The Effects of High CO_2 and Elevated Temperature on Intestinal Physiology, Ichthyocarbonate Production and Dissolution in the Gulf Toadfish (*Opsanus beta*)

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Introduction

- Teleost fish form carbonate precipitates in the intestine as an osmoregulatory process that allows them to stay hydrated (1)
- Teleosts comprise 3-15% of the marine inorganic carbon cycle, with predictions that their contribution will increase with climate change (2)
- The fate of teleost carbonates upon excretion under climate change conditions can have implications for global carbon cycling (3)
- **Hypotheses:**
- We hypothesized that Gulf toadfish would exhibit no change in carbonate production when acclimated to high CO_2 but would increase production with high temperature
- We also hypothesized that there would be increased carbonate dissolution under high CO_2 and high temperature
- This was the first study to examine the combined effects of high CO_2 and high temperature, and we predicted that both production and dissolution would increase

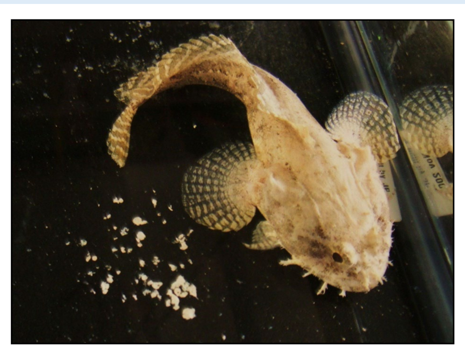

Figure 1: The Gulf toadfish and excreted carbonate precipitates

Methods

Carbonate Production and Intestinal Physiology

- Toadfish were acclimated in one of four treatments:

Control 410 µatm CO_2; 26-28°C	**High CO_2 (HCO2)** 1900 µatm CO_2; 26-28°C
High Temperature (HT) 410 µatm CO_2; 32°C	**High CO_2 and Temperature (HCO2xHT)** 1900 µatm CO_2; 32°C

Figure 2: Each treatment in the production experiment and their target CO_2 concentrations and temperatures

- Trial 1 ran for 14-20 days and Trial 2 ran for 26 days
- Following acclimation, the following endpoints were measured:
- Carbonates and intestinal fluid were removed from the intestine and weighed, and fluid was removed to obtain the weight of the carbonates
- The pH, osmolality, and total CO_2 of the intestinal fluid was measured

Dissolution

- Carbonates were collected from the tank bottoms and used in pH stat titrations to obtain the mass-specific dissolution rates

Control 410 µatm CO_2; 28°C	**HCO2** 1754 µatm CO_2; 28°C
HT 410 µatm CO_2; 32°C	**HCO2xHT** 1754 µatm CO_2; 32°C

Figure 3: Conditions used for the dissolution experiment, including CO_2 concentrations and temperatures

