ICES Journal of Marine Science Advance Access published May 8, 2015

ICES Journal of Marine Science

ICES Journal of Marine Science; doi:10.1093/icesjms/fsv085

Foraging behaviour of the epaulette shark *Hemiscyllium ocellatum* is not affected by elevated CO_2

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Heinrich, D. D. U., Watson, S-A., Rummer, J. L., Brandl, S. J., Simpfendorfer, C. A., Heupel, M. R., and Munday, P. L. Foraging behaviour of the epaulette shark *Hemiscyllium ocellatum* is not affected by elevated CO_2. – ICES Journal of Marine Science, doi: 10.1093/icesjms/fsv085.

Received 12 November 2014; revised 15 April 2015; accepted 16 April 2015.

Increased oceanic uptake of atmospheric carbon dioxide (CO_2) is a threat to marine organisms and ecosystems. Among the most dramatic consequences predicted to date are behavioural impairments in marine fish which appear to be caused by the interference of elevated CO_2 with a key neurotransmitter receptor in the brain. In this study, we tested the effects of elevated CO_2 on the foraging and shelter-seeking behaviours of the reef-dwelling epaulette shark, *Hemiscyllium ocellatum*. Juvenile sharks were exposed for 30 d to control CO_2 (400 µatm) and two elevated CO_2 treatments (615 and 910 µatm), consistent with medium- and high-end projections for ocean pCO_2 by 2100. Contrary to the effects observed in teleosts and in some other sharks, behaviour of the epaulette shark was unaffected by elevated CO_2. A potential explanation is the remarkable adaptation of *H. ocellatum* to low environmental oxygen conditions (hypoxia) and diel fluctuations in CO_2 encountered in their shallow reef habitat. This ability translates into behavioural tolerance of near-future ocean acidification, suggesting that behavioural tolerance and subsequent adaptation to projected future CO_2 levels might be possible in some other fish, if adaptation can keep pace with the rate of rising CO_2 levels.

Keywords: carbon dioxide, climate change, elasmobranch, foraging behaviour, hypercapnia.

Introduction

Increased uptake of anthropogenic carbon dioxide (CO_2) at the ocean surface is a serious threat to marine organisms and ecosystems (Fabry *et al.*, 2008; Doney *et al.*, 2009). Approximately 30% of the CO_2 released by humans since the industrial revolution has been absorbed by the oceans, causing a 0.1-unit reduction in global ocean pH at a rate many times faster than any time over the past 800 000 years (Hoegh-Guldberg *et al.*, 2007; Lüthi *et al.*, 2008). If global CO_2 emissions continue on the current trajectory, atmospheric CO_2 is projected to exceed 900 ppm by 2100 (Meinshausen *et al.*, 2011) and ocean pH will decline by another 0.3–0.4 units (Caldeira and Wickett, 2005: Collins *et al.*, 2013). Elevated CO_2 levels can affect the behaviour of teleost fish (Briffa *et al.*, 2012; Branch *et al.*, 2013; Jutfelt *et al.*, 2013; Munday *et al.*, 2014). A range of sensory, cognitive, and behavioural abnormalities have been reported in reef fish that have been reared at CO_2 levels projected to occur by the end of this century (Munday *et al.*, 2009, 2012). For example, the innate response of juvenile reef fish to predator odours and conspecific alarm cues is impaired at higher CO_2 levels, causing them to become attracted to these odours rather than repelled from them (Dixson *et al.*, 2010; Welch *et al.*, 2014). Auditory preferences are altered (Simpson *et al.*, 2011), behavioural lateralization declines (Domenici *et al.*, 2011; Welch *et al.*, 2014), and the ability to learn is lost (Ferrari *et al.*, 2012; Chivers *et al.*, 2014). In addition, newly settled juveniles become more active and exhibit riskier behaviour, which increases mortality rates due to predation in natural coral reef habitat (Munday *et al.*, 2010). The effects of elevated CO_2 on reef fish behaviour are not limited to larval and juvenile fish. Exposure to elevated CO_2 reduced homing success of adult cardinal fish (Devine *et al.*, 2012) and altered the attraction of a coral reef meso-predator (*Pseudochromis fuscus*) to the olfactory stimulus released by injured prey (Cripps *et al.*, 2011). Not all species or all behaviours are affected, but those behavioural changes that occur can affect the outcome of key ecological processes, such as predator–prey (Ferrari *et al.*, 2011) and competitive interactions (McCormick *et al.*, 2013), with implications for the structure and function of marine ecosystems in a high CO_2 world.

