

Effects of ocean acidification on visual risk assessment in coral reef fishes

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Summary

1. With the global increase in CO₂ emissions, there is a pressing need for studies aimed at understanding the effects of ocean acidification on marine ecosystems. Several studies have reported that exposure to CO₂ impairs chemosensory responses of juvenile coral reef fishes to predators. Moreover, one recent study pointed to impaired responses of reef fish to auditory cues that indicate risky locations. These studies suggest that altered behaviour following exposure to elevated CO₂ is caused by a systemic effect at the neural level.

2. The goal of our experiment was to test whether juvenile damselfish *Pomacentrus amboinensis* exposed to different levels of CO₂ would respond differently to a potential threat, the sight of a large novel coral reef fish, a spiny chromis, *Acanthochromis polyacanthus*, placed in a watertight bag.

3. Juvenile damselfish exposed to 440 (current day control), 550 or 700 μ atm CO₂ did not differ in their response to the chromis. However, fish exposed to 850 μ atm showed reduced antipredator responses; they failed to show the same reduction in foraging, activity and area use in response to the chromis. Moreover, they moved closer to the chromis and lacked any bobbing behaviour typically displayed by juvenile damselfishes in threatening situations.

4. Our results are the first to suggest that response to visual cues of risk may be impaired by CO₂ and provide strong evidence that the multi-sensory effects of CO₂ may stem from systematic effects at the neural level.

Key-words: carbon dioxide, coral reefs, ocean acidification, *Pomacentrus amboinensis*, predator–prey interactions, risk assessment, visual cues

Introduction

The rapid increase in the concentration of atmospheric greenhouse gases during the past century, and the concomitant change in climate patterns, has led the scientific community to invest considerable effort towards understanding their effects on terrestrial and aquatic ecosystems (Orr *et al.* 2005; The Royal Society 2005; Fabry *et al.* 2008; Doney *et al.* 2009; Kerr 2010). The atmosphere and shallow oceans are in approximate gas equilibrium. Therefore, as CO₂ concentrations increase in the atmosphere, more CO₂ is absorbed by the ocean. Additional CO₂ reacts with water to generate carbonic acid and hydrogen ions, which increases

the acidity of the water. Increasing hydrogen ions bond with carbonate ions to form more bicarbonate, leading to a reduction in carbonate ion concentration (Orr *et al.* 2005; Fabry *et al.* 2008). The current emissions trajectory indicates that atmospheric CO₂ will exceed 500 μ atm by mid-century and could reach 850 μ atm by the end of this century (Meehl *et al.* 2007; Raupach *et al.* 2007). The biological consequences of this reduction in carbonate ion availability have led most of the research on ‘ocean acidification’ to focus on calcifying organisms such as corals, echinoderms and crustaceans (Kleypas *et al.* 2006; Hofmann *et al.* 2010). Much less is known about how changes in ocean chemistry may affect other species such as fishes (Ishimatsu, Hayashi & Kikkawa 2008). A recent meta-analysis (Kroeker *et al.* 2010) shows that only 25% of the 198 tests reporting ocean

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acidification effects were performed on non-calcifiers, with only 2% of the studies being conducted on fishes. Despite the apparent similarities between CO₂-induced ocean acidification and acid-rain-induced freshwater acidification, these two phenomena are markedly different. The ocean acidification issues are believed to stem from the carbonate imbalance, rather than from a pH decrease *per se*. For this reason, we will abstain from bringing freshwater literature into this study.

