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Odor tracking in sharks is reduced under future ocean acidification conditions

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Abstract

Recent studies show that ocean acidification impairs sensory functions and alters the behavior of teleost fishes. If sharks and other elasmobranchs are similarly affected, this could have significant consequences for marine ecosystems globally. Here, we show that projected future CO_2 levels impair odor tracking behavior of the smooth dogfish (*Mustelus canis*). Adult *M. canis* were held for 5 days in a current-day control (405 ± 26 μatm) and mid (741 ± 22 μatm) or high CO_2 (1064 ± 17 μatm) treatments consistent with the projections for the year 2100 on a 'business as usual' scenario. Both control and mid CO_2-treated individuals maintained normal odor tracking behavior, whereas high CO_2-treated sharks significantly avoided the odor cues indicative of food. Control sharks spent >60% of their time in the water stream containing the food stimulus, but this value fell below 15% in high CO_2-treated sharks. In addition, sharks treated under mid and high CO_2 conditions reduced attack behavior compared to the control individuals. Our findings show that shark feeding could be affected by changes in seawater chemistry projected for the end of this century. Understanding the effects of ocean acidification on critical behaviors, such as prey tracking in large predators, can help determine the potential impacts of future ocean acidification on ecosystem function.

Keywords: behavior, climate change, dogfish, *Mustelus canis*, ocean acidification, olfaction

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Introduction

Anthropogenic emissions of carbon dioxide (CO_2) have driven atmospheric CO_2 concentrations to their highest level (>395 ppm; Dlugokencky & Tans, 2013) in the past 800 000 years (Lüthi *et al.*, 2008), a level that would be much higher if not for the absorption capacity of the oceans, which sink approximately 25% of all additional CO_2 (Ballantyne *et al.*, 2012). Ocean uptake of anthropogenic CO_2 has reduced average pH by 0.1 units since preindustrial times, through a process known as ocean acidification. Atmospheric CO_2 concentrations are projected to exceed 900 ppm by 2100 if emission rates maintain their current trajectory (Meishausen *et al.*, 2011), and pH is projected to decrease further over the next one to two centuries as CO_2 emissions continue to rise (Caldeira & Wickett, 2005). On this 'business as usual' scenario, ocean pH would decline an additional 0.3–0.4 units by 2100 (Meehl *et al.*, 2007; Meishausen *et al.*, 2011), with a rate of change faster than experienced in at least a million years (Hönisch *et al.*, 2012).

Concern that rising CO_2 levels and ocean acidification will severely impact marine ecosystems has escalated (Fabry *et al.*, 2008; Wittmann & Pörtner, 2013). Initial research on this subject primarily focused on understanding the effects that reduced carbonate ion concentrations in a low pH ocean would have on organisms that build their shell and skeletons from calcium carbonate (Kleypas *et al.*, 2006). More recently, evidence has accumulated that rising dissolved CO_2 levels can impact a wide range of biological processes (Wittmann & Pörtner, 2013), including the sensory performance and behavior of marine fish (Munday *et al.*, 2009; Dixson *et al.*, 2010). Exposure to CO_2 levels within the range projected to occur in the ocean by 2100 have been shown to impair decision-making (Domenici *et al.*, 2012; Jutfelt *et al.*, 2013) and the response to auditory (Simpson *et al.*, 2011) and olfactory cues (Munday *et al.*, 2009; Dixson *et al.*, 2010) in marine fishes. The use of chemical cues is essential to the ecology of many marine species and impaired olfactory performance in reef fishes at elevated CO_2 has been shown to affect homing ability, habitat selection and risk of predation (Munday *et al.*, 2009; Dixson *et al.*, 2010; Briffa *et al.*, 2012; Leduc *et al.*, 2013). Whether near-future CO_2 levels could affect critical ecological activities in other groups of marine organisms, particularly in species that rely heavily on chemical signals, has only just started to be investigated (Meredith & Kajiura, 2010; Manríquez *et al.*, 2013).

