Friedlander and Parrish 1998). Many reef fish have specific microhabitat associations, with some of the most habitat specialised fishes forming obligate, and often symbiotic, associations with just one or a few coral species (Sale 1977; Munday et al. 1997; Elliott and Mariscal 2001; Pratchett et al. 2006; Wong 2011). As a result, the distributions and abundance of coral-associated fishes may be dictated by the distributions and availability of preferred coral species (Bell and Galzin 1984; Kuwamura et al. 1994; Chabanet et al. 1997; Munday 2002; MacNeil et al 2009). Occupying preferred habitats can be essential for individual fitness, with growth, reproductive success, and survival often dependent on associating with preferred substrates (Jones 1988; Clarke 1992; Wellington 1992; Caley and Munday 2003).

Global climate change is predicted to increase the frequency and intensity of environmental disturbances, with coral reefs and other shallow water ecosystems potentially at greatest risk (Hughes et al. 2003; Donner et al. 2005). Rising sea surface temperature has already caused mass coral-bleaching events that have seriously impacted coral communities in many parts of the world (Glynn 1993; Hoegh-Guldberg 1999). Ocean acidification due to absorption of excess carbon dioxide from the atmosphere is expected to further impact coral health through reduced calcification rates (Leclercq et al. 2000; Langdon et al. 2003; Hoegh-Guldberg et al. 2007; Kleypas and Yates 2009). Consequently, climate-induced perturbations are likely to further weaken coral reef habitats already impacted by other anthropogenic disturbances including sedimentation, overfishing, and pollution (Hughes et al. 2003). Coral decline has been shown to directly affect the diversity and abundance of coral reef fishes, with the effects most apparent for coral-associated species that depend on live coral for shelter or nutritional purposes (Jones et al. 2004; Munday 2004a; Bellwood et al. 2006; Pratchett et al. 2008; Wilson et al. 2006, 2007).

Rising CO_2 levels can also have direct effects on coral reef fishes. Recent studies show that increased levels of dissolved CO_2 disrupt sensory mechanisms and behaviour in coral reef fishes. Damselfish larvae exposed to elevated CO_2 were unable to discriminate between various olfactory (Munday et al. 2009; Dixson et al. 2010) and auditory (Simpson et al. 2011) cues that may be necessary for site selection at settlement, and exhibit more active and riskier behaviour following settlement to natural coral reef habitat leading to an increase in post-settlement mortality (Munday et al. 2010, Ferrari et al. 2011). Elevated CO_2 may also affect brain function, with alteration in behavioural lateralization observed in CO_2 exposed damselfish (Domenici et al. 2011). One study has demonstrated exposure to elevated CO_2 can impair the homing behaviour of adult cardinalfishes (Devine et al. 2011); however, if adults of other species are similarly affected, and the ecological consequences of behavioural changes in reef fishes, are currently unknown. Furthermore, how direct effects of elevated CO_2 on fish behaviour might interact with changes to coral reef habitat caused by global warming and ocean acidification has yet to be investigated.

Coral-dwelling gobies are among the most habitat specialised fishes on coral reefs, possessing specific preferences for a limited number of coral habitats, and in some cases a single coral species (Tyler 1971; Munday et al. 1997; Dirnwöber and Herler 2007). Habitat availability is believed to be the primary determinant of the distribution and abundance of most coral goby populations (Kuwamura et al. 1994, Munday et al. 1997, Munday 2002, 2004a) and competition for preferred coral habitat has been observed in many species (Munday et al. 2001, Munday 2004b, Hobbs and Munday 2004). Acquisition of preferred microhabitat can influence individual fitness in coral gobies, as growth and survival are enhanced on the most suitable habitats (Munday 2001; Caley and Munday 2003). Any impairment in the ability to identify preferred habitats or alterations to patterns of habitat use caused by rising CO_2 levels could have a significant effect on individual performance. However, a unique consequence of the close association of coral gobies with their host coral colonies includes periodic exposure to fluctuations in pH and CO_2 concentrations within coral branches at night due to respiration of their host coral colony (Suzuki et al. 1995; Yates and Halley 2006; Nilsson et al. 2007). Yates and Halley (2006) found nightly increases in pCO_2 as high as ~800-1200µatm on in situ patch reefs with 10-22% coral cover, therefore this nightly exposure to elevated CO_2 might enhance tolerance to future oceanic conditions.