Most previous research on the effects of elevated CO₂ on fish has been conducted at CO₂ levels much higher than predicted to occur over the next 50–100 years because of anthropogenic greenhouse gas emissions and has focused on detecting physiological tolerance limits (Ishimatsu, Hayashi & Kikkawa 2008). However, a few recent studies have examined the effects of environmentally relevant sublethal concentrations of CO₂ on the ecology of coral reef fishes. Munday *et al.* (2009) first demonstrated that upon exposure to *c.* 1000 p.p.m. CO₂, clownfish *Amphiprion percula*, tested in a two-channel flume chamber, displayed impaired ability to distinguish between the odours of different habitats, becoming attracted to the chemical cues they normally avoided. Moreover, they also became unable to distinguish between the odour of kin and non-kin. This olfactory impairment was also demonstrated in a predation context, whereby clownfish exposed to *c.* 1000 p.p.m. CO₂ preferred swimming on the side of the flume that contained predator odour, while control clownfish preferred staying on the seawater control side, away from the predator odour (Dixon, Munday & Jones 2010). Both Munday *et al.* (2010) and Ferrari *et al.* (2011a) showed similar olfactory impairments in juvenile damselfish and demonstrated a dramatic increase in mortality rate under natural conditions for fish exposed to 850 p.p.m. CO₂. Chemosensory tests performed at different CO₂ levels indicate that impairment occurs at *c.* 700 p.p.m. (Dixon, Munday & Jones 2010). Until recently, the behavioural effects of CO₂ have been associated with chemosensory impairment. However, Simpson *et al.* (2011) just documented that damselfish exposed to *c.* 600 p.p.m. CO₂ failed to avoid reef noise during the day in the same manner as control fish not exposed to CO₂. It is unknown whether ocean acidification may impact other senses used in risk assessment.

Fishes use a variety of information sources to avoid being captured by predators. In coral reefs, fishes are known to rely on auditory and chemosensory cues when making decisions on where to settle (Simpson *et al.* 2005; Vail & McCormick 2011). However, once settled on the reef, the fishes rely heavily on visual and chemical sources of information. Chemical cues, both predator odours and alarm cues from conspecifics, are particularly important at night and in highly structured habitats, but visual cues have the advantage in that they provide the most accurate information about risk in both space and time (Ferrari, Wisenden & Chivers 2010).

The goal of our experiment was to test whether CO₂ effects would manifest themselves in predation contexts that provided primarily visual information for risk assessment. Our experiment purposefully excluded chemosensory information

that could be used by the fish to assess risk. If CO₂ effects on risk assessment are manifest in multiple sensory systems, then the problem likely does not rest with impaired sensory perception but rather with a more generalized alteration in cognitive processing. Here, we exposed juvenile damselfish *Pomacentrus amboinensis* to a range of CO₂ treatments (440 µatm – present-day level control, 550, 700 and 850 µatm CO₂), following methodologies similar to Ferrari *et al.* (2011a), and exposed them to the sight of an adult spiny chromis *Acanthochromis polyacanthus* in a sealed plastic bag. The chromis is a larger coral reef fish to which the juvenile damselfish are naive and hence may represent a significant predation threat to the damselfish.

Materials and methods

FISH COLLECTION AND CO₂ TREATMENT

The experiment was conducted at the Lizard Island research station (14°40'S, 145°28'E), on the Great Barrier Reef, Australia, in November 2010. Juvenile *P. amboinensis* (16–21 days old) were caught overnight using light traps (Meekan *et al.* 2001) moored *c.* 100 m off the fringing reef. These traps collect fish at the end of their pelagic phase, immediately prior to their settlement to the reef (Meekan, Milicich & Doherty 1993). Fishes caught in the traps were brought back to the station just after dawn and sorted by species, and small groups of *P. amboinensis* were transferred into 35-L aquaria maintained at one of four CO₂ concentrations. Previous experiments have demonstrated that the behavioural effects of elevated CO₂ are evident within 4 days of exposure to relevant CO₂ treatments and that longer exposures result in identical behavioural impairments compared to larvae raised under the same CO₂ levels from birth (Munday *et al.* 2010). Therefore, larvae were maintained in the CO₂ treatments for four consecutive days, and experiments were completed within 48 h of the final day of exposure to CO₂ treatments. The fish were fed freshly hatched *Artemia nauplii* three times a day.

CO₂ treatments were maintained by CO₂ dosing to a set pH_{NBS} following standard techniques for ocean acidification research, as set out in the Best Practices Guides for Ocean Acidification Research (Gattuso *et al.* 2010). Seawater was pumped from the ocean into 4 × 60 L sumps, where it was diffused with ambient air (control) or CO₂ to achieve a pH of *c.* 8.15 (control), 8.06, 7.97 or 7.89. The reduced pH values were selected to achieve the approximate CO₂ conditions required, based on preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH controller (Tunze Aquarientechnik, Penzberg, Germany) was attached to each of the CO₂-treated sumps to maintain pH at the desired level. A solenoid injected a slow stream of CO₂ into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. The powerhead rapidly dissolved CO₂ into the seawater and also served as a vigorous stirrer. Equilibrated seawater from each sump was supplied at a rate of *c.* 500 mL s⁻¹ to four replicate 35-L aquaria, each housing a group of larval fishes. To maintain oxygen levels and the required pCO₂ levels, aquaria were individually aerated with air (control *c.* 390 p.p.m.) or CO₂-enriched air (*c.* 550, 700 or 850 µatm). The concentration of CO₂-enriched air was controlled by a scientific-grade pressure regulator and precision needle valve and measured continuously with an infrared CO₂ probe (Vaisala GM70, Vaisala, Helsinki, Finland). Temperature and pH_{NBS} of each aquarium were measured each morning and afternoon, using an HQ40d pH

