Supporting Information

MacMillan et al. 10.1073/pnas.1212788109

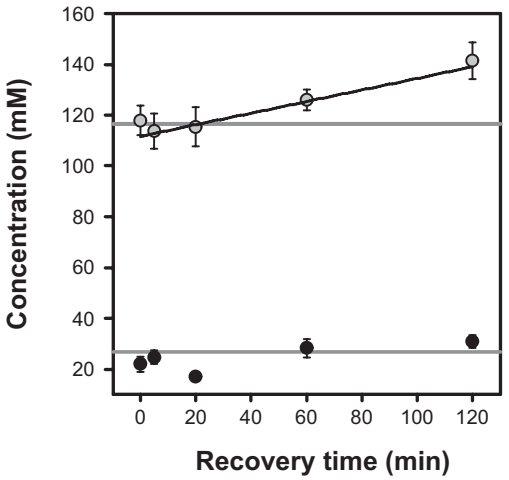


Fig. S1. Muscle concentration of Na^+ (black) and K^+ (gray) during chill-coma recovery at 22 °C following 24 h at 0 °C. Gray lines indicate mean values of control crickets (those that did not receive any cold exposure). Cold exposure did not significantly affect Na^+ or K^+ concentration of the muscle. Solid black line indicates a significant positive relationship between recovery time and K^+ concentration.

