Responses of a coral reef shark acutely exposed to ocean acidification conditions

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Abstract Anthropogenic ocean acidification (OA) is a threat to coral reef fishes, but few studies have investigated responses of high-trophic-level predators, including sharks. We tested the effects of 72-hr exposure to OA-relevant elevated partial pressures of carbon dioxide (pCO_2) on oxygen uptake rates, acid–base status, and haematology of newborn tropical blacktip reef sharks (*Carcharhinus melanopterus*). Acute exposure to end-of-century pCO_2 levels resulted in elevated haematocrit (i.e. stress or compensation of oxygen uptake rates) and blood lactate concentrations (i.e. prolonged recovery) in the newborns. Conversely, whole blood and mean corpuscular haemoglobin concentrations, blood pH, estimates of standard and maximum metabolic rates, and aerobic scope remained unaffected. Taken together, newborn blacktip reef sharks appear physiologically robust to end-of-century pCO_2 levels, but less so than other, previously investigated, tropical carpet sharks. Our results suggest peak fluctuating pCO_2 levels in coral reef lagoons could still physiologically affect newborn reef sharks, but studies assessing the effects of long-term exposure and in combination with other anthropogenic stressors are needed.

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Introduction

The oceans are absorbing approximately 30% of atmospheric carbon dioxide (CO_2)—a phenomenon referred to as ocean acidification (OA)—that is predicted to increase CO_2 partial pressures (pCO_2) from 400 µatm to 1000 µatm by the year 2100 if current rates of greenhouse gas emissions remain unabated (Meinshausen et al. 2011). There are many documented cases of deleterious physiological responses of marine ectotherms to elevated pCO_2 (Heuer and Grosell 2014; Hannan and Rummer 2018). Coral reef fishes were thought to be resilient to OA because of exposure to much higher pCO_2 during their evolutionary history; however, recent evidence suggests that these fishes remain vulnerable to current unprecedented rates of environmental change, including OA (Rummer and Munday 2017; Munday et al. 2019). To date, studies have focused on identifying behavioural (e.g. foraging, activity, cognitive ability) and physiological responses (e.g. metabolic rate, acid–base status, haematology) to simulated OA conditions in teleost fishes (reviewed in Heuer and Grosell 2014), with few studies addressing responses of elasmobranch fishes, the sharks and rays (Rosa et al. 2017; Hannan and Rummer 2018). However, elasmobranchs may respond to ocean acidification differently than teleosts due to their excellent acid–base buffering capacity (Green and Jutfelt 2014; Heinrich et al. 2014; Dziergwa et al. 2019).

Three species of coral reef sharks—reef-dwelling carpet sharks (family *Hemiscylliidae*), including epaulette sharks (*Hemiscyllium ocellatum*; Heinrich et al. 2014, 2016; Johnson et al. 2016), white-spotted bamboo sharks (*Chiloscyllium plagiosum*; Lopes et al. 2018; Pegado et al. 2018), and brown-banded bamboo sharks (*C. punctatum*; Rosa et al. 2014, 2016a, b)—have been investigated as of 2018, each exhibiting unique responses to ocean acidification conditions. It is important to note that carpet sharks are small mesopredators that can be functionally redundant in coral reef ecosystems (Roff et al. 2016). Understanding the impact of OA-relevant pCO_2 levels on larger, ecologically important meso- and apex predator species will be pivotal to the health of coral reef ecosystems (Nagelkerken and Munday 2016).

The purpose of this study was to provide a preliminary understanding as to the effects of ocean acidification conditions on the physiology of a large-bodied coral reef mesopredator. Our objective was to characterize metabolic rates, acid–base status, and haematological status of newborn blacktip reef sharks (*Carcharhinus melanopterus*) following acute exposure to OA-relevant elevated pCO_2 conditions. Studies of this nature can inform ecological risk assessments to better manage coral reef fish populations under OA through an understanding of species' vulnerabilities.

Materials and methods

All experiments were approved by James Cook University's Animal Ethics Committee (protocol 2089) and the French Polynesian Ministère de la Promotion des Langues, de la Culture, de la Communication et de l'Environnement (Arrêté 9524).

Newborn blacktip reef sharks (Table 1) were collected using monofilament gill nets on fringing reefs around the island of Moorea in French Polynesia in November 2014. Sharks were transported to the CRIOBE research station on Moorea, where they were tagged (Hallprint, Hindmarsh Valley, SA, Australia) and housed in 1250-l flow-through tanks (four sharks per tank). Feeding (5.0% body mass in fresh tuna) occurred every other day, except for a 100-hr fast before experimentation. Following experimentation, all sharks were released at their original capture sites.