This study aimed to determine if exposure to elevated CO_2 affects habitat preferences of coral-dwelling gobies. Habitat preferences were compared between gobies exposed to current-day ambient CO_2 (440 µatm) and an elevated CO_2 level (880 µatm) that could occur in the shallow oceans by 2100 based on the current CO_2 emissions trajectory (Meehl et al. 2007; Raupach et al. 2007). Pair-wise olfactory tests were performed using *Paragobiodon xanthosomus*, a species that inhabits just one coral species, to determine if gobies could discriminate between odours cues of preferred (*Seriatopora hystrix*) and non-preferred (*Pocillopora damicornis*) coral species. Habitat choice experiments were then conducted in the field using *Gobiodon histrio* to assess habitat selections between preferred (*Acropora nasuta*) and non-preferred (*Acropora tenuis*) coral species following placement on dead coral habitat. This experiment simulated a potential future scenario in which the preferred host coral dies from coral bleaching or other stress, and gobies must search for another suitable colony to occupy.

4.3 MATERIALS AND METHODS

4.3.1 Study species and location

This study was conducted at Lizard Island in the Great Barrier Reef, Australia (145º27'E, 14º41'S) in December 2010. Two goby species were used in the experiments; yellow-green gobies, *Paragobiodon xanthosomus*, were used in the laboratory-based olfactory discrimination tests and broad-barred gobies, *Gobiodon histrio*, were used in the field-based habitat choice experiment (Figure 4.1). These two goby species were selected based on their degree of coral specialisation. *Paragobiodon xanthosomus* is exclusively associated with a single coral species, *Seriatopora hystrix* (Thompson et al. 2007; Wong 2011), making it an ideal species for testing olfactory discrimination ability. *Gobiodon histrio* was selected for the field-based habitat choice experiments as it occupies a broader range of coral species. *G. histrio* are known to prefer and are most commonly associate with *Acropora nasuta*, however they will occasionally occupy colonies of other *Acropora* species, including *A. tenuis* (Munday et al. 1997).

Gobies collected from various locations in the Lizard Island lagoon were transported to the research station and placed in replicate 35 L aquaria at either ambient or elevated CO_2 (see below). Previous studies demonstrate that behavioural alterations due to elevated CO_2 are present after 4 consecutive days in treatment (Munday et al. 2010; Ferrari et al. 2011; Devine et al. 2011), consequently all gobies were held in CO_2 treated seawater for 4 days prior to testing. Gobies were fed newly hatched *Artemia* nauplii three times daily.

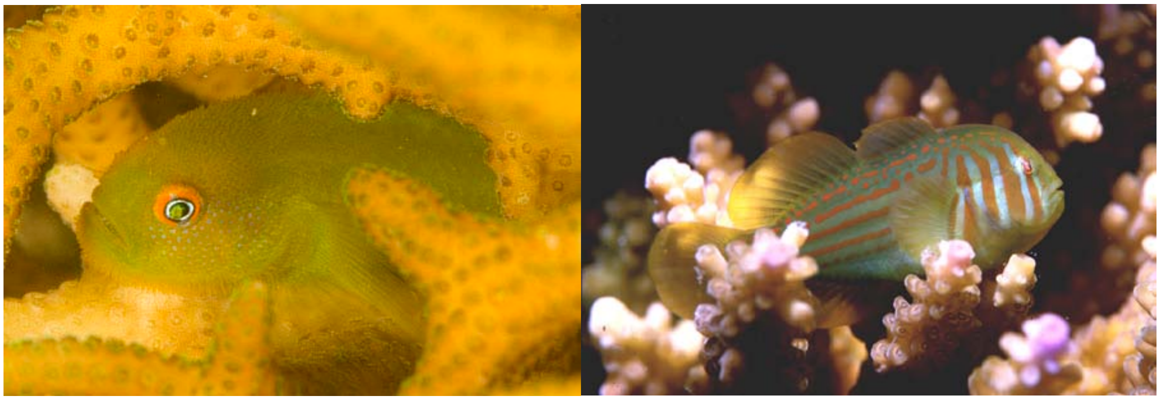

Figure 4.1 Study species: *Paragobiodon xanthosomus* (left) used for olfactory trials and *Gobiodon histrio* (right) used for field habitat choice tests.