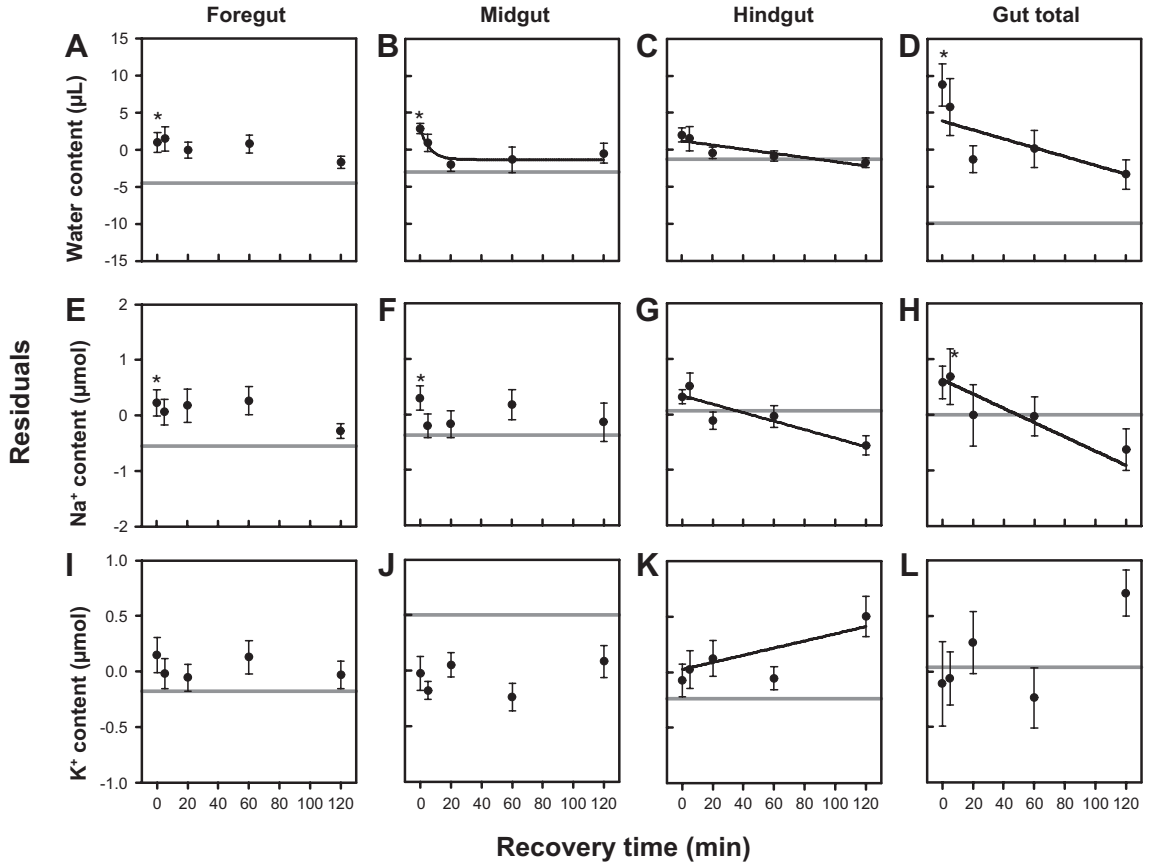


Fig. S2. Residual water (*A–D*), Na^+ (*E–H*), and K^+ (*I–L*) content of each of the three gut segments and total gut of crickets during chill-coma recovery at 22 °C following 24 h at 0 °C. Solid black lines indicate a significant relationship between recovery time and gut volume or ion content. Gray lines indicate means of control crickets (those that did not receive any cold exposure). Asterisks denote a significant effect of cold exposure on ion concentration or content.

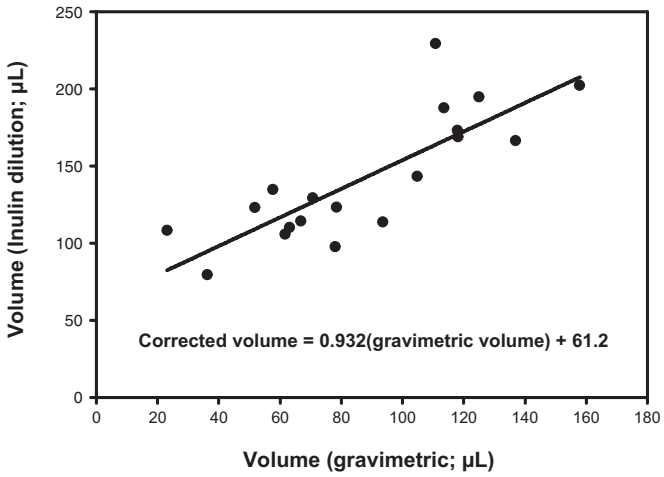


Fig. S3. The relationship between gravimetric estimates of hemolymph volume and volume quantified using the inulin dilution method in *G. pennsylvanicus*. Gravimetric estimates of hemolymph volume significantly correlated ($P_{intercept} < 0.001$; $P_{slope} < 0.001$, $R^2 = 0.66$) with estimates obtained on the same individuals using the inulin dilution method. Inulin dilution was completed on 19 crickets at 22 °C to generate correction factors for hemolymph (equation shown) and muscle (4.14 ± 0.66% hemolymph by volume) ion and water content. A 5% solution of FITC inulin in 150 mM NaCl was dialyzed in the dark and at room temperature against 150 mM NaCl (to remove unbound FITC) in benzoylated dialysis tubing with a molecular weight cutoff of 2,000 kDa (Sigma Aldrich) and the resulting (~3% FITC–inulin) solution was used for injection. Crickets were immobilized using plasticine and a small amount of vacuum grease was applied to the injection site to minimize bleeding. A 2-µL bolus of the inulin solution was injected at the base of the right wing under the pronotum. Fifteen minutes later the crickets were sampled as above to collect hemolymph and muscle tissue. Muscle tissue from one femur was used to measure water content gravimetrically. The hemolymph and muscle tissue from the other femur were weighed and placed in 20 volumes of 500-mM Hepes buffer (pH 7.4), homogenized using a bullet blender with 1-mm glass beads (Next Advance), and centrifuged at 7,000 × *g* for 10 min. Fifty-microliter aliquots of the resulting supernatant were loaded onto black 96-well plates and fluorescence was measured (excitation = 485 nm, emission = 538 nm) in a Spectramax M2e spectrophotometer (Molecular Devices). Sample fluorescence was compared with dilutions of the 3% injection solution in 500-mM Hepes and corrected by comparison with hemolymph and muscle tissue of three control crickets that did not receive an injection.

