Rising CO2 concentrations affect settlement behavior of larval damselfishes

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Rising CO$_2$ concentrations affect settlement behaviour of larval damselfishes

B. M. Devine · P. L. Munday · G. P. Jones

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 (700 and 850 ppm) 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 850 ppm CO$_2$ displaying highest settlement rates around the full moon. 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.

Keywords Climate change · Ocean acidification · Habitat preferences · Pomacentridae · Settlement behaviour · Lunar periodicity

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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 towards 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 among 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 utilising 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 (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 risk from rising CO_2, as increased 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 (700–850 ppm) could occur in the oceans by the end of this century according to predicted 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 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 440 ppm (current-day control), 700 or 850 ppm 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 whether 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.

Materials and methods

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 and 0630 hours 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. *Pomacentrus moluccensis* is a live 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.

Seawater manipulation

Seawater was pumped directly from the ocean into 3×60 l sumps and diffused with either ambient air (control) or CO_2-enriched air to achieve concentrations of ~ 700 or 850 ppm. Equilibrated seawater from each sump was supplied to four replicate aquaria at a rate of ~ 200–250 ml min^{-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 ($\pm$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 as follows: 449.76 ± 6.84 ($\pm$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 programme CO2SYS (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 ($\pm$ 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.

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. Subjects were presented with pair-wise choice tests of (1) hard coral versus soft coral, (2) hard coral versus coral rubble, (3) soft coral versus coral rubble and a blank seawater versus seawater as a control. To attain cues, habitat structures approximately 10 cm in diameter were placed in 8 l containers of untreated seawater for 2 h. Habitat structures were removed and the conditioned water was gravity fed into the chamber at 100 ml min^{-1}. For each trial, a single fish was introduced in the downstream end of the chamber and allowed to acclimate for 2 min. The position of the fish within the chamber was then recorded at 5 s intervals for 2 min. 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.

Habitat choice experiment

To test whether 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 300 l cylindrical outdoor aquaria contained the same three habitats used in olfactory trials: hard coral, soft coral, and coral rubble. Habitat patches were 15–20 cm in diameter and placed 30 cm apart on 2–3 cm of sand covering the aquarium base. At the start of each trial, a single fish was allowed to acclimate for 1 h in an opaque PVC holding tube (diameter 20 cm) placed in the centre of the testing aquaria. Fish were introduced to the holding tube in the evening between 1730 and 1830 h and released into the test arena after dark. The position of each fish relative to habitats was recorded at first light (0530–0545 hours) and every 30 min afterwards until 1130 h. 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 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.

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 850 ppm CO_2-treated fish to determine whether alterations in settlement patterns occur following exposure to elevated CO_2. Fish were placed individually in open-air 300 l cylindrical outdoor aquaria containing settlement habitat in the centre of each arena. Habitat structures were $\sim$15 cm 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 $\sim$20 cm) for 1 h prior to testing. Each fish was introduced to the acclimation tube in the evening at 1830 h, with release into the test arena occurring after dark. The following morning, each aquarium was surveyed at first light (0530–0540 hours) to record whether overnight settlement had occurred. Ten aquaria were used throughout the study, providing approximately 5 replicates per treatment daily across two lunar cycles ($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.

Results

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 (Fig. 1). Larvae from 700 and 850 ppm treatments displayed a general decrease in attraction to hard coral odour, with significant differences observed in hard coral versus soft coral trials (700 ppm: $\chi^2 = 6.83$ $df = 2$, $P < 0.04$; 850 ppm: $\chi^2 = 9.33$, $df = 2$, $P < 0.01$). In soft coral versus coral rubble trials, control larvae displayed no preference for either cue, whereas larvae in 700 and 850 ppm treatments swam in the soft coral cue for the majority of the test period, with a significant attraction to soft coral exhibited by 850 ppm larvae ($\chi^2 = 6.97$, $df = 2$, $P < 0.03$).

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 (Fig. 1). Although not significant, *P. amboinensis* larvae in 700 and 850 ppm 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 700 ppm CO_2 treated individuals in soft coral versus coral rubble trials ($\chi^2 = 9.64$, $df = 2$, $P < 0.01$).

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 (Fig. 1). Larvae exposed to elevated CO_2 treatments exhibited a general trend of increased attraction to hard and soft coral habitats in all trials, with 850 ppm CO_2-treated fish significantly avoiding their preferred habitat odour ($\chi^2 = 10.14$, $df = 2$, $P < 0.01$), spending less than 40% of the test period within the coral rubble cue in hard coral versus coral rubble trials.

