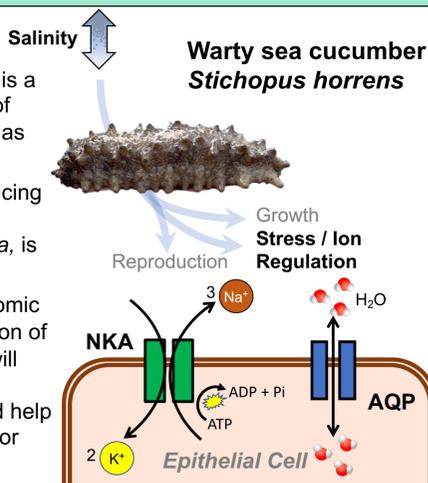


Introduction

Background

- In the state of Hawai'i, there is a rising interest in the culture of native sea cucumbers, such as *Stichopus horrens*.
- A major constraint to introducing and rearing *S. horrens* in Hawaiian fishponds or *loko ia*, is freshwater intrusion.
- Quantifying changes in coelomic fluid osmolality and expression of key ion regulatory proteins will elucidate this species' osmoregulatory capacity and help define tolerance thresholds for management.



Goal: Characterize the response of *S. horrens* to hyposmotic stress.

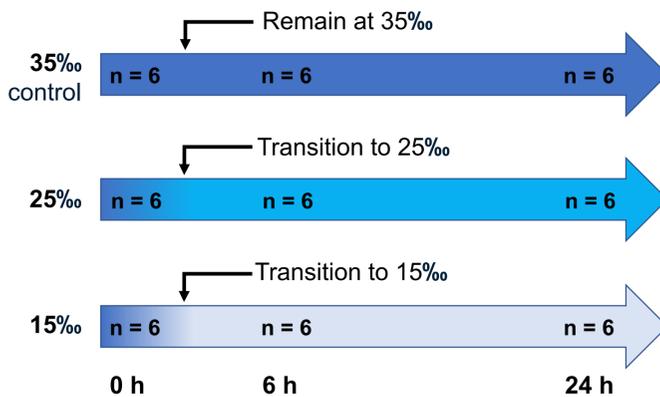
Objectives:

- To measure changes in coelomic fluid osmolality in response to a salinity challenge.
- To characterize the mechanism that allow *S. horrens* to tolerate osmotic stress.

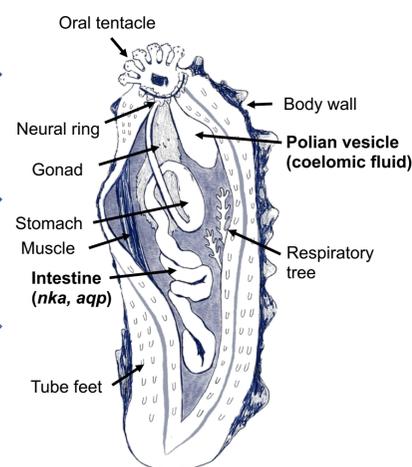
Hypothesis: Exposure to hyposmotic stress will decrease coelomic fluid osmolality, which will be accompanied by transcriptional changes of *nka* and *aqp* in the intestine.

Experimental Design

Salinity Challenge



Tissue Collection

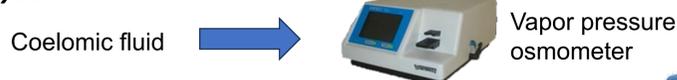


Tissues were snap frozen in liquid nitrogen and stored in a -80°C freezer until analysis.