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Elasmobranch fishes (sharks, skates, and rays) are widely considered to possess superior olfactory sensitivities compared with bony fishes and have long been referred to in the popular media as 'swimming noses' (Schluessel *et al.*, 2008). This reputation is partly based on anecdotal observations, but also due to their particularly large olfactory structures, including surface area of the olfactory epithelia (Lisney *et al.*, 2007) and volume of the olfactory bulb (Parker, 1914). Olfaction plays an integral role in food localization (Kajiura *et al.*, 2000; Gardiner & Atema, 2007), mating (Johnson & Nelson, 1978; Rasmussen & Schmidt, 1992), predator detection (Heuter *et al.*, 2004), homing, and navigation (Edrén & Gruber, 2005) in elasmobranchs. Olfaction is considered especially important as a distant sense because chemical signals can become entrained in currents and transported much further in the marine environment than visual, mechanical, or electrical signals (Atema, 2012). This enables elasmobranchs to detect chemical cues emanating from distant sources in their expansive environment. If acidification affects the cognitive system of sharks in a similar way to that of teleost fishes, the consequences for shark populations, and the function of marine ecosystems globally, could be far-reaching.

According to the latest ICUN reports, one-third of oceanic shark species are threatened by extinction, with 64 species placed on the Red List (Camhi *et al.*, 2009). Elasmobranchs are characterized by slow growth, late maturity, and low fecundity, making them extremely vulnerable to any rapid perturbations in their natural environment. Increasing pressure from overfishing, pollution, and habitat loss is causing the rapid decline of many shark populations (Schindler *et al.*, 2002; Myers *et al.*, 2007; Hisano *et al.*, 2011). Numerous reports have shown that the decline of apex predators has cascading effects throughout ocean ecosystems (Stevens *et al.*, 2000; Schindler *et al.*, 2002; Myers *et al.*, 2007; Hisano *et al.*, 2011) and changes in the behavior of predators can have fundamental effects on prey species (Madin *et al.*, 2010). Yet, whether ocean acidification will affect the behavior of sharks and other elasmobranchs, with potentially fundamental consequences for the structure and function of marine ecosystems, remains unknown.

The objective of this study was to determine the behavioral effect of ocean acidification on the odor tracking ability of the smooth dogfish shark, *Mustelus canis*. We use the term 'odor' to refer to chemical stimuli; prior experiments with this species (Parker, 1914; Gardiner & Atema, 2007) and several other sharks (Gardiner *et al.*, 2014) have shown that 'nose plugs' block odor access to the nose and abolish food odor detection. The smooth dogfish is amenable to behavioral and physiological studies in captivity and controlled conditions. Using a 10 000 l 8 × 2 m, recirculating Atema choice flume (Fig. 1, Gardiner & Atema, 2007), designed specifically for testing chemical preferences of large aquatic animals, we compared odor tracking and feeding behavior of *M. canis* held for 5 days in either a moderate (741 ± 22 μatm) or high (1064 ± 17 μatm) CO_2 treatment to that of sharks kept under present-day control conditions (405 ± 26 μatm CO_2). Previous studies have shown that fish exposed to elevated CO_2 for 3–4 days exhibit sensory and behavioral abnormalities (Munday *et al.*, 2009, 2010, 2013; Dixson *et al.*, 2010; Simpson *et al.*, 2011; Briffa *et al.*, 2012; Domenici *et al.*, 2012; Leduc *et al.*, 2013) identical to those in animals that have been exposed for weeks to months (Munday *et al.*, 2013, 2014). The elevated CO_2 treatments were set to match the upper range of projections for atmospheric and ocean CO_2 concentrations by 2100 (Caldeira & Wickett, 2005; Meehl *et al.*, 2007). Using a concentrated squid rinse as prey odor, individual sharks were monitored for: (i) the percentage of time spent in the odor plume side compared to ambient seawater side of the flume, (ii) the percentage of time spent near the odor source, where the concentration of odor was highest, compared to the downstream end of the flume, and (iii) the attack level displayed toward the odor source.