4.3.2 Seawater manipulation

Seawater was pumped from the ocean into 60 L sumps and diffused with ambient air (control) or CO_2 to achieve a pH_{NBS} of approximately 8.15 (control) or 7.89 (treatment). 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 in the sump whenever the pH of the seawater rose above the set point. Equilibrated seawater from each sump was supplied at a rate of ~500ml min^{-1} to four replicate 35 L aquariums. Temperature and pH_{NBS} of each aquarium was measured each morning and afternoon using a HQ40d pH meter (Hach, Colorado, USA) calibrated with fresh buffers. Total alkalinity of seawater was estimated by Gran titration from water samples taken twice weekly from control and treatment aquaria. Alkalinity standardizations achieved accuracy within 1% of certified reference material (Dr. A. Dickson, Scripps Oceanographic Institute). Average seawater $p\mathrm{CO_2}$ was calculated from seawater parameters in the program CO2SYS (Lewis and Wallace 1995) and using the constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987) (Table 4.1).

Table 4.1 Mean (±SD) seawater parameters in the experimental system. Temperature, pH salinity, and total alkalinity (TA) were measured directly and $p\mathrm{CO_2}$ estimated from these parameters using CO2SYS.

$\mathrm{pH_{NBS}}$	Temp (ºC)	Salinity (ppt)	TA (µmol.kg^{-1}SW)	$p\mathrm{CO_2}$ (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)

4.3.3 Olfactory trials

The effect of exposure to elevated $\mathrm{CO_2}$ on the ability of *Paragobiodon xanthosomus* to discriminate between coral species using olfactory cues was determined using odour preference tests in the laboratory. Gobies were tested in 300 L cylindrical aquaria containing two odour sources, the preferred coral *Seriatopora hystrix* (Pocilloporidae), and a non-preferred confamilial coral species, *Pocillopora damicornis,* positioned on opposite sides of the test arena. Each odour source consisted of a coral 8-10 cm in diameter housed within a porous, opaque mesh cylinder, allowing emission of chemical cues into the test arena while visually concealing the coral from test individuals. Each goby was introduced to the centre of the aquarium and allowed to acclimate to the two odours for 30 minutes. Following the acclimation period the position of the test subject in relation to each odour source was recorded, with preference for a particular cue indicated by association within 30 cm of the odour source. All gobies not within 30 cm of an odour source were denoted as making ‘no choice’ in preference for either habitat odour. Twenty individuals were tested from both the control and elevated CO_2 treatment. Test water was changed between each trial and there was no water flow during the test period. Prior to each trial test water was maintained still for 5 minutes to allow concentration gradients to form before introduction of the test subject. A chi-square test of homogeneity was used to test if the frequency of individuals associated with each habitat odour source differed between control and elevated CO_2 treated fish.

4.3.4 Habitat preference experiment

A field experiment was used to determine if *Gobiodon histrio* exposed to elevated CO_2 could successfully locate alternative habitat following death of their host coral. Gobies were placed on a dead colony of *Acropora nasuta* (preferred habitat) and given the choice of a similar sized healthy colony of *A. nasuta* and *A. tenuis* (non-preferred habitat). As the frequency of coral bleaching events is predicted to increase with rising global temperatures (Hoegh-Guldberg 1999), this experimental design simulated a potential future scenario for obligate coral dwellers where it may be necessary to select a new coral colony following death of a host coral. Each experimental replicate consisted of equal-sized colonies (20-30 cm diameter) of live *Acropora nasuta*, live *Acropora tenuis*, and dead *A. nasuta* collected throughout the lagoon and placed 0.5 m apart. Experimental replicates were placed 10 m apart on sand substrate at 6 m depth and 15 m away from the nearest reef on the leeward side of Lizard Island. Each morning (0900-1100 h), gobies from the control and elevated CO_2 treatment were placed individually on dead *A. nasuta* habitats. In the afternoon (1500-1700 h) and the following morning (0800-1000 h) each experimental replicate was surveyed and habitat association for each goby recorded. All *G. histrio* were initially collected from *A. nasuta* colonies to ensure *A. nasuta* was indeed the preferred coral species for all individuals tested. Twenty fish from the control treatment and twenty fish from the elevated CO_2 treatment were tested. Chi-square tests of homogeneity were used to compare frequency of individuals associated with each available habitat option, and to compare the timing of transition to new habitats between control and CO_2 treated fish.

