

Acid-base adjustments and first evidence of denticle corrosion caused by ocean acidification conditions in a demersal shark species

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INTRODUCTION

- Global ocean acidification → lowering the pH to 7.3 by 2300
- Acute hypercapnia in the South African west and south coasts → upwelling and low oxygen events
- Impact of hypercapnia on the endemic demersal shark *Haploblepharus edwardsii*
- Acid-base regulation and effects of chronic hypercapnia on growth rates and denticle structure and composition
- Hydrodynamics and skin protection



- Absorption of anthropogenic $\text{CO}_2 \rightarrow$ ocean acidification
- Effects on the biology, distribution, morphology, behaviour and physiology of marine organisms
- $+\text{CO}_2 \rightarrow +\text{H}_3\text{O}^+ \rightarrow -\text{CO}_3^{2-} \rightarrow -\text{pH}$

The South African west and south coasts

- Environmental dynamics in the Benguela Large Marine Ecosystem (BCLME)
- Episodes of hypercapnia with pH levels of 7.4 – 7.6 (sometimes even 6.6)
- Characteristics:
 - I. Coastal upwelling
 - II. Algal decay and bacterial respiration
 - III. Low-oxygen

Upwelling conditions

- 3-10 days cycle in spring and summer
- Cold and hypercapnic water moving to the surface
- After the season: collapse of phytoplankton blooms \rightarrow low-oxygen levels
- Result of climate change: longer, more frequent and severe

- Cartilaginous fishes – slow rate of evolution, low phenotypic plasticity, low adaption and low fecundity
- Embryo survival and development time are not affected, but body condition, growth, aerobic potential and behaviour are

Details for experiment on *H. edwardsii*

- Hypercapnic levels below the range of what occurs during upwelling
- Small, easy to rear and can be easily obtained in relevant numbers
- Acid-base regulation during the exposure to acute (32h) and chronic (9 weeks) hypercapnia
- Effects on growth rates and denticle structure
- Hypothesis: the sharks are able to acclimatize to acute and chronic hypercapnia, physiological compensation will come at an energetic cost that decreases somatic growth, low pH has detrimental effects on denticle structure (similar to human dental corrosion after the exposure to carbonated drinks)

MATERIALS AND METHODS

Experimental animals

- Permission of the Research Ethics Committee and in accordance with the relevant guidelines and regulations
- 80 specimens caught in a basin
- Caught by SCUBA divers by hand
- Bait: sardines in perforated 5 l plastic bottles
- Transferred into an 800 l tank on a car trailer
- Water in the tank continuously provided with oxygen from a cylinder
- Transported to a Research Aquarium in Cape Town
- Weighted and maintained in round flow-through holding tanks (4500 l) for four months prior to experimentation
- Fed rations of 5% of average body mass with pieces of squid
- Not fed in the week of experimentation

Experimental procedures

- Acute exposure: 66 larger sharks were acclimatized for 48h prior to experimentation in smaller round tanks which were mixed by propellers and aerated by compressed air
 - Individuals distributed between normocapnic and hypercapnic tanks with pH levels of about 7.3 to 8.0
 - Blood sampling: individuals removed from tanks after 1.5, 3, 6 and 24 hours
 - Analysis carried out in the statistical software environment R
 - The experiment was carried out in a room with stable ambient air temperature
 - During the acute experiment, seawater conditions were tested five times in each replicate tank and did not differ significantly between replicates of each treatment
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- Chronic exposure: 14 remaining smaller sharks, weighted, measured (total length) and tagged left on the dorsal fin
 - Transferred into normocapnic and hypercapnic tanks (1000 l plastic tanks) well mixed and aerated
 - Sharks were re-weighted and measured after 4, 6 and 9 weeks
 - Seawater $p\text{CO}_2$, H_3O^+ , CO_3^{2-} were calculated using measured pH, salinity, ambient temperature and total alkalinity

