Environmental Stress and RNAi Effect on Osmoregulation of the Yellow Fever Mosquito, *Aedes aegypti*

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Abstract

Cation chloride cotransporters (CCCs) play an essential osmoregulatory role in the larval life stage of the yellow fever mosquito, *Aedes aegypti*. Three putative Na-coupled CCCs have previously been observed to be expressed in different digestive tissues such as the mid-gut and hindgut. One Na-coupled CCC, AAEL006180 (aeCCC1), is hypothesized to play a secretory role and found predominantly in Malpighian tubules. To determine the effect of a changing external environment on larval hemolymph I compared hemolymph ion concentration of larvae reared in tap water and 30% seawater. Larvae were reared in either tap water or 30% seawater and then transferred to the other salinity for 24 and 48 hours. Freshwater reared larvae experienced an approximately 30% increase and a six-fold increase in mean hemolymph sodium and ammonia, respectively. Seawater reared larvae experienced approximately a 35% decrease in mean hemolymph sodium within 24 hours of transfer and then rose back up to freshwater mean hemolymph sodium after 48 hours, and mean hemolymph ammonia significantly increased after 24 hours and then reduced back to initial concentrations. In both transfers there was not a significant change in potassium concentrations. I also used RNAi to reduce expression of the aeCCC1 transporter and measured hemolymph ion concentrations. I exposed first instar larvae to aeCCC1 dsRNA for 2 hours. Negative controls were no dsRNA and a dsRNA that had no sequence homology to *A. aegypti*. Larvae exposed to aeCCC1 dsRNA had a five-fold increase in mean hemolymph total ammonia, approximately a 20% decrease in mean hemolymph total potassium, and no consistent change in mean hemolymph sodium compared to negative controls. The process in which *A. aegypti* regulate hemolymph ion concentrations remains unknown, but the changes observed as a result of changes in environment and exposure to siRNA provide further insight in to possible osmoregulatory mechanisms.

Introduction

Background

- The yellow fever mosquito, *Aedes aegypti,* is the main vector for arboviral diseases yellow fever and dengue(2012).
- *A. aegypti* experience different osmotic stress larval and adult life stages. Larvae are freshwater obligates, but experience varying levels of ion concentrations. Adult mosquitoes experience osmotic stress when taking a blood meal.
- Mosquito larvae osmoregulate by transporting salt across the epithelia of the alimentary canal, especially the anal papillae and Malpighian tubules.
- The main function of Malpighian tubules is to secrete fluid containing ions from hemolymph to the lumen, but this secretion process must be able to adapt with the changing environment.
- Na-dependent cation-chloride cotransporters (CCC) are localized to the Malpighian tubules. They are found in other blood-sucking invertebrates as well as vertebrates. A CCC gene, AAEL006180 (aeCCC1), has previously been observed to be present in high quantities in larvae.
- We measured larvae hemolymph of larvae exposed to freshwater and 30% sea water for different time intervals and larvae exposed to dsRNA to knock down aeCCC1.

Hypotheses

- Larvae reared in freshwater and transferred to 30% sea water will experience an increase in mean sodium hemolymph concentration within 24 hours.
- Larvae reared and exposed to 30% sea water will have a higher mean ammonium hemolymph concentration.
- Larvae exposed to dsRNA will have increased amounts of sodium, potassium, and ammonium.

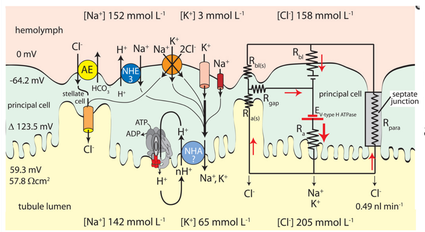

Figure 1. Beyenbach model of transepithelial NKCC in Malpighian tubule of adult female *A. aegypti*. Image adapted from Beyenbach and Piermarini, 2011.

Methods

Insects: Wild-type *Aedes aegypti* were raised from egg to adult stage as described (Pannabecker *et al.*, 1993). Eggs (Liverpool) were hatched under a vacuum chamber to induce low O_2. Larvae were raised in ~100 mL of freshwater or 30% seawater (Instaocean) at 28 °C, 80% RH and 12hr light/day diurnal cycle. Larvae were fed *ad libitum* with finely ground Total Goldfish® (Secaucus, New Jersey). After or until 4th instar, larvae were collected and their hemolymph was sampled.

Transfer: Larvae were reared as described above. 48 hours and 24 hours prior to hemolymph collection, freshwater larvae were transferred to 30% seawater and vice versa. Following transfer, larvae were raised the same as control group being fed once a day.

dsRNA Exposure: Larvae were hatched in freshwater and fed immediately following hatching. 48 hours after hatching, ~50 1st-2nd instar larvae were transferred to a 1.5 mL microcentrifuge tube. Excess water was carefully removed using a series of pipettes. Larvae were exposed to 100 µL of respective treatment (0.2-0.6 µg/µL DST7a-4 dsRNA, 0.2-0.6 µg/µL DST7b-3 dsRNA, 0.2-0.6 µg/µL negative control dsRNA, or dH_2O) for 2 hours and tubes were placed back in 28 °C, 80% RH.