4.4 RESULTS

4.4.1 Olfactory preferences

There was a significant difference in olfactory discrimination ability between *Paragobiodon xanthosomus* exposed to control versus elevated CO_2 conditions (χ^2_2=11.65, p=0.03). Control individuals strongly preferred the odour of *Seriatopora hystrix*, with 70% of gobies exhibiting a strong association with the odour source of their host coral species. In contrast, gobies exposed to 880µatm CO_2 appeared unable to distinguish between habitat odour cues. Only 16% of the elevated CO_2 treatment fish preferred the odour of their host coral *S. hystrix*. The remaining 84% were equally associated with the non-preferred coral species *Pocillopora damicornis* or exhibiting no preference for the odour of either coral species (Figure 4.2).

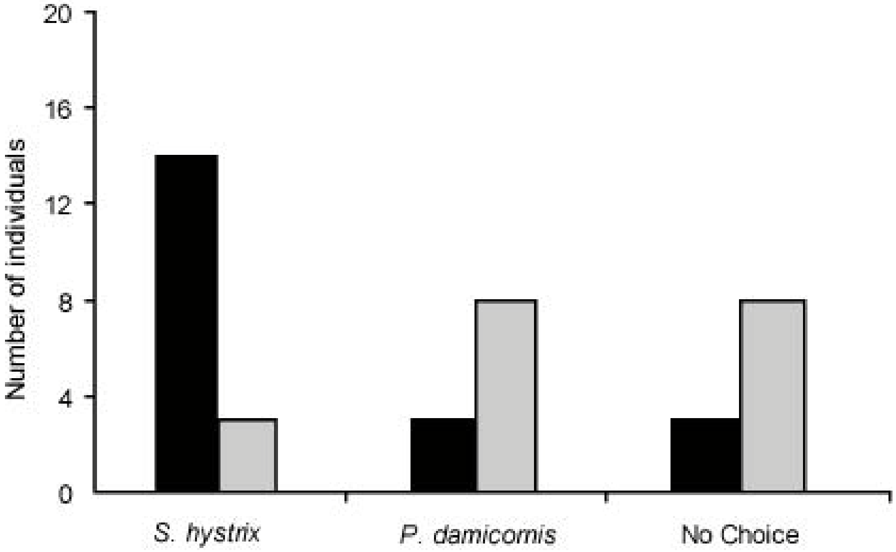

Figure 4.2 The number of control and elevated CO_2 treated *Paragobiodon xanthosomus* associating with each odour source or displaying no preference for either odour. Black bars represent control gobies; white bars, 880 µatm CO_2 exposed gobies.

4.4.2 Habitat choice experiments

Habitat choices by *Gobiodon histrio* on patch reefs significantly differed between control and 880µatm CO_2 treated individuals (χ^2_2=15.17, p=0.01). Within 24 hours of placement on dead *Acropora nasuta* all control *G. histrio* successfully migrated to live *A. nasuta* habitat. In contrast gobies held at 880µatm CO_2 were associated with all available habitats types (Figure 4.3). While 45% of CO_2 treated *G. histrio* moved to *A. nasuta*, an equal proportion did not vacate the dead coral habitat and the remaining 10% moved to the non-preferred *A. tenuis* habitat. In addition, there was a significant difference in the timing of transition to new habitats (χ^2_1=6.14, p=0.02). Ninety-percent of 880µatm CO_2 treated *G. histrio* made final habitat selections by the afternoon observation, whereas 45% of control individuals delayed movement until night with final selections established by the following morning observation.

