



## Research

**Cite this article:** Green L, Jutfelt F. 2014 Elevated carbon dioxide alters the plasma composition and behaviour of a shark. *Biol. Lett.* **10**: 20140538.  
<http://dx.doi.org/10.1098/rsbl.2014.0538>

Received: 10 July 2014  
Accepted: 27 August 2014

### Subject Areas:

behaviour, ecology, environmental science

### Keywords:

behaviour, respirometry, aerobic scope, blood chemistry, carbon dioxide, Davenport diagram

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2014.0538> or via <http://rsbl.royalsocietypublishing.org>.

# Elevated carbon dioxide alters the plasma composition and behaviour of a shark

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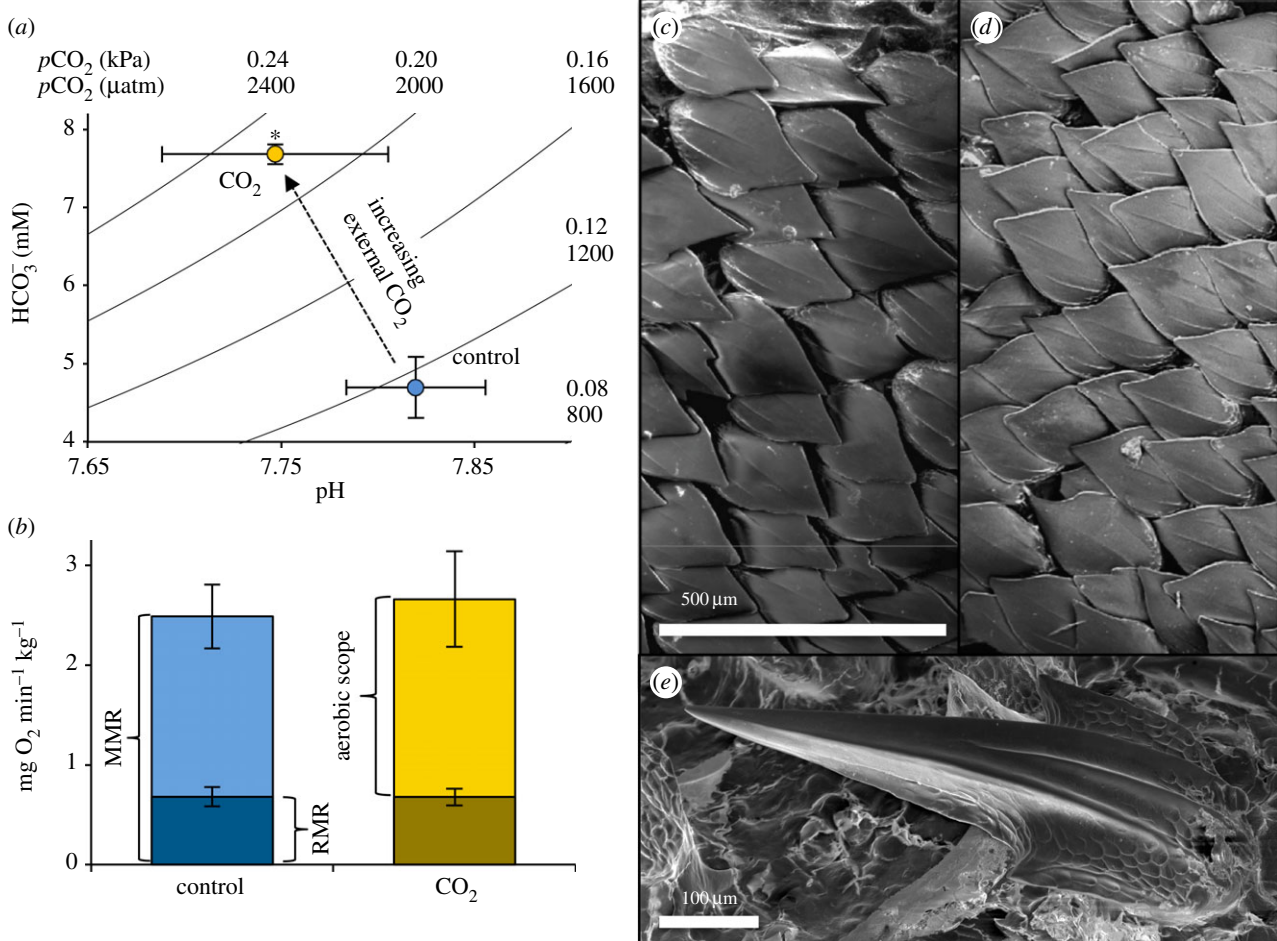
Increased carbon emissions from fossil fuels are increasing the  $p\text{CO}_2$  of the ocean surface waters in a process called ocean acidification. Elevated water  $p\text{CO}_2$  can induce physiological and behavioural effects in teleost fishes, although there appear to be large differences in sensitivity between species. There is currently no information available on the possible responses to future ocean acidification in elasmobranch fishes. We exposed small-spotted catsharks (*Scyliorhinus canicula*) to either control conditions or a year 2100 scenario of 990  $\mu\text{atm } p\text{CO}_2$  for four weeks. We did not detect treatment effects on growth, resting metabolic rate, aerobic scope, skin denticle ultrastructure or skin denticle morphology. However, we found that the elevated  $p\text{CO}_2$  group buffered internal acidosis via  $\text{HCO}_3^-$  accumulation with an associated increase in  $\text{Na}^+$ , indicating that the blood chemistry remained altered despite the long acclimation period. The elevated  $p\text{CO}_2$  group also exhibited a shift in their nocturnal swimming pattern from a pattern of many starts and stops to more continuous swimming. Although  $\text{CO}_2$ -exposed teleost fishes can display reduced behavioural asymmetry (lateralization), the  $\text{CO}_2$ -exposed sharks showed increased lateralization. These behavioural effects may suggest that elasmobranch neurophysiology is affected by  $\text{CO}_2$ , as in some teleosts, or that the sharks detect  $\text{CO}_2$  as a constant stressor, which leads to altered behaviour. The potential direct effects of ocean acidification should henceforth be considered when assessing future anthropogenic effects on sharks.

