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The Effects of Chronic Hypercarbia on Morphological and Ventilatory Development in Crayfish

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Background

Instances of abnormally high CO_2 levels are becoming increasingly common in freshwater ecosystems undergoing eutrophication. Chronic hypercarbia (long-term elevation of the partial pressure of carbon dioxide (Pco_2)) is pervasive in these eutrophic ecosystems.

Elevated Pco_2 increases the ventilation and metabolism of some tadpoles and aquatic frogs and hinders their morphological development[2,3]. We expect that chronic hypercarbia will affect most aquatic organisms in the same way, but it remains to be verified that chronic hypercarbic conditions also alter aquatic organisms similarly to intermittent and acute hypercarbic challenges.

Hypothesis

Chronic hypercarbia hinders morphological development and alters the development of ventilatory responses to ⬆Pco_2.

Methodology

Freshwater crayfish (*Procambarus clarkii*) were reared in chronic hypercarbic or normocarbic water. Chronic hypercarbia was achieved by equilibrating the water with 5% CO_2 (21% O_2, balance N_2) and quantified by measuring pH.

Crayfish were fed ad libitum to maximize growth. Weekly morphological measurements included body mass, length from the rostrum to the tip of the telson, and width across the carapace. Ventilatory and cardiovascular measurements were taken in randomly chosen individuals using impedance electrodes surgically placed within their carapace[1]. During ventilatory measurements, the water was equilibrated with the following: 0%, 2.5%, 5%, 8%, 12%, and 0% CO_2 (21% O_2, balance N_2 for all).

References

1. Bierbower, S.M., Cooper, R.L. 2009. Measures of Heart and Ventilatory Rates in Freely Moving Crayfish. Journal of Visualized Experiments 32: 1594.
2. Fonseca, E.M., da Silva, G.S.F., Fernandes, M., Giusti, H., Noronha-de-Souza, C.R., Glass, M.L., Bícego, K.C., Gargaglioni, L.H. 2012. The breathing pattern and the ventilatory response to aquatic and aerial hypoxia and hypercarbia in the frog *Pipa carvolhoi*. Comparative Biochemistry and Physiology 162: 281-287.
3. Simard, E., Trépanier, G., Larochelle, J. 2003. Intermittent hypoxia and plasticity of respiratory chemoreflexes in metamorphic bullfrog tadpoles. Respiratory Physiology & Neurobiology 135: 59-72.