meter (Hach, Loveland, CO, USA) calibrated with fresh buffers. Total alkalinity of seawater was estimated by Gran titration from water samples taken twice weekly from each CO₂ treatment. Alkalinity standardisations performed before processing each batch achieved accuracy within 1% of certified reference material from Dr A. Dickson (Scripps Oceanographic Institute). Average seawater pCO₂ was calculated using these parameters in the program CO2SYS and using the constants of Mehrbach *et al.* (1973) refit by Dickson & Millero (1987). Estimated seawater parameters are shown in Table 1.

EXPERIMENTS

Following CO₂ conditioning, fish were transferred individually into 20-L clear plastic flow-through tanks (32 × 16 × 16 cm), containing a sand substrate, a coral object (shelter) and an airstone, placed on the opposite side of the coral, to which was attached a piece of tubing used as an injection hose to introduce food. Having the hose attached to the air stone allows a rapid diffusion of the *Artemia* throughout the tank. Damselfish juveniles treated with elevated CO₂ retain their impaired behavioural responses for at least 48 h after being transferred back into control water (Munday *et al.* 2010); hence, the tanks were filled with freshly pumped ocean water (mean temperature: 28 °C). Each tank was covered on three sides with black plastic to ensure visual isolation from neighbouring tanks. A 4 × 4 cm grid was drawn in the front of the tank to allow the observer to record behavioural parameters. The fish were fed *ad libitum* and left undisturbed over night. Food was provided again in the morning, 1 h prior to testing.

Behavioural observations followed established protocols (Ferrari, Wisenden & Chivers 2010; Holmes & McCormick 2010b) and consisted of a 4-min pre-stimulus presentation period followed by a 4-min post-stimulus presentation period. The two observation periods were separated by a 45-sec stimulus introduction period, during which an adult *A. polyacanthus* (12.4 ± 1.1 cm SD fork length), placed in a watertight clear plastic bag (20 × 10 cm) containing oxygenated water, was gently introduced at the end of the tank on the opposite side of the coral object. The bag was oriented such that the side of *A. polyacanthus* was facing the fish (the long side of the bag was parallel to the short side of the tank). The bag also contained a thin layer of gravel to ensure it would settle on the bottom of the tank.

To stimulate activity, we introduced small quantities of *Artemia* into the tank on the opposite side of the coral, via the injection hose. Turbulence created by the airstone allowed the food to spread throughout the tank within a few seconds. The fish were fed 2.5 mL of food (solution containing *c.* 250 freshly hatched *Artemia* per mL) 5 min prior to the start of the trial and immediately prior to the two observation phases. During each observation period, we collected data on: (i) foraging, measured as the total number of feeding strikes in the 4-min period, regardless of success; (ii) activity level, measured

as the total number of lines crossed; a line was crossed when the entire body of the fish crossed the line; and (iii) area use, measured as the total number of 4 × 4 cm squares visited. During the post-stimulus presentation only, we also measured (iv) minimum approach distance, as the smallest distance (in cm, assessed using the grid) between the fish and the bag containing the *A. polyacanthus*; and (v) the occurrence of bobbing behaviour (fish exposed to threats will often display rapid, small-amplitude vertical movements). Decreases in foraging, activity and area use and an increase in frequency of bobbing are common antipredator behaviours displayed by animals (Ferrari, Wisenden & Chivers 2010). We tested 22–25 fish in each treatment (mean ± SD standard length: 1.4 ± 0.1 cm). The same number of fish from each treatment was tested on a given day, the order of testing was randomized, and the observer was blind with respect to the CO₂ treatment groups.

