iScience, Volume *27*

Supplemental information

Evolution of ion transporter Na^+/K^+-ATPase expression in the osmoregulatory maxillary glands of an invasive copepod

Teresa E. Popp, Sophie Hermet, Jacob Fredette-Roman, Emma McKeel, William Zozaya, Corentin Baumlin, Guy Charmantier, Carol Eunmi Lee, and Catherine Lorin-Nebel

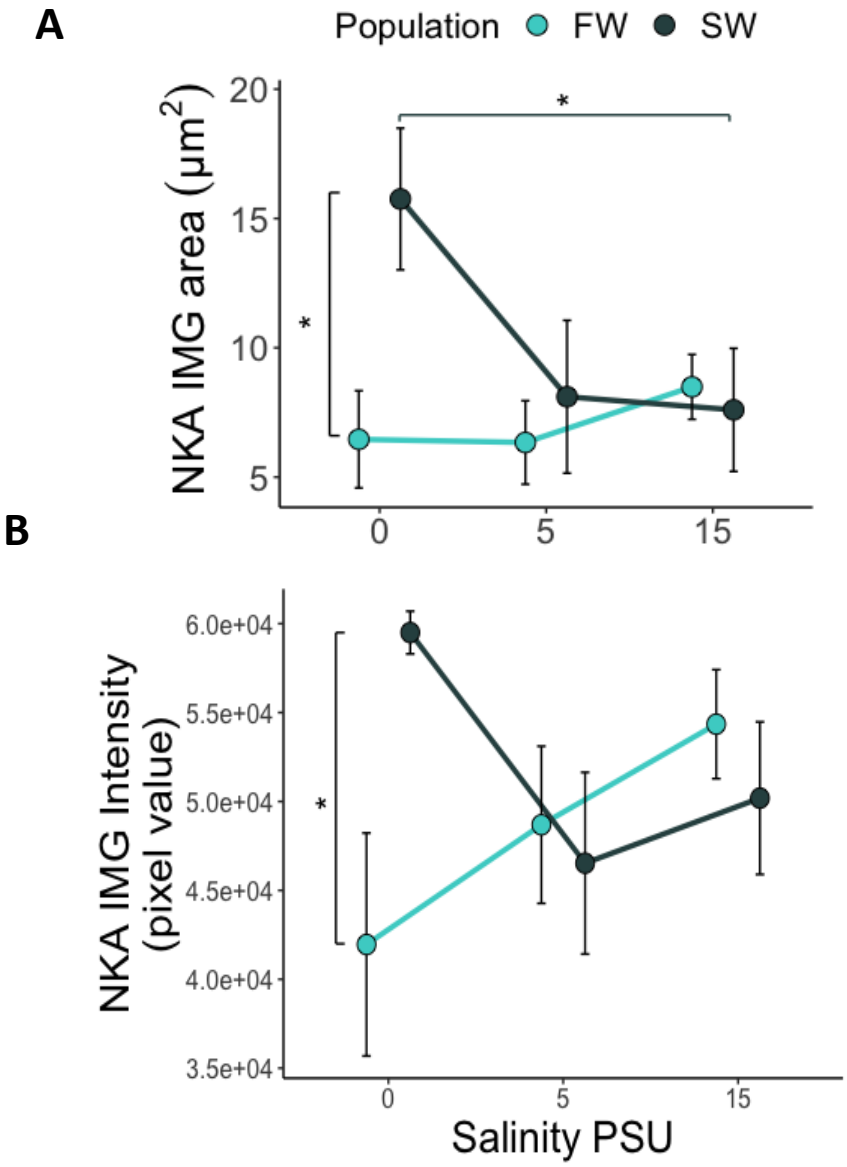

Figure S1. NKA *in situ* expression in response to treatment salinities (0, 5, 15 PSU) for saline (SW) and freshwater (FW) populations, measured under common garden conditions, related to Figure 7. (A) Total NKA immunolabeled maxillary gland (IMG) area calculated as the sum of area (μm^2) in all cuts multiplied by the thickness of each cut (5 μm) divided by the prosome length (μm) of that individual. **(B)** The maximum NKA IMG intensity (in pixel value). Data are represented as Mean ± SE. $P < 0.05$ = ∗. Mean values and standard errors are reported in Table S3.