Morphological Results

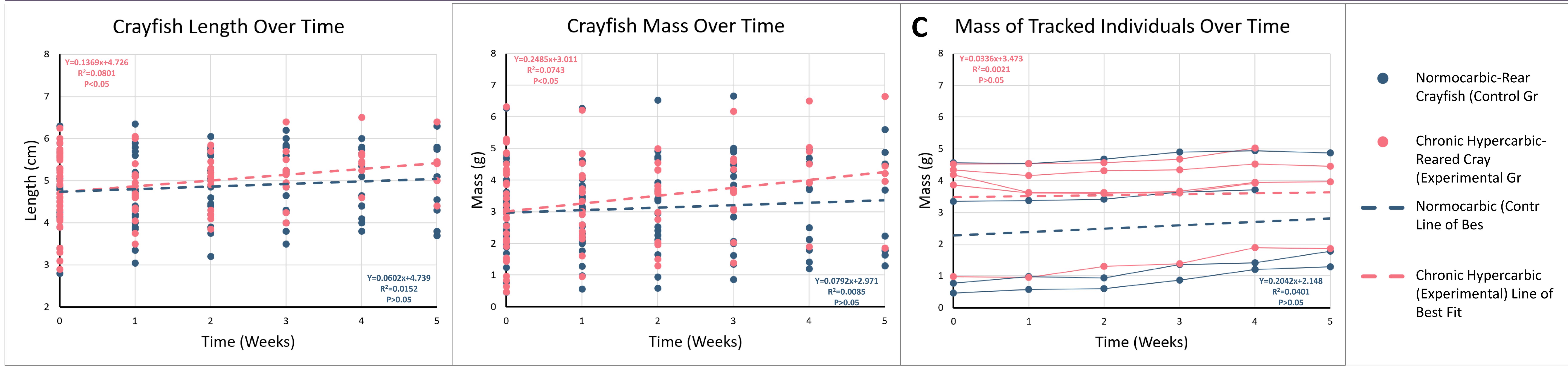

Figure 1. (A) Body length increases over 5 weeks of development in crayfish reared in chronic hypercarbia (5% CO_2) (P = 0.01; linear regression) but not in crayfish reared in normocarbia (P = 0.20). Further, there is no significance between the two slopes (P = 0.27). **(B)** Body mass increases over 5 weeks of development in crayfish reared in chronic hypercarbia (5% CO_2) (P = 0.01) but not in crayfish reared in normocarbia (P = 0.33). Further, there is no significance between the two slopes (P = 0.18). **(C)** For tracked individuals, there was no effect on body mass across 5 weeks of development by chronic hypercarbia (5% CO_2) (P = 0.82) nor by normocarbia (P = 0.35). Further, there is no significance between the two slopes (P = 0.50).

Ventilatory and Cardiovascular Measurements in Progress

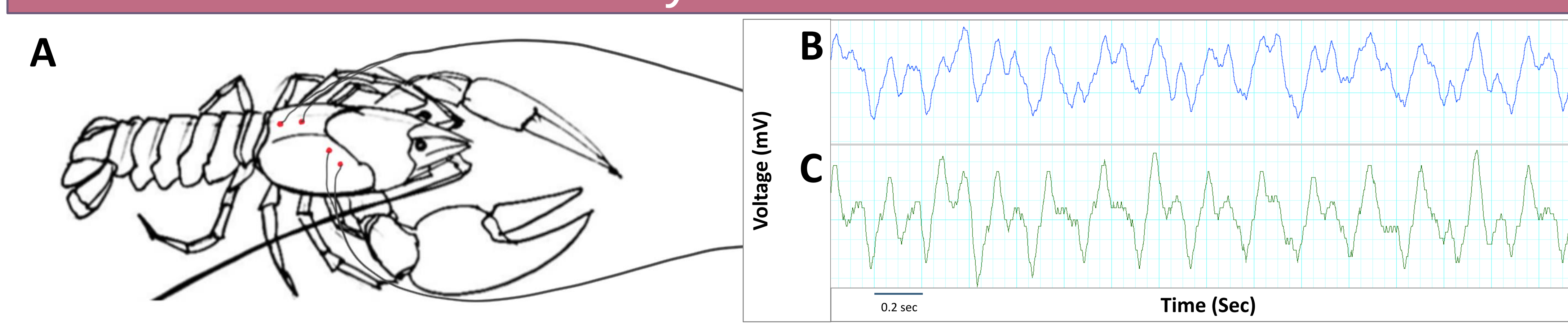

Figure 2. (A) Cartoon showing placement of electrodes inserted for recording an electrocardiogram **(B)** to quantify heart rate and an electromyogram **(C)** to record scaphognathite beating frequency. This is a sample trace only; measurements are currently in progress.