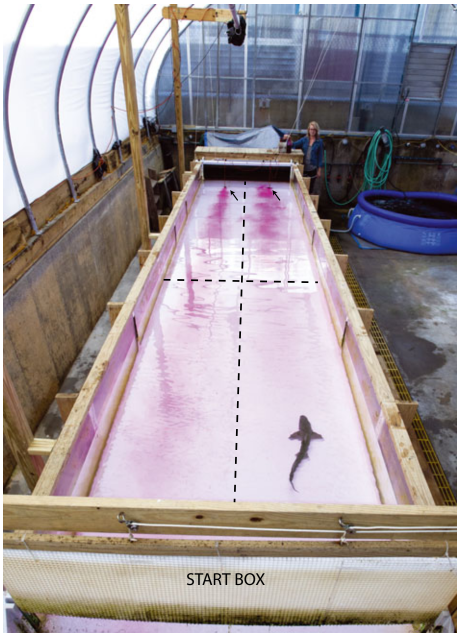

Fig. 1 An individual shark in the choice flume. Both plumes are visualized here for photographic purposes using 0.1 g L-1 of Rhodamine B dye dissolved in ambient seawater. Dashed lines are added to the image to indicate the flume quadrants used to determine activity level through number of lines crossed. Start box holding area and brick baffles (arrows) are also labeled.

Understanding the effects of ocean acidification on critical behaviors such as tracking and feeding attacks is critical for determining the potential impacts of future ocean acidification on these key species, thereby providing insight into the impact on ecosystem function.

Materials and methods

Animal collection and husbandry

Smooth dogfish (*Mustelus canis,* order Carcharhiniformes, family Triakidae) is a coastal demersal species of elasmobranch widely distributed through the North Western Atlantic Ocean (Conrath *et al*., 2002). This small benthic shark, 150 cm maximum length, migrates seasonally between the coasts of New England and South Carolina, United States in response to changes in water temperature (Roundtree & Able, 1996). The diet of *M. canis* consists of invertebrates and small fish, hunting mainly as an opportunistic predator searching for sick and injured prey items (Roundtree & Able, 1996; Gelsleichter *et al*., 1999). This is an ideal species to test the impacts of ocean acidification on tracking as both the food search behavior (Parker, 1922; Gardiner & Atema, 2007) and olfactory structures (Northcut, 1978) are well described. *M. canis* is currently listed as near threatened by the International Union for Conservation of Nature and has been proposed as an addition to the species management list for the National Oceanic and Atmospheric Association Fisheries Service.

Mustelus canis were purchased from the Marine Biology Laboratory's Marine Research Resources Center (MBL) in Woods Hole, Massachusetts USA, or from the local fishing industry. Sharks were caught through trawling in Nantucket Sound (n = 19; MBL) or near-shore hook and line fishing (n = 5; local fishing effort). Sharks taken from MBL were held for less than 1 month in MBL aquaria before being brought to our lab at the Woods Hole Oceanographic Institution (WHOI) for experimental use. While held at MBL, animals were fed a diet of local frozen squid (*Loligo pealeii*) and frozen capelin (*Mallotus villosus*). Fish obtained through local fisherman were brought directly to the research lab on the day of collection.

Sharks were housed in three, 1000 l 6-foot diameter soft-sided pools, each maintained at a different CO_2 level (n = 8 sharks per treatment; Table 1). To ensure proper replication among CO_2 treatments, sharks were treated in small groups of between 2–4 animals in the same pool at any period of time, and CO_2 treatments were rotated among the pools. On the day of arrival, sharks were randomly allocated to a CO_2 treatment and placed in the corresponding soft-sided pool. The day after arrival, sharks were sexed, measured (average total length 93.2 cm ± 1.1SE; fork length 79.3 cm ± 1.0SE) and given two colored tags for individual identification using T-bar anchor tags through the dorsal fin. Sharks were fed 2–3 cm pieces of thawed caplin (*M. villosus)* after tagging and then three times a week until their olfactory responses were tested. To ensure sharks were motivated to track a food odor, sharks were not fed 2 days before testing.

Simulated ocean acidification treatment

Sharks were kept at current-day CO_2 levels for the study location (405 ± 26 SE μatm), or at one of two elevated CO_2 treatments: mid (741 ± 22 SE μatm CO_2) and high (1064 ± 17 SE μatm CO_2) consistent with projections for the end of this century (Table 1). Fresh seawater was pumped into the three holding pools at a rate of ~1 L s^{-1}. Elevated CO_2 treatments were maintained by CO_2 dosing into the two treatment pools. A pH controller (TUNZE aquarientechnik) attached to each treatment pool was set to the pH level corresponding with the desired partial pressure of CO_2 (pCO_2).