STATISTICAL ANALYSES

Pre- and post-stimulus data for foraging, activity and area use were computed into a per cent change from the pre-stimulus baseline ((post-pre)/pre). Owing to the interdependency of the three behaviours, we analysed the three variables together using a one-way MANOVA. Subsequent Tukey *post hoc* tests were performed to assess the differences in behavioural responses between the different CO₂ levels. Foraging data did not follow parametric assumptions; hence, we used rank-transformed foraging data in the analyses. The data for minimum distance to the chromis were analysed using a one-way ANOVA, followed by *post hoc* Tukey tests. The occurrence of bobbing behaviour was analysed using a chi-square test, followed by *post hoc* comparisons.

Results

There was no effect of CO₂ treatment on the behaviour of juvenile *P. amboinensis* measured during the pre-stimulus period (MANOVA: Pillai's trace: $F_{9,276} = 0.84$, $P = 0.58$). However, there was a statistically significant effect of CO₂ concentration on the responses of fish to the presentation of the chromis (Pillai's trace: $F_{9,276} = 3.31$, $P = 0.001$, Fig. 1). *Post hoc* tests revealed the same patterns for the three behavioural measures: fish exposed to 440, 550 or 700 µatm CO₂ did not differ in their response to the chromis. However, fish exposed to 850 µatm CO₂ showed a weaker antipredator response. That is, they displayed higher foraging rate, higher activity levels and greater area use than fish exposed to lower CO₂ concentrations.

The one-way ANOVA also revealed a statistically significant effect of CO₂ on the minimum approach distance ($F_{3,92} = 5.31$, $P = 0.002$, Fig. 1). *Post hoc* tests revealed that fish exposed to increasing concentrations of CO₂ decreased their minimum approach distance.

The chi-square test performed on the 2 × 4 contingency table revealed a significant interaction between CO₂ concentration and the occurrence of bobbing ($\chi^2_3 = 9.7$, $P = 0.021$; bobbing frequencies of 63%, 62.5%, 56% and 0% for 440, 550, 700 and 850 µatm CO₂ fish, respectively). *Post hoc* comparisons revealed no difference in the occurrence of bobbing between fish exposed to 440 and 550 (two-tailed Fisher's exact test, $P > 0.99$), or 550 and 700 µatm CO₂ (Fisher's Exact

Table 1. Mean (±SD) seawater parameters in the experimental system. Temperature, pH salinity and total alkalinity (TA) were measured directly. pCO₂ was estimated from these parameters using CO2SYS

pH _{NBS}	Temp °C	Salinity ppt	TA (µmol kg ⁻¹ SW)	pCO ₂
8.15 (0.04)	27.66 (0.98)	35	2269.66 (15.01)	440.53 (44.46)
8.06 (0.05)	27.37 (0.93)	35	2265.04 (27.00)	554.04 (81.69)
7.97 (0.06)	27.59 (0.97)	35	2259.87 (11.55)	718.37 (110.82)
7.89 (0.06)	27.74 (0.99)	35	2261.23 (14.92)	879.95 (140.64)

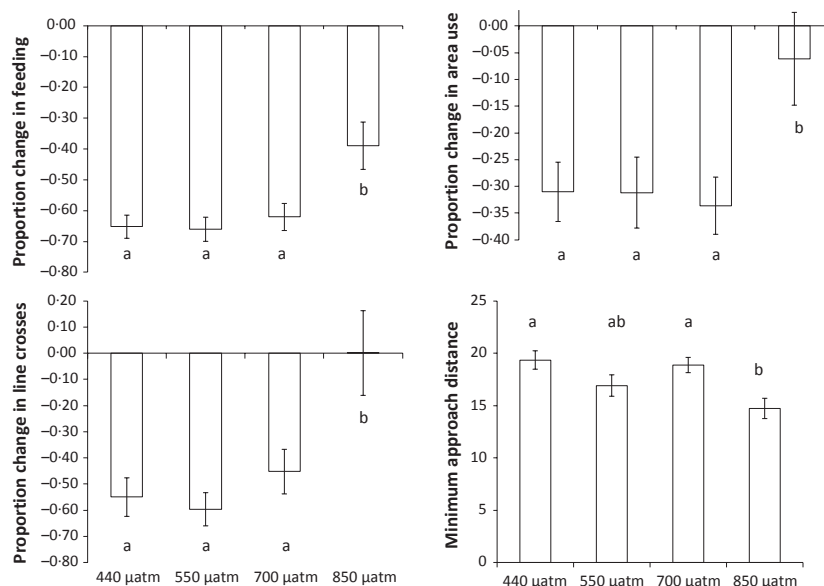


Fig. 1. Mean proportion change (\pm SE) in feeding strikes (top left panel), area use (top right panel), line crosses (bottom left panel) and mean (\pm SE) minimum approach distance (bottom right panel) for juvenile damselfish treated with different CO₂ concentration and exposed to the sight of a spiny chromis. Different letters refer to statistical differences at a 0.05 α level.