Elevating pCO_2 to simulate ocean acidification conditions was achieved following established protocols (Dickson et al. 2007). Header tanks were equipped with pH controllers (AT Control System, AB Aqua Medic GmbH, Germany) that dosed CO_2 to achieve a desired pH on the National Bureau of Standards scale (pH_{NBS}). Seawater pCO_2 was calculated as per Watson and colleagues (Watson et al. 2017) in CO2SYS (Table 1) using pH_{NBS} and total alkalinity (A_T) as inputs. We measured pH_{NBS} daily (Seven2Go Pro, Mettler Toledo, Switzerland) and A_T weekly from water samples collected weekly using Gran titrations and certified reference materials (Dr. A.G. Dickson, Scripps Institution of Oceanography).

Aquaria were randomly assigned to ambient ($pCO_2 = 600$ µatm) or end-of-century ($pCO_2 = 1000$ µatm) conditions. Sharks (ambient $n = 8$, end-of-century $n = 8$) were acutely exposed to treatment conditions for 72 hrs. We then quantified sharks' oxygen uptake rates ($\dot{M}O_2$, in mg O_2 kg^{-1} h^{-1}) as proxies of metabolic rates using intermittent-flow respirometry. The identical respirometry system, protocols, and $\dot{M}O_2$ analyses are described elsewhere (Rummer et al. 2016; Bouyoucos et al. 2018). Briefly, sharks were exercised (individually, 3-min chasing with 1-min air exposure) in treatment water so that their maximum $\dot{M}O_2$ (a proxy of maximum metabolic rate; MMR) could be measured immediately after introducing

Table 1 Shark morphometrics and experimental seawater carbonate chemistry

Treatment	Tank (sharks)	Mass (kg)	Total length (cm)	Temperature (°C)	Salinity	pH_{NBS}	Total alkalinity (µmol kg^{-1} SW)	pCO_2 (µatm)
Ambient pCO_2	1 $n = 4$	0.8 ± 0.1	57.5 ± 2.4	28.7 ± 0.8	34.5 ± 0.7	8.03 ± 0.02	2278.3 ± 9.9	608 ± 34.8
	2 $n = 4$	1.1 ± 0.2	61.5 ± 2.4	28.7 ± 0.8	34.5 ± 0.7	8.04 ± 0.04	2285.3 ± 4.8	599 ± 74.5
End-of-century pCO_2	1 $n = 4$	1.2 ± 0.1	59.5 ± 1.3	28.7 ± 0.7	34.5 ± 0.7	7.83 ± 0.03	2294.7 ± 7.6	1048 ± 148.7
	2 $n = 4$	1.1 ± 0.3	59.7 ± 5.4	28.7 ± 0.7	34.5 ± 0.7	7.83 ± 0.05	2274.6 ± 22.4	1045 ± 78.9

Values are presented as mean ± standard deviation. Water quality metrics (temperature, salinity, pH_{NBS}, and total alkalinity) were measured directly to calculate CO_2 partial pressures (pCO_2). Values for pCO_2 were calculated from directly measured metrics using CO2SYS sharks to respirometry chambers. During the 1-min air exposure period, sharks were weighed to the nearest 10 g in a soft plastic bag suspended from a digital scale, as body mass during respirometry is required for $\dot{M}O_2$ calculations. Then, sharks were allowed to recover in respirometry chambers maintained at their pCO_2 treatment conditions for up to 24 hrs to achieve minimum $\dot{M}O_2$ (a proxy of standard metabolic rate, SMR), which would allow us to calculate absolute aerobic scope (AAS = MMR − SMR).