A solenoid injected a slow stream of CO_2 into a pump (Eheim 3400) at the bottom of the treatment pools whenever the pH of the water rose above the set point. A precision needle valve inserted between the solenoid and the pump ensured a slow steady delivery of CO_2 into the water. The pump rapidly dissolved the CO_2 into the water and also acted as a vigorous mixer of water around the pool. The control treatment pool also used a pump to mix and move water. Each pool was covered with a clear plastic sheet to reduce CO_2 off gassing. The CO_2 system was able to efficiently maintain a stable pH throughout the treatment.

Water pH_{NBS} was measured twice daily with a pH meter (TPS WP80) calibrated daily with fresh pH buffers (Merk, Darmstadt, Germany). Temperature was recorded at least twice daily, averaging 19.6 °C ± 1.5 SE. Oxygen levels were measured with a WTW OXI 340i electrode and remained high (>90%) throughout the study. Total alkalinity was measured by Gran titration to within 2% of certified reference material (Prof. A. Dickson, Scripps Institution of Oceanography) from

Table 1 Summary of seawater parameters in control and acidification treatments. pCO_2 was calculated using the program CO_2SYS from measured values of pH_{NBS} and total alkalinity. Values are mean ± SE

Location	Treatment	Salinity (ppt)	Temperature (°C)	pH_{NBS}	Total alkalinity (μmol.kg^{-1} SW)	pCO_2 (μatm)
Holding Pools	Control	30.8	19.3 (±0.4)	8.11 ± 0.04	2077 (±32)	405 (±26)
	Mid	30.8	19.3 (±0.4)	7.80 ± 0.01	1962 (±28)	741 (±22)
	High	30.8	19.3 (±0.4)	7.69 ± 0.01	2009 (±17)	1064 (±17)
Flume	Control	30.8	19.3 (±0.1)	8.08 ± 0.01	2073 (±21)	412 (±9)
	Mid	30.8	19.2 (±0.1)	7.84 ± 0.01	1936 (±25)	734 (±16)
	High	30.8	19.6 (±0.1)	7.71 ± 0.05	2022 (±19)	1071 (±18)

replicate samples taken weekly. Seawater pCO_2 was estimated in the program CO2SYS using already established constants (Mehrbach *et al.*, 1973; Dickson & Millero, 1987).

Odor trials

All experiments were conducted in a 10 000 l, recirculating odor choice flume maintaining two parallel plumes not mixing until the downstream exit (Fig. 1, Gardiner & Atema, 2007). The flume had a working area of 8.0 m × 2.0 m (length × width) and was filled to a depth of 40 cm. This apparatus was designed to conduct pair-wise choice experiments, with sharks able to freely choose between two sides of the flume: one side contained a plume of tank water with food odor, and the other side contained a plume of only flume water. The plumes were generated from identical point sources near the bottom at the upstream end of the flume. The mean current velocity, measured visually with Rhodamine B dye, in the cross-sectional center of the flume was ~1.5 m min^{-1}. Prior to any experiments, these plumes were visualized and video recorded with 0.1 g l^{-1} of Rhodamine B dye dissolved in ambient seawater. The plumes remained spatially separate along the entire length of the flume (Fig. 1).

'Squid juice' was used as an odor attractant for all experiments, prepared by soaking 500 g wet mass of freshly thawed squid, cut into 2–3 cm pieces, for 24 h in 1 l of seawater. The solution/emulsion was strained through cheesecloth prior to use and stored at 3 °C for up to 48 h after straining. Prior to use, the squid juice was brought up to ambient water temperature. During the trials, squid juice was delivered onto one side of the flume and a control flume water source without odor was delivered to the other side of the flume. Both stimuli were delivered through soft PVC tubing (3.16 mm i.d.) at a rate of 0.4 ml s^{-1}, controlled by a Gilson Minipuls three peristaltic pump with a two channel pump head (Gilson, Inc. Middletown, WI, USA). The tubing outflow nozzles were fixed to a plastic board on the flume floor, to keep them in the same position throughout all experimental trials. Wake turbulence was created by placing a brick (20 cm × 9 cm × 6 cm) across the mean flow, 15 cm downstream from each nozzle (Gardiner & Atema, 2007). These bricks acted both as a baffle, widening the chemical plume, and a target for the sharks to 'attack' at the conclusion of chemical tracking.