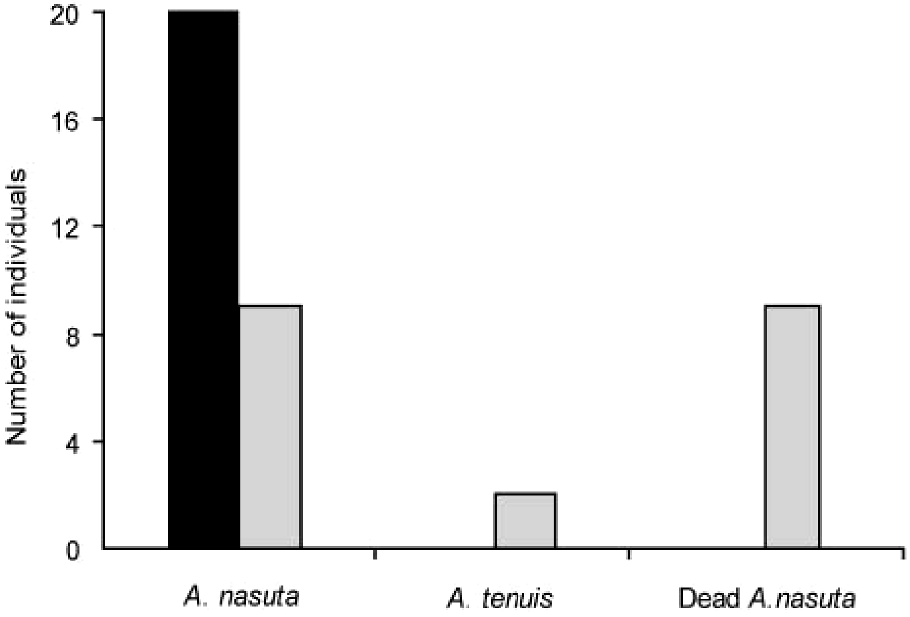

Figure 4.3 The number of control and elevated CO_2 exposed *Gobiodon histrio* associating with each habitat type the morning following placement on dead *Acropora nasuta*. Black bars represent control gobies; white bars, 880 µatm CO_2 exposed gobies.

4.5 DISCUSSION

Despite periodic exposure to high CO_2 levels through residence within respiring coral branches (Nilsson et al. 2004), coral gobies experienced sensory and behavioural impairment following prolonged exposure to elevated CO_2. Consistent with previous studies on larval fishes (Munday et al. 2009), CO_2 treated *Paragobiodon xanthosomus* were unable to discriminate between olfactory cues relevant to habitat selection. While *P. xanthosomus* exposed to current day CO_2 levels were strongly attracted to odours from their host coral *Seriatopora hystrix*, gobies exposed to elevated CO_2 displayed an inability to distinguish between preferred and non-preferred habitat odours, with the majority of individuals exhibiting no preference for either cue or electing to associate with odours of non-preferred habitat (*Pocillopora. damicornis*). Similarly, *Gobiodon histrio* exposed to elevated CO_2 displayed impaired ability to select preferred habitat in a natural setting. Control fish placed on dead coral habitat were able to successfully locate live *Acropora nasuta* habitat, whereas gobies exposed to elevated CO_2 selected both preferred and non-preferred habitats. These results suggest that CO_2 levels predicted to occur in the ocean by the end of this century could significantly affect the ability of highly specialised species to locate appropriate habitat following disturbances such as coral bleaching.

If the frequency of coral bleaching events increases with rising temperatures, and additional stressors such as ocean acidification impede recovery of coral habitats from disturbances, gobies will likely encounter a future in which movement from degraded host corals to more suitable habitats would be necessary with increased frequency. Coral gobies, especially breeding pairs, exhibit strong host coral fidelity, but will leave highly degraded host corals and are capable of migration to new habitats upwards of 2 m away (Feary 2007; Wall and Herler 2008). All control *G. histrio* left the dead coral colony and moved to a live colony of *A. nasuta* within 24 hours in the field experiment. This is consistent with the observation that *G. histrio* always leave dead coral colonies following coral bleaching in the field (Bonin et al. 2009). However, many of the *G. histrio* exposed to elevated CO_2 preferred to remain on the dead coral habitat, and others moved to a non-preferred coral host. This willingness to persist on degraded coral habitat could impact individual fitness of coral-associated fishes in future seas.

Coral-dwelling gobies exhibit clear habitat preferences (Munday et al. 1997, 2001; Herler 2007), and inhabiting preferred coral species has been shown to strongly influence growth rates and survival (Munday 2001; Caley and Munday 2003). For example, growth of juvenile *G. histrio* was 3 times higher and survival 5 times higher on *A. nasuta* than on *A. loripes* (Munday 2001). Coral-dwelling gobies never persist on dead coral, which suggests live coral is important for their long-term survival. Dead coral structure can quickly become covered in algae, and gobies inhabiting degraded or dead corals might be more vulnerable to predation (Coker et al. 2009). Furthermore, *Gobiodon* are also known to feed on the tissue of their host corals (Brooker et al. 2010) and dead or degraded corals may not provide essential nutritional resources, although to what extent feeding on coral tissue influences goby success is unknown. Although only a few *G. histrio* selected the non-preferred coral species *A. tenuis*, even a slight alteration in habitat preferences of this species could impact populations and distributions of other coral goby species. *G. histrio* are a relatively large, aggressive species that are competitively dominant to most other species of coral gobies (Munday et al. 2001), therefore species occurring naturally on *A. tenuis* could easily be displaced if *G. histrio* exhibit changes in habitat choices in an elevated CO_2 environment.