Treatment	T_A °C	pH_w	A_T $\mu\text{mol kg}^{-1}$	$\text{O}_2\%$	Salinity ‰	Ca^{2+} mmol l^{-1}	Mg^{2+} mmol l^{-1}	$p\text{CO}_2$ Torr (μatm)	HCO_3^- mmol l^{-1}	CO_3^{2-} mmol l^{-1}
Acute exposure										
Acclimation	17.8 ± 0.0	8.05 ± 0.02	2000 ± 3	94.7 ± 0.1	34.9 ± 0.0	10.1 ± 0.4	52.3 ± 4.3	0.3 ± 0.0 (337 \pm 15)	1.7 ± 0.0	0.2 ± 0.0
Normocapnia	17.3 ± 1.0	7.99 ± 0.07	1963 ± 22	91.8 ± 0.3	34.9 ± 0.0	11.2 ± 0.4	54.0 ± 2.1	0.3 ± 0.0 (386 \pm 8)	1.7 ± 0.0	0.1 ± 0.0
Hypercapnia	17.0 ± 1.1	7.31 ± 0.06	2010 ± 50	90.0 ± 0.0	35.0 ± 0.0	10.7 ± 0.1	52.7 ± 1.4	1.7 ± 0.0 (2184 \pm 45)	2.0 ± 0.0	0.0 ± 0.0
Recovery	17.2 ± 0.2	8.01 ± 0.01	1991 ± 12	92.3 ± 0.2	35.0 ± 0.0	10.9 ± 1.0	53.2 ± 2.3	0.3 ± 0.0 (371 \pm 9)	1.7 ± 0.0	0.2 ± 0.0
Chronic exposure										
Normocapnia	16.4 ± 0.0	7.93 ± 0.06	1900 ± 300	90.7 ± 0.2	35.0 ± 0.0	10.8 ± 0.2	52.4 ± 1.9	0.3 ± 0.0 (437 \pm 11)	1.7 ± 0.2	0.1 ± 0.0
Hypercapnia	16.7 ± 1.1	7.36 ± 0.05	1980 ± 60	90.1 ± 0.6	35.0 ± 0.0	11.0 ± 0.1	53.5 ± 2.0	1.4 ± 0.0 (1904 \pm 22)	1.9 ± 0.0	0.0 ± 0.0

Sampling

- After the acute experiment and at termination of the chronic experiment the sharks were removed
- Heads (eyes) covered with a soaked cloth
- Head and tail held tight by hand
- 1 ml of blood immediately withdrawn from the caudal vein and animals returned to the tank
- Animals from the chronic experiment sacrificed
- Skin samples taken dorso-laterally next to the dorsal fin and frozen

Analysis of denticles

- Scanning of shark skin and denticles by SEM
- Ratios of damaged and intact denticles quantified by counting

Blood acid-base balance

- Blood pH measured within 20 s after sampling using a micro pH electrode
- A blood subsample injected into a de-gassing chamber
- Handerson Hasselbalch equation (I and II – derivations) for measuring pH and $c\text{CO}_2$ values, $p\text{CO}_2$, and HCO_3^-

$$p\text{CO}_2 = \frac{c\text{CO}_2}{10^{pH-pK^{\text{H}}} \times \alpha\text{CO}_2 + \alpha\text{CO}_2}$$

$$\text{HCO}_3^- = c\text{CO}_2 - \alpha\text{CO}_2 \times p\text{CO}_2$$

- Ca^{2+} and Mg^{2+} concentrations determined spectrophotometrically by commercial kits

Haematocrit

- Subsamples of blood transferred for measurement of haematocrit
- The vessels were closed and the samples shaken
- Subsequently quantified using a Micro Haematocrit reader

RESULTS

Blood acid-base balance during acute hypercapnia

- Normocapnic control group:
 - No significant changes in pH
 - $c\text{CO}_2$ levels in a narrow range of between 5 and 6 mmol l^{-1}
 - Calculated values of $\text{HCO}_3^- + \text{CO}_3^{2-}$ and $p\text{CO}_2$ showed very little change
- Hypercapnic treatment:
 - Extracellular pH increased by 0.1 units in the first 3h
 - After 24h there was an over-compensation of pH by approximately 0.15 pH units
 - pH increased by another 0.05 units during the subsequent 8h of recovery in normocapnic seawater
 - The extracellular $c\text{CO}_2$ levels rose by 114% during 24h hypercapnic exposure, after a normocapnic recovery there was a sharp decline by 58%
 - The values for $\text{HCO}_3^- + \text{CO}_3^{2-}$ followed a similar trend