Odor trials were always run on the fifth day of CO_2 exposure, or equivalent holding time for control sharks. Sharks were tested in their corresponding CO_2 treatment level; the flume was treated with CO_2 in the same way as described above for the pools (Table 1).

Sharks were individually placed into the flume for behavioral testing. A single trial consisted of a 10 min habituation period where the shark was free to swim throughout the entire flume; no odor was introduced to the flume during this period. Following the habituation time, the shark's baseline swimming behavior was recorded for a 10 min period. Swimming patterns were recorded through overhead video surveillance cameras. These baseline data allowed for any potential side bias by individual sharks to be accounted for in the statistical analysis. Preliminary trials indicated a 10 min habituation period was sufficient for sharks to display normal swimming behavior, with 93% of sharks tested displaying no significant difference in swimming behavior after 3 min of being introduced into the flume. Preliminary experiments were conducted by recording shark movement in 10 min time blocks every hour for 10 sharks over a 12 h period. Using the same methods described for the experimental analysis, 10 min blocks were compared against each other; no statistical difference was found ($P > 0.10$).

Following the 10 min baseline recording, the shark was gently herded into the downstream start box (2 m × 1 m), separated from the testing area with a retractable mesh gate (see labeled picture Fig. 1). Then the odor plume was started along with a timer and the overhead video cameras. The side of the flume that initially contained the odor stimulant was randomly assigned to each individual shark. The shark was held in the start box for 6 min, the time needed for the odor to reach the start box, from the upstream end of the flume, as indicated by a series of flow rates and dye tests. Three minutes into the holding time, the shark's gill ventilation rate was recorded, along with the pH and temperature of the flume. After the 6 min holding period, the gate was lifted and the shark was able to freely explore the flume for 4 min. During this time, behavioral traits were recorded, including: the number of bumps or bites at the odor baffle; the number of times the shark circled the odor source, and any notes on specific unique behaviors that should be assessed when the video trials were analyzed. At the conclusion of the 4 min trial period, the odor was stopped and both the brick baffle and odor delivery lines and nozzles were switched to the opposite side of the flume for the second part of the trial; this allowed the assessment of nonodor-induced side bias. While the odor was switched, the shark was given a 10 min period to freely swim throughout the flume without odor being injected. Following this time, the trial period was repeated including the 6-min start box period to re-establish the odor plume, now on the opposite side, and the 4-min experimental trial. At the conclusion of the second experimental trial, one 2–3 cm piece of squid was placed in the center of the flume and the time to feed was recorded.

Statistical analysis

During baseline recordings, individual sharks displayed strong side preferences, with untreated sharks spending greater than 83% (±6.6 SE) of their time on one side of the flume. The side bias displayed was not consistent among individuals and therefore the preferred side was individually determined and accounted for in the statistical analysis.

A repeated measures ANOVA, with a between-subjects factor (CO_2 level) and a within-subject factor (side of odor release in relation to side preference), was used for three comparisons: (i) time spent by sharks on their preferred side of the whole flume to determine if CO_2 changed the shark's behavioral response to odor; (ii) time spent in the upstream end of the flume to determine if CO_2 affected behavior near the odor source, and (iii) number of feeding attacks defined as the combined number of 'bumps' or