Habitat choices at settlement

Log-linear analysis revealed that the proportion of larvae settling overnight varied significantly for all species, and
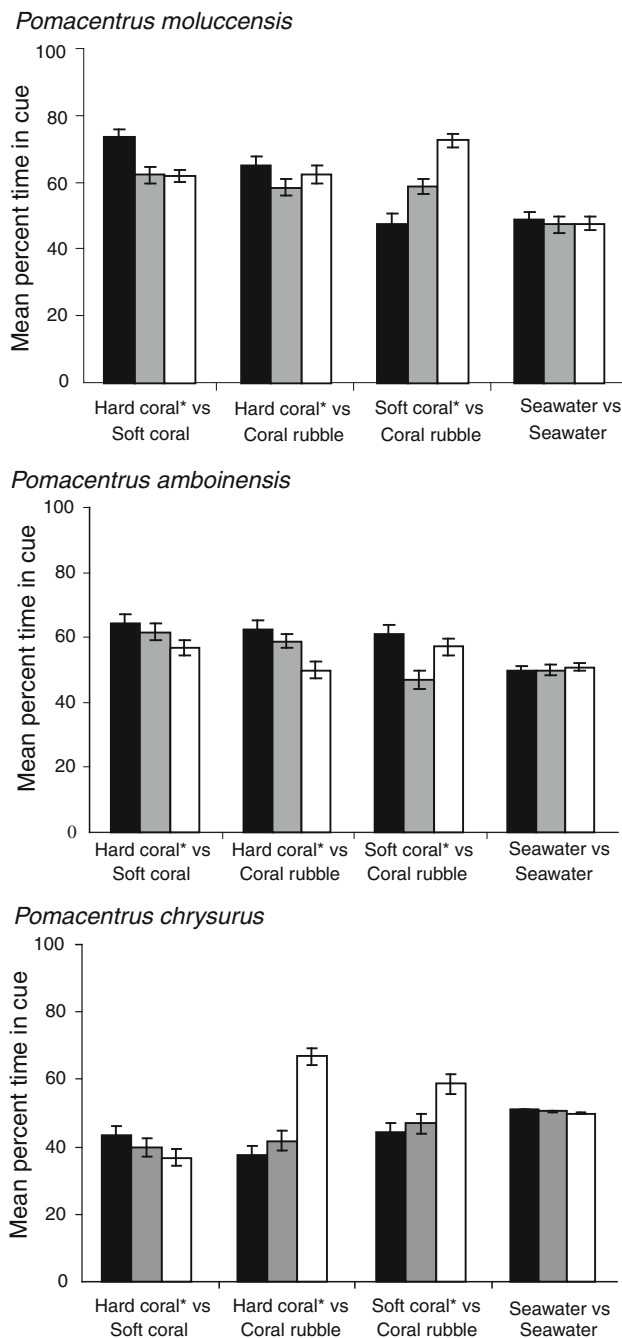
Fig. 1 Mean percent time larvae spent in a given cue (*asterisk*) for each odour comparison test for *Pomacentrus moluccensis*, *P. amboinensis* and *P. chrysurus*. *Black bar*: control; *grey bar*: 700 ppm; *white bar*: 850 ppm

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 1, 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 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 (Fig. 2). 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.

Table 1 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C) and settlement rates (S) by larval *Pomacentrus moluccensis*

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

NS not significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 2 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C) and settlement rates (S) by larval *Pomacentrus amboinensis*

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

NS not significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 3 Log-linear analysis of relationships between observation time (T), CO_2 treatment level (C) and settlement rates (S) by larval *Pomacentrus chrysurus*