Mortality Results

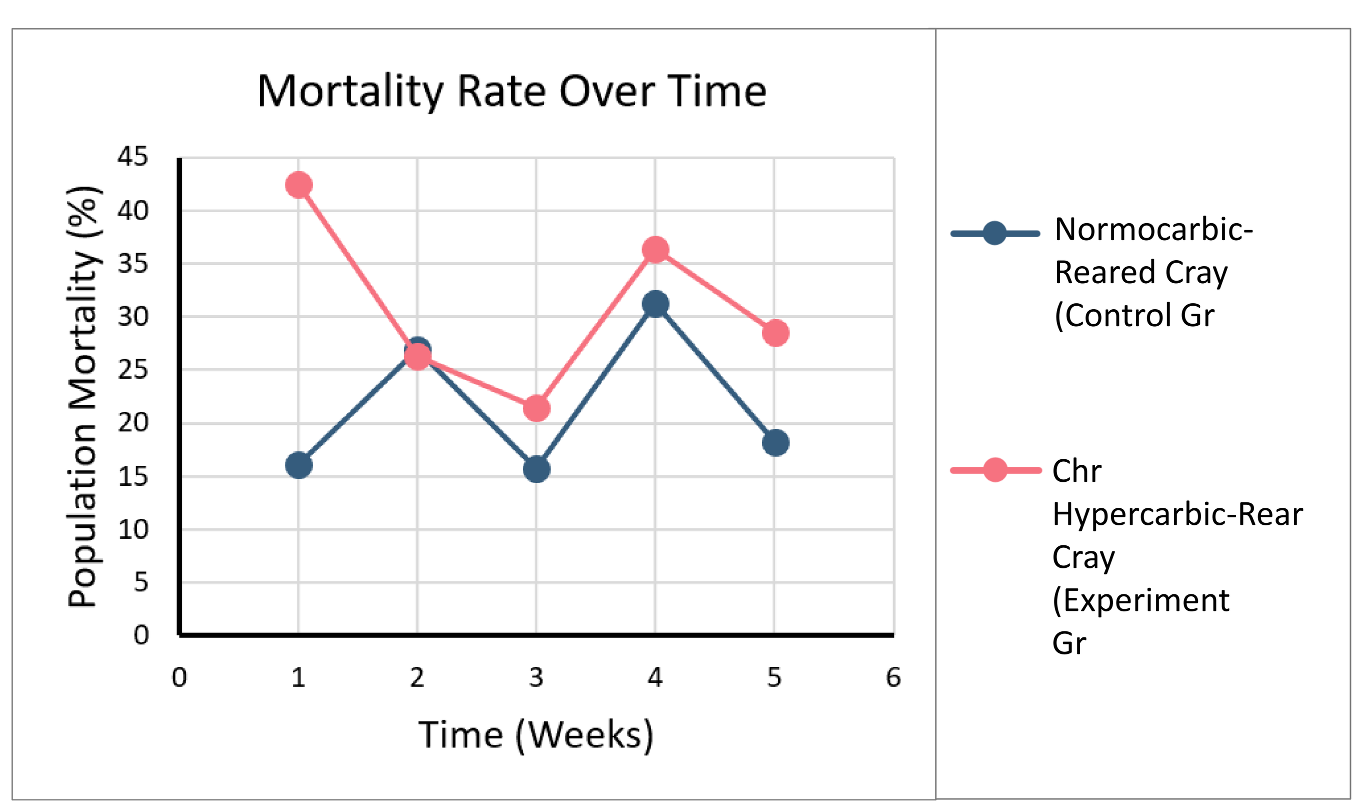

Figure 3. Weekly mortality rates for normocarbia-reared and chronic hypercarbic-reared crayfish. Qualitative observations suggested a higher mortality rate of small crayfish in the experimental group. A chi-squared test indicated that first-week mortality rates differed significantly between the control and experimental groups (**$^{**}P$ = 0.01**).

Discussion

- Morphometric data (Fig. 1) show no effect of chronic hypercarbia within five weeks of exposure.
- An initial high mortality rate (Fig. 3) in the first week of chronic hypercarbia confounds our ability to quantify the effects of chronic hypercarbia on growth rates. This is pertinent to ecologists concerned with environmental impacts on aquatic species' survival and aquaculturists' goal to maximize growth.

Questions Driving Future Directions

- Could slower metabolic rates be a physiological response to chronic hypercarbia, mitigating its apparent effects on morphological development?
- How does chronic hypercarbia affect the development of human fetuses when mothers smoke or live in areas of heavy air pollution?
- Do fetuses exposed to chronic hypercarbia also have higher mortality rates?

Acknowledgements

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