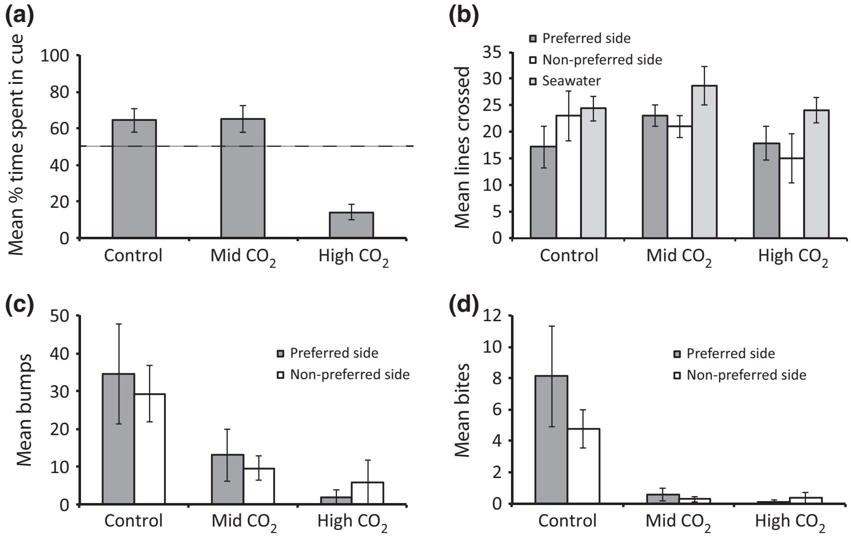

Fig. 2 (a) Mean (±SE) percent time sharks from control and elevated CO_2 treatments spent on the food odor side (dashed line indicates no preferences, values >50% indicate attraction; values <50% indicate avoidance), (b) mean number of lines crossed by sharks as a measure of activity, (c) mean number of bumps and (d) mean number of bites on the brick as a measure of food attack.

'bites' taken on the brick baffle directly in front of the odor source. The number of feeding attacks was also compared with a Kruskal–Wallis test, only considering the number of bumps. Prior to each of these three analyses, data were examined for normality and homogeneity of variances using D'Agostino–Pearson and residual analysis, and sphericity with the Mauchly's test. In addition, a repeated measure ANOVA, with the same design described above, was used to compare time spent by sharks near the source of the odor plumes compared to the downstream end of the flume. In addition, for the third analysis, the number of shark feeding strikes was considered as the combined number of bites and bumps taken by each individual shark during the trials.

Results

Under present-day control conditions, sharks adjusted their position in the choice flume based on odor, spending a greater amount of time on the side containing the food odor plume, than the side containing only a seawater plume (Fig. 2a, Table 2). This happened regardless of the individual's natural side preference, with all control sharks spending a significantly greater amount of time on the nonpreferred side of the flume when the odor plume was presented there (Fig. 3). Sharks held in the mid level CO_2 treatment showed a similar trend as the control group (Fig. 2a, Fig. 3). That is, they tended to spend more time in the flume side containing the odor plume.

In contrast, there was a clear shift in the tracking response of sharks from the high CO_2 treatment. Sharks in this treatment significantly avoided the odor plume area, instead, spending more time on the seawater side (Fig. 2a, Table 2). This avoidance behavior in high CO_2 was further emphasized when the odor plume was moved to the individual shark's naturally preferred swimming side, resulting in individuals spending significantly more time in their nonpreferred side of the fume (Fig. 3), as indicated by the significant interaction between CO_2 level and side of odor release (Table 2). The importance of the odor stimuli on the behavior of the shark is highlighted by the control treated sharks spending greater than 75% of their time in the

Table 2 Repeated measures ANOVA for time spent by sharks on their preferred side among treatments under three levels of CO_2 (control, medium, and high) and three conditions of odor release: on shark's preferred side, on shark's nonpreferred side, and shark swimming behavior prior to the odor release (baseline data). This analysis had a between-subjects factor (CO_2 level) and a within-subject factor (side of odor release). Bold values indicate significance ($P < 0.05$)

Source of variation	SS	df	MS	F	*P*
Between subjects					
CO_2 level	6819.19	2	3409.60	6.30	**0.007**
Error	11361.12	21	541.01		
Within subjects					
Side of odor release	5727.44	2	2863.72	9.97	**<0.001**
CO_2 level * side of odor release	4577.89	4	1144.47	3.94	**0.008**
Error	1288	42	290.19		

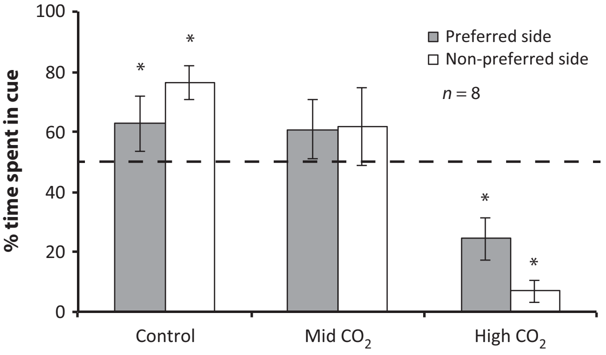

Fig. 3 Mean (±SE) time spent on the food odor side when presented on either the preferred or nonpreferred side for sharks exposed to control or elevated CO_2 conditions. Dashed line indicates no preferences, values >50% indicate attraction; values <50% indicate avoidance. Asterisks indicate bars that are statistically different from 50% as determined through a resampling test.

nonpreferred side of the flume when the odor was presented on this side (Fig. 3). Sharks treated in control and mid CO_2 water spent greater than 60% of their time in the water stream with the food stimulus, but this value fell below 15% in high CO_2-treated fish (Fig. 2a). Exposure to elevated CO_2 did not significantly affect the activity level of the sharks (Fig. 2b). Sharks in all treatments displayed normal swimming behavior, making slow steady, up and down the length of the flume parallel to the current.