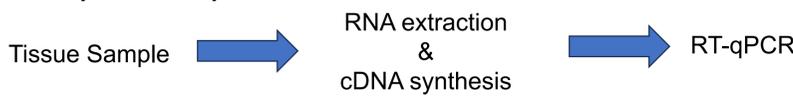
Abbreviations

NKA	Na ⁺ /K ⁺ -ATPase	AQP	Aquaporin
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Osmolality Analysis



Gene Expression Pipeline



Results

S. horrens Exhibit Capacity to Retain Ions During Salinity Challenge

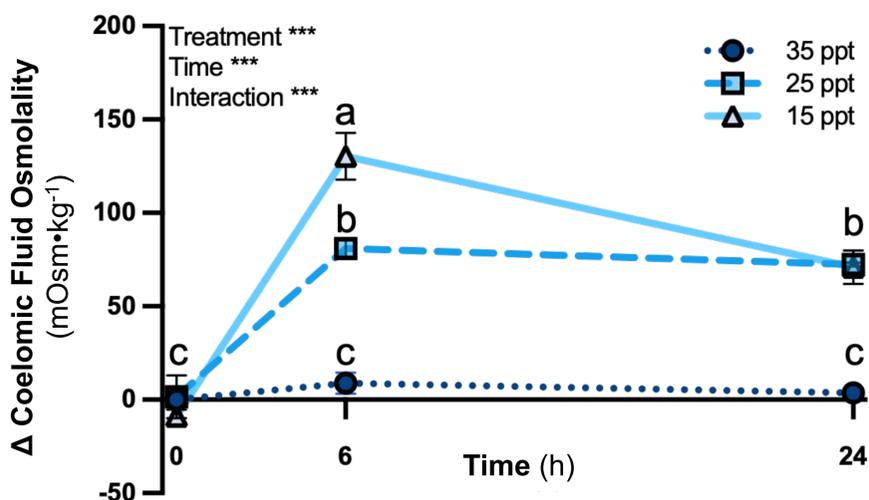


Figure 1: Changes in coelomic fluid osmolality during a salinity challenge. Mean difference compared to environmental osmolality ± S.E.M (n=6). A two-way ANOVA followed by Fisher's LSD. *** $P < 0.001$.

Males Exhibit Greater Ion Retention at Lower Salinities

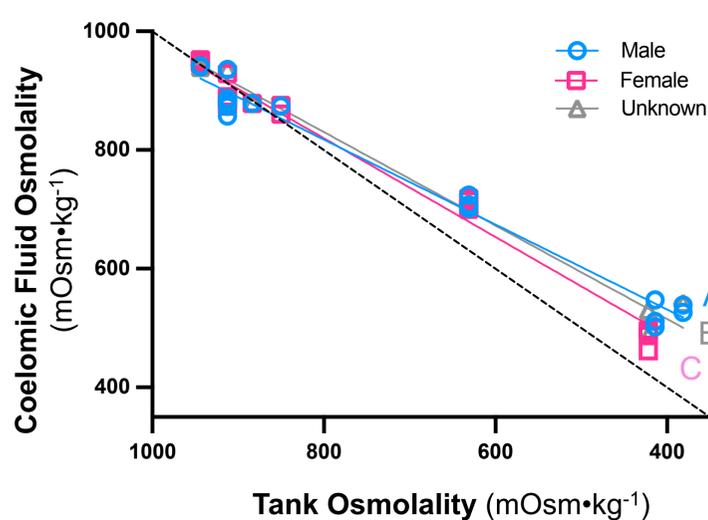


Figure 2: Internal and external osmolality correlations by sex. Slopes differed significantly among sexes (ANCOVA, $F_{2,145} = 5.39$, $p = 0.0080$).