Test, $P = 0.77$). However, fish exposed to 850 μatm CO₂ showed a significant decrease in the occurrence of this behaviour as compared to fish exposed to 700 μatm (Bonferroni-corrected α : $0.05/3 = 0.017$; Fisher's exact test, $P = 0.003$, Fig. 1).

Discussion

Our results clearly show differential threat responses of fish treated with different concentrations of CO₂. Juvenile fish exposed to 440, 550 and 700 μatm CO₂ did not differ in their behavioural response to the adult chromis. They all showed strong decreases in foraging, activity and area use, typical responses observed by individuals responding to a potentially threatening situation (Ferrari, Wisenden & Chivers 2010). However, fish treated at 850 μatm CO₂ showed a decrease in the intensity of response compared to fish with lower CO₂ exposure; they did not reduce feeding, activity and area use as much, indicating that they perceived the chromis as less threatening than other conspecifics. All fish showed an increased vigilance upon introduction of the chromis bag in the tank, which likely provided mechanical and visual disturbances. However, the fish exposed to 850 μatm seem to overcome this disturbance and very quickly ignored or became attracted to it. In addition, the 850 μatm juveniles did not display bobbing behaviour as typical observed in risky situations but rather approached the chromis.

The decrease in response reported here supports previous results showing a decrease in antipredator response by CO₂-treated fish. However, because of the absence of chemosensory cues, we can infer that effect documented in the present study is not mediated by an alteration of the olfactory system. We suspect that vision was the main sense used by the juvenile

fish to assess risk, although we cannot completely rule out other senses such as auditory cues, given that we do not have information about whether *A. polyacanthus* produce sounds that could be used in risk assessment by the damselfish. The occurrence of bobbing behaviour is typically a visually mediated behaviour in our test species. Juvenile damselfishes recruiting to the reef have no experience with the diversity of predators that they will encounter on the reef; hence, the recruits often need to learn which large fish pose a threat to them (Mitchell *et al.* 2011). Spiny chromis are not typically considered predators of juvenile damselfish. However, we chose the spiny chromis because of its docile behaviour during stimulus presentation. Other piscivores such as dottybacks, wrasses or lizardfishes, which are common predators of recruit fishes (Holmes & McCormick 2010a), were not as compliant in the holding bag, striking the bag and trying repeatedly to get out, hence confounding the source of information available to the damselfish. Spiny chromis were rarely in contact with the bag.

Another interesting result is the threshold at which CO₂ effects became apparent. In previous studies of chemosensory assessment, marked behavioural effects appeared after exposure to 700 μatm CO₂ (Dixon, Munday & Jones 2010; Munday *et al.* 2010). In the one study on auditory responses, Simpson *et al.* (2011) documented significant effects at 600 μatm CO₂. However, in the present study, fish exposed to 850 μatm CO₂ were affected, while those exposed to lower concentrations were not. Moreover, the pattern of negative effects was consistent for all behavioural measures. Given that we are using the same CO₂ system and the same test species in the same location as other studies reporting those effects (Munday *et al.* 2010; Ferrari *et al.* 2011a), it is reasonable to compare the CO₂ thresholds. The relatively high

threshold effect found in the present study may indicate that CO₂ affects the visual systems independently of the chemosensory and auditory systems, i.e. via different pathways. Alternatively, the same physiological response may be responsible for changes in the chemosensory, auditory and visual responses, but the visual pathway is less sensitive to changes in CO₂ concentrations.