Table S1. Statistical results for comparisons of body size (prosome length *μ*m) and Whole-Animal Na^+/K^+-ATPase expression between saline *versus* freshwater populations and between salinity treatments, related to Figure 2 and 3 Two-way ANOVA tests were performed, followed by Tukey post hoc tests. Additional *a priori* pairwise comparisons were performed between populations at each treatment salinity using Student's t-tests. Results show the effects of the factors *Population* (saline vs. freshwater populations), *Salinity* (0, 5, 15 PSU), and the interaction of *Population* × *Salinity* on body size and Na^+/K^+-ATPase expression. df = degrees of freedom. Significant results are indicated as ∗ = $P < 0.05$, ∗∗∗ = $P < 0.0001$.

Comparison (greater value indicated by > and <)	Test	Test statistic	df	*P*-value
A. Body Size (*μm*) – log transformed				
Population	Two-way ANOVA	$F = 0.693$	1,284	0.406
0 PSU	*a priori* t-test	$t = 0.40$	111	0.60
5 PSU	*a priori* t-test	$t = -1.13$	77	0.262
15 PSU, fresh > saline population	*a priori* t-test	$t = 1.95$	85	0.050*
Salinity	Two-way ANOVA	$F = 39.67$	2,283	<0.0001***
Saline population, 0 < 5 PSU	Tukey HSD			<0.0001***
Saline population, 0 < 15 PSU	Tukey HSD			<0.0001***
Saline population, 5 *vs* 15 PSU	Tukey HSD			0.472
Freshwater population, 0 < 5 PSU	Tukey HSD			0.00319*
Freshwater population, 0 < 15 PSU	Tukey HSD			<0.0001***
Freshwater population, 5 < 15 PSU	Tukey HSD			0.0360*
Population x *Salinity* interaction	Two-way ANOVA	$F = 3.02$	2,283	0.050*
B. Whole-Animal NKA – cube root transformed				
Population	Two-way ANOVA	$F = 0.805$	1,35	0.376
0 PSU	*a priori* t-test	$t = -0.650$	14	0.526
5 PSU	*a priori* t-test	$t = 0.929$	9	0.373
15 PSU, saline > fresh population	*a priori* t-test	$t = -2.17$	11	0.050*
Salinity	Two-way ANOVA	$F = 3.30$	2,34	0.0490*
Saline population, 0 *vs* 5 PSU	Tukey HSD			0.892
Saline population, 0 < 15 PSU	Tukey HSD			0.0169*
Saline population, 5 < 15 PSU	Tukey HSD			0.0444*
Freshwater population, 0 *vs* 5 PSU	Tukey HSD			0.0607
Freshwater population, 0 *vs* 15 PSU	Tukey HSD			0.361
Freshwater population, 5 *vs* 15 PSU	Tukey HSD			0.350
Population x *Salinity* interaction	Two-way ANOVA	$F = 2.32$	2,34	0.114

Table S2: Statistical results for comparisons of *in situ* Na^+/K^+-ATPase expression in the maxillary glands between saline *versus* freshwater populations and between salinity treatments, related to Figure 7. Analysis was done using a two-way ANOVA or Kruskal-Wallis test with *a priori* pairwise Student's t-tests and *a priori* Wilcoxon ranked sum tests. Results show effects of the factors *Population* (saline vs. freshwater populations), *Salinity* (0, 5, 15 PSU), and the interaction of *Population* × *Salinity* on Na^+/K^+-ATPase expression. df = degrees of freedom. *Post-hoc* comparisons are reported if significant differences were observed at $P < 0.05 = *$.