The behavioural changes observed in marine teleosts at high CO_2 appear to be caused by an interference with the GABA-A receptor, the primary inhibitory neurotransmitter receptor in the vertebrate brain (Nilsson *et al.*, 2012; Chivers *et al.*, 2014; Hamilton *et al.*, 2014). The GABA-A receptor is an ion-channel with conductance for Cl^- and HCO_3^-. Under normal conditions, ion gradients over the neuronal membrane result in an inflow of Cl^- and HCO_3^- upon binding of the GABA-A receptor, which then leads to hyperpolarization and inhibition of the neuron (Lambert and Grover, 1995; Nilsson *et al.*, 2012). However, when exposed to elevated CO_2, marine teleosts excrete Cl^- from their bodies to accumulate HCO_3^- from seawater to buffer the pH disturbance and prevent an acidosis (Brauner and Baker, 2009; Esbaugh *et al.*, 2012; Heuer and Grosell, 2014). The changes in the gradient of these ions over the neuronal membrane could alter the function of the receptor, leading to impaired behavioural responses. Depending on the magnitude of changes in HCO_3^- and Cl^- during acid-base regulation, the resultant alterations of ion gradients could either potentiate the GABA-A receptor function or reverse its action, making it excitatory rather than inhibitory (Nilsson *et al.*, 2012; Hamilton *et al.*, 2014; Heuer and Grosell, 2014).

In contrast to the many studies that have been conducted into the effects of near-future ocean acidification on the behaviour of teleost fish, especially on coral reefs, much less is known about the potential consequences of increasing levels of CO_2 on large predators, such as sharks (Rosa *et al.*, 2014). Elasmobranchs have the same GABA-A neurotransmitter receptor found in teleost brains (Lambert and Grover, 1995) and they also accumulate HCO_3^- from the seawater in exchange for Cl^- from the body to buffer an environmental pH disturbance (Heisler *et al.*, 1988; Claiborne *et al.*, 2002; Brauner and Baker, 2009). Elasmobranchs may also increase branchial ammonia excretion rates to further ameliorate an acidosis (King and Goldstein, 1983; Claiborne and Evans, 1992). We recently demonstrated that epaulette sharks, upon 90 d exposure to near-future CO_2 levels, exhibit an increase in plasma $[HCO_3^-]$, and although this did not affect metabolic performance (Heinrich *et al.*, 2014), altered ion gradients might have affected neurotransmitter function and thus behaviour. Indeed, two recent studies have observed significant effects of elevated CO_2 on shark behaviours. Odour tracking of the smooth dogfish (*Mustelus canis*) declined following 5 d exposure to 1064 µatm CO_2 (Dixson *et al.*, 2014), and the nocturnal swimming pattern of the spotted catshark (*Scyliorhinus canicula*) changed from starts and stops to a more continuous swimming pattern after 28 d exposure to 990 µatm CO_2 (Green and Jutfelt, 2014). Furthermore, the catshark exhibited an accumulation of plasma HCO_3^-, which could be consistent with an effect of high CO_2 on neurotransmitter function, leading to behavioural changes.

The aim of this study was to test the effects of near-future CO_2 levels on the behaviour of a reef-dwelling shark that periodically experiences high CO_2 levels in its natural habitat. The epaulette shark (*Hemiscyllium ocellatum*) is a small, benthic, relatively sedentary species of shark that inhabits shallow coral reef flats and lagoons (Randall *et al.*, 1997). It frequently shelters in small caves and holes within the reef matrix. Due to this pattern of habitat use, epaulette sharks may experience episodes of short-term environmental hypoxia and hypercapnia, especially during nocturnal low tides (Kinsey and Kinsey, 1967; Routley *et al.*, 2002; Nilsson and Renshaw, 2004; Diaz and Breitburg, 2009). CO_2 levels in shallow coral reef habitat, such as in lagoons and on reef flats, can exceed 1000 µatm overnight during low tides (Shaw *et al.*, 2012) and may be further elevated within the reef matrix due to biological respiration. The epaulette shark is metabolically adapted to these conditions (Heinrich *et al.*, 2014), and we predicted that the behaviour of this small shark may be similarly tolerant of CO_2 levels that induce abnormal behaviour in reef fish and other sharks. We compared foraging and shelter-seeking behaviour of juvenile epaulette sharks acclimated to elevated CO_2 for over 30 d with individuals kept at current-day control conditions (∼400 µatm) for the same period. Elevated CO_2 treatments were consistent with medium- (∼615 µatm) and high-end projections (∼910 µatm) for ocean pCO_2 by 2100 (Meinshausen *et al.*, 2011). Specifically, each shark's activity level and the time spent away from shelter over the course of 1 h in the presence of food were investigated. Furthermore, we determined activity levels while foraging and recorded the time required to locate and reach the food source. We then examined each individual's responses to a disturbance by recording activity and time required to find a new shelter upon disturbance.