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

NS not significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
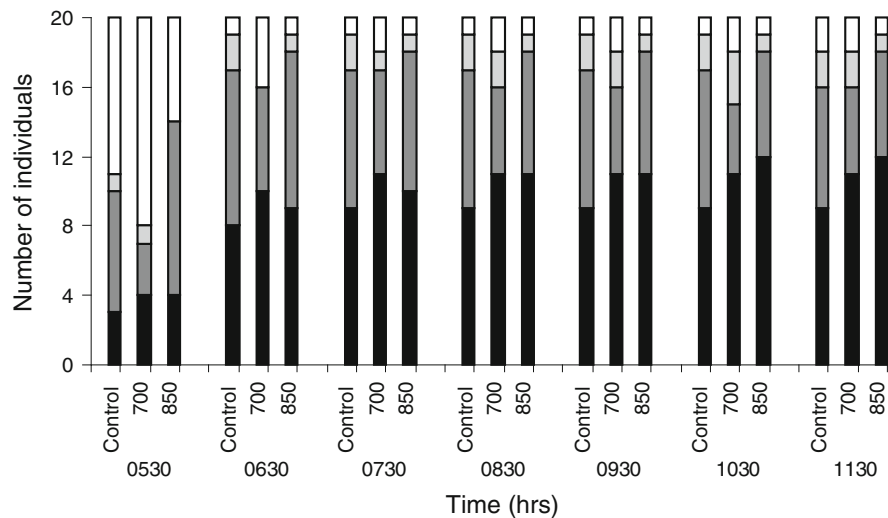
Fig. 2 Number of larvae associated with each microhabitat in overnight habitat choice experiments for control and CO$_2$ treatments at each observation time. Colours within each *bar* represent hard coral (*black*), soft coral (*dark grey*), coral rubble (*light grey*) and a no choice (*white*) category for larvae not associated with habitat structure at each time
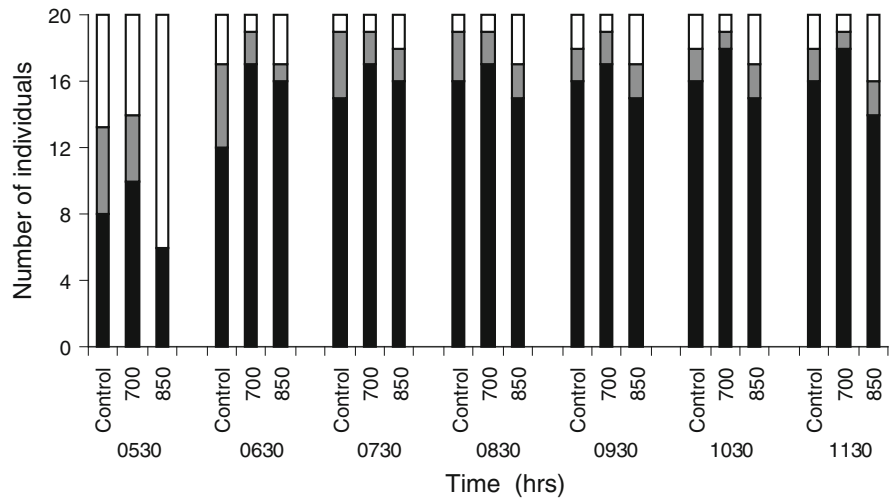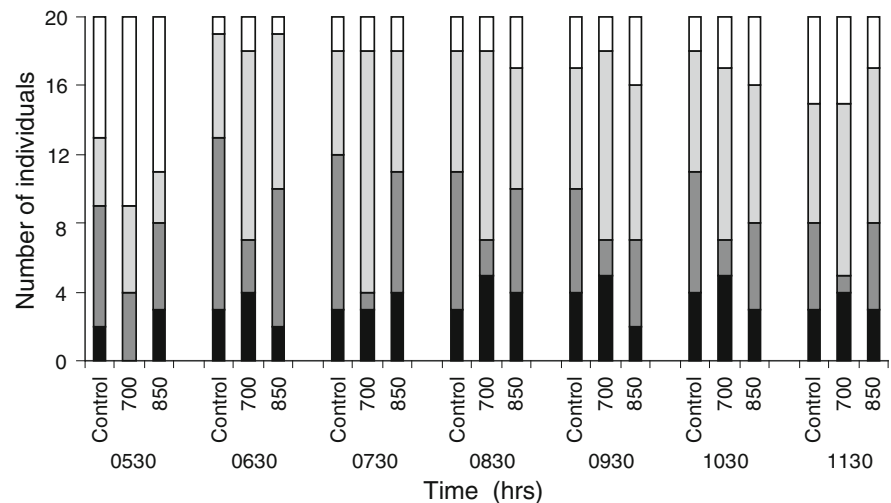
Table 4 Summary of Chi-square statistics for habitat choice comparisons between treatments for each species

|	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	$P < 0.0001$

Between species comparisons based on control preferences for each species

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 (Fig. 2; Table 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, although 35–60% of individuals were associated with coral rubble habitat throughout the day.

Lunar settlement patterns

Exposure to elevated CO_2 caused a dramatic shift in settlement timing in relation to moon phases in *P. chrysurus* (Fig. 3). The saturated model containing the terms moon phase, CO_2 treatment, settlement rate, and their interactions best described the relationship between lunar phase and CO_2 exposure on settlement rates. Removal of the higher-level interaction caused a significant increase in deviance
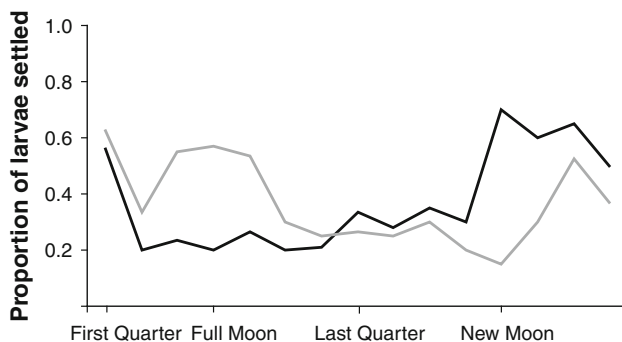
Fig. 3 Proportion of larvae settling overnight in each CO_2 treatment for each lunar phase. Proportions are based on combined data from two complete lunar cycles at 2 days intervals. Each data point represents approximately 15–20 individuals. *Black line* represents control treatment; *grey line*, 850 ppm CO_2

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 (Fig. 3). 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 (Fig. 3).

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 dual 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 850 ppm CO_2, possibly relying on elevated moonlight in order to utilise 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 minimise 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, maximising 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 (Doherty et al. 2004; Almany and Webster 2006). 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.

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