We then used 23-gauge hypodermic needles and heparinized syringes to collect blood samples (1 ml) immediately upon removing sharks from respirometry chambers. We have found in preliminary studies that this is when the sharks are most amenable to this procedure; indeed, sharks achieve a quiescent, minimally stressed state during measurement of SMR (Bouyoucos et al. 2018). Moreover, because sharks are coming out of respirometry chambers, minimal handling is required to place them into tonic immobility to draw the blood sample. The entire process takes less than one minute. A VetScan i-STAT (Abaxis, Union City, CA, USA) with a CG4 + cartridge was used to measure pH and lactate concentrations (in mmol l^{-1}). Blood pH measurements were adjusted using the i-STAT's temperature correction function (Harter et al. 2015). Haematocrit (Hct = erythrocyte volume · whole blood volume^{-1}) was measured after centrifuging whole blood (3 min at $17{,}000 \times g$). Haemoglobin concentration ([Hb], in g l^{-1}) was measured using a HemoCue Hb 201 System (Australia Pty Ltd.) and established correction curves (Rummer et al. 2013). Mean corpuscular haemoglobin concentration (MCHC, in mmol l^{-1}) was calculated as [Hb] · Hct^{-1}.

All analyses were conducted in R (R Core Team 2018). Body mass (ANOVA, $F_{3,12} = 2.24$, $P = 0.136$) and water temperature ($F_{3,76} = 0.52$, $P = 0.984$) values were not significantly different between tanks and were therefore excluded from models. Tank effects were investigated using Welch two-sample t-tests and a standard $\alpha = 0.05$.

Table 2 General linear model output describing the effects of 72-hr exposure to two different CO_2 partial pressures (pCO_2) on various physiological parameters

Response	D.F.	F-value	P value
Standard metabolic rate	1, 14	1.19	0.293
Maximum metabolic rate	1, 14	0.63	0.440
Absolute aerobic scope	1, 14	0.34	0.571
Haematocrit	**1, 14**	**10.50**	**< 0.001**
Haemoglobin concentration	1, 14	2.49	0.136
Mean corpuscular haemoglobin concentration	1, 14	4.12	0.062
Blood pH	1, 14	2.19	0.161
Blood lactate	**1, 14**	**23.39**	**< 0.001**

Statistically significant effects of pCO_2 are indicated in bold

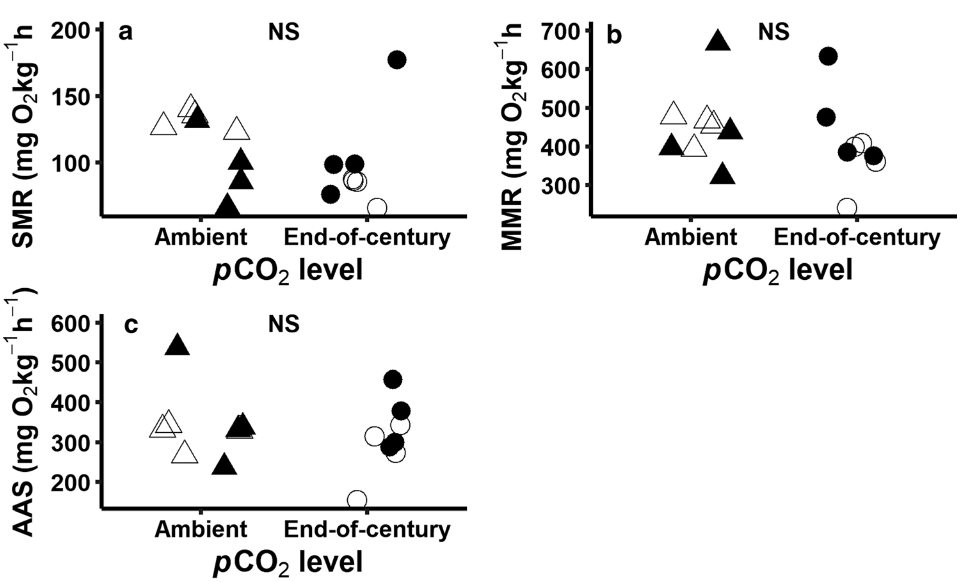

Fig. 1 Effects of 72-hr exposure to pCO_2 (ambient = 600 µatm, end-of-century = 1000 µatm) on estimates of metabolic rates in newborn blacktip reef sharks (*Carcharhinus melanopterus*). Different shapes represent different pCO_2 levels, and black and white symbols refer to separate holding tanks within treatments. Comparisons between pCO_2 levels that are not significantly different are denoted "NS". Abbreviations: absolute aerobic scope, AAS; maximum metabolic rate, MMR; standard metabolic rate, SMR