Material and methods

Experimental animals

Hemiscyllium ocellatum is a small benthic elasmobranch common on reef flats and in lagoons on the Great Barrier Reef (GBR), Australia. Their relatively small size and benthic lifestyle make them well suited for laboratory experiments. Furthermore, they are not highly territorial and may shelter together in coral reef habitat, making them ideal to maintain in captivity (Michael, 2003). Animals were collected from the GBR under an A1 commercial harvest licence and were supplied by Northern Barrier and Cairns Marine (Cairns, Australia). Thirty sharks were shipped to James Cook University (JCU) where they were kept in groups of five individuals in six 700 l tanks (230 *l* × 105 *W* × 50 *D* cm) supplied with a continuous flow of seawater (Figure 1). Individuals were measured [standard length: 33.38 ± 7.29 cm (mean ± SD); weight: 232.47 ± 117.98 g] to ensure an equal distribution of sizes among tanks. *Hemiscyllium ocellatum* matures around 60 cm (Last and

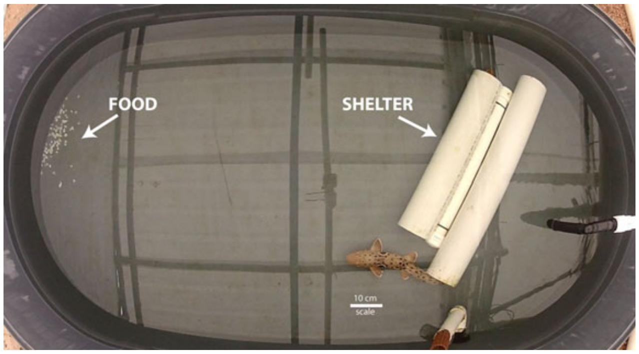

Figure 1. Photograph of holding tank with PVC shelter at one end and food placed at the opposite end. Tank dimensions (internal): 230 *l* × 105 *W* × 50 *D* cm. Scale bar = 10 cm.

Stevens, 2009); therefore, all individuals were large juveniles. For identification, each individual was given a unique fin clip along the margins of pectoral, pelvic, and dorsal fins. Sharks were habituated to laboratory holding conditions (control CO_2) for at least 30 d before commencing CO_2 treatments. Sections of PVC pipe were placed in the tanks to provide shelter. Food was provided once every 24 h and consisted of raw prawn meat (4% of shark biomass). The epaulette shark is mostly a crepuscular feeder, but will readily feed throughout the day, especially in captivity.

Experimental conditions

The experimental system consisted of three 8000 l recirculating seawater systems each of which was set to simulate one of three CO_2 scenarios, current-day control (~400 μatm), medium (~615 μatm), and high (~910 μatm) CO_2. The experimental system supplied the 6 × 700-l rearing tanks containing sharks, plus an additional three experimental tanks, giving a total water volume of >10 000 l in each system. Although replicated treatment systems are preferable, this was not logistically or financially possible due to the large size of each system. Seawater in each system received constant particle filtration (50 μm), protein extraction, biological filtration (fluidised sand-bed), and ultraviolet filtration.

Two of the six tanks containing sharks were assigned to each CO_2 treatment and supplied with CO_2-equilibrated seawater at a rate of 25 l min^{-1}. A plastic cover was placed on each tank to reduce CO_2 loss. Target CO_2 levels, initial total alkalinity (TA), temperature, and salinity were entered in CO2SYS (Pierrot *et al*., 2006) to generate pH set points for the experiment. Seawater pCO_2 was then established by adjusting and maintaining seawater pH within 0.05 units of the determined set-point using a pH computer (Aqua Medic AT-Control, Bissendorf, Germany). Electronic solenoids dosed CO_2 into a 3000-l sump on each system whenever the measured pH in the sump rose above the desired pH. This central approach of pH manipulation allowed for greater stability in seawater pH within the holding tanks. Temperature was maintained at 28.5°C with a heater/chiller unit attached to each system.

The pH_{TOTAL} of each tank was recorded daily by comparing the mV reading from a Hach HQ40d meter (Hach Company, Loveland, CO, USA) with the mV reading of Tris buffer (Dr A. G. Dickson, Scripps Institution of Oceanography) at the same temperature. Salinity and TA were measured weekly. TA was determined by Gran titration using certified reference materials (Dr A. G. Dickson, Scripps Institution of Oceanography). Measured pH_{TOTAL} and TA were used in CO2SYS (Pierrot *et al.*, 2006) to estimate seawater pCO_2 using the constants K1 and K2 from Mehrbach *et al.* (1973) refit by Dickson and Millero (1987), and Dickson (1990) for $KHSO_4$. Seawater carbonate chemistry parameters are shown in Table 1.

Experimental protocol

Sharks were maintained in CO_2 treatment for a minimum of 30 d before experimentation. Behavioural comparisons between treatment groups were made in two separate experiments, described below. Physiological traits measured in the same sharks are reported in Heinrich *et al.* (2014). This study was conducted under JCU animal ethics approval A1779 and all animal husbandry, and experimental procedures were consistent with Australian guidelines for animal care.

