

Research



pressure due to just CO₂ gas. Ocean is the world's biggest carbon sink absorbing half of CO₂ from burning fossil fuels. As ocean warms it takes less CO₂. Carbonate ions, when CO₂ reacts with sea water, it releases these hydrogen ions which compete with shells for carbonate, so these clams/coral can't build themselves with carbonate.

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Global change biology

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 μatm $p\text{CO}_2$ 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 HCO_3^- accumulation with an associated increase in 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 CO_2 -exposed teleost fishes can display reduced behavioural asymmetry (lateralization), the CO_2 -exposed sharks showed increased lateralization. These behavioural effects may suggest that elasmobranch neurophysiology is affected by CO_2 , as in some teleosts, or that the sharks detect 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.

elasmobranch-sharks and rays. Detect and avoid predators. Teleosts make up about 96% of all species of fish.

1. Introduction

Since the beginning of the industrial revolution, anthropogenic use of fossil carbon resources has increased the atmospheric CO_2 concentration, reaching 400 μatm $p\text{CO}_2$ as of 2013 [1]. The atmospheric CO_2 concentration will continue to increase, possibly reaching 940 μatm 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 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 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 CO_2 levels affect the behaviour and

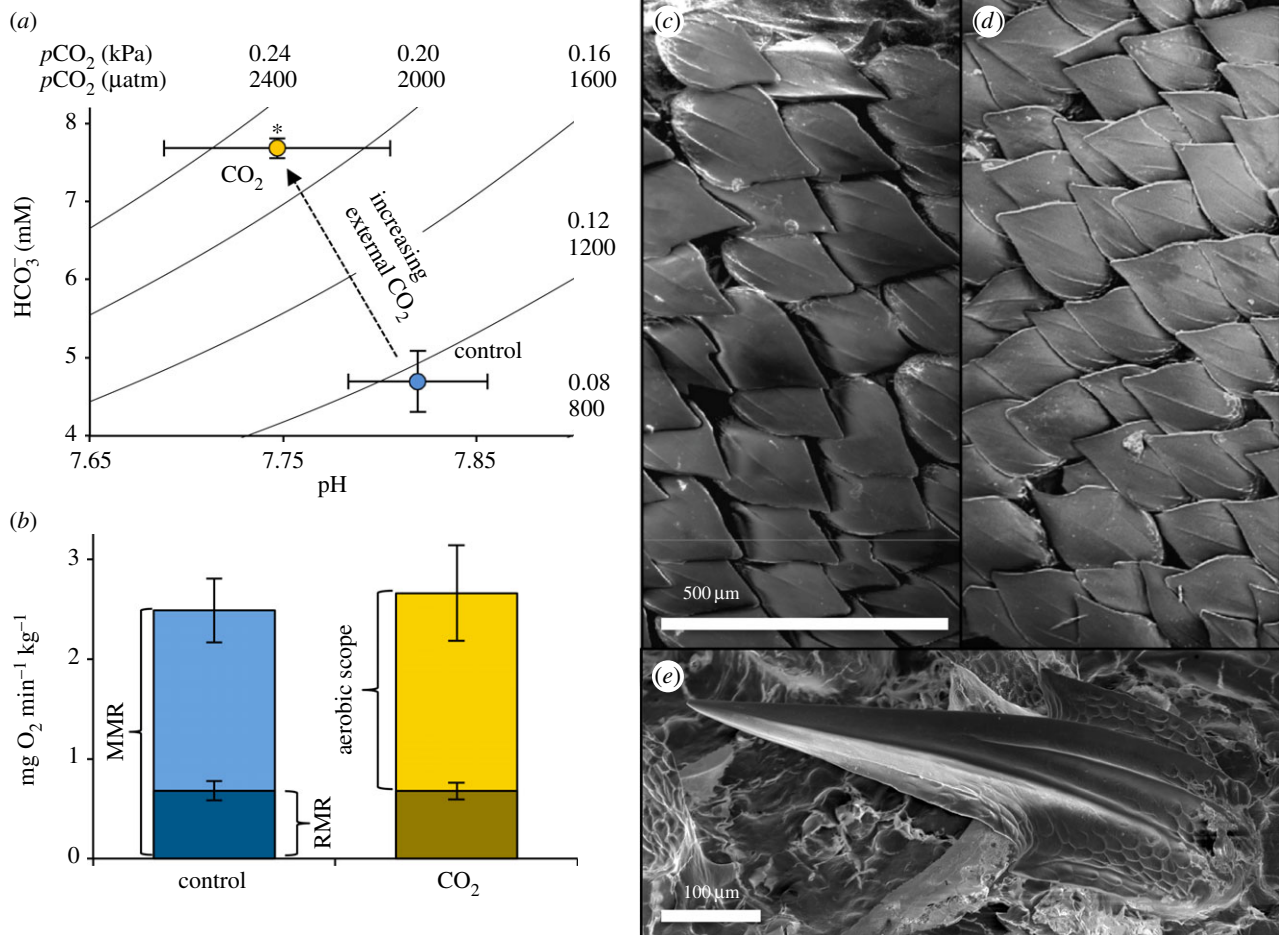


Figure 1. Effects of $p\text{CO}_2$ on the physiology of *S. canicula*. (a) Davenport diagram showing plasma HCO_3^- levels, $p\text{CO}_2$ and pH after 4 weeks of exposure to control water (400 μatm CO_2) or ocean acidification conditions (990 μatm CO_2). Asterisks indicate a significant difference ($p < 0.05$). (b) Oxygen consumption rates. Control $n = 7$, CO_2 $n = 9$. Resting metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope are indicated by brackets. Dentine scanning electron micrographs showing control (c) and CO_2 -exposed (d) pectoral fin denticles from adults. Image (e) shows a single dorsal denticle from the CO_2 -exposed group with no visible damage or divergent growth. (Online version in colour.)