## 1. Introduction

Since the beginning of the industrial revolution, anthropogenic use of fossil carbon resources has increased the atmospheric  $\text{CO}_2$  concentration, reaching 400  $\mu\text{atm } p\text{CO}_2$  as of 2013 [1]. The atmospheric  $\text{CO}_2$  concentration will continue to increase, possibly reaching 940  $\mu\text{atm}$  by 2100 according to the RCP8.5 high-emission scenario [2]. The increase in atmospheric  $p\text{CO}_2$ , with the concurrent rise in surface water  $p\text{CO}_2$ , is shifting the oceanic carbonate ion balance and lowering the pH (i.e. ocean acidification) [3]. Elevated water  $p\text{CO}_2$  affects a range of organisms, and their responses are highly varied [4].

Teleost fishes can show behavioural disturbances when exposed to elevated  $\text{CO}_2$  levels. Behaviours such as activity, boldness, lateralization, and responses to olfactory, auditory and visual cues are reported to be altered in some species [5–8], whereas other species appear less sensitive [9,10]. Very little is known about possible effects of ocean acidification on elasmobranchs. It was recently reported that 5 days of exposure to elevated  $\text{CO}_2$  altered the response to olfactory cues in smooth dogfish (*Mustelus canis*) [11]. Shark populations are currently in sharp decline globally, and further negative effects of ocean acidification could therefore lead to an increased risk of shark species extinctions as well as reduced ecosystem function [12].