Foraging behaviour

Foraging behaviour was recorded within each of the six tanks on 10 consecutive days using high-resolution video cameras (GoPro II) installed centrally above the tanks with the lens parallel with the water surface. Video was recorded at a resolution of 1080 p for 60 min following the introduction of food by hand at the end of the tank opposite to the shelter location (Figure 1). Foraging behaviour was examined in groups within the treatment tanks, rather than individually, for several reasons. First, this eliminated handling stress that may have affected behaviour. Second, *H. ocellatum* exhibits highly social behaviour, and a reduced commitment to foraging when held in isolation (DDUH, pers. obs.); consequently, small groups provided a better representation of their natural behaviour.

Video files were viewed unconverted on a computer screen using Windows Media Player Version 12 (Microsoft, Redmond, WA, USA) in the slow playback mode. Information collected from the video included: (i) each individual's latency to the first bite, (ii) the activity level of each individual between leaving the shelter and reaching the food source, (iii) the total time spent outside of the shelters, and (iv) the total activity level over the course of 1 h. Individuals could be unambiguously tracked throughout the experimental period by the tags on their fins. The activity level was estimated by counting the number of times each animal crossed a line, using the animal's snout as a reference. To do this, a 10 × 10 cm grid was added to each video, post-recording. The number of line crossings was then converted into activity level, defined as the number of lines crossed per minute for foraging activity and the number of lines crossed in 1 h for total activity. Data on foraging behaviour were collected for 10 consecutive days for each individual.

Shelter-seeking behaviour

Following the last day of foraging behaviour trials, the response to a disturbance was tested. Individuals were transferred to a separate experimental tank (same dimensions as the holding tanks and filled with water from the same CO_2 treatment) containing a PVC shelter at one end. Following transfer and 10 min habituation to the new tanks and shelter, the shelter was removed and an alternative shelter was provided at the opposite end of the tank. The response of the animal was recorded using a high-definition video camera. The time the animal took to find and enter the new shelter, and each individual's activity during that time was determined from the video. Activity was determined in the same way as the foraging behaviour experiment described above. Following the behaviour trials, the length of each individual was measured to include in statistical analyses due to the potential for body size to influence behaviour.

Statistical analyses

Generalized linear mixed-effects models (GLMMs) were used to test for the effects of elevated CO_2 and standard length on foraging and shelter-seeking behaviours. For foraging behaviour, four separate models were performed on: (i) the time spent outside shelters, (ii) latency to the first bite, (iii) the number of line crossings between food introduction and the first bite, and (iv) the overall number of line crossings. As data were integer counts of events or seconds, the exponential family was used. In all models, the variance exceeded the mean in a Poisson model and, therefore, a negative binomial error distribution with a log-link function was used in all instances. In addition, due to the high prevalence of zero values in the time spent outside shelters and the total activity, models were specified with zero inflation as a single constant term across the model. For the variables describing latency to the first bite and line crossings before feeding, cases in which the shark did not feed during the observation period were omitted from the model. Zero-inflated models outperformed models lacking the specification for zero

Table 1. Mean (± s.d.) pCO_2, pH_T, temperature, salinity, and TA for experimental tanks.

Treatment	pCO_2 (μatm)	pH_T	Temperature (°C)	Salinity	TA (μmol kg^{-1} SW)
Control					
Tank 1	408 ± 46	8.01 ± 0.04	28.6 ± 0.33	35.6 ± 0.70	2154 ± 34
Tank 2	394 ± 52	8.02 ± 0.05	28.7 ± 0.42	35.6 ± 0.70	2154 ± 34
Medium					
Tank 1	621 ± 86	7.85 ± 0.05	28.8 ± 0.41	35.8 ± 0.64	2081 ± 30
Tank 2	613 ± 80	7.85 ± 0.05	28.6 ± 0.39	35.8 ± 0.64	2081 ± 30
High					
Tank 1	915 ± 73	7.70 ± 0.03	28.7 ± 0.20	36.0 ± 0.28	2083 ± 40
Tank 2	895 ± 83	7.71 ± 0.04	28.7 ± 0.26	36.0 ± 0.28	2083 ± 40

pCO_2 was estimated in CO2SYS from the other measured variables.

inflation. Given the non-independence of measurements made within tanks and length measurements on individuals, both tank ID and shark ID were included as a random factor, with individual sharks implicitly nested within tanks. In addition, we fitted a random intercept to the sequential days to account for potential systematic variation among days. Both activity variables (activity until the first bite and total activity) were modelled using the corresponding time measurements (latency to the first bite and time outside shelters) as offset on the log scale. Always, the inclusion of an interaction term between CO_2 treatment and standard length did not improve the model fit significantly (Supplementary Table S1).