Hemolymph Collection: Larvae were collected with a Pasteur pipette and placed on filter paper to absorb any external medium. The larva was placed on a parafilm under a compound microscope and its cuticle was punctured using fine tipped forceps. Hemolymph flowed out and 50.6 nL of hemolymph was collected using a glass capillary needle (diameter 50-70 µm) filled with mineral oil prior to being fitted on a Nanoject II microinjector (Drummond Scientific, Broomall, PA). The hemolymph was diluted (~3,000 fold) with 150 µL of deionized water. Concentrations of sodium, ammonium and potassium were measured with a Dionex 500-DX chromatography system (Sunnyvale, CA) fitted with a CS12a cation-exchange column, CSRS-Ultra 4-mm recyclable suppressor, and an AS50 autosampler with 25-µL injection loop (Bowles and Gillen, 2001). Samples were eluted with methanosulfonic acid (18 mM) at a 1-mL/min flow rate. Hemolymph ion concentrations were analyzed with Dionex PeakNet®-PA software v 5.21 (Dionex Corp., Sunnyvale, CA) (Akuma, unpublished).

Results

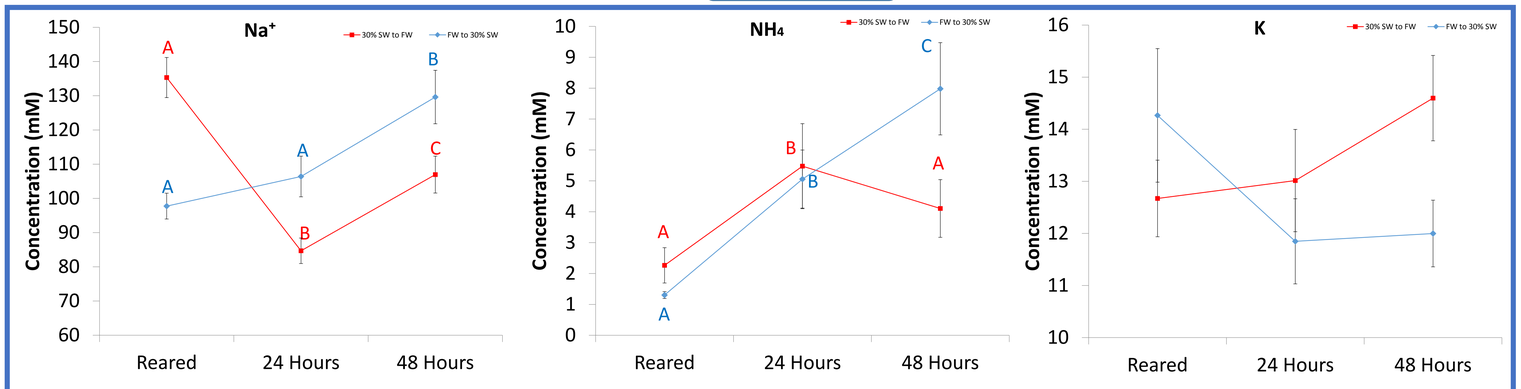

Figure 2. Hemolymph concentrations of sodium, ammonium, and potassium of 4th instar *A. aegypti* larvae. N(freshwater for Life) = 50. N(30% SW for Life)= 50. N(FW 24 Hours)= 28. N(30% SW 24 Hours)= 28. N(FW 48 Hours)= 28. N(30% SW 48 Hours)= 28. One-Way ANOVA with Tukey's Comparison. Error bars = SEM. Sodium (FW to 30% SW): P-value < 0.001. Sodium (30% SW to FW): P-Value < 0.001. Ammonium (FW to 30% SW): P-Value < 0.001. Ammonium (30% Sw to FW): P-Value < 0.05. Potassium (FW to 30% SW): P-Value > 0.05. Potassium (30% SW to FW): P-Value > 0.05. Within treatments, means that share a similar letter are not statistically significant.