Comparison (greater value indicated by >)	Test	Test statistic	df	*P*-value
A. NKA IMG Area -square root transformed				
Population	Two-way ANOVA	$F = 2.33$	1, 35	0.137
0 PSU, saline > fresh	*a priori* t-test	$t = -2.83$	10	0.0181*
5 PSU	*a priori* t-test	$t = -0.287$	10	0.780
15 PSU	*a priori* t-test	$t = 0.600$	11	0.560
Salinity	Two-way ANOVA	$F = 1.32$	2, 34	0.281
Saline population, 0 *vs* 5 PSU	*a priori* t-test	$t = 2.10$	10	0.0620
Saline population, 0 > 15 PSU	*a priori* t-test	$t = 2.47$	11	0.0332*
Saline population, 5 *vs* 15 PSU	*a priori* t-test	$t = 0.0117$	11	0.991
Freshwater population, 0 *vs* 5 PSU	*a priori* t-test	$t = -0.140$	10	0.891
Freshwater population, 0 *vs* 15 PSU	*a priori* t-test	$t = -1.12$	11	0.286
Freshwater population, 5 *vs* 15 PSU	*a priori* t-test	$t = -1.22$	11	0.249
Population x *Salinity* interaction	Two-way ANOVA	$F = 3.17$	2, 34	0.050*
B. NKA IMG Intensity				
Population	Kruskal Wallis	$H = 0.577$	1, 35	0.447
0 PSU, saline > fresh	*a priori* Wilcoxon ranked sum	$W = 5$	n = 6	0.0411*
5 PSU	*a priori* Wilcoxon ranked sum	$W = 20$	n = 6	0.818
15 PSU	*a priori* Wilcoxon ranked sum	$W = 27$	n = 6	0.445
Salinity	Kruskal Wallis	$H = 1.23$	2, 34	0.542
Saline population, 0 *vs* 5 PSU	*a priori* Wilcoxon ranked sum	$W = 30$	n = 6	0.0649
Saline population, 0 *vs* 15 PSU	*a priori* Wilcoxon ranked sum	$W = 27$	n = 6	0.179
Saline population, 5 *vs* 15 PSU	*a priori* Wilcoxon ranked sum	$W = 13$	n = 6	0.485
Freshwater population, 0 *vs* 5 PSU	*a priori* Wilcoxon ranked sum	$W = 14$	n = 6	0.589
Freshwater population, 0 *vs* 15 PSU	*a priori* Wilcoxon ranked sum	$W = 10$	n = 6	0.1375
Freshwater population, 5 *vs* 15 PSU	*a priori* Wilcoxon ranked sum	$W = 15$	n = 6	0.445
Population x *Salinity* interaction	Kruskal Wallis	$H = 7.70$	5	0.174

Table S3. Values of body size and Na^+/K^+-ATPase (NKA) expression of freshwater and saline populations across three salinities (0, 5, 15 PSU), related to Figure 2, 3, and 7. Response variables include *Body Size*, *Whole-Animal NKA* band signal intensity, *NKA IMG Area*, *NKA IMG Intensity*. Mean values and standard errors of the mean (SE) are reported.

Population	Salinity (PSU)	Mean	SE
A. Body Size (*μm)*			
Freshwater	0	764.5	9.66
Freshwater	5	834.7	13.6
Freshwater	15	900.7	15.3
Saline	0	758.6	8.17
Saline	5	863.2	17.2
Saline	15	857.0	19.2
B. Whole-Animal NKA (band signal intensity)			
Freshwater	0	$8.16x10^{-5}$	$1.97x10^{-5}$
Freshwater	5	$2.79x10^{-4}$	$1.11 x10^{-4}$
Freshwater	15	$1.38x10^{-4}$	$3.44 x10^{-5}$
Saline	0	$1.44x10^{-4}$	$4.90 x10^{-5}$
Saline	5	$1.66x10^{-4}$	$8.28 x10^{-5}$
Saline	15	$4.10x10^{-4}$	$1.05 x10^{-4}$
C. NKA IMG Area (μm^2)			
Freshwater	0	6.46	1.88
Freshwater	5	6.34	1.618
Freshwater	15	8.49	1.258
Saline	0	15.8	2.74
Saline	5	8.11	2.95
Saline	15	7.60	2.38
D. NKA IMG Intensity (pixel intensity)			
Freshwater	0	$2.03x10^4$	$1.15 x10^4$
Freshwater	5	$2.45x10^4$	$2.40x10^4$
Freshwater	15	$2.16x10^4$	$1.08x10^4$
Saline	0	$2.67x10^4$	$2.17x10^4$
Saline	5	$2.48x10^4$	$2.09x10^4$
Saline	15	$2.45x10^4$	$1.76x10^4$

Comparison of Integument Permeability between Saline and Freshwater Populations, related to Figures 3 and 7.