For the two shelter-seeking variables, the time lapsed until a shelter was reached was again modelled using a GLMM with a negative binomial error distribution and a log-link function, whereas the number of line crossings per minute was approximately normally distributed and was therefore modelled using a Gaussian error distribution and an identity link function. As in the foraging models, the non-independence of measurements from the same tank was accounted for by including tank as a random factor. Likewise, performance of models with and without an interaction term between CO_2 treatment and standard length was assessed and no significant improvement was found when the interaction term was included. Model comparison was performed using Akaike's Information Criterion. Model validation was performed using residual plots. All analyses were performed in *R* (R Development Core Team, 2014) using the packages *lme4* (Bates *et al.*, 2012), *glmmadmb* (Skaug *et al.*, 2014), and *ggplot2* (Wickham, 2009). As behavioural variables associated with foraging and shelter-seeking behaviour are not fully independent, the results of this study require the contextual interpretation of the model outputs. The nature of the data rendered a multivariate approach impossible, making our statistical treatment the most robust extrapolation of the data.

Results

Foraging behaviour

Neither the time sharks spent outside of shelters (GLMM: $p = 0.98$ and 0.32 for medium and high CO_2, respectively) or the total activity level over the course of 1 h in the presence of food (GLMM: $p = 0.80$ and 0.51) were affected by CO_2 treatment (Figure 2a and b; Supplementary Table S1). There was a significant linear relationship between standard length of the sharks and the time spent outside of the shelters (GLMM: $p < 0.0001$), with larger individuals tending to spend more time away from shelters, but size had no effect on the overall activity (GLMM: $p = 0.08$).

When foraging, neither the time required to reach the food source (GLMM: $p = 0.99$ and 0. 19) or the level of activity during the search (GLMM: $p = 0.79$ and 0.13) were affected by CO_2 treatment. There was, however, a significant effect of standard length on the time and activity required to find food, with smaller individuals requiring more time and line-crossings to locate and reach the food source (GLMMs: $p < 0.0001$ and $p < 0.0001$, respectively; Figure 2c and d; Supplementary Table S1).

All models identified substantial individual variation among sharks, but the tank identity and the sequence of days during which observations were performed contributed little to the overall variation explained by the model (Supplementary Table S1).

Shelter-seeking behaviour

Shelter-seeking behaviour was not significantly affected by CO_2 treatment. Neither the time required to re-enter a shelter (GLMM: $p = 0.92$ and 0.99; Figure 3a; Supplementary Table S2) or activity before reaching the new shelter (GLMM: $p = 0.84$ and 0.42; Figure 3b; Supplementary Table S2) were affected by CO_2 treatment. In contrast to foraging behaviour, these traits were not influenced by body size (GLMMs: $p = 0.42$ and $p = 0.65$, respectively; Figure 3; Supplementary Table S2). The effect of tanks on the variance explained by the two models was negligible (Supplementary Table S2).

Discussion

In contrast to the behavioural effects of high CO_2 in some marine teleosts, projected near-future CO_2 levels did not affect foraging or shelter-seeking behaviours of epaulette sharks. *Hemiscyllium ocellatum* held under elevated CO_2 conditions for 30 d did not exhibit riskier behaviours by spending more time outside shelter than control counterparts, and likewise, the ability to successfully locate a familiar food source or shelter site when disturbed was unaffected. Our results suggest that the behaviour of the epaulette shark, and maybe other reef-dwelling benthic elasmobranchs, will be unaffected by elevated CO_2 and reduced seawater pH predicted for the end of this century. However, our results also contrast with two other recent studies that have observed significant effects of high CO_2 on response to olfactory cues (Dixson *et al.*, 2014) and swimming patterns (Green and Jutfelt, 2014) of small benthic or epibenthic sharks. The mechanistic basis underlying the behavioural effects of ocean acidification on marine teleosts and elasmobranchs appears to be associated with changes to ion concentrations across neuronal membranes. Specifically, changes in HCO_3^- and Cl^- gradients that occur when fish are exposed to elevated CO_2 can affect the function of GABA-A neurotransmitter receptors in the brain (Nilsson *et al.*, 2012; Chivers *et al.*, 2014; Hamilton *et al.*, 2014). Elasmobranchs are thought to use similar acid-base regulatory mechanisms to teleosts (Claiborne *et al.*, 2002; Brauner and Baker, 2009) and, consequently, could be sensitive to the effects of elevated

