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Effects of Rapid pH and Salinity Change on the Physiology of a Local Estuarine Fish Species, Fundulus heteroclitus

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Effects of varying pH and salinity on the physiology of an estuarine fish species, *Fundulus heteroclitus heteroclitus*

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Introduction

- *Fundulus heteroclitus* (Atlantic Killifish or Mummichog) have evolved coping strategies that allow them to withstand and thrive in highly variable environments.
- In laboratory experiments, *F. heteroclitus* have shown the ability to tolerate wide ranges of salinity (Griffith, 1974) and pH (Picard & Schulte, 2004).
- Sapelo Island, Georgia offers a unique environment to study coping strategies of *F. heteroclitus* to multiple environmental stressors as they inhabit streams and ponds with variable salinity (1–25 ppt) and pH (4.5 -7).
- *F. heteroclitus* show increases in plasma cortisol levels when exposed to altered salinity levels (Marshall et al., 1999).
- Changes in expression patterns of ion transport protein encoding genes in gills of *F. heteroclitus* occur starting at 24 hours post salinity change (Scott et al., 2004).

Objective and Hypotheses

Objective: Tease apart the individual and multistressor effects of low salinity and low pH on the physiology of *F. heteroclitus* in a controlled, lab based study.

Hypothesis 1: Plasma cortisol levels will increase in response to salinity and pH stress (individually). Fish exposed to the multistressor treatment of low pH and low salinity will exhibit a synergistic increase in cortisol compared to individual stressor treatments.

Hypothesis 2: Ion transport genes will show altered expression patterns in response to salinity and pH stress (individually). Multistressor treatments will have additive effects on gene regulation. See analysis table for specific gene predictions.

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Experimental Design

Treatment 1 Control	Treatment 2 pH Stressor	Treatment 3 Salinity Stressor	Treatment 4 Multistressor
Neutral pH (6.8)	Neutral pH (6.8)	Low pH (4.5)	Low pH (4.5)
Moderate Salinity (15-16 ppt)	Low Salinity (1-2 ppt)	Moderate Salinity (15-16 ppt)	Low Salinity (1-2 ppt)

- All fish were caught on Sapelo Island and transported to Georgia Southern University
- Fish were acclimated to control treatment conditions that replicated capture site salinity and pH for 30 days before being transferred to experimental conditions
- 3 tanks/treatment
- 6 fish sampled per treatment (2/tank) on Day 1, 5, and 7 post transfer
- Gills collected for gene expression analysis
- Whole body instantly frozen in liquid nitrogen for cortisol analysis
- Cortisol levels determined via Enzyme Linked Imnnunosorbant Assay (ELISA)

Results

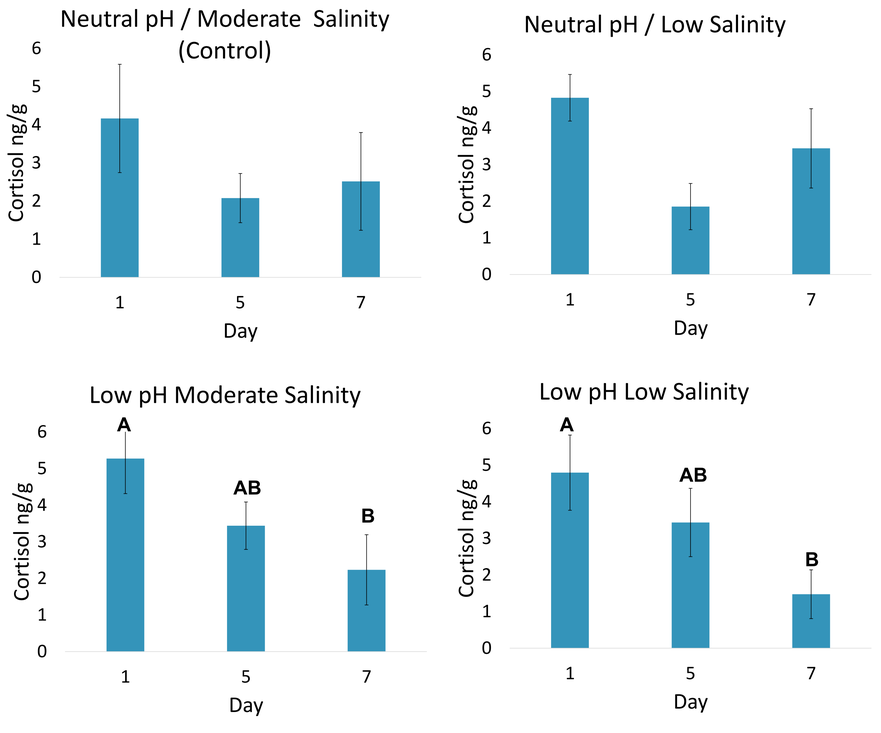

Figure 1. Effect of varying salinity and/or pH on whole body cortisol (ng/g) in *F. heteroclitus*. Data presented as mean ± SE (n=6). Differing letters represent significant differences between sample days within treatment. P ≤ 0.05 considered significant.

Upcoming Analyses

RNA extracted from gill tissue and cDNA synthesized for gene expression analysis using QRT-PCR.

Gene	Function	Expression Prediction
Na^+-K^+-ATPase	Sodium-potassium pump-pumps sodium out of cells and potassium into cells	• Upregulation in low salinity, neutral pH • Highest upregulation in low salinity and low pH
CFTR	Channel for movement of chloride ions in and out of cells	• Downregulation in low salinity, neutral pH • Highest downregulation in low salinity and low pH
EF1α	Involved in elongation, used as a house-keeping gene because it is unaffected by salinity or pH challenges	• Stay constant in all treatments

Conclusions

- There was a significant difference in whole body cortisol levels between Days 1 and 7 in the two treatments with low pH
- Our results suggest that environments with low pH (4.5) may be more favorable than environments with neutral pH
- Our results also suggest that low salinity was not stressful to the fish given there was no difference in cortisol levels with variation in salinity

Significance

The information gained from this project will provide a better understanding of the stress this species exhibits due to rapid salinity change as well as whether fish can rapidly adapt to changing conditions. Given the ubiquity of the common killifish, and its importance in estuarine food webs, these data may provide insight into how we can manage and maintain diverse fish populations and support ecosystem functioning.

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