To measure attack behavior, a brick was placed at the source of the odor to act both as a baffle for the odor cue as well as a focal point for feeding attacks (labeled in Fig. 1). The control group attacked the 'food' source by either bumping the odor brick with their snout (Fig. 2c) or biting it (Fig. 2d) significantly more frequently than sharks held in either of the elevated CO_2 treatments (Table 3). There was a significant interaction between CO_2 level and side of odor release for feeding attacks (Table 3), with sharks from the high CO_2 treatment displaying more attack behaviors when the odor was on their nonpreferred side, compared with sharks from the control and moderate CO_2 treatment, which tended to show more feeding strikes when the odor was on their preferred side (Fig. 2c).

Table 3 Repeated measures ANOVA for the feeding strikes (bumps and bites combined) by shark individuals among treatments under three levels of CO_2 (control, medium, and high) and two conditions of odor release: on shark's preferred side, and on shark's nonpreferred side, and shark swimming behavior prior to the odor release (baseline data). This analysis had a between-subjects factor (CO_2 level) and a within-subject factor (side of odor release). Bold values indicate significance ($P < 0.05$)

Source of variation	SS	df	MS	F	*P*
Between subjects					
CO_2 level	43125	2	21562	1.15	0.335
Error	392849	21	18707		
Within subjects					
Side of odor release	391699	2	195849	15.97	**<0.001**
CO_2 level * side of odor release	368606	4	92151	7.52	**<0.001**
Error	514928	42	12260		

There was no significant difference among treatments in the amount of time spent in the upstream (higher odor concentration) vs. the downstream end (lower odor concentration) of the flume, likely due to the repeated upstream–downstream swimming pattern, which all individuals maintained ($P > 0.05$). There was no difference in the gill rates of sharks held in the different water conditions ($F_{(2,\ 8)} = 2.0665$, $P > 0.15$), suggesting that stress to the animals was unlikely to be driving the patterns described above.

Discussion

This study is the first to investigate the effects of ocean acidification on the behavior of a large predator and elasmobranch. We show that the ability of a common coastal, temperate shark to properly respond to prey odor is negatively affected by CO_2 levels projected to occur in the open ocean by the end of this century, or earlier in CO_2-enriched coastal regions (Feely *et al.*, 2008; Hofmann *et al.*, 2011). *Mustelus canis* naturally exhibited a preference for the chemical cues of squid odor; however, following exposure to ~1000 μatmCO_2 individuals switched to a strong avoidance of food odor. This change in reaction to chemical cues is consistent with previous studies showing that larval and juvenile reef fish exposed to elevated CO_2 were unable to discriminate between ecologically important cues, becoming attracted to cues they normally avoid (Munday *et al.*, 2009, 2010, 2013; Dixson *et al.*, 2010) and exhibiting reduced preferences for the cues they normally favor (Munday *et al.*, 2009). Our current results demonstrate that sharks also exhibit the behavioral impairment of odor plume tracking and food attacks caused by elevated CO_2 that were previously found in teleost fishes.

Our results for a large predator are similar to those for a small predatory teleost found on coral reefs, the dottyback, *Psuedochromis fusucs* (Cripps *et al.*, 2010). The dottyback exhibited a 20% reduction in attraction to chemical signals that indicate food after exposure to moderate (630 μatm) and high (948 μatm) CO_2 for 7 days (Cripps *et al.*, 2010). It was hypothesized that the diminished preference could indicate a reduced ability to recognize the chemical cues presented. This conclusion is further supported by our results, where CO_2-treated sharks displayed a 45% reduction in time spent in the stimulus plume and attacked food less aggressively in response to chemical stimuli when compared to the control individuals. In the dottyback study, individuals held at high CO_2 displayed higher activity levels compared with controls and the moderate CO_2 treatment. In contrast, elevated CO_2 had no effect on the swimming speed or activity level of the shark. While effects on behavior in dottybacks occurred at slightly lower $p\mathrm{CO}_2$ compared with those observed in this study, the time of exposure (7 days) was similar to the 5 day exposure time tested here, suggesting a similar mechanism may be responsible for the shift in odor tracking observed in the shark species used for this experiment.