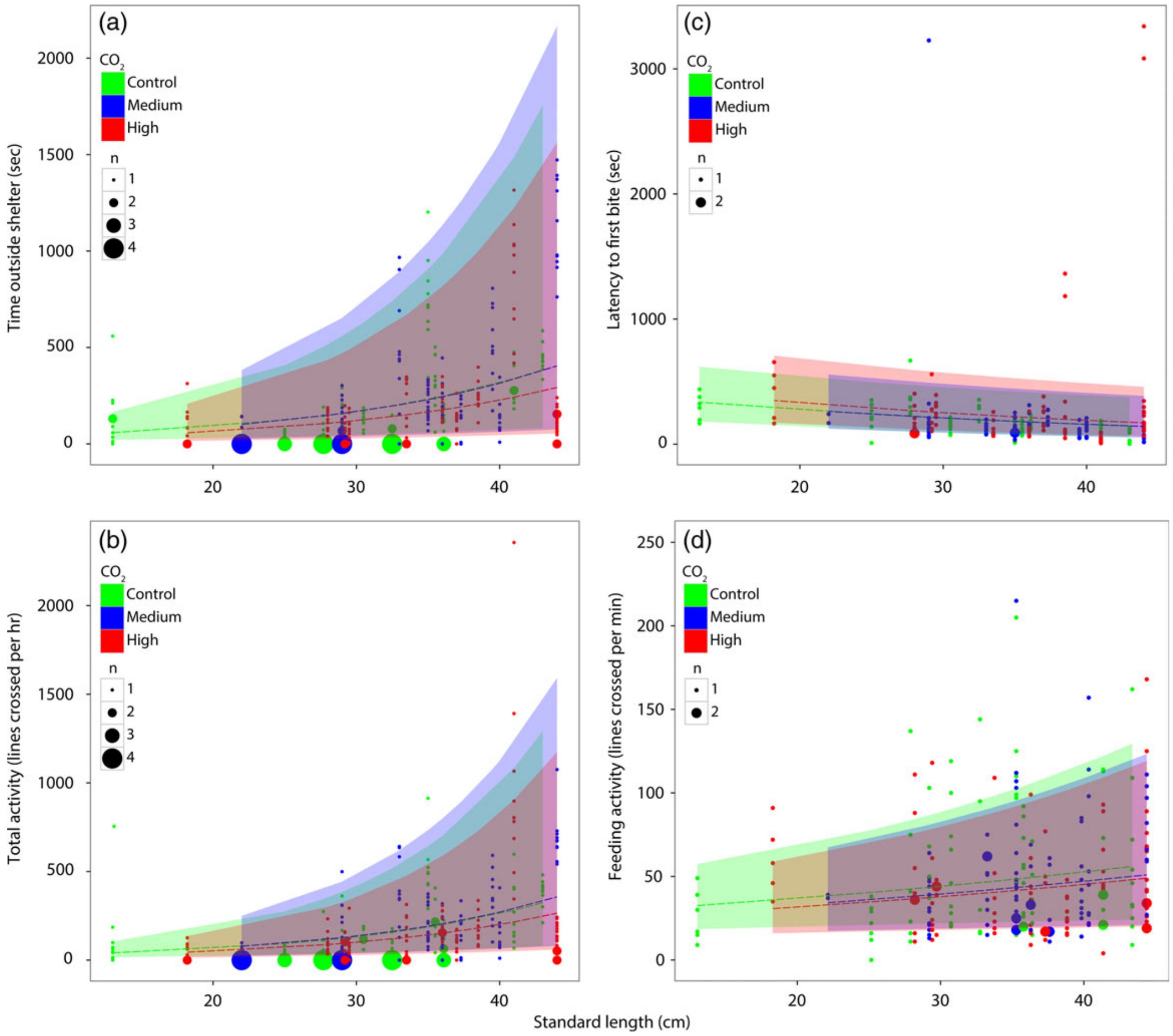

Figure 2. Individual foraging behaviour of epaulette sharks *H. ocellatum* with the predicted fit from GLMMs on the response scale superimposed (± 95% confidence intervals). Time that individual sharks spent outside of shelters over the course of 1 h in the presence of food (a), total activity in the presence of food expressed as the number of line crossings per minute on a 10 × 10 cm grid during 1 h (b), time in minutes required by individuals to reach a food source and commence feeding (c), and foraging activity, calculated as the number of line crossings on a 10 × 10 cm grid, from when a shark left a shelter until it reached food (d). Behavioural traits are plotted against the standard length of each individual, with ten daily observations for each individual. Point size is adjusted based on the number of overlaying observations for each individual. Colours indicate the three CO$_2$ treatments: control =400 µatm, medium =615 µatm, high =910 µatm.