Results

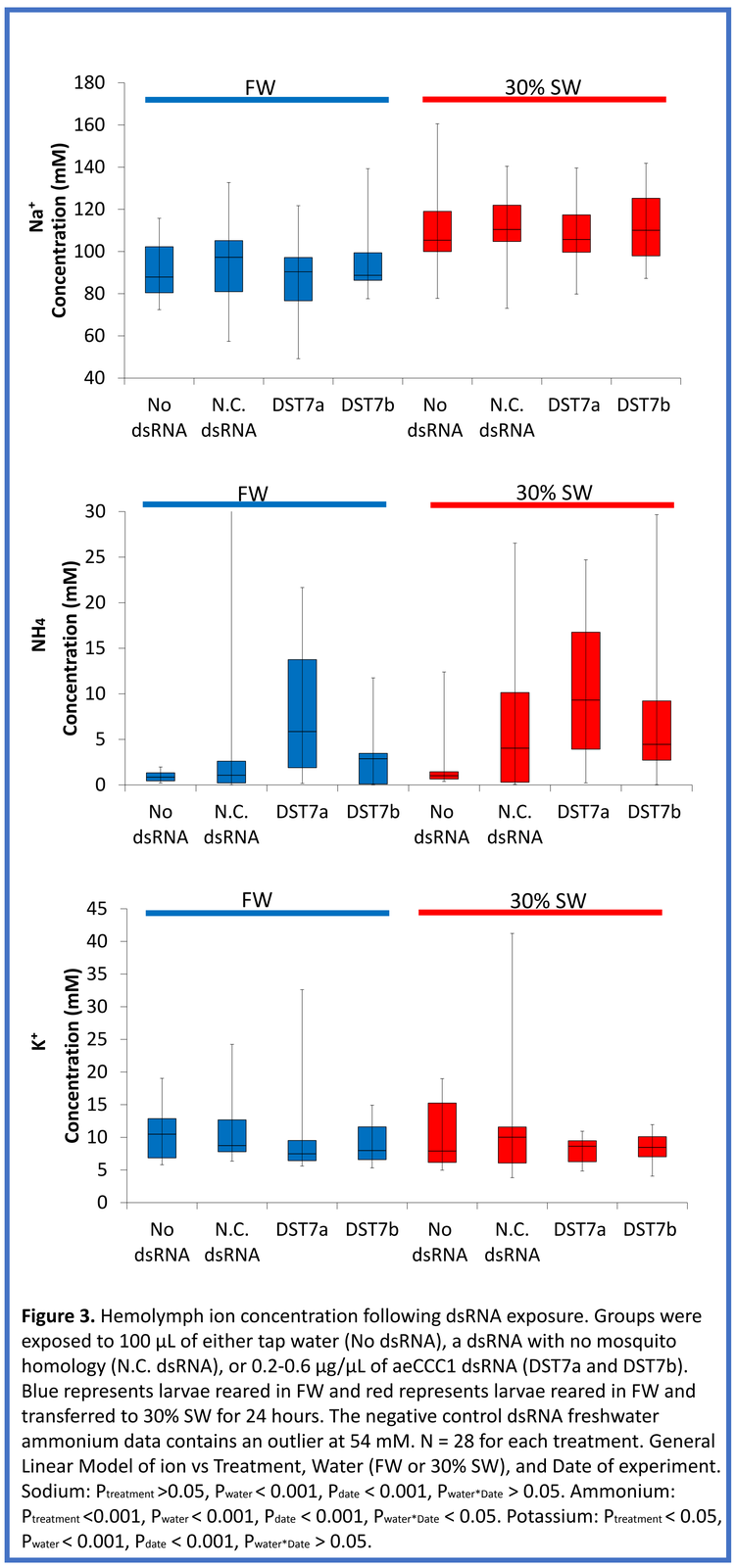

Figure 3. Hemolymph ion concentration following dsRNA exposure. Groups were exposed to 100 µL of either tap water (No dsRNA), a dsRNA with no mosquito homology (N.C. dsRNA), or 0.2-0.6 µg/µL of aeCCC1 dsRNA (DST7a and DST7b). Blue represents larvae reared in FW and red represents larvae reared in FW and transferred to 30% SW for 24 hours. The negative control dsRNA freshwater ammonium data contains an outlier at 54 mM. N = 28 for each treatment. General Linear Model of ion vs Treatment, Water (FW or 30% SW), and Date of experiment. Sodium: $P_{treatment} > 0.05$, $P_{water} < 0.001$, $P_{date} < 0.001$, $P_{water*Date} > 0.05$. Ammonium: $P_{treatment} < 0.001$, $P_{water} < 0.001$, $P_{date} < 0.001$, $P_{water*Date} < 0.05$. Potassium: $P_{treatment} < 0.05$, $P_{water} < 0.001$, $P_{date} < 0.001$, $P_{water*Date} > 0.05$.

Summary

- Larvae transferred from FW to 30% SW experienced a 33% increase in mean sodium hemolymph concentration after 48 hours. Larvae transferred from 30% SW to FW experienced a 37% decrease in mean sodium hemolymph concentration within 24 hours. Mean sodium hemolymph concentrations increased back to observed FW concentrations after 48 hours (**Figure 2**).
- Larvae reared in 30% SW had higher mean ammonium hemolymph concentrations. FW larvae transferred to 30% SW experienced about a 5 fold increase in mean ammonium hemolymph concentrations (**Figure 2**).
- Larvae exposed to aeCCC1 dsRNA experienced about a 15% reduction in hemolymph potassium compared to controls (**Figure 3**). This trend was not observed in larvae transferred to 30% SW for 24 hours.
- Larvae exposed to aeCCC1 dsRNA experienced up to a 5 fold increase in hemolymph ammonium compared to controls (**Figure 3**). A similar trend remained when larvae were exposed to dsRNA and transferred to 30% SW for 24 hours.

Conclusion/Future Questions

- Increasing salinity of the environment increases sodium and ammonium hemolymph concentrations.
- Knock down of hypothesized cation chloride cotransporter aeCCC1 leads to a large increase in ammonium hemolymph concentrations.
- These preliminary studies necessitate the quantification of expression of aeCCC1 and other transport proteins following exposure to dsRNA.
- How does knock-down affect movement of ions when larvae are transferred between environments?

References

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