Exposure time	n	pH	cCO ₂	pCO ₂		[HCO ₃ ⁻ + CO ₃ ²⁻]	Ca ²⁺ mmol.l ⁻¹	Mg ²⁺ mmol.l ⁻¹
			mmol.l ⁻¹	Torr	(kPa)	mmol.l ⁻¹		
Normocapnia (h)								
0	5	7.90 ± 0.03	5.3 ± 0.6	1.5 ± 0.1	(0.2 ± 0.0)	5.2 ± 0.6	7.9 ± 0.9	3.5 ± 0.2
1.5	5	7.85 ± 0.07	5.0 ± 0.5	2.1 ± 0.5	(0.2 ± 0.0)	4.9 ± 0.5	7.7 ± 1.6	3.4 ± 0.1
3	5	7.82 ± 0.06	5.1 ± 0.9	1.7 ± 0.6	(0.2 ± 0.1)	5.0 ± 0.9	7.5 ± 0.7	3.3 ± 0.3
6	5	7.86 ± 0.27	5.5 ± 1.9	1.7 ± 0.5	(0.2 ± 0.1)	5.4 ± 1.9	7.8 ± 1.1	3.4 ± 0.3
24	5	7.96 ± 0.04	6.0 ± 1.2	1.5 ± 0.3	(0.2 ± 0.0)	5.9 ± 1.2	8.2 ± 1.0	3.6 ± 0.4
32 (Recovery)	5	7.94 ± 0.03	5.2 ± 0.5	1.3 ± 0.2	(0.2 ± 0.0)	5.1 ± 0.5	8.1 ± 0.8	3.4 ± 0.3
Hypercapnia (h)								
0	6	7.76 ± 0.10	6.3 ± 0.7	2.4 ± 0.4	(0.3 ± 0.1)	6.2 ± 0.7	7.5 ± 0.9	3.2 ± 0.3
1.5	6	7.83 ± 0.03*	10.6 ± 1.1*	3.4 ± 0.2*	(0.4 ± 0.0)	10.5 ± 1.1*	7.4 ± 1.5	3.2 ± 0.1
3	6	7.86 ± 0.02*	11.0 ± 1.1*	3.3 ± 0.3*	(0.4 ± 0.0)	10.9 ± 1.1*	6.6 ± 1.0	3.2 ± 0.3
6	6	7.84 ± 0.04*	10.0 ± 0.5*	3.1 ± 0.2*	(0.4 ± 0.0)	9.9 ± 0.5*	7.2 ± 1.2	3.3 ± 0.1
24	6	7.91 ± 0.02*	13.5 ± 1.6*	3.6 ± 0.4*	(0.5 ± 0.1)	13.3 ± 1.6*	8.9 ± 0.8	3.6 ± 0.2
32 (Recovery)	6	7.96 ± 0.04*	5.7 ± 0.5	1.4 ± 0.20	(0.2 ± 0.0)	5.6 ± 0.5	8.0 ± 1.6	3.3 ± 0.2

