Supplementary Materials for:

Physiological Responses to Gravity in an Insect

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Table S1. Data used for testing the effect of body orientation and region on the percent of the image occupied by air sacs or tracheae. Animals labeled with A were adults, while those labeled with a J were 3rd instars.

Animal	Region	Structure	Head-Up	Head-Down	Prone
A1	Head	Air Sacs	88	2	79
A2	Head	Air Sacs	100	22	39
A3	Head	Air Sacs	100	0	77
A4	Head	Air Sacs	97	3	53
J1	Head	Air Sacs	41	72	J1
J2	Head	Air Sacs	39	0	j2
J3	Head	Air Sacs	71	0	j3
J4	Head	Air Sacs	0	0	j4
J5	Head	Air Sacs	0	0	j5
J6	Head	Air Sacs	0	0	j6
J7	Head	Air Sacs	81	14	j7
A1	Abdomen	Air Sacs	10	83	32
A2	Abdomen	Air Sacs	23	41	53
A3	Abdomen	Air Sacs	18	61	11
A4	Abdomen	Air Sacs	29	58	80
J1	Abdomen	Air Sacs	0	4	
J2	Abdomen	Air Sacs	7	6	
J3	Abdomen	Air Sacs	4	6	
J4	Abdomen	Air Sacs	6	12	
J5	Abdomen	Air Sacs	14	10	
J6	Abdomen	Air Sacs	2	3	
J7	Abdomen	Air Sacs	2	6	
A1	Head	Tracheae	0	13	9
A2	Head	Tracheae	0	5	0
A3	Head	Tracheae	0	5	44
A4	Head	Tracheae	6	3	5
J1	Head	Tracheae	0	10	
J2	Head	Tracheae	15	18	
J3	Head	Tracheae	0	18	
J4	Head	Tracheae	0	24	
J5	Head	Tracheae	24	1	
J6	Head	Tracheae	27	14	
J7	Head	Tracheae	0	0	
A1	Abdomen	Tracheae	38	20	12
A2	Abdomen	Tracheae	0	43	12
A3	Abdomen	Tracheae	0	0	11
A4	Abdomen	Tracheae	10	3	2
J1	Abdomen	Tracheae	39	0	
J2	Abdomen	Tracheae	23	30	
J3	Abdomen	Tracheae	57	30	
J4	Abdomen	Tracheae	34	17	
J5	Abdomen	Tracheae	28	30	
J6	Abdomen	Tracheae	23	23	
J7	Abdomen	Tracheae	32	33	

Table S2. Data used for testing the effect of N_2-anesthesia on the logit-transformed proportion of head images occupied by air sacs.

animal	orientation	condition	Logit airsacs
17	down	normoxia	-0.55
17	down	anoxia	-1.69
14	down	normoxia	-0.75
14	down	anoxia	-3.99
13	up	normoxia	0.219
13	up	anoxia	2.99
15	up	normoxia	-0.27
15	up	anoxia	2.99
16	up	normoxia	0.912
16	up	anoxia	1.69

Table S3. Difference in percent of the insect body cross-sectional area occupied by the tracheal system at each of the 13 equidistant locations along the length of the 8 grasshoppers imaged using μCT. Location 1 is the most anterior, and location 13 the most posterior. Positive values indicate that head-up animals had a greater percent of the image area occupied by tracheal system structure than head-down animals, whereas negative values indicate the converse.

	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5	Specimen 6	Specimen 7	Specimen 8
Location 1	14.36	12.46	14.01	14.01	11.97	-2.73	0.61	-8.13
Location 2	24.09	21.95	9.44	10.19	20.10	24.94	8.28	-24.10
Location 3	-0.07	29.30	28.51	22.07	14.08	21.14	-4.56	-21.07
Location 4	-10.65	9.16	7.90	7.34	7.18	-4.64	4.86	-22.68
Location 5	-9.22	2.46	-1.19	6.57	2.08	7.65	-11.93	-19.97
Location 6	-34.25	13.49	20.36	30.31	-20.05	8.56	-7.08	29.02
Location 7	-53.19	-29.17	-11.80	12.49	-25.15	-15.22	-3.08	11.50
Location 8	-16.79	-32.53	-25.32	-2.43	-49.52	-16.41	-0.63	-38.42
Location 9	-27.80	-4.62	-18.99	-13.80	-20.77	-3.19	-7.78	23.88
Location 10	-38.28	-2.29	-18.12	-8.58	-2.59	-1.70	-3.86	16.86
Location 11	-23.82	-10.22	-27.03	-24.71	-1.24	-0.71	-10.63	-8.50
Location 12	-13.12	-27.65	-41.86	-25.13	-2.66	-5.96	-20.65	30.29
Location 13	-2.49	-0.66	-18.50	-1.20	-5.69	-0.21	-24.57	15.72

Table S4. Results of 3-way ANOVA, testing effects of condition (normoxia vs. anoxia), orientation (up or down) and segment (cranial or caudal third) on the proportion of hemolymph in each segment (logit-transformed to achieve normality), using prone, head-up and head-down animals, with animal as a random factor. There was a highly significant interaction between orientation and segment, indicating that orientation significantly affected hemolymph distribution, and a significant three-way interaction between orientation, condition and segment, indicating that anoxia influenced the effect of orientation on hemolymph distribution.

