Evolutionary Ecology Research — Volume 17 (2016)

Osmoregulatory physiology and rapid evolution of salinity tolerance in threespine stickleback recently introduced to fresh water

APPENDIX

Jeffrey N. Divino[1], Michelle Y. Monette[2], Stephen D. McCormick[3], Paul H. Yancey[4], Kyle G. Flannery[4], Michael A. Bell[5], Jennifer L. Rollins[5], Frank A. von Hippel[6], and Eric T. Schultz[1]

[1]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA

[2]Department of Biological and Environmental Sciences, Western Connecticut State University, Danbury, Connecticut, USA

[3]USGS, Conte Anadromous Fish Research Center, Turners Falls, Massachusetts, USA

[4]Biology Department, Whitman College, Walla Walla, Washington, USA

[5]Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York, USA

[6]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

Correspondence: J.N. Divino, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, 06269-3043. *e-mail*: jeffrey.divino@uconn.edu

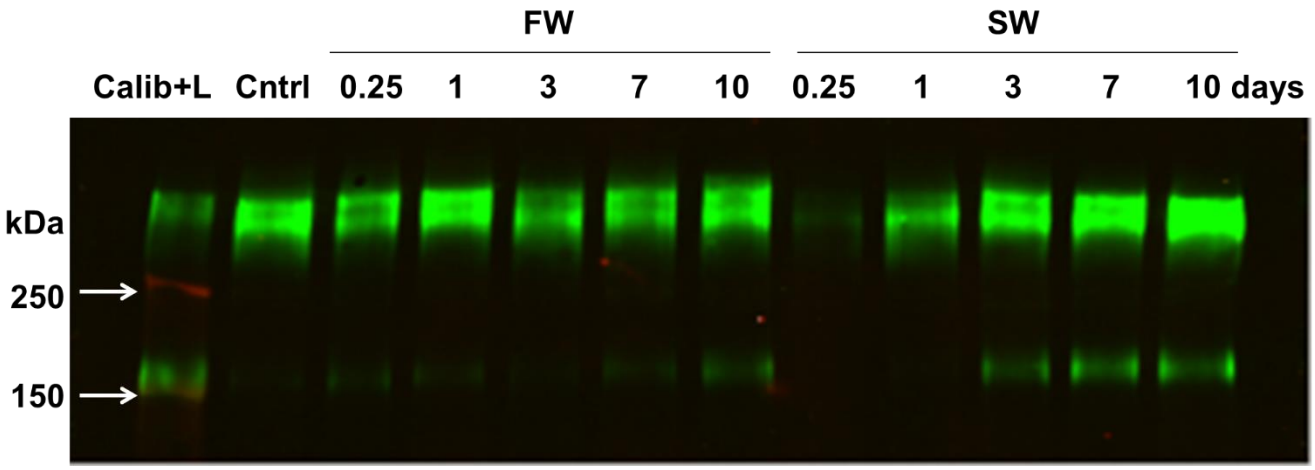

Figure S1. Representative immunoblot of NKCC protein levels in anadromous stickleback gills. NKCC was visualized by incubating blots in the T4 antibody and probing with an IR-fluorescent conjugate (green bands). Blots were digitized by high-resolution infrared scanning and fluorescence intensity of smaller (monomeric) and larger (multimeric) bands was quantified for total NKCC abundance. Each blot (n = 16) comprised a panel of homogenized gill samples selected from each time point and salinity (FW = 0.2 ppt; SW = 35 ppt). Potential blot-to-blot variation in signal strength was accounted for by normalizing to a common calibrator (Calib) consisting of gill tissue from pooled samples and run in the first lane of each blot. Molecular masses of the protein ladder (L; red bands) are labeled in kilodaltons.