

Table S1. Results of false discovery rate corrected *t*-tests and general linear models of hemolymph and muscle ion concentrations and calculated muscle equilibrium potentials of *Gryllus pennsylvanicus* exposed to 0°C

Ion	Test	Variable	d.f.	Statistic	<i>P</i>
Na ⁺	<i>t</i> -test	Hemolymph concentration	10	<i>t</i> =3.26	0.024
		Muscle concentration	10	<i>t</i> =4.385	0.008
		Equilibrium potential	10	<i>t</i> =0.77	0.461
	General linear model	Hemolymph concentration	2,58	<i>F</i> =13.35	<0.001
		Muscle concentration	2,58	<i>F</i> =0.27	0.606
		Equilibrium potential	2,64	<i>F</i> =0.91	0.345
	<i>t</i> -test	Hemolymph concentration	11	<i>t</i> =4.13	0.008
		Muscle concentration	10	<i>t</i> =0.12	0.907
		Equilibrium potential	10	<i>t</i> =2.11	0.244
K ⁺	General linear model	Hemolymph concentration	2,58	<i>F</i> =31.23	<0.001
		Muscle concentration	2,64	<i>F</i> =0.01	0.786
		Equilibrium potential	2,64	<i>F</i> =45.74	<0.001
	<i>t</i> -test	Hemolymph concentration	11	<i>t</i> =2.85	0.032
		Muscle concentration	10	<i>t</i> =0.84	0.563
		Equilibrium potential	10	<i>t</i> =1.17	0.357
	General linear model	Hemolymph concentration	2,64	<i>F</i> =0.70	0.398
		Muscle concentration	2,64	<i>F</i> =0.01	0.955
		Equilibrium potential	2,64	<i>F</i> =1.83	0.181
Ca ²⁺	<i>t</i> -test	Hemolymph concentration	10	<i>t</i> =1.86	0.144
		Muscle concentration	9	<i>t</i> =0.15	0.907
		Equilibrium potential	9	<i>t</i> =1.24	0.357
	General linear model	Hemolymph concentration	2,59	<i>F</i> =16.03	<0.001
		Muscle concentration	2,60	<i>F</i> =0.56	0.458
		Equilibrium potential	2,52	<i>F</i> =5.51	0.023

t-tests were used to compare ion concentrations and equilibrium potentials between control crickets and crickets exposed to 0°C for 12 h. General linear models were then used to examine the effect of prolonged cold exposure (up to 120 h) on ion concentrations and equilibrium potentials and include control crickets only if there was no difference between control and 12 h. *P*-values in bold denote significant effects of cold exposure on the response variable.

Table S2. Results of false discovery rate corrected *t*-tests and general linear models of hemolymph and tissue water content of *Gryllus pennsylvanicus* exposed to 0°C

Test	Tissue	d.f.	Statistic	<i>P</i>
<i>t</i> -test	Hemolymph	11	<i>t</i> =1.35	0.603
	Muscle	10	<i>t</i> =0.70	0.782
	Gut total	11	<i>t</i> =0.55	0.782
	Foregut	13	<i>t</i> =1.97	0.070
	Midgut	13	<i>t</i> =2.15	0.051
	Hindgut	13	<i>t</i> =3.11	0.008
General linear model	Hemolymph	2,49	<i>F</i> =88.68	<0.001
	Muscle	2,63	<i>F</i> =1.20	0.277
	Gut total	2,51	<i>F</i> =7.13	0.010
	Foregut	2,60	<i>F</i> =2.12	0.038
	Midgut	2,60	<i>F</i> =44.68	<0.001
	Hindgut	2,52	<i>F</i> =0.28	0.597

t-tests were used to compare water content between control crickets and crickets exposed to 0°C for 6 h. General linear models were then used to examine the effect of prolonged cold exposure (up to 120 h) on water content, and include control crickets only if there was no difference between control and 6 h. *P*-values in bold denote significant effects of cold exposure on water content.

Table S3. Results of false discovery rate corrected t-tests and general linear models of hemolymph, foregut, midgut and hindgut ion content of *Gryllus pennsylvanicus* exposed to 0°C

Ion	Test	Tissue	d.f.	Statistic	P
Na ⁺	t-test	Hemolymph	13	$t=2.61$	0.068
		Foregut	13	$t=1.60$	0.322
		Midgut	13	$t=4.10$	0.006
		Hindgut	13	$t=5.42$	0.001
	General linear model	Hemolymph	2,66	$F=97.78$	<0.001
		Foregut	2,57	$F=2.77$	0.008
		Midgut	2,60	$F=4.83$	0.032
		Hindgut	2,52	$F=12.96$	<0.001
K ⁺	t-test	Hemolymph	13	$t=1.98$	0.085
		Foregut	14	$t=0.65$	0.631
		Midgut	14	$t=2.91$	0.044
		Hindgut	14	$t=0.11$	0.915
	General linear model	Hemolymph	2,67	$F=0.10$	0.758
		Foregut	2,57	$F=2.03$	0.160
		Midgut	2,52	$F=4.95$	0.031
		Hindgut	2,60	$F=3.21$	0.078
Mg ²⁺	t-test	Hemolymph	13	$t=2.37$	0.068
		Foregut	13	$t=1.27$	0.405
		Midgut	14	$t=0.91$	0.572
		Hindgut	12	$t=1.85$	0.264
	General linear model	Hemolymph	2,66	$F=61.60$	<0.001
		Foregut	2,53	$F=0.48$	0.491
		Midgut	2,60	$F=19.44$	<0.001
		Hindgut	2,60	$F=2.54$	0.116
Ca ²⁺	t-test	Hemolymph	13	$t=1.863$	0.085
		Foregut	13	$t=1.46$	0.334
		Midgut	14	$t=0.78$	0.597
		Hindgut	14	$t=0.44$	0.727
	General linear model	Hemolymph	2,65	$F=67.53$	<0.001
		Foregut	2,55	$F=0.85$	0.359
		Midgut	2,60	$F=21.58$	<0.001
		Hindgut	2,60	$F=1.64$	0.205

t-tests were used to compare ion content between control crickets and crickets exposed to 0°C for 6 h. General linear models were then used to examine the effect of prolonged cold exposure (up to 120 h) on ion content, and include control crickets only if there was no difference between control and 6 h. P-values in bold denote significant effects of cold exposure on ion content.