Intestine Exhibits High *nka* Expression and Moderate *aqp* Expression

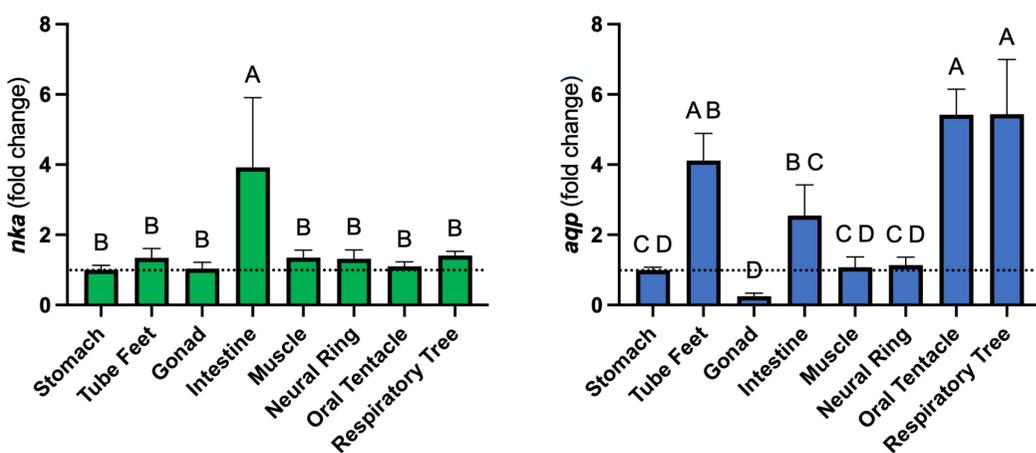


Figure 3: Tissue distribution analysis of *nka* and *aqp*. Bars represent mean fold-change ± S.E.M. relative to the stomach. One-way ANOVA followed by a Fisher's LSD test. Means that do not share a letter are different from one another (n = 9; $P < 0.05$).

Salinity Challenge Does Not Alter *nka* or *aqp* Expression in Intestines

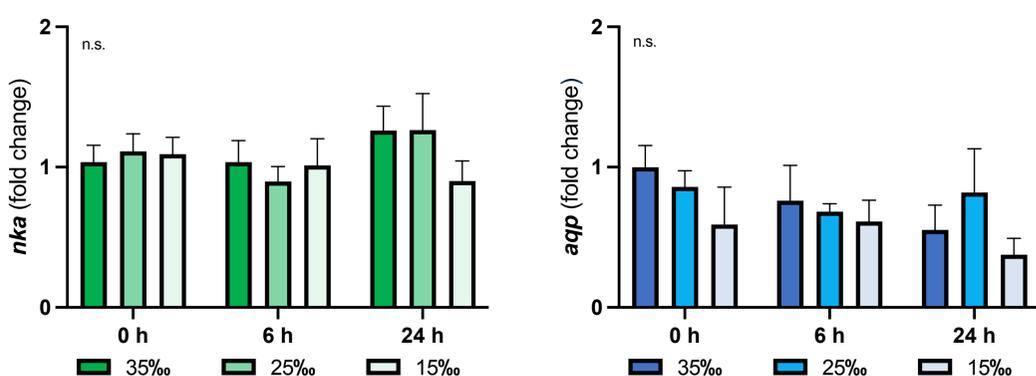


Figure 4: Intestinal expression of *nka* and *aqp* following a salinity challenge. Bars represent mean fold-change ± S.E.M. relative to the control group (35 ppt) at time 0 h. A two-way ANOVA followed by a Fisher's LSD test was used (n = 6).

Conclusion

- Stichopus horrens* partially regulates coelomic fluid osmolality under acute hyposmotic stress.
- No change in intestinal *nka* or *aqp* expression were detected.
- Regulation likely occurs via alternative pathways or in other tissues.
- These findings highlight *S. horrens* as a resilient candidate for aquaculture production.**

Next Steps

- Analyze expression of other ionoregulatory proteins.
- Investigate expression of target proteins in the respiratory tree, oral tentacles, and Polian vesicle.
- Determine combined impact of thermal and osmotic stress.

Acknowledgements

This work was funded in part by grants from the National Oceanic and Atmospheric Administration (NA14OAR4170071) which is sponsored by the University of Hawai'i Sea Grant College Program, SOEST (Projects YR 55 R/SS-38 and 57 R/SS-39), and the National Institute of Food and Agriculture Hatch no. HAW02051-H to A.P.S.