The ability of marine species to persist through the challenges presented by elevated dissolved CO₂ will depend on the adaptability of those species to their novel environmental conditions and the speed at which the environment will be changing. Regardless of the exact reason for the observed effects of dissolved CO₂, different sensory inputs may be sensitive to different thresholds of CO₂, and this may prove important for the survival of those species. If one sensory system is impaired at a given level of CO₂, could another sense compensate for this loss? If so, this may slow down the functional effects of CO₂ on individuals. Munday *et al.* (2010) and Ferrari *et al.* (2011a) both showed a graded effect of CO₂ on the mortality of juvenile damselfishes, with an increase in mortality occurring at 700 p.p.m. (c. 700 µatm) and heavier mortality occurring at 850 p.p.m. (c. 850 µatm) compared to control fish. This implies that the loss of chemosensory assessment of risk at 700 p.p.m. CO₂ and the impaired responses to auditory responses at 600 p.p.m. cannot be fully compensated by normal responses to visual stimuli at that same level.

Most coral reef fishes have a bipartite life history made of an initial pelagic stage whereby larvae reside in the plankton for a period of weeks to months (Leis 2007), followed by a benthic phase, for which juvenile fish must locate suitable habitat and in doing so face a new and abundant array of predatory reef fishes. It is during this transitioning period that predation pressure is intense; predators may remove at least 60% of newly settling fish in a single night (Almany & Webster 2006), creating population bottlenecks. Although these newly settled fish have juvenile form and coloration, they are largely naïve to the suite of predators that await them on the reef. Their ability to detect predators visually, chemically and mechanically is hence crucial for their survival, and our results suggest that CO₂ has the ability to dramatically alter the dynamics of predator–prey interactions taking place in coral reefs. Indeed, Ferrari *et al.* (2011b) recently conducted a mesocosm experiment showing that the pattern of predator selectivity for different species of damselfishes was influenced by exposure to elevated CO₂. An increase in the consumption of juvenile fishes by predators during their transition to benthic life will impact the replenishment of reefs and could have far-reaching implications on the biodiversity of coral reefs (2011b).

Our experiment was short term and ignored any potential for adaptation to dissolved CO₂. While laboratory experiments showed that maintaining juveniles in elevated CO₂ from birth does not further the effects of CO₂ (Munday *et al.* 2010), it is possible that species may adapt through selection over several generations. Longer-term experiments will be needed to answer questions about adaptation and selection, even if laboratory conditions cannot fully replicate the

selective environment exerted by predators on different CO₂-sensitive phenotypes in the wild. We currently do not know the exact cause of behavioural alterations mediated by CO₂; however, the accumulating experimental evidence shows that altered behaviour following exposure to elevated CO₂ is caused by a systemic effect at the neural level. Behavioural lateralization (tendency of individuals to turn left or right) is a non-sensory-related behaviour and an expression of brain functional asymmetries. Recent evidence suggests that such lateralization in coral reef fishes is also affected by elevated exposures to CO₂ (Domenici *et al.* 2011), which strengthens the idea that elevated CO₂ affects brain function in larval fishes. It seems likely that such effects are related to ionic changes associated with acid–base regulation, but additional studies are required to pinpoint the exact mechanisms involved (Munday *et al.* 2010; Simpson *et al.* 2011).

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References

- Almany, G.R. & Webster, M.S. (2006) The predation gauntlet: early post-settlement mortality in reef fishes. *Coral Reefs*, **25**, 19–22.
- Dickson, A.G. & Millero, F.J. (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Research*, **34**, 1733–1743.
- Dixon, D.L., Munday, P.L. & Jones, G.P. (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters*, **13**, 68–75.
- Domenici, P., Allan, B., McCormick, M.I. & Munday, P.L. (2011) Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biology Letters*, doi: 10.1098/rsbl.2011.0591.
- Doney, S.C., Fabry, V.J., Feely, R.A. & Kleypas, J.A. (2009) Ocean acidification: the other CO₂ problem. *Annual Review of Marine Science*, **1**, 169–192.
- Fabry, V.J., Seibel, B.A., Feely, R.A. & Orr, J.C. (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science*, **65**, 414–432.
- Ferrari, M.C.O., Wisenden, B.D. & Chivers, D.P. (2010) Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, **88**, 698–724.
- Ferrari, M.C.O., Dixon, D.L., Munday, P.L., McCormick, M.I., Meekan, M.G., Sih, A. & Chivers, D.P. (2011a) Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. *Global Change Biology*, **17**, 2980–2986.
- Ferrari, M.C.O., McCormick, M.I., Munday, P.L., Meekan, M.G., Dixon, D.L., Lonnstedt, O. & Chivers, D.P. (2011b) Putting prey and predator into the CO₂ equation – qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecology Letters*, **14**, 1143–1148.
- Gattuso, J.-P., Kunshan, G., Lee, K., Rost, B. & Schulz, K.G. (2010) Approaches and tools to manipulate the carbonate chemistry. *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (eds U. Riebesell, V.J. Fabry, L. Hansson & J.-P. Gattuso), pp. 41–52. Publications Office of the European Union, Luxembourg.
- Hofmann, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T. & Sewell, M.A. (2010) The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective. *Annual Review of Ecology and Systematics*, **41**, 127–147.