Following exposure to increased CO_2, *P. xanthosomus* were unable to distinguish between olfactory cues of preferred versus non-preferred coral hosts. An inability to differentiate habitat types through odour cues could impact habitat selections of both larvae and adults. As settlement of most larval reef fishes, including gobies, occurs at night (Dufour and Galzin 1993; Leis and McCormick 2002; Doherty et al. 2004), settling larvae potentially rely heavily on accurate olfactory information in the absence of visual cues. Larval coral gobies possess highly developed olfactory organs (Arvedlund et al. 2007) suggesting larvae utilise odour cues in order to locate preferred coral hosts at settlement. If elevated CO_2 impairs the ability of larvae to discriminate between habitat types using olfactory cues, settlement to non-host coral species might occur, which could influence post-settlement growth and mortality (Munday 2001). Habitat choice experiments in this study suggest adult gobies similarly rely on olfactory cues to locate suitable habitat, as it is likely gobies placed on the dead coral would have been able to visually detect the different coral habitats situated only 0.5 m away. Adult coral gobies may also depend on well developed olfactory senses to aid in feeding and reproductive behaviours and to defend occupied corals from intruders.

Highly specialised coral associates dependent on the survival of only a few preferred coral species are believed to be at increased risk of extinction if coral cover continues to decline (Munday 2004; Wilson et al 2007). Approximately 10% of reef fish species have an obligate association with live corals throughout their life (Jones et al. 2004). The abundance of these species typically declines as coral cover diminishes, resulting in local extinction for some coral-specialist species (Jones et al. 2004; Munday 2004a). Elevated atmospheric CO_2 at concentrations causing sensory impairment in fishes (>500ppm) will undoubtedly exacerbate coral declines worldwide, with net calcification of branching coral species used by coral-dwelling gobies (families Acroporidae and Pocilloporidae) expected to cease if oceanic CO_2 reaches 800ppm (Hoegh-Guldberg et al. 2007; Veron et al. 2009). Therefore, while end of the century CO_2 concentrations potentially alter habitat associations in reef fishes, there may also be relatively few corals remaining that are suitable habitat for coral-dwelling fishes.

This study demonstrates possible alterations in habitat associations of coral-dwelling gobies at CO_2 concentrations that could occur in oceans by the end of this century. Although coral gobies are diurnally exposed to elevated CO_2 within the branches of host corals, *G. histrio* and *P. xanthosomus* exhibited no tolerance following continuous exposure to high-CO_2 and display similar sensory and behavioural disruptions as other previously tested reef fishes (Munday et al. 2009, 2010; Dixson et al. 2010; Devine et al. 2011). Our results suggest that alterations in habitat preferences and possible shifts in species distributions could occur due to rapidly rising CO_2 levels. Whether selection of CO_2 tolerant genotypes will occur with sufficient speed to mitigate the behavioural and sensory impairment detected here, and in earlier studies, is unknown. Use of dead or non-preferred habitats versus preferred habitat will generate a strong selective gradient and it is possible that more tolerant genotypes could rapidly spread throughout the population, especially given that most gobies are relatively short lived (Hernaman and Munday 2005; Depczynski and Bellwood 2006). Regardless, it is clear that coral-associated fishes face a period of extreme selective pressure as their ability to locate increasingly less abundant coral habitats is impaired by the same factor (rising CO_2) that is causing massive changes to coral reef habitat.

CHAPTER 5: GENERAL DISCUSSION

As CO_2 concentrations continue to rise in the atmosphere and oceans over the coming century (Meehl et al. 2007; Raupach et al. 2007; Doney 2010), marine organisms will be subjected to alterations in water chemistry unprecedented previously for millions of years (Royal Society 2005; Hoegh-Guldberg et al. 2007). This thesis investigated the effects that such changes in ocean chemistry have on the behaviour of coral reef fishes. Habitat associations and sensory behaviour of multiple fish taxa and life history stages were disrupted by CO_2 levels predicted to occur in the ocean over coming decades (550-950ppm), highlighting the potential for ocean acidification to have significant ecological impacts on reef fish communities.