The small-spotted catshark (*Scyliorhinus canicula*) is a benthic temperate shark with a wide distribution in a broad range of habitats [13]. Here, we tested the hypothesis that elevated  $\text{CO}_2$  levels affect the behaviour and



**Figure 1.** Effects of  $p\text{CO}_2$  on the physiology of *S. canicula*. (a) Davenport diagram showing plasma  $\text{HCO}_3^-$  levels,  $p\text{CO}_2$  and pH after 4 weeks of exposure to control water ( $400 \mu\text{atm CO}_2$ ) or ocean acidification conditions ( $990 \mu\text{atm CO}_2$ ). Asterisks indicate a significant difference ( $p < 0.05$ ). (b) Oxygen consumption rates. Control  $n = 7$ ,  $\text{CO}_2$   $n = 9$ . Resting metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope are indicated by brackets. Denticle scanning electron micrographs showing control (c) and  $\text{CO}_2$ -exposed (d) pectoral fin denticles from adults. Image (e) shows a single dorsal denticle from the  $\text{CO}_2$ -exposed group with no visible damage or divergent growth. (Online version in colour.)

**Table 1.** Effects of  $\text{CO}_2$  on blood parameters of *S. canicula* after 4 weeks of treatment. Control  $n = 6$ ,  $\text{CO}_2$   $n = 6$ . Italic numbers indicate a significant difference ( $p < 0.05$ ).

blood parameter	control	$\text{CO}_2$	significance
pH	$7.82 \pm 0.04$	$7.75 \pm 0.06$	$p = 0.313$
$\text{HCO}_3^-$ (mmol $\text{l}^{-1}$ )	$4.70 \pm 0.39$	$7.68 \pm 0.13$	$p < 0.0001$
$\text{K}^+$ (mmol $\text{l}^{-1}$ )	$3.28 \pm 0.13$	$3.27 \pm 0.25$	$p = 0.969$
$\text{Ca}^{2+}$ (mmol $\text{l}^{-1}$ )	$2.89 \pm 0.02$	$2.88 \pm 0.04$	$p = 0.804$
$\text{Cl}^-$ (mmol $\text{l}^{-1}$ )	$236.28 \pm 3.67$	$243.33 \pm 1.74$	$p = 0.113$
$\text{Na}^+$ (mmol $\text{l}^{-1}$ )	$257.39 \pm 2.29$	$266.17 \pm 2.74$	$p = 0.034$
haemoglobin (g $\text{l}^{-1}$ )	$29.11 \pm 1.69$	$27.75 \pm 0.93$	$p = 0.534$
haematocrit	$15.58 \pm 1.24$	$15.17 \pm 0.28$	$p = 0.740$
MCHC (g $\text{l}^{-1}$ )	$19.44 \pm 2.05$	$18.34 \pm 0.72$	$p = 0.660$

physiology of *S. canicula*, similar to the effects reported in teleost fishes. We exposed sharks to either control or a high  $p\text{CO}_2$  water treatment for 4 weeks, and subsequently examined the behaviour and physiology of the sharks.

## 2. Material and methods

See the supplementary material for details. In short, 20 small-spotted catsharks were exposed to either control  $p\text{CO}_2$

( $401 \mu\text{atm}$ ) or high  $p\text{CO}_2$  ( $993 \mu\text{atm}$ ) conditions for 1 month prior to behavioural and physiological testing. The water  $p\text{CO}_2$  was measured using infrared (IR) absorbance [9]. The swimming pattern of the sharks was filmed overnight using IR light and IR cameras, and behaviour parameters were calculated and statistically analysed. Lateralization was measured in a double T-maze according to Jutfelt *et al.* [6] with 40 turning decisions per shark. Relative and absolute lateralization indexes were calculated [14]. Oxygen consumption rates (resting and maximum) were obtained using intermittent flow respirometry, and aerobic scope was

**Table 2.** Effects of CO<sub>2</sub> on growth and mortality of *S. canicula*. Control  $n = 6$ , CO<sub>2</sub>  $n = 8$  for standard growth rates (SGR; W, weight; L, length; BL, body length) and Fulton's condition. Control  $n = 10$ , CO<sub>2</sub>  $n = 10$  for mortality measurements.

parameter	control	CO <sub>2</sub>	significance
SGR W (% W d <sup>-1</sup> )	0.21 ± 0.04	0.40 ± 0.08	$p = 0.098$
SGR L (% BL d <sup>-1</sup> )	0.04 ± 0.02	0.11 ± 0.04	$p = 0.090$
mortality (%)	20	10	$p = 0.343$
Fulton's condition	0.30 ± 0.01	0.30 ± 0.01	$p = 0.768$

calculated [15]. Blood pH was analysed immediately after collection by venepuncture, and plasma was analysed for total CO<sub>2</sub>. Plasma concentrations of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup> were measured using an ion radiometer biosensor. Pectoral fin skin samples were analysed for denticle morphology and surface imperfections using scanning electron microscopy. The sharks used in this study were returned to the public aquarium from which they were borrowed. Data are presented as mean ± SE.