Recent experiments have found that treatment with the $\mathrm{GABA_A}$ antagonist, gabazine, reverses the effects of elevated CO_2 on the response to chemical stimuli in coral reef fish (Nilsson *et al.*, 2012). It was proposed that changes in Cl^- and/or HCO_3^- gradients over the neuronal membranes, due to acid–base regulation in CO_2 exposed fishes, interfere with the function of $\mathrm{GABA_A}$ receptors in some of the neuronal circuits, leading to abnormal behaviors. Fish compensate for CO_2-induced acidosis by accumulating HCO_3^-, which is coupled with an equimolar decline in Cl^- (Nilsson *et al.*, 2012; Hamilton *et al.*, 2014). HCO_3^- and Cl^- are also the key ions involved in the (de)activation of $\mathrm{GABA_A}$ receptors and prolonged changes in the concentrations of these ions in fish exposed to elevated CO_2 appear to interfere with receptor function (Nilsson *et al.*, 2012). The interference of $\mathrm{GABA_A}$ receptor function by acid–base regulatory process could provide a mechanistic understanding of the widespread behavioral changes reported in teleost fish exposed to high CO_2, and now in a shark. Additional research is required to determine if the $\mathrm{GABA_A}$ receptor is responsible for the behavioral changes demonstrated here; however, due to the conserved nature of this receptor it is likely the same mechanism.

Mustelus canis inhabit bays and inshore shallow waters, acting as a scavenger and opportunistic predator, primarily feeding nocturnally on large crustaceans including small lobsters, shrimp, and crabs as well as small fish and mollusks (Bigelow & Schroeder, 1953; Roundtree & Able, 1996; Gelsleichter *et al.*, 1999). Their nocturnal feeding habit and frequent turbidity of the environment limits the effectiveness of locating prey via visual information over long distances (Bigelow & Schroeder, 1953). Chemical cues are often important or even essential in initiating a foraging response in predators, particularly elasmobranchs (Gardiner & Atema, 2007; Gardiner *et al.*, 2014). A shift in the response intensity to food stimuli could result in a decrease in the predator activity and reduced ability to respond to fluctuations in food availability. In addition, these coastal habitats also experience naturally high CO_2 levels from upwelling or enhanced acidification from eutrophication (Hönisch *et al.*, 2012). Consequently, the high CO_2 levels used in this study may occur earlier in these shallow water ecosystems than they are predicted to occur in the open ocean (Melzner *et al.*, 2013).

This study was carried out under laboratory conditions and thus does not allow for the full evaluation of the potential effects of ocean acidification on predatory abilities and vulnerability of *M. canis* in their natural environment. Nevertheless, the effects observed suggest that the outcome of predator–prey interactions could be influenced by elevated $p\mathrm{CO}_2$ in nature. Furthermore, as these experiments were short term, involving acute exposure to elevated CO_2, the potential for acclimation and/or adaptation was not tested. The potential of long-lived marine organisms, such as sharks, to adapt to rapid ocean acidification remains unknown (Sunday *et al.*, 2014). Whether the shift in behavior exhibited by sharks held in acidified water could be mitigated by adaption to a slow increase in CO_2 over several generations remains to be seen. Sharks are an ancient phylogenetic lineage that have survived periods of high CO_2 in the geologic past that were much greater than projected CO_2 levels for the next couple of centuries (Hönisch *et al.*, 2012). Consequently, there is good reason to suspect that they have ample adaptive capacity, but it remains to be determined if they can adapt fast enough to keep pace with the unprecedented rapid rise in atmospheric and ocean CO_2 occurring now. This is particularly relevant for elasmobranchs, which are characterized as classic K-selected species displaying slow growth rate and production of few offspring. This might reduce the potential for evolution to rescue populations from the demographic effects of rising CO_2 levels.

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