Results

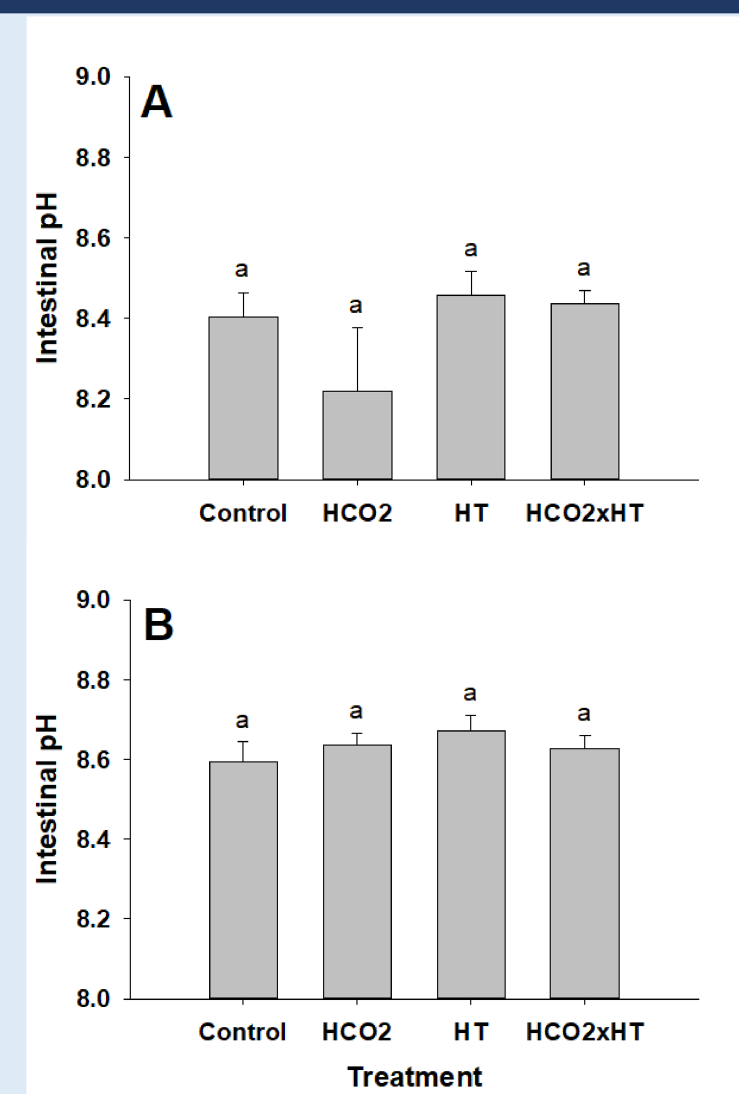

Figure 4: Average pH of intestinal fluid in trial 1 (A) and trial 2 (B)

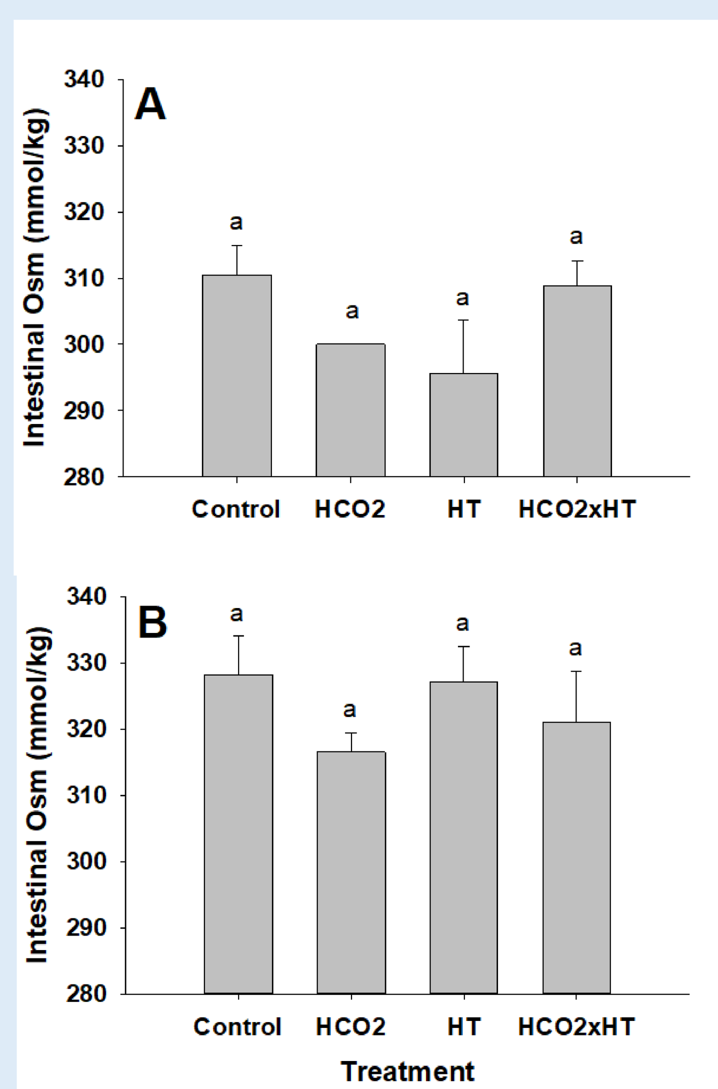

Figure 5: Average osmolality of intestinal fluid in trial 1 (A) and trial 2 (B)