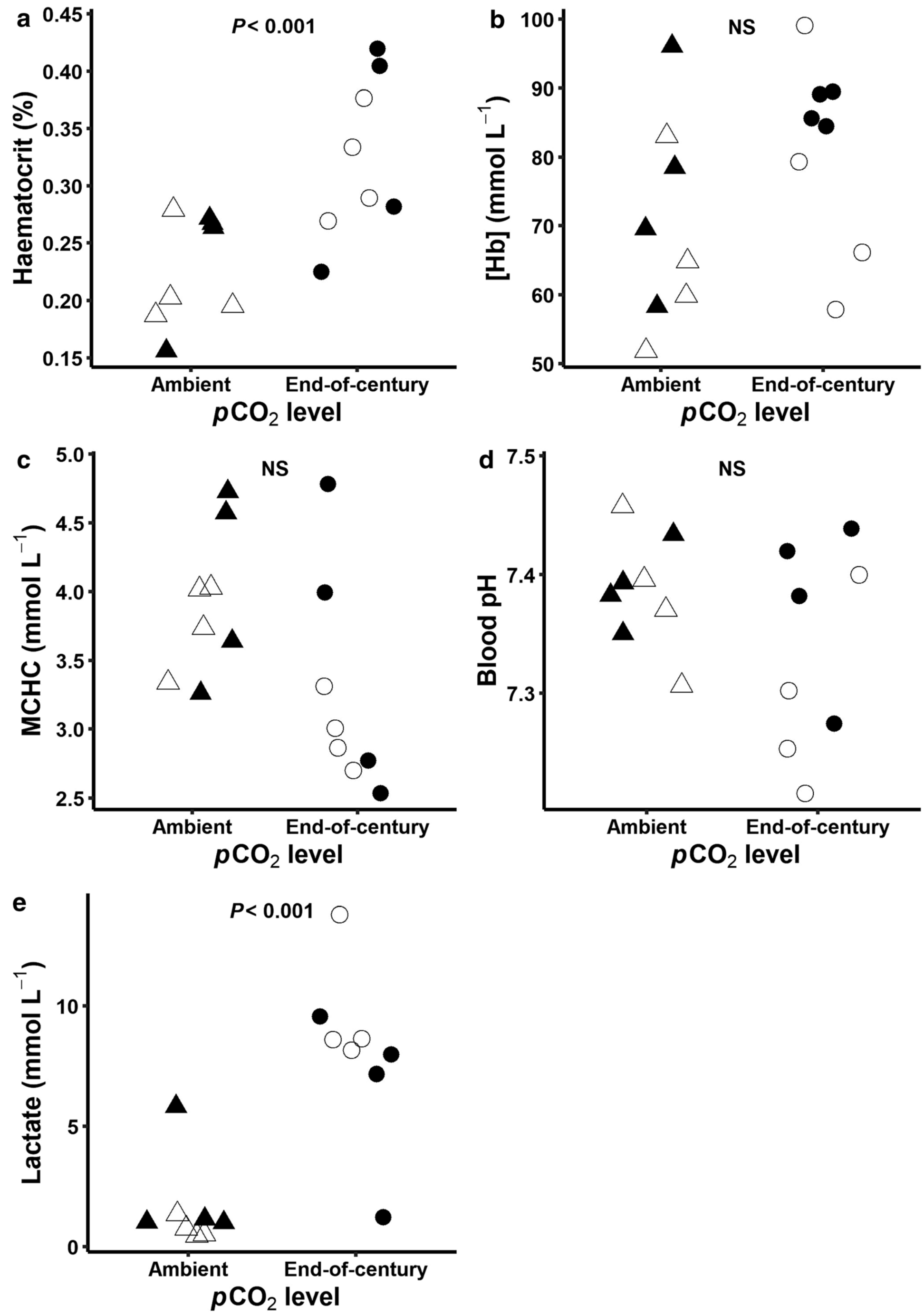

Fig. 2 Effects of 72-hr exposure to pCO_2 (ambient = 600 µatm, end-of-century = 1000 µatm) on blood-based metrics in newborn blacktip reef sharks (*Carcharhinus melanopterus*). Different shapes represent different pCO_2 levels, and black and white symbols refer to separate holding tanks within treatments. Comparisons between pCO_2 levels that are not significantly different are denoted "NS." Abbreviations: haemoglobin concentration, [Hb]; mean corpuscular haemoglobin concentration, MCHC

Physiological variables were then modelled as a function of pCO_2 level using general linear models.

Results and discussion

The purpose of this study was to understand the effects of acute exposure to end-of-century OA conditions on the physiology of a large-bodied coral reef mesopredator. Exposure to end-of-century elevated pCO_2 levels did not affect estimates of metabolic rates (Table 2; Fig. 1). However, blood-based metrics revealed various responses to elevated pCO_2 (Table 2; Fig. 2).