### 3. Results

#### (a) Physiology

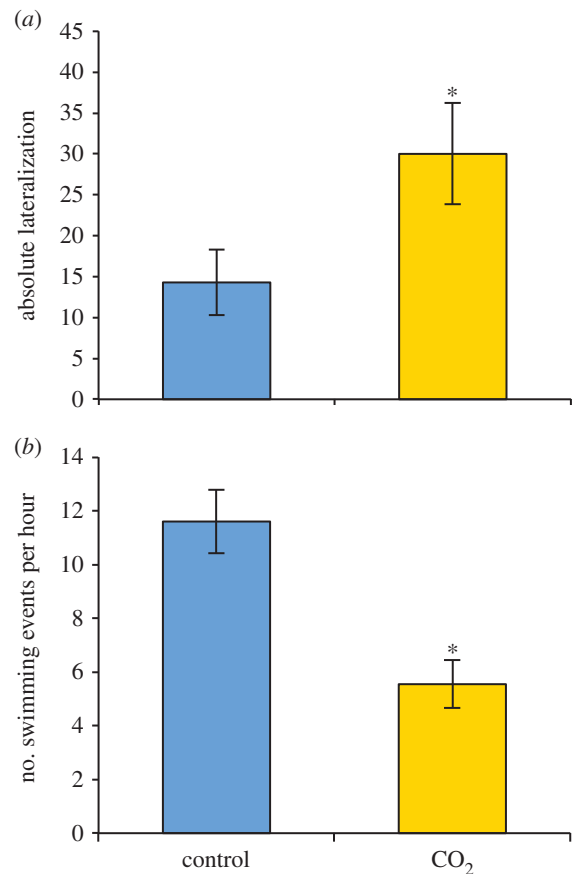
Blood plasma values are provided in table 1. HCO<sub>3</sub><sup>-</sup> levels were significantly different ( $p < 0.0001$ ) between control and CO<sub>2</sub>-exposed sharks (figure 1a). Plasma Na<sup>+</sup> concentrations were elevated ( $p = 0.034$ ) in the CO<sub>2</sub> treatment. Growth, Fulton's condition and mortality did not differ significantly (table 2). The metabolic parameters (resting and maximum oxygen consumption, absolute and factorial aerobic scope) did not differ between treatments (figure 1b). The denticles lacked significant differences in length ( $p = 0.666$ ) and width ( $p = 0.584$ ) between the control and CO<sub>2</sub>-exposed animals (figure 1c). Females displayed significantly larger denticles than did the males ( $p_{\text{width, length, ratio}} < 0.0001$ , mixed model analysis); and there was no correlation with shark weight. No ultrastructure damage or unusual growth of denticles was observed for any of the sharks.

#### (b) Behaviour

The absolute lateralization was significantly different ( $p < 0.05$ ) between the control and CO<sub>2</sub>-exposed sharks (figure 2a). The CO<sub>2</sub>-exposed animals expressed a longer swimming time per swimming event (figure 2b), with significantly fewer swimming events compared with the control animals ( $p = 0.014$ , mixed model analysis). Sharks in the CO<sub>2</sub>-exposed group had a tendency towards a higher proportion of swimming events reaching the surface ( $p = 0.082$ , mixed model analysis).

