Comparative Characterization of Na^+ Transport in *Cyprinodon variegatus variegatus* and *Cyprinodon variegatus hubbsi*: A Model Species Complex for Studying Teleost Invasion of Freshwater

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The euryhaline fish *Cyprinodon variegatus variegatus* (*Cvv*) tolerates salinities ranging from 0.3-160 psu, but cannot survive in freshwater (<2 mM Na^+). A population (*C.v. hubbsi*; *Cvh*) has been isolated in several freshwater (0.4-1 mM Na^+) lakes for ~150 ky. Characterization of Na^+ transport kinetics in these subspecies when acclimated to different Na^+ concentrations reveals *Cvv* and *Cvh* have qualitatively similar low affinity Na^+ uptake kinetics (K_m = 7,000-38,000 μM) when acclimated to 7 mM Na^+, but *Cvh* switches to a high affinity system (K_m = 100-140 μM) in freshwater. Inhibitor experiments show EIPA-sensitive Na^+ uptake in both subspecies regardless of ambient Na^+ concentration. This suggests *Cvh* is utilizing a Na^+/H^+ exchanger for Na^+ uptake even in low Na^+ (0.1 mM) environments despite theoretical thermodynamic constraints. Characterization of mitochondrial rich cell (MRC) size and density in fish acclimated to different Na^+ concentrations revealed a linear relationship between the fractional area of MRCs and Na^+ uptake rate. However, *Cvh* have higher Na^+ uptake rates at a given MRC fractional area indicating enhanced Na^+ uptake at low ambient Na^+ concentrations is due to differential expression of proteins involved in Na^+ uptake. This differential protein expression is what allows *Cvh* to osmoregulate in freshwater.