Acid-base balance and other blood parameters during chronic hypercapnia

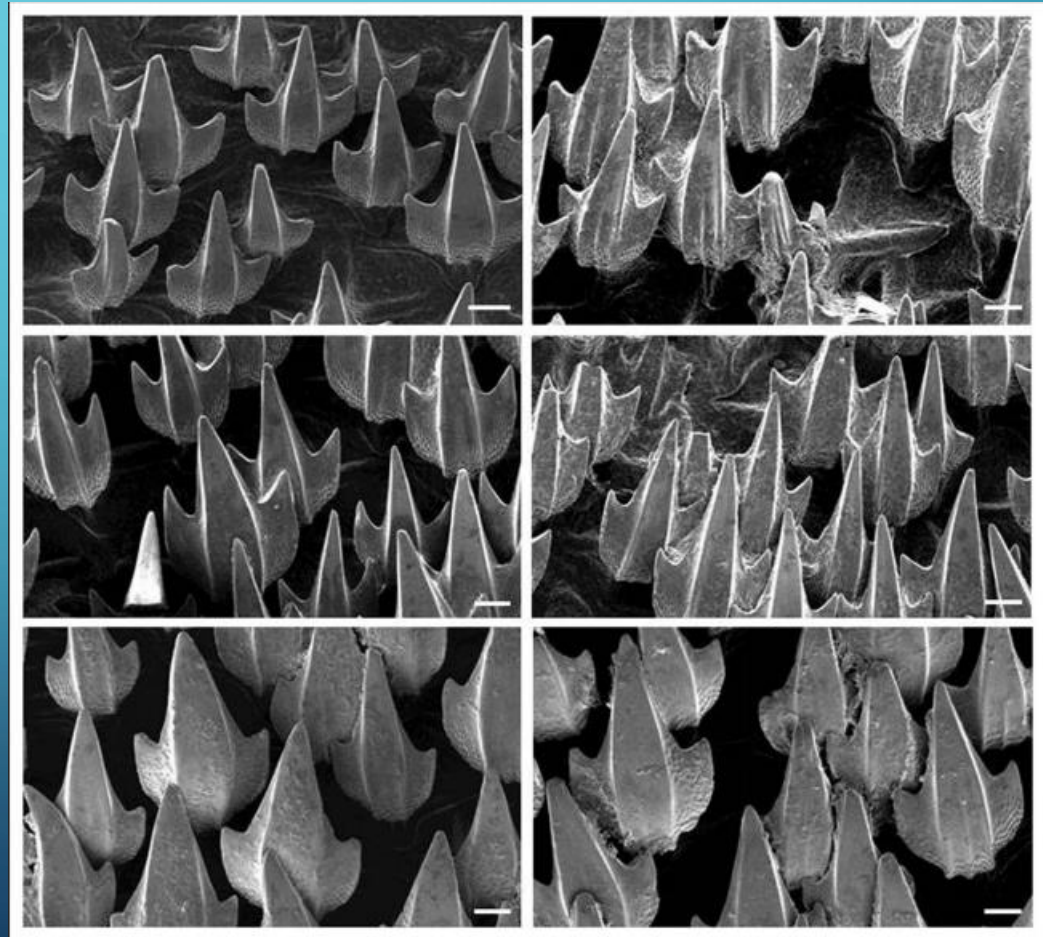
- The pH levels measured in the blood of hypercapnic and normocapnic incubated sharks were identical
- cCO₂ had approximately doubled to 8.3 mM under hypercapnia compared to normocapnia

Exposure time	n	pH	cCO ₂ mmol·l ⁻¹	pCO ₂		[HCO ₃ ⁻ + CO ₃ ²⁻] mmol·l ⁻¹	Ca ²⁺ mmol·l ⁻¹	Mg ²⁺ mmol·l ⁻¹	Haematocrit %
				Torr	(kPa)				
Normocapnia (h)	7	7.87 ± 0.04	4.3* ± 0.2	1.1 ± 0.2	(0.1 ± 0.0)	4.2* ± 0.2	8.1 ± 0.8	3.6 ± 0.1	30 ± 5
Hypercapnia (h)	6	7.88 ± 0.08	8.3 ± 0.9	2.2 ± 0.7	(0.3 ± 0.1)	8.1 ± 0.9	8.8 ± 0.8	3.6 ± 0.2	27 ± 3

- Elevation of pCO₂ and HCO₃⁻ + CO₃²⁻ caused a vetical shift in hypercapnic sharks, indicating bicarbonate buffering that results in slight alkalosis

Analysis of denticles following chronic hypercapnia

- Denticles from normocapnic sharks were mostly intact and had a shiny surface with sharp edges
- Many denticles from hypercapnic sharks were damaged and their surface appears corroded and edges less sharp
- Quantitative analysis of denticles from SEM micrographs revealed that significantly less denticles were damaged (pieces broken off) on normocapnic sharks than on hypercapnic ones
- Elemental composition of denticles from both treatments revealed some significant differences:
 - I. Two of the elements that form fluoroapatite and hydroxyapatite, Ca and P, have a lower proportion in denticles from hypercapnic sharks
 - II. The proportions of C and O are elevated



Impact of chronic hypercapnia on body size

- Sharks from chronic normocapnic treatment:
 - Total length increase → 0.5%
 - Mass increase → 6.2%
- Sharks from hypercapnic treatment:
 - Total length relatively stable
 - Mass increase → 0.7%
- Nothing significant