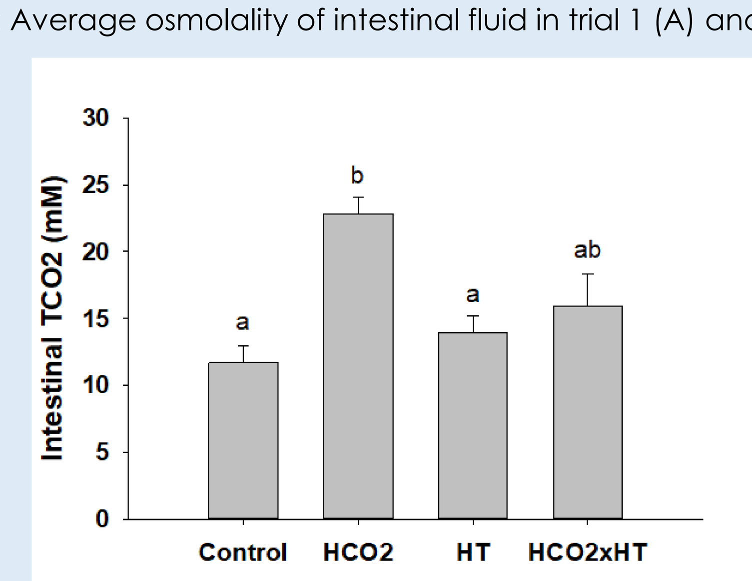

Figure 6: Average total CO_2 of the intestinal fluid in trial 2

Results

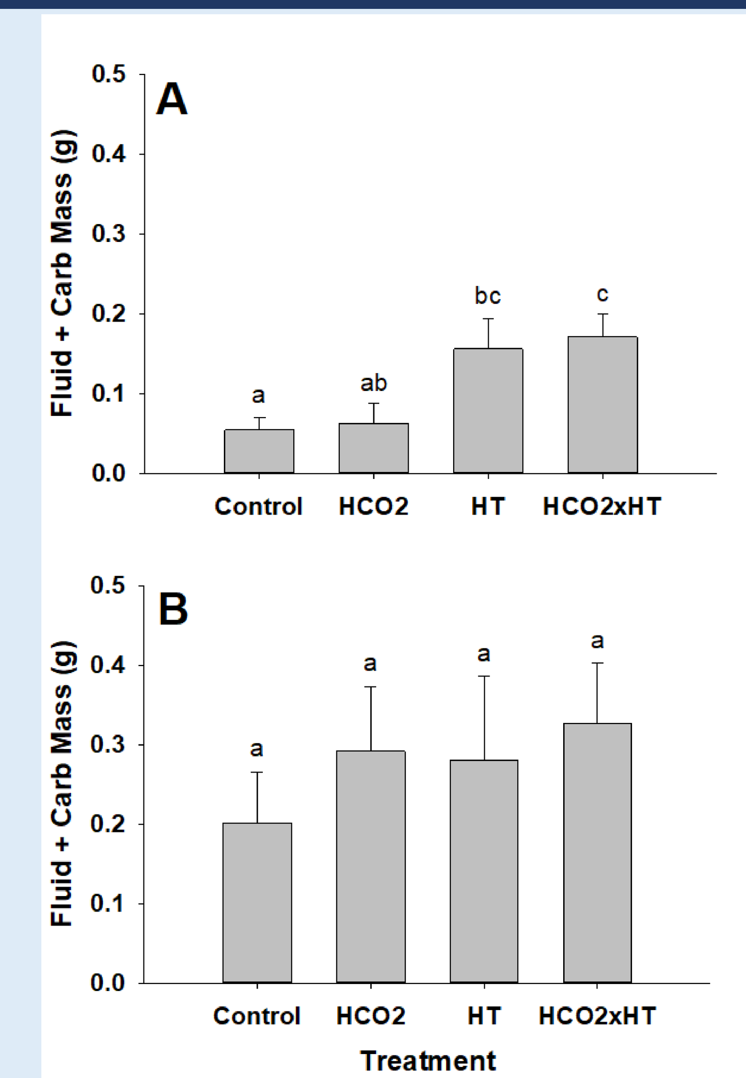

Figure 7: Average mass of intestinal fluid and carbonates in trial 1 (A) and trial 2 (B)