CO$_2$ on GABA-A receptor function. This raises the question of why behavioural effects were not detected in the epaulette shark. The most likely explanation as to why the behaviour of *H. ocellatum* was unaffected by higher CO$_2$ levels is that they are adapted to high CO$_2$ levels frequently encountered in their natural environment. This species is known for its exceptionally high tolerance to short-term hypoxia and possesses the lowest critical oxygen tension (an indicator of hypoxia tolerance) measured in any elasmobranch tested to date (Wise *et al.*, 1998; Routley *et al.*, 2002). Commonly found on shallow reef flats, epaulette sharks shelter within coral heads and other small crevices or holes (Last and Stevens, 2009). The oxygen concentration within these microhabitats can drop to very low levels, even nearing complete anoxia during low tides at night, due to the respiration of reef organisms (Kinsey and Kinsey, 1967; Diaz and Breitburg, 2009). This adaptation to hypoxic conditions allows *H. ocellatum* to reside in tide pools and on reef flats during nocturnal low tides, potentially providing protection from larger reef predators (Routley *et al.*, 2002; Nilsson and Renshaw, 2004; Last and Stevens, 2009). Seawater pCO$_2$ on shallow reef flats can exceed 1000 µatm for several hours overnight (Shaw *et al.*, 2012) and may be even higher in small caves, reef crevices, and other restricted habitats due to reef respiration (Gagliano *et al.*, 2010). Respiration by the shark itself is likely to further exacerbate local hypoxia and hypercapnia within the caves and holes where it shelters. The ability of *H. ocellatum* to routinely tolerate a very wide range of O$_2$ and CO$_2$ conditions, including very high CO$_2$ levels at night and in its shelter sites, suggests that this species may not engage acid-base regulation at the same pCO$_2$ threshold as fish from other habitats. *Hemiscyllium ocellatum* may

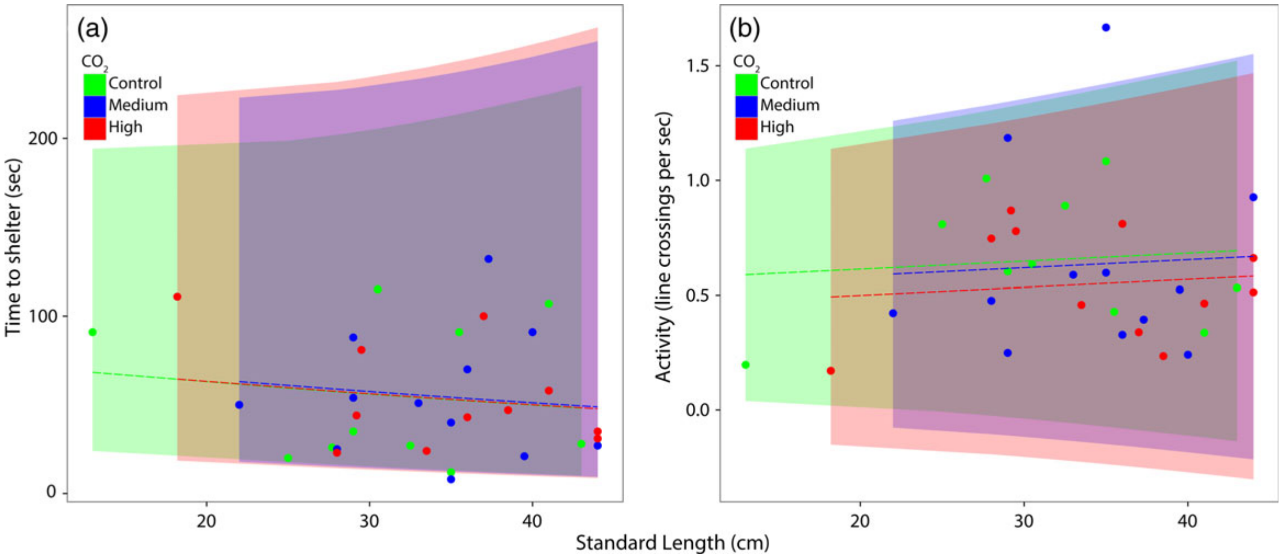

Figure 3. Individual shelter-seeking behaviour of epaulette sharks *H. ocellatum* with the predicted fit from the GLMM on the response scale (±95% confidence intervals) superimposed. Time in minutes required for sharks from control, medium, and high CO_2 treatments to reach an alternative shelter (a) and activity level expressed as number of line crossings on a 10 × 10 cm grid (b) after the removal of their original shelters. Both time and activity are plotted against the standard length of each individual. Each point represents a single individual. Colours indicate the three CO_2 treatments: control =400 μatm, medium =615 μatm, high =910 μatm.

simply allow inter- and intracellular pCO_2 and pH to fluctuate more broadly than in other reef fish. If so, there may be smaller changes to ion gradients that could interfere with the function of GABA-A receptors, and therefore, neural function and behaviour, at the relatively low pCO_2 levels studied here. Alternatively, *H. ocellatum* might regulate acid-base status to the same degree as other species, but may rely more heavily on alternative mechanisms, such as enhanced ammonia excretion or non-bicarbonate buffering (King and Goldstein, 1983; Claiborne and Evans, 1992). Either way, there would be smaller changes in HCO_3^- and Cl^- concentrations, which appear to be the underlying reason for behavioural disturbance of fish exposed to elevated CO_2.