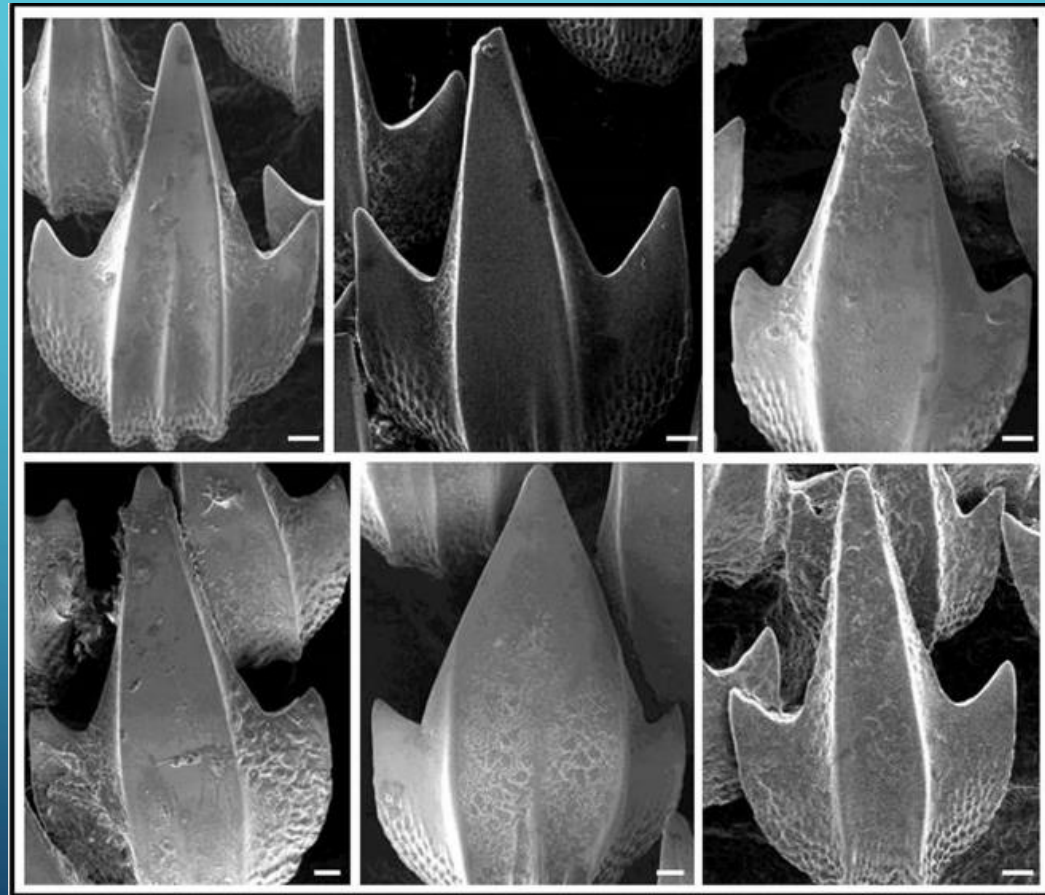
DISCUSSION

- *Main findings:*

1. *Haploblepharus edwardsii* adjusts well physiologically to acute hypercapnia
 2. This regulation can be maintained during chronic hypercapnic exposure
 3. The prolonged regulation is likely to be energetically costly but in the present study no significant depression of somatic growth was observed
 4. Although the sharks can maintain their acid-base balance, prolonged exposure to hypercapnia has detrimental chemical effects that cannot be compensated (denticles' surface)
- Compensation mechanisms exist in the form of elevation of bicarbonate levels to return pH to the original values
 - The observed effects on denticles are a result of chemical dissolution
 - Shark denticles have been attributed a number of different functions (protection against skin abrasions during hunting and mating, improvement of hydrodynamics)

CONCLUSION

- *H. edwardsii* is well adapted to hypercapnic conditions due to coastal upwelling and subsequent low-oxygen events
- Negative consequences on denticles during hypercapnia
- Hydrodynamics and skin protection could be compromised
- Chondrichthyans are particularly susceptible to ocean acification on denticles and additional studies are needed to elucidate the extent of this effect



The background is a blue gradient with faint concentric circles. White circuit-like lines with circular nodes are positioned in the corners: top-left, top-right, bottom-left, and bottom-right.

THANK YOU FOR LISTENING!