### 4. Discussion

Here, we show that  $p\text{CO}_2$  levels possible for the end of the century disturb the behaviour of small-spotted catsharks. The control sharks showed a nocturnal swimming pattern characterized by many starts and stops, whereas sharks in the high CO<sub>2</sub>-exposed group displayed more continuous



**Figure 2.** Effects of  $p\text{CO}_2$  on the behaviour of *S. canicula*. (a) Absolute lateralization. Control  $n = 7$ , CO<sub>2</sub>  $n = 10$ . The total average number of swimming events during the dark period is shown in figure (b). Asterisks indicate a significant difference ( $p < 0.05$ ). Control  $n = 6$ , CO<sub>2</sub>  $n = 5$ . (Online version in colour.)

swimming with fewer but longer swimming bouts. It is therefore possible that behavioural disturbance could become a consequence of future ocean acidification. The increased swimming duration mimics the altered behaviour that has been previously described in some teleost fishes, as hyperactivity has been recorded as one common behavioural symptom after exposure to elevated CO<sub>2</sub> levels [5,8,16]. In teleosts, behavioural disturbances from CO<sub>2</sub> exposure are hypothesized to be caused by a dysfunction of GABAergic neurons in the central nervous system (CNS) [17]. This change in behaviour may be caused by a shift in plasma Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> ion concentrations, which leads to reduced hyperpolarization of neurons of high CO<sub>2</sub>-exposed fish. The GABA<sub>A</sub> receptor is present in all vertebrates, and the altered shark behaviour could therefore be caused by reduced inhibition of CNS motor regions. The plasma levels of HCO<sub>3</sub><sup>-</sup> were dramatically elevated in the sharks, whereas the Cl<sup>-</sup> levels were not significantly affected, suggesting that HCO<sub>3</sub><sup>-</sup> is responsible for the majority of the behavioural shift. However, an alternative explanation for the altered swimming behaviour is that the sharks were continuously detecting the high CO<sub>2</sub> levels and altered their swimming behaviour to search for better water quality. Such CO<sub>2</sub> avoidance behaviour has recently been described in Atlantic cod [9].

Asymmetric behaviour, where an individual prefers one side, is common in vertebrates and teleost fishes exposed to elevated CO<sub>2</sub> typically show reduced lateralization [6,14]. The sharks displayed the opposite effect, with significantly

increased lateralization. This effect could be due to a disruption of the almost perfect behavioural symmetry observed in the control group, leading to a random distribution of behavioural asymmetry in the elevated CO<sub>2</sub> group.

The sharks appeared physiologically robust and were able to maintain normal oxygen consumption at high pCO<sub>2</sub>, both at rest and after a swim challenge, and the aerobic scope was not affected. In teleost fishes, the aerobic scope can be reduced [18], unaffected [19] or higher [20,21] in high pCO<sub>2</sub> water, and it is possible that there could be similar species differences within elasmobranchs. Denticles consist of hydroxyapatite, which has a low solubility and is not likely affected by weak acids [22]. The larger denticle size of females is likely for protection from male aggression during courtship [23].

Sharks, like teleosts [24], counter acidosis by gill H<sup>+</sup>/Na<sup>+</sup> and Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> counter-exchange, as previously demonstrated for sharks after short-term exposure to very high pCO<sub>2</sub> levels [25]. The significant increases in plasma Na<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> concentrations in this study show that the same mechanism is responsible for pH regulation under

near-future CO<sub>2</sub> levels and that the ion concentrations remain altered after long-term exposure. It is possible that the new plasma ion levels are within the tolerance range for the sharks, but sub-lethal long-term impacts of altered ion levels cannot be excluded.

In conclusion, we demonstrated that plasma pH during long-term exposure to ocean acidification conditions was maintained via chronic elevation of HCO<sub>3</sub><sup>-</sup> concentrations, with an associated decrease in Na<sup>+</sup>. Furthermore, shark behaviour was altered and resulted in increased lateralization and a shift in swimming from a pattern of many starts and stops to more continuous swimming. Elasmobranchs may therefore be susceptible to the effects of future ocean acidification.

**Acknowledgements.** We are grateful to Emma Nohrén and Helle Andersen at Havets Hus, Lysekil, Sweden, for lending us the sharks. We also thank Isabel Casties and Charlotte Alvord for excellent technical assistance. We are grateful to Paolo Domenici for constructive discussions regarding our data.

**Funding statement.** The Swedish Research Council Formas funded the study.

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