Table S1. Results of linear and nonlinear regression of cricket chill-coma recovery time and cold exposure duration

Index	Model	df	*F*	*P*	*F* (exponent)	*P* (exponent)	Log likelihood	AIC	*P*.adj.	Sig.
Abdomen	Linear	38	958.9	<0.001			−83.9	175.8		
	Exponential	**38**	**2,603.5**	**<0.001**	**67.8**	**<0.001**	**−77.5**	**165.0**	**<0.001, <0.001**	***
Forelegs	Linear	38	363.3	<0.001			−106.6	221.1		
	Exponential	**38**	**6,863.7**	**<0.001**	**8.7**	**<0.001**	**−89.2**	**188.4**	**<0.001, <0.001**	***
Hindlegs	Linear	38	155.1	<0.001			−113.5	234.9		
	Exponential	**38**	**5,851.3**	**<0.001**	**0.1**	**<0.001**	**−97.3**	**204.5**	**<0.001, 0.001**	***
Righting (<24 h)	Linear	38	267.7	<0.001			−151.1	310.3		
	Exponential	**38**	**1,675.5**	**0.002**	**3.2**	**0.073**	**−147.0**	**304.1**	**0.002, 0.073**	**
Activity detection	Linear	30	306.9	<0.001			−93.4	192.9		
	Exponential	**30**	**814.7**	**<0.001**	**13.2**	**0.001**	**−82.3**	**172.6**	**<0.001, 0.001**	***

Four visual indices of chill-coma recovery were recorded: first coordinated contraction of the abdomen, movement of the forelegs, movement of the hind legs, and the ability of a cricket to independently right itself (righting). *F* and *P* values of the exponent parameter are shown separately for exponential models. Models of best fit (boldface) were chosen based on Akaike information criterion (AIC), with an AIC difference of 2 required for selection of the more complex exponential model. Statistical significance (Sig.) was determined on adjusted *P* values (*P*.adj.) following false discovery rate correction (see *Materials and Methods* for details).