Consistent with earlier experiments (Munday et al. 2009b), larval damselfishes exposed to elevated CO_2 exhibited impaired ability to distinguish between odours of common reef microhabitats. However, when all sensory cues were available CO_2 treated larvae still successfully located preferred settlement habitat (Chapter 2). This suggests that visual cues can compensate for the loss of olfactory function during habitat selection by larval fishes. Nevertheless, exposure to elevated CO_2 could disrupt the settlement process, with fundamental changes observed in the timing of settlement. The increased preference to settle during the full moon instead of the new moon in larvae exposed to elevated CO_2 is consistent with the hypothesis that they rely on visual cues when olfactory function is impaired. Consequently, despite the potential for compensation of olfactory impairment through visual habitat assessment, larvae could still suffer increased mortality in a high CO_2 environment as a result of settling during unfavourable lunar periods.

Research conducted to date has focussed on exploring the developmental and behavioural consequences of elevated CO_2 on the early life history stages of damselfishes (Munday et al. 2009b, 2010, 2011; Dixson et al. 2010; Ferrari et al. 2011), with potential effects on adult individuals and throughout various species groups largely unknown. My research showed that sensory impairment and behavioural alterations from elevated CO_2 also occur in adult reef fishes, leading to significant changes in habitat associations. Olfactory discrimination of resting sites and navigational capabilities of adult cardinalfish were compromised following exposure to elevated CO_2 at concentrations as low as 550ppm (Chapter 3), which could occur in oceans within 40 years (Meehl et al. 2007). Adult coral gobies were similarly affected, contrary to the assumption that exposure to natural diurnal fluctuations in dissolved CO_2 in water surrounding coral colonies might make this group more tolerant to rising CO_2 levels. In addition to olfactory disruption, adult *G. histrio* exposed to high CO_2 exhibited alterations in habitat preferences and were more willing to persist on unfavourable habitats (Chapter 4). These disruptions to critical adult behaviours in response to elevated CO_2 could significantly affect the distribution and viability of reef fish populations.

Olfactory impairment occurred in both larval and adult stages across multiple reef taxa, suggesting sensory disruptions due to elevated CO_2 could be widespread among marine fishes, and potentially other organisms. Olfaction is an important sensory system for the establishment and maintenance of habitat associations in reef fishes, utilised during the initial selection of settlement sites (Danilowicz 1996; Elliott et al. 1995; Lecchini et al. 2005, 2007b; Ben-Tzvi et al. 2010) and subsequent recognition of selected habitat (Colin 1996; Dittman and Quinn 1996; Døving et al. 2006). Olfactory disruption has the potential to alter habitat selections early in life and impact habitat associations essential for critical adult behaviours such as foraging and spawning. Here it is suggested that settling larvae may be able to compensate for olfactory disruption through reliance on alternative sensory systems, such as vision. However, limiting the use of olfactory cues during overnight settlement caused larvae exposed to increased CO_2 to alter lunar settlement patterns to periods when visual acuity was highest, a behavioural impairment that could lead to higher mortality. Recent research has shown auditory behaviours are also affected, as reef fish larvae no longer avoid reef sounds during daylight hours following exposure to elevated CO_2 (Simpson et al. 2011). Impairment of this important auditory response could exacerbate the risks encountered during settlement if larvae shift attraction to reef noise from night to day after exposure to high CO_2, which could further impact mortality and subsequently recruitment rates of reef fish larvae.

The mechanisms responsible for impaired sensory function in fishes exposed to elevated CO_2 are not fully understood. However, new research has documented interference of elevated CO_2 on behavioural lateralization (Domenici et al. 2011), a trait that is directly related to cognitive function (Dadda and Bisazza 2006) and spatial orientation (Sovrano et al. 2005). This supports the hypothesis that while the actual reception of sensory cues is unaffected by elevated CO_2 (Munday et al. 2009b, 2010; Simpson et al. 2011), the interpretation and cognitive analysis of sensory cues within the brain used to make competent responses and decisions based on environmental stimuli may be affected by high CO_2. Such brain dysfunction could explain the inability of some fishes throughout this study to successfully utilise senses, like vision, that appear unaffected at low concentrations tested here in order to locate preferred habitats. Although olfaction is potentially the key sensory mechanism utilised during homing, it is likely that fish also use visual landmarks for mapping the area surrounding home sites, as spatial memory is well-documented in many fish species (Noda et al. 1994; Braithwaite 1998; Hughes and Blight 1999). Habitat choice tests involving coral gobies suggest similar interference with cognitive ability, as it is improbable gobies could not visually detect live coral colonies only 50cm away. Therefore, an inability to accurately interpret perceived environmental cues is potentially the source of sensory disruption due to elevated CO_2; suggesting changes in water chemistry might disrupt general cognitive functions making fish less perceptive to sensory information necessary for behaviours such as habitat selection and predator avoidance.