- Holmes, T.H. & McCormick, M.I. (2010a) Size-selectivity of predatory reef fish on juvenile prey. *Marine Ecology Progress Series*, **399**, 273–283.
- Holmes, T.H. & McCormick, M.I. (2010b) Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish. *Behavioural Processes*, **83**, 299–305.
- Ishimatsu, A., Hayashi, M. & Kikkawa, T. (2008) Fishes in high-CO₂, acidified oceans. *Marine Ecology Progress Series*, **373**, 295–302.
- Kerr, R.A. (2010) Ocean acidification unprecedented, unsettling. *Science*, **328**, 1500–1501.
- Kleypas, J., Feely, R.A., Fabry, V.J., Langdon, C., Sabine, C.L. & Robbins, L.L. (2006) *Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research*. NOAA/Pacific Marine Environmental Laboratory, Seattle.
- Kroeker, K.J., Kordas, R.L., Crim, R.N. & Singh, G.G. (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, **13**, 1419–1434.
- Leis, J.M. (2007) Behaviour as input for modelling dispersal of fish larvae: behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. *Marine Ecology Progress Series*, **347**, 185–193.
- Meehl, G.A., Stocker, T.F., Collins, P., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J. & Zhao, Z.-C. (2007) Global climate projection. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report on the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller). Cambridge University Press, Cambridge.
- Meekan, M.G., Milicich, M.J. & Doherty, P.J. (1993) Larval production drives temporal patterns of larval supply and recruitment of a coral reef damselfish. *Marine Ecology Progress Series*, **93**, 217–225.
- Meekan, M.G., Wilson, S.G., Halford, A. & Retzel, A. (2001) A comparison of catches of fishes and invertebrates by two light trap designs, in tropical NW Australia. *Marine Biology*, **139**, 373–381.
- Mehrbach, C., Culberson, C.H., Hawley, J.E. & Pytkowicz, R.M. (1973) Measurements of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, **18**, 897–907.
- Mitchell, M.D., McCormick, M.I., Ferrari, M.C.O. & Chivers, D.P. (2011) Coral reef fishes rapidly learn to identify multiple unknown predators upon recruitment to the reefs. *PLoS ONE*, **6**, e15764.
- Munday, P.L., Dixon, D.L., Donelson, J.M., Jones, G.P., Pratchett, M.S., Devitsina, G.V. & Doving, K.B. (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1848–1852.
- Munday, P.L., Dixon, D.L., McCormick, M.I., Meekan, M., Ferrari, M.C.O. & Chivers, D.P. (2010) Replenishment of fish populations is threatened by ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12930–12934.
- Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.F., Yamanaka, Y. & Yool, A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.
- Raupach, M.R., Marland, G., Ciais, P., Le Quere, C., Canadell, J.G., Klepper, G. & Field, C.B. (2007) Global and regional drivers of accelerating CO₂ emissions. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 10288–10293.
- Simpson, S.D., Meekan, M.G., Montgomery, J., McCauley, R. & Jeffs, A. (2005) Homeward bound. *Science*, **308**, 221.
- Simpson, S., Munday, P.L., Wittenrich, M.L., Manassa, R., Dixon, D.L., Gagliano, M. & Yan, H.Y. (2011) Ocean acidification erodes crucial auditory behaviour in a marine fish. *Biology Letters*, **7**, 917–920.
- The Royal Society (2005) *Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide* (ed. The Royal Society). The Royal Society, London.
- Vail, A.L. & McCormick, M.I. (in press) Metamorphosing reef fishes avoid predator scent when choosing a home. *Biology Letters*.

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