Table S2. Results of *t* tests of the effects of 24 h of exposure to 0 °C on hemolymph, muscle, and gut ion and water content

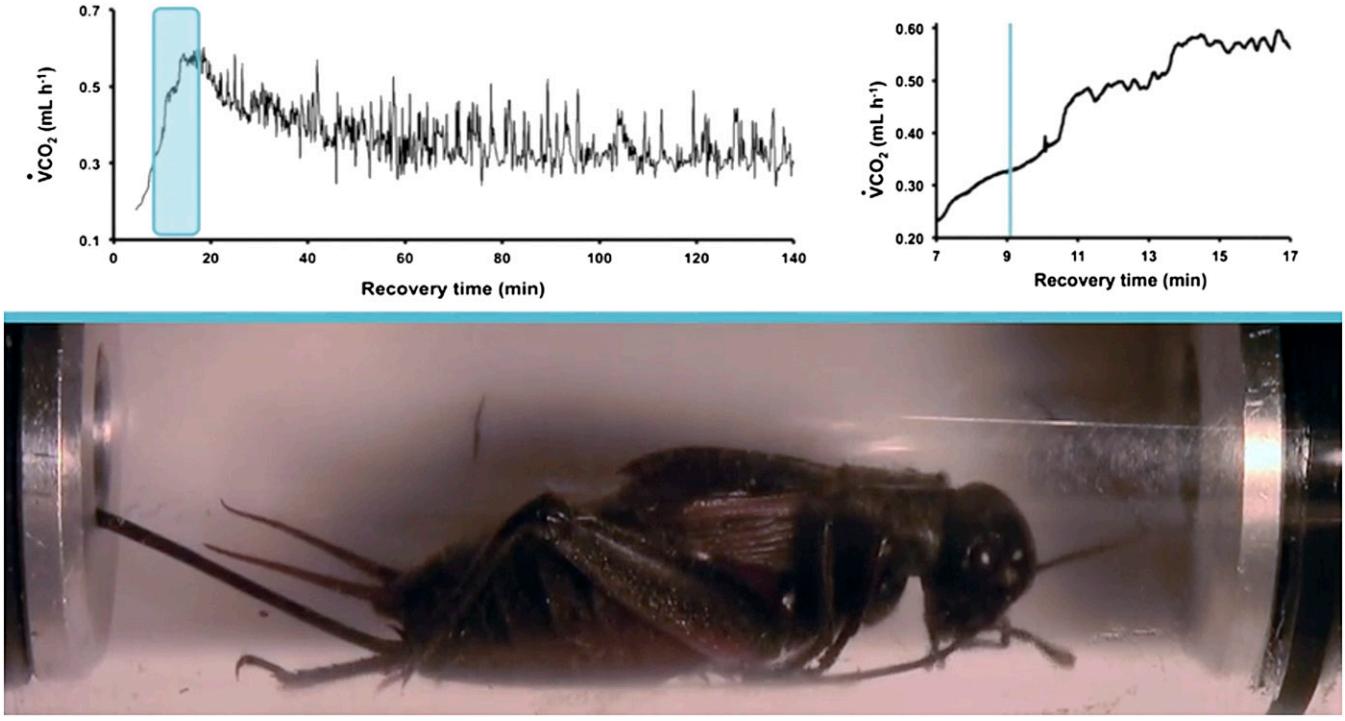
Tissue	Variable	df	*t*	*P*	*P*.adj.	Sig.	Dir.
Hemolymph	**Volume**	**12**	**6.55**	**<0.001**	**0.001**	**	–
	Na^+ (μmol)	**18**	**4.65**	**<0.001**	**0.001**	**	–
	K^+ (μmol)	18	1.01	0.322	0.322		
	[Na^+]	**14**	**3.10**	**<0.001**	**0.017**	*	–
	[K+]	**10**	**2.80**	**0.019**	**0.024**	*	+
Muscle	Water content	17	0.16	0.873	0.873		
	[Na^+]	18	1.12	0.277	0.582		
	[K+]	15	0.19	0.844	0.873		
	Ena	17	0.96	0.349	0.582		
	Ek	**13**	**3.37**	**0.005**	**0.025**	*	+
Foregut	**Water content**	**18**	**3.04**	**0.007**	**0.021**	*	+
	Na^+ content	**17**	**2.71**	**0.017**	**0.025**	*	+
	K^+ content	18	1.39	0.182	0.182		
Midgut	**Water content**	**16**	**5.53**	**<0.001**	**<0.001**	***	+
	Na^+ content	**17**	**2.37**	**0.030**	**0.045**	*	+
	K^+ content	15	2.03	0.061	0.061		
Hindgut	Water content	17	2.52	0.022	0.066		
	Na^+ content	15	1.51	0.151	0.227		
	K^+ content	17	0.70	0.488	0.488		
Gut total	**Water content**	**17**	**4.29**	**<0.001**	**0.002**	**	+
	Na^+ content	**14**	**3.33**	**0.005**	**0.008**	**	+
	K^+ content	17	0.30	0.772	0.772		