Newborn blacktip reef sharks exhibited a haematological response to OA conditions. Specifically, sharks that were acutely exposed to end-of-century pCO_2 levels had significantly higher Hct (Fig. 2a) but the same [Hb] (Fig. 2b) and MCHC (Fig. 2c) as their ambient counterparts. Elevated Hct is generally not observed among sharks exposed to OA-relevant elevated pCO_2 levels (Heinrich et al. 2014), but this trend has been documented as a result of exercise stress (i.e. gill net capture) in blacktip reef sharks (Schwieterman et al. 2019). However, documented effects on [Hb] and MCHC have been variable (Green and Jutfelt 2014; Heinrich et al. 2014). Increasing Hct suggests red blood cell swelling (i.e. if MCHC decreases) or the release of smaller, immature erythrocytes (i.e. in the case of low [Hb]) into circulation (Wood 1991). Changes in Hct may be a compensatory mechanism to maintain SMR or MMR (Hannan and Rummer 2018). Indeed, in line with the results from this study, metabolic rates in carpet sharks were also not affected by end-of-century pCO_2 levels (Heinrich et al. 2014; Rosa et al. 2014). Alternatively, elevated Hct could reflect a respiratory adjustment to elevated pCO_2 and stationary respiration during 24-hr respirometry, where free-swimming, ram-ventilating animals could respond differently, a topic worthy of further investigation.

Ocean acidification conditions may have affected sharks' recovery from an exercise stress. Blood pH was not affected (Fig. 2d), but sharks exhibited higher blood lactate concentrations upon exposure to end-of-century pCO_2 levels (Fig. 2e). Indeed, arterial blood pH/plasma proton (H^+) loads return to pre-exercise levels faster than plasma lactate concentrations in exercised sharks (Richards et al. 2003). Sharks typically experience an acute drop in blood pH during exposure to elevated pCO_2 that is buffered by bicarbonate in the plasma and by haemoglobin (Green and Jutfelt 2014; Heinrich et al. 2014; Dziergwa et al. 2019). Here, blacktip reef sharks did not exhibit a measurable increase in the cost of acid–base regulation (i.e. SMR), which suggests proficiency in acid–base regulation. However, the combination of exercise and exposure to elevated pCO_2 prolonged lactate clearance in blacktip reef sharks, as lactate values were high, in between peak and baseline concentrations for this species (Bouyoucos et al. 2018). While stationary ventilation may be confounding in a species that routinely ram-ventilates, exposure to OA conditions has been shown to prolong recovery from exercise in stationary ventilating skates (Di Santo 2016).

In conclusion, our work suggests that newborn blacktip reef sharks may experience a physiological stress response upon brief exposure to end-of-century pCO_2 levels. Given the fluctuating nature of pCO_2 in shallow, nearshore and coral reef habitats (Hannan et al., 2020), and that this population of newborn blacktip reef sharks has a very small home range (Bouyoucos et al., 2020), it is likely that these sharks experience elevated pCO_2 conditions on a diurnal basis. Moreover, these newborn reef sharks may undergo similar physiological responses to those observed in this study during periods of peak pCO_2 that may not be evident in the more physiologically robust, previously studied carpet sharks. Indeed, the fluctuating nature of pCO_2 has been documented in coral reef lagoons (diel, ranging 188–1697 µatm; Silverman et al. 2012), mid-shelf reefs (diel, ranging 275–542 µatm; Albright et al. 2013), and even among different microhabitats within a reef (386–445 ± 250 µatm, depending on reef exposure, wind speed, and time of day; Hannan et al. 2020) on the Great Barrier Reef, Australia. Albeit less exhaustively, the fluctuating nature of pCO_2 (ranging 240–546 µatm) has also been documented in the shallows (0.61–1.3 m) off of this study site in Moorea, French Polynesia (Frankignoulle et al. 1996; Gattuso and Frankignoulle 1998). In the future, not only will the average pCO_2 of these systems increase, but the natural pCO_2 cycles may also be amplified. Moving forward, studies should define long-term effects of ocean acidification on the physiology and behaviour of reef sharks, including this species' potential for reversible acclimation. Indeed, studies of this nature will be important steps towards understanding the extent that climate change is a conservation issue for top predators in coral reef ecosystems.

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