**Results**

Insects: Wild-type *Aedes aegypti* were raised from egg to adult stage as described (Pannabecker et al., 2007). Eggs (Puntarenas, Costa Rica) were hatched under a vacuum chamber to induce low O2. Larvae were raised in 30% sea water, with no changes in aeCCC1 and aeCCC2. Overall, across stages of development, aeCCC3 was expressed 500 times higher in larvae than in pupae or adults; aeCCC2 was expressed 35-fold higher in larvae and adults than in pupae, whereas aeCCC1 was expressed in similar amounts across all stages. The different gene expression patterns of aeCCC1, aeCCC2, and aeCCC3 in the above conditions suggest diverse functional roles for these transporters.

**Hypotheses**

- aeCCC isoforms are differentially expressed across tissues along the alimentary tract of *A. aegypti*.
- The expression levels of aeCCC isoforms vary at different stages of development (i.e. larvae, pupae and adults) due to osmoregulatory function of each isoform (secretory or absorptive).
- Expression levels of aeCCC isoforms in larvae depend on salinity of surrounding water.

**Methods**

RNA isolation and qPCR: Tissue-specific or total RNA was isolated using the TRIzol prep according to the manufacturer’s instructions. RNA was quantified via NanoDrop-1000 (Thermo Scientific, Wilmington, DE) and decontaminated of genomic DNA using the TURBO DNA-free kit (Ambion/Applied Biosystems, Austin, TX). RNA was further purified using the RNA Clean & Concentrator<sup>TM</sup>-25 kit (Zymo Research Corp., Irvine, CA). Total RNA (10 μg) or tissue-specific RNA (1 μg) was reverse-transcribed using the TaqMan Reverse Transcription kit (Applied Biosystems, Life Technologies, Carlsbad, CA). Primers were designed using the Primer Express software v2.0 (Invitrogen). For all qPCR experiments, expression levels were quantified using the fluorescence marker SYBR Green (Applied Biosystems, Austin, TX) and the ribosomal protein S5 (aeRP55) was used as internal control (Ribeiro et al., 2007). q-PCR was run in triplicate on a 96-well plate. Gene expression levels were quantified by threshold cycle differences (dCT) on an ABI prism 7500 sequence detector.

**Conclusion/Future Questions**

- aeCCC1 shares closer homology to vertebrate NKCCs than other aeCCCs (Fig. 1). aeCCC1 is constitutively expressed across developmental stages (Fig. 2) and more highly expressed in the adult head than any osmoregulatory tissue studied (Fig. 3). aeCCC2 is downregulated during the larva-to-pupa metamorphosis but upregulated again in adults (Fig. 2). In adults, aeCCC2 is expressed more than 200-fold higher in the hindgut than in other osmoregulatory tissues (Fig. 6). But in larvae, aeCCC2 expression is not affected by 30% sea water growth conditions (Fig. 3). aeCCC3 is more highly expressed in larva than in pupae and adult females (Fig. 2). In larvae, aeCCC3 expression is higher in the anal papillae than in Malpighian tubules (Fig. 4). However, this tubular expression is upregulated in 30% sea water growth conditions (Fig. 3).
- The different tissue and developmental expression patterns of aeCCC1, aeCCC2 and aeCCC3 suggest diverse functional roles for these transporters.
- These preliminary results necessitate immunohistochemical studies with isoform-specific antibodies to localize the aeCCC proteins.
- The current study has not shown the immediate effect of a blood meal on aeCCC expression levels. In the future, it will be necessary to determine the time-dependent expression levels of aeCCC isoforms along the female alimentary canal after a blood meal.

**References**


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