Disruption of brain pathways leading to sensory impairment and alterations in habitat associations could have far-reaching impacts on sustainability of reef fish populations. Larval recruitment could be significantly hindered if higher mortality due to predation occurs following a shift of settlement timing to full moon phases, or fitness consequences for larvae experiencing difficulty in locating preferred habitat at night with the absence of accurate olfactory cues. If critical adult behaviours such as homing are altered due to elevated CO_2 fish may be unable to navigate between sites necessary for the replenishment of marine populations. Fishes with compromised cognitive abilities may also be unable to cope with a rapidly changing environment, and may be forced to persist in unsuitable or degraded habitats.

Variability in responses to elevated CO_2 indicates that some fish species may be more sensitive to changes in ocean chemistry than others (Ferrari et al. 2011). The apogonids tested here experienced full sensory impairment at a lower CO_2 concentration (550ppm) than observed in previous studies (>700ppm). This suggests that behavioural alterations due to rising CO_2 could occur much sooner than previously believed, possibly by mid-century when atmospheric CO_2 concentrations are projected to pass 500ppm. As cardinalfishes are an important dietary component for many reef predators (Chystal et al. 1985; Beukers-Stewart and Jones 2004), their susceptibility to elevated CO_2 could impact more tolerant taxa through alterations in the distribution and abundance of prey. The short generation time observed in most apogonid and gobiid species might contribute to development of adaptations over time, particularly if shifts in habitat use generate adequate selection gradients for adaptation to occur. However, the low variability in behavioural responses of cardinalfishes observed within CO_2 treatments in my experiments suggests there may be relatively limited opportunity for selection of tolerant individuals.

The results of this project indicate important directions for future research. Impaired interpretation of cues from multiple sensory systems in fish exposed to elevated CO_2 suggests interference with critical neural pathways, although the physiological processes responsible for this impairment have yet to be determined. Understanding the mechanism inducing disruption of brain function in response to elevated CO_2 would provide valuable insight to the range of senses and fish behaviours that could potentially be affected by ocean acidification. Elevated CO_2 appears to affect a range of sensory systems and behaviours, but at different concentrations. If each sensory system displays sensitivity to elevated CO_2 at different levels, with olfactory and auditory interpretation affected at relatively low concentrations, future experiments should explore the thresholds at which more resilient senses such as vision are affected in high CO_2 environments. Determining the CO_2 concentration at which visually-mediated behaviours are impaired could be an essential next step in understanding how fish behaviours may be altered in future seas.

Behavioural alterations observed in this study were derived from acute exposure to near future CO_2 concentrations, however it is essential for further research to explore acclimation and adaptation potential of reef fishes to high CO_2 environments, using longer term and multi-generational studies. While short-term studies such as this are a vital first-step is assessing potential ecological processes that may be impacted by ocean acidification, long-term studies are crucial for understanding how species will cope with rising CO_2 over a number of decades. Testing offspring of individuals held in future CO_2 conditions should explore the potential for physiological and behavioural adaptation to determine if fishes may become more tolerant to elevated CO_2 over an extended period of time. The extension of long-term studies to integrate multiple climate change elements, such as elevated sea temperatures and habitat disturbance leading to changes in habitat and food availability, will provide a better understanding of the effects that rising CO_2 levels will have on fish populations and communities. The interactive effects of climate change stressors could exacerbate impacts of elevated CO_2 on fishes, especially if ocean warming causes thermal optima to be exceeded in the future (Pörtner and Farrell 2008; Munday et al. 2009a). This integrative testing approach would provide the most accurate depiction of ecological scenarios to come.

Although life history traits of reef fishes (e.g. growth, development and survival) do not appear to be affected by CO_2 concentrations predicted to occur in the ocean this century (Ishimatsu et al. 2008; Munday et al. 2011), the research conducted here adds significantly to the growing evidence that rising atmospheric CO_2 is a significant threat to marine fish through effects on individual behaviour. This project addresses critical gaps in our knowledge of how elevated CO_2 might interfere with habitat preferences of reef fishes, exploring sensory mechanism utilised to discriminate habitat sites in a variety of reef taxa and at both larval and adult life history stages, and in natural coral reef habitats. These findings not only pertain to habitat associations of coral reef fishes, but present an array of high-CO_2 effects that have the potential to affect other marine organisms. Testing if other taxa are similarly affected and what capacity they have to adapt to rising CO_2 levels over multiple generations will be key areas for future research.

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