Methods

To compare the capacity of copepods from both the saline and freshwater populations to retain ions at low salinities, ion efflux was measured using radioactive sodium (^{22}Na). Individual *Eurytemora carolleeae* copepods were placed in native salinities with ^{22}Na for 12 hours (freshwater population n = 7 at 0 PSU, saline population n = 5 at 15 PSU). Individual copepods were then transferred to 0 PSU containing no ^{22}Na. Ion (^{22}Na) efflux was measured in the new water using a scintillation counter for 3 hours after transfer. The variable calculated was the proportion of radioactive sodium retained = [^{22}Na retained / (^{22}Na effluxed + ^{22}Na retained)]. This variable was tested for normality and comparisons between the two populations was performed using a Student's t-test.

Results

After transfer to 0 PSU for 3 hours, two saline copepods went comatose, and one died (Table S4). The ion efflux values for these individuals were kept for analysis because ion efflux is a passive process and does not require conscious or active excretion. Comparisons between the two populations revealed that there were significant differences in the proportion of radioactive sodium retained after three hours in 0 PSU (Student's t-test; t = 2.33, df = 9.42, P = 0.0219) with the freshwater population retaining more ions than the saline population (Figure S2). Sample sizes for this study are low, and while the findings are significant, further data collection is necessary to establish conclusive results.

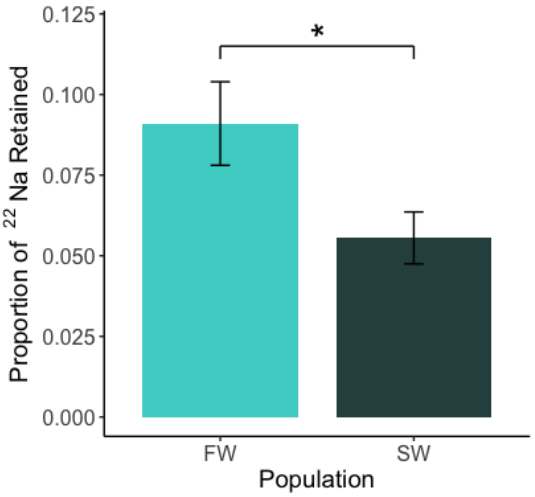

Figure S2. Proportion of radioactive sodium (^{22}Na) retained after transfer to 0 PSU for 3 hours for both the freshwater (FW) and saline (SW) populations, related to Figures 3 and 7.

Proportion of ^{22}Na retained was calculated as [^{22}Na retained / (^{22}Na effluxed + ^{22}Na retained)]. Data are represented as Mean ± SE.

Table S4: Ion efflux (^{22}Na) measurements obtained after 3 hours in 0 PSU for both saline (SW) and freshwater (FW) populations, related to Figures 3 and 7. Measures include ion efflux, ion retention, and the proportion of ions retained (ions retained / total). Copepod status reports whether a copepod went comatose or died after extended time in 0 PSU.

Population	Efflux	Retained	Retained/Total	Copepod status
FW	7828.9	317.8	0.039	
FW	5761.9	354.8	0.058	
FW	14171.0	2194.9	0.134	
FW	23878.0	2523.9	0.096	
FW	9197.9	877.8	0.087	
FW	15282.0	1614.9	0.096	
FW	5496.9	803.8	0.128	
SW	21215.0	1470.9	0.065	Comatose
SW	5662.9	512.8	0.083	
SW	1188.9	55.0	0.044	Died
SW	2314.9	105.3	0.044	
SW	7031.9	308.8	0.042	Comatose