Consistent with this hypothesis, HCO_3^- accumulation in the catshark, which exhibited altered nocturnal swimming behaviour at 990 μatm CO_2, was 0.5 mmol l^{-1} for every 100 μatm increase in CO_2 over the range 401–993 μatm (Green and Jutfelt, 2014). In contrast, the epaulette sharks in this study accumulated HCO_3^- at 0.39 mmol l^{-1} for every 100 μatm CO_2 over the range 390–870 μatm (Heinrich *et al.*, 2014). Furthermore, the average concentration of plasma HCO_3^- was 7.68 mmol l^{-1} in the catshark at 990 μatm CO_2, but is estimated to be 5.06 mmol l^{-1} in the epaulette shark at the same CO_2 level based on the observed rate of HCO_3^- accumulation. Although there were some differences in the sampling and analytical methods used in the two studies, these comparisons suggest that the epaulette shark may reach a lower final concentration of plasma HCO_3^- in an equivalent CO_2 environment than the catshark. This could influence the relative change in ion gradients at the neurotransmitter receptors, and thus the extent of behavioural changes (Heuer and Grosell, 2014).

In contrast to our findings for *H. ocellatum*, the benthic sharks *M. canis* and *S. canicula* exhibited significant changes in prey detection and swimming behaviour, respectively, when exposed to similar CO_2 levels used in this study. Although these two species of shark are benthic or epibenthic, they are more mobile than *H. ocellatum* and often rest on the open benthos rather than deep within small crevices and holes. Female *S. canicula* may shelter in shallow water caves when reproducing (Sims *et al.*, 2001), but they move to deep, well oxygenated water at night. Consequently, both these species are unlikely to routinely experience the same severe hypoxia and hypercapnia experienced by *H. ocellatum*. The reef-dwelling and shelter-seeking habits of the epaulette shark could be the reason its behaviour was unaffected by near-future CO_2 levels, whereas the two other species of small shark exhibit behavioural changes at similar CO_2 levels. Alternatively, our study could have come to different conclusions because of differences in the behaviours tested or differences in the experimental design. We focused on the foraging and shelter-seeking behaviour during the day, whereas Green and Jutfelt (2014) examined nocturnal swimming patterns. Dixson *et al.* (2014) investigated a more similar behaviour to our study, but their exposure period (5 d) was much less than ours (30 d). Perhaps, *H. ocellatum* might have exhibited altered behaviours after 5 d of exposure to elevated CO_2, but acclimated to a normal behaviour after 30 d. However, we consider this unlikely because reef fish exposed to high CO_2 for a month or more exhibit very similar changes in behaviour to those observed after just 4–5 d exposure (Munday *et al.*, 2013a, 2014). Furthermore, Green and Jutfelt (2014) observed significant effects on swimming behaviour in *S. canicula* after a similar exposure period to that used here.

Our results suggest that adaptation to shallow reef habitats could protect *H. ocellatum* from near-future ocean acidification, as observed in some other animals that occupy habitats that naturally experience episodes of high CO_2 (Melzner *et al.*, 2009). Maintaining vital behavioural traits under elevated CO_2 also suggests that there is potential for the adaptation of behaviours to a high CO_2 environment—but the rate of change in CO_2 will be a key. Epaulette sharks have adapted to their environment over millions of years, whereas the uptake of CO_2 into the ocean is occurring at a rate unprecedented in the recent geological record (Hoegh-Guldberg *et al.*, 2007). Whether marine organisms can adapt their acid-base regulatory mechanisms or the sensitivity of neurotransmitter receptors at a rate that will keep pace with climate change must be determined. Since *H. ocellatum* is exceptionally tolerant of fluctuating environmental conditions, it may not be a general representation of how all elasmobranchs will respond to ocean acidification. Less-tolerant elasmobranchs may suffer physiological (Rosa *et al.*, 2014) or behavioural impacts (Dixson *et al.*, 2014; Green and Jutfelt, 2014) from rising CO_2 conditions, similar to those documented in teleosts. Earlier life stages in some elasmobranch may also be more sensitive to elevated CO_2 compared with adults (Rosa *et al.*, 2014). Future experiments on early life stages and pelagic elasmobranchs that may be less tolerant of elevated CO_2 are needed. Importantly, studies that use short-term experiments to predict the impacts of high CO_2 on fish and other marine organisms must consider the potential for acclimation and adaptation over the time frame that CO_2 levels will rise in the future (Munday *et al.*, 2013b; Sunday *et al.*, 2014; Welch *et al.*, 2014). Inferences about the impacts of future high CO_2 levels on animal populations, based on the results of studies that acutely expose animals to future conditions, must be made with the appropriate caution because they do not account for any adaptation that could occur as CO_2 levels rise over coming decades.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Acknowledgements

Thanks to staff from JCU's Marine and Aquaculture Research Facility Unit (MARFU) for advice and assistance with maintenance of the experimental system. Shannon McMahon assisted with the preparation of the figures. This research was funded by the ARC Centre of Excellence for Coral Reef Studies and the JCU's Centre for Sustainable Tropical Fisheries and Aquaculture.

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Handling editor: Howard Browman