Statistical significance (Sig.) (boldface) was determined on adjusted *P* values (*P*.adj.) following false discovery rate correction (see *Materials and Methods* for details). Dir, direction of change.

Table S3. Results of linear and nonlinear (NL) models of *G. pennsylvanicus* hemolymph, muscle, and gut ion and water content during chill-coma recovery following 24 h at 0 °C

Tissue	Variable	Model	df	*F*	*P*	LL	AIC	*P*.adj.	Sig.	Dir.
Hemolymph	Volume	**Linear**	**44**	**1,629.3**	**<0.001**	**−141.6**	**291.2**	**<0.001**	***	**+**
		NL	44	4,004.2, 354.1	0.590, 0.658	−141.0	292.1			
	Na^+ content	**Linear**	**42**	**644.6**	**<0.001**	**−86.7**	**181.5**	**<0.001**	***	**+**
		NL	42	900.5, 91.7	0.652, 0.729	−86.7	183.5			
	K^+ content	Linear	39	117.7	0.602	−47.9	103.8	0.602		
		NL	39	81.0, 32.5	0.347, 0.755	−49.2	108.4			
	$[Na^+]$	Linear	34	1,309.1	0.513	−152.2	312.4	0.602		
		NL	N/A	N/A	N/A	N/A	N/A	N/A		
	$[K^+]$	Linear	34	161.3	<0.001	−116.0	239.9			
		NL	**34**	**87.4, 41.3**	**0.004, 0.158**	**−111.0**	**232.1**	**0.008, 0.237**	**	**–**
Muscle	H_2O content	Linear	47	1.33	0.255	−52.8	113.5	0.306		
		NL	47	0.2, 1.4	0.651, 0.241	−53.2	116.4			
	$[Na^+]$	**Linear**	**43**	**224.2**	**0.011**	**−147.8**	**303.6**	**0.022**	*	**+**
		NL	43	88.9, 40.6	0.997, 0.999	−151.8	313.7			
	$[K^+]$	**Linear**	**43**	**815.1**	**0.002**	**−183.0**	**374.0**	**0.006**	**	**+**
		NL	43	1,875.7, 2.2	0.903, 0.886	−183.0	375.9			
	E_{Na}	Linear	42	167.7	0.812	−170.6	349.2	0.812		
		NL	42	85.9, 88.7	0.994, 0.974	−170.3	350.7			
	E_k	Linear	43	546.7	<0.001	−164.6	347.1			
		NL	**43**	**760.0, 56.9**	**<0.001, 0.201**	**−163.3**	**336.6**	**0.001, 0.301**	**	**–**
Foregut	H_2O content	Linear	47	0.6	0.115	−128.0	264.0	0.345		
		NL	47	0.1, 5.2	0.711, 0.023	−128.1	266.2			
	Na^+ content	Linear	47	0.5	0.500	−51.9	111.8	0.750		
		NL	47	0.1, 0.1	0.742, 0.721	−52.3	114.7			
	K^+ content	Linear	47	0.1	0.887	−26.7	61.4	0.887		
		NL	47	0.1, 0.1	0.724, 0.963	−26.9	63.7			
Midgut	H_2O content	Linear	43	0.7	0.143	−115.5	239.0			
		NL	**43**	**8.5, 4.4**	**0.001, 0.302**	**−111.7**	**233.3**	**0.005, 0.378**	**	**–**
	Na^+ Content	Linear	42	1.0	0.0.532	−43.6	95.3			
		NL	42	2.4, 1.9	0.030, 0.178	−41.2	92.3	0.075, 0.297		
	K^+ Content	Linear	40	0.1	0.478	−13.8	35.7	0.478		
		NL	40	0.1, 0.1	0.911, 0.994	−14.2	38.4			
Hindgut	H_2O content	**Linear**	**45**	**3.4**	**0.010**	**−112.3**	**232.6**	**0.010**	*	**–**
		NL	45	1.2, 7.8	0.005, 0.294	−111.0	232.2			
	Na^+ content	**Linear**	**43**	**8.1**	**<0.001**	**−37.4**	**82.8**	**0.001**	**	**–**
		NL	43	0.2, 41.0	0.654, <0.001	−37.3	84.5			
	K^+ content	**Linear**	**42**	**9.3**	**0.004**	**−25.9**	**59.8**	**0.012**	*	**+**
		NL	42	253.6, 0.2	<0.001, 0.683	−25.6	61.3			
Gut total	H_2O content	**Linear**	**43**	**0.8**	**0.018**	**−144.0**	**296.0**	**0.027**	*	**–**
		NL	43	5.1, 4.0	0.005, 0.267	−142.2	294.4			
	Na^+ Content	**Linear**	**42**	**3.1**	**0.007**	**−70.4**	**148.8**	**0.022**	*	**–**
		NL	42	0.1, 29.7	0.603, <0.001	−69.9	149.8			
	K^+ Content	Linear	40	3.85	0.192	−48.8	105.6	0.192		
		NL	40	43.4, 0.3	<0.001, 0.996	−49.3	108.6			

Models of best fit (boldface) were chosen based on Akaike information criterion (AIC), with an AIC difference of 2 required for selection of the more complex exponential model. Variables that could not be effectively fit to a given model are marked (N/A) and are considered nonsignificant. *F* and *P* values of the exponent parameter are shown separately for exponential models. Statistical significance (Sig.) was determined on adjusted *P* values (*P*.adj.) following false discovery rate correction. Dir., direction of change; LL, log likelihood ratio.



Movie S1. Example video recording of a cricket (8× speed) inside the respirometry chamber with accompanying $\dot{V}CO_2$ trace (*Upper Left*) during chill-coma recovery at 22 °C following 16 h at 0 °C. The activity detector was removed to enable video recording. Three 10-min periods are shown in sequence, with the time-matched $\dot{V}CO_2$ trace shown in detail (*Upper Right*). As metabolic rate peaks early in the overshoot, there is minimal movement of the cricket inside the chamber, demonstrating that the overshoot is not simply a product of cricket activity during recovery. Later in the overshoot, the cricket has a high and variable $\dot{V}CO_2$that was often associated with high apparent activity in crickets measured using the activity detector. From this video it is clear that this activity is modest, and in the form of abdominal contractions that may serve to supply oxygen and expel CO_2 more effectively during a period of high metabolic demand and/or actively mix the hemolymph, the composition of which is changing. After the overshoot is complete, true periods of high activity (such as attempts by the cricket to turn around in the chamber) cause deviations in $\dot{V}CO_2$that are small and fleeting relative to the overshoot.

Movie S1