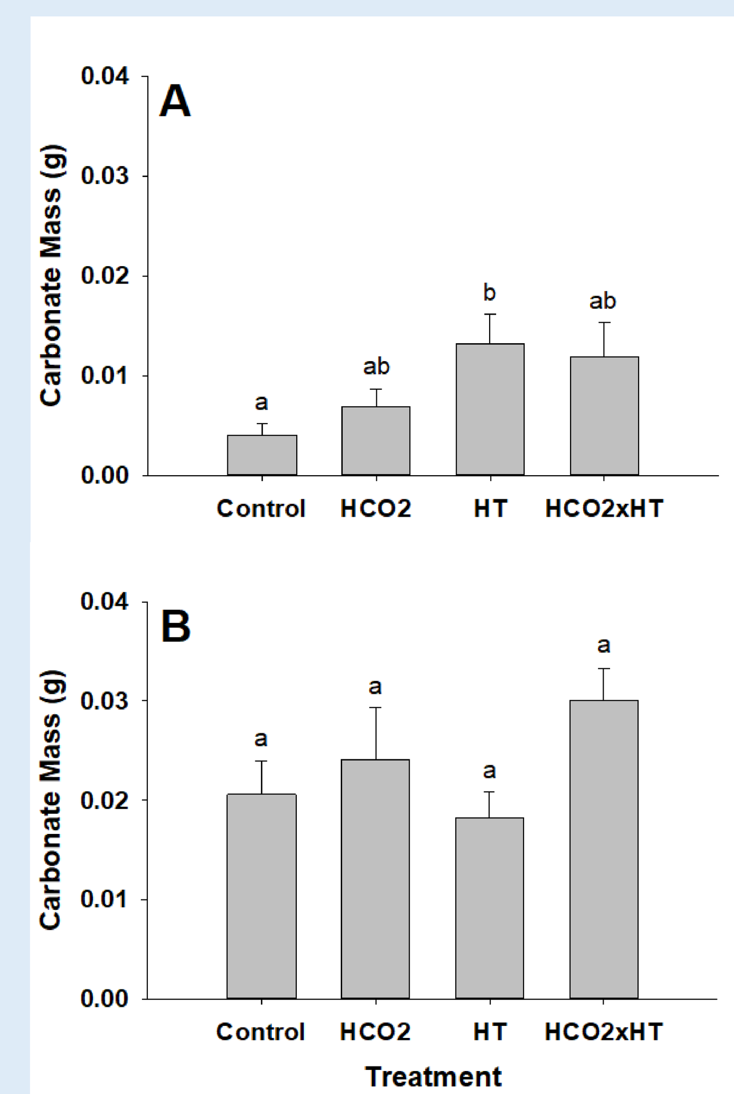

Figure 8: Average mass of carbonates in trial 1 (A) and trial 2 (B)

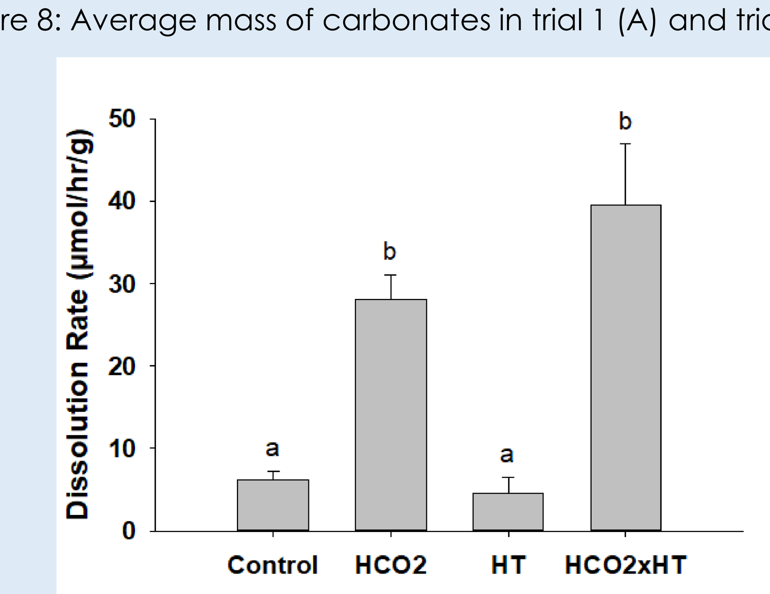

Figure 9: Average dissolution rate

Discussion

Intestinal Physiology

- There was a slight trend for a decrease in osmolality in the HCO2 group (4,5)
- The significant increase in total CO_2 in the HCO2 group compared to the control may indicate increased HCO_3^- secretion
- None of the metrics suggest that the HCO2xHT group displays evidence of interactive effects

Carbonate Production

- There was no significant increase in carbonate production in the HCO2 group (4,5)
- There was some evidence that temperature increased carbonate production in the intestine (Trial 1) which could be based on increased drinking rate associated with increased metabolic rate (2,5)
- Trial 2 HT production was not significantly different from other treatments, and the reason behind this different result is unknown
- There was no evidence of interactive effects on production in the HCO2xHT group

Dissolution

- Dissolution rate did not significantly change in HT
- Dissolution was significantly higher in HCO2 and HCO2xHT groups, and there was a trend to suggest interactive effects for dissolution rate
- Under future climate change conditions, carbonates could dissolve more rapidly, raising the saturation state in near surface waters and potentially making it easier for calcifying organisms to maintain their carbonate skeletons (6)

Future Directions

- Increasing the sample size and running more trials would confirm if trends could have some validity
- Examining the activity or expression of other transporters in the intestinal epithelium under the same conditions could aid in understanding physiological mechanisms
- Differences between trials could potentially be reduced if future studies select fish of similar sizes, sample fish the same number of days post feeding, and control flowthrough seawater temperatures more tightly
- Results from this study suggest that ichthyocarbonates may be of increasing importance to the marine inorganic carbon cycle in future climate change conditions

References

(1) Whittamore et al. 2010, (2) Wilson et al. 2009, (3) Orr et al. 2005, (4) Heuer et al. 2012, (5) Heuer et al. 2016, (6) Salter et al. 2019

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