Table 1. Effects of CO_2 on blood parameters of *S. canicula* after 4 weeks of treatment. Control $n = 6$, CO_2 $n = 6$. Italic numbers indicate a significant difference ($p < 0.05$).

blood parameter	control	CO_2	significance
pH	7.82 ± 0.04	7.75 ± 0.06	$p = 0.313$
HCO_3^- (mmol l^{-1})	4.70 ± 0.39	7.68 ± 0.13	$p < 0.0001$
K^+ (mmol l^{-1})	3.28 ± 0.13	3.27 ± 0.25	$p = 0.969$
Ca^{2+} (mmol l^{-1})	2.89 ± 0.02	2.88 ± 0.04	$p = 0.804$
Cl^- (mmol l^{-1})	236.28 ± 3.67	243.33 ± 1.74	$p = 0.113$
Na^+ (mmol l^{-1})	257.39 ± 2.29	266.17 ± 2.74	$p = 0.034$
haemoglobin (g l^{-1})	29.11 ± 1.69	27.75 ± 0.93	$p = 0.534$
haematocrit	15.58 ± 1.24	15.17 ± 0.28	$p = 0.740$
MCHC (g l^{-1})	19.44 ± 2.05	18.34 ± 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 μatm) or high $p\text{CO}_2$ (993 μatm) 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 CO₂ on growth and mortality of *S. canicula*. Control $n = 6$, CO₂ $n = 8$ for standard growth rates (SGR; W, weight; L, length; BL, body length) and Fulton's condition. Control $n = 10$, CO₂ $n = 10$ for mortality measurements.

parameter	control	CO ₂	significance
SGR W (% W d ⁻¹)	0.21 ± 0.04	0.40 ± 0.08	$p = 0.098$
SGR L (% BL d ⁻¹)	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 CO₂. Plasma concentrations of K⁺, Na⁺, Ca²⁺ and Cl⁻ 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. HCO₃⁻ levels were significantly different ($p < 0.0001$) between control and CO₂-exposed sharks (figure 1a). Plasma Na⁺ concentrations were elevated ($p = 0.034$) in the CO₂ 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 CO₂-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 CO₂-exposed sharks (figure 2a). The CO₂-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 CO₂-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 pCO₂ 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 CO₂-exposed group displayed more continuous

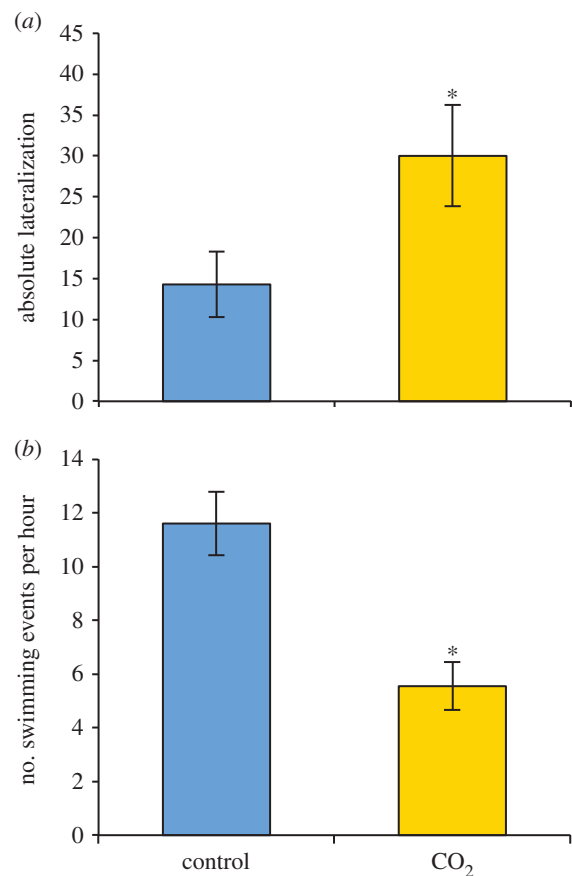


Figure 2. Effects of pCO₂ on the behaviour of *S. canicula*. (a) Absolute lateralization. Control $n = 7$, CO₂ $n = 10$. The total average number of swimming events during the dark period is shown in figure (b). Asterisks indicate a significant difference ($p < 0.05$). Control $n = 6$, CO₂ $n = 5$. (Online 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 CO₂ levels [5,8,16]. In teleosts, behavioural disturbances from CO₂ 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⁻ and HCO₃⁻ ion concentrations, which leads to reduced hyperpolarization of neurons of high CO₂-exposed fish. The GABA_A 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 HCO₃⁻ were dramatically elevated in the sharks, whereas the Cl⁻ levels were not significantly affected, suggesting that HCO₃⁻ 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 CO₂ levels and altered their swimming behaviour to search for better water quality. Such CO₂ 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 CO₂ 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 CO₂ group.

The sharks appeared physiologically robust and were able to maintain normal oxygen consumption at high pCO₂, 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 pCO₂ water, and it is possible that there could be similar species differences within elasmobranchs. Denticles consist of hydroxylapatite, 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⁺/Na⁺ and Cl⁻/HCO₃⁻ counter-exchange, as previously demonstrated for sharks after short-term exposure to very high pCO₂ levels [25]. The significant increases in plasma Na⁺ and HCO₃⁻ concentrations in this study show that the same mechanism is responsible for pH regulation under

near-future CO₂ 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 HCO₃⁻ concentrations, with an associated decrease in Na⁺. 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.

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