Parameter	Df	SS	MS	F	P
Orientation	2	0.000	0.000	0.000	1.0
Condition	1	0.000	0.000	0.000	1.0
Segment	1	6.044	6.044	93.621	7.40e-16 ***
orientation:condition	1	0.000	0.000	0.000	1.0
orientation:segment	2	2.205	1.103	17.080	4.51e-07 ***
condition:segment	2	0.320	0.320	4.963	0.028*
orientation:condition:segment	1	0.478	0.478	7.402	0.0077**
Residuals	96	6.198	0.065		

Table. S5.

Results of 3-way ANOVA, using animal as a random factor, testing effects of condition (normoxia vs. anoxia), orientation (up or down) and segment (cranial or caudal thirds) on the proportion of hemolymph in each segment (logit-transformed), using only head-up and head-down animals. There was a highly significant interaction between orientation and segment, indicating that orientation significantly affected hemolymph distribution, and a significant three-way interaction between orientation, condition and segment, indicating that anoxia influenced the effect of orientation on hemolymph distribution.

Parameter	DF	SS	MS	F	P
Orientation	1	0.000	0.000	0.000	1.0
Condition	1	0.000	0.000	0.00	1.0
Segment	1	4.528	4.528	80.307	1.97e-13***
orientation:condition	1	0.00	0.000	0.000	1.0
orientation:segment	1	2.200	2.200	39.019	2.41e-08 ***
condition:segment	1	0.320	0.320	5.683	0.0197*
orientation:condition:segment	1	0.478	0.478	8.476	0.005**
Residuals	74	4.173	0.056		

Table S6. Data used for testing effect of body orientation and N_2-anesthesia on the distribution of hemolymph, tracked using ^{3}H-inulin. The column labeled "propcounts" indicates the proportion of ^{3}H counts (disintegrations per min) for that animal in each segment (animals were divided into thirds).

condition	orientation	animal	segment	counts	propcounts	logitcounts
normoxia	prone	V	cranial	2854.98	0.156	-0.731
normoxia	prone	V	caudal	11246.4	0.616	0.206
normoxia	prone	V	mid	4132.73	0.226	-0.533
normoxia	prone	X	cranial	6760.23	0.462	-0.0658
normoxia	prone	X	caudal	2516.94	0.172	-0.682
normoxia	prone	X	mid	5350.35	0.365	-0.239
normoxia	prone	Z	cranial	9408.98	0.574	0.131
normoxia	prone	Z	caudal	3773.74	0.230	-0.523
normoxia	prone	Z	mid	3183.52	0.194	-0.617
normoxia	down	W	cranial	3497.12	0.253	-0.469
normoxia	down	W	caudal	1805.96	0.130	-0.822
normoxia	down	W	mid	8508.53	0.616	0.205
normoxia	down	Y	cranial	24116.23	0.829	0.688
normoxia	down	Y	caudal	2553.66	0.0878	-1.016
normoxia	down	Y	mid	2390.5	0.0822	-1.048
normoxia	down	Alpha	cranial	11796.68	0.310	-0.346
normoxia	down	Alpha	caudal	2313.57	0.0609	-1.188
normoxia	down	Alpha	mid	23841.58	0.628	0.228
normoxia	prone	P	cranial	2260.68	0.260	-0.453
normoxia	prone	P	caudal	571.41	0.0658	-1.152
normoxia	prone	P	mid	5846.69	0.673	0.315
normoxia	prone	R	cranial	944.39	0.440	-0.104
normoxia	prone	R	caudal	291.73	0.136	-0.803
normoxia	prone	R	mid	908.03	0.423	-0.134
normoxia	prone	T	cranial	2880.4	0.367	-0.236
normoxia	prone	T	caudal	2337.22	0.297	-0.372
normoxia	prone	T	mid	2627.15	0.334	-0.298
normoxia	down	Q	cranial	1946.56	0.174	-0.674
normoxia	down	Q	caudal	2825.82	0.253	-0.469
normoxia	down	Q	mid	6370.38	0.572	0.125
normoxia	down	S	cranial	3746.5	0.409	-0.159
normoxia	down	S	caudal	2399.12	0.262	-0.450
normoxia	down	S	mid	3009.25	0.329	-0.310
normoxia	down	U	cranial	3158.57	0.413	-0.153
normoxia	down	U	caudal	1906.54	0.249	-0.479

normoxia	down	U	mid	2590.31	0.338	-0.291
normoxia	up	D	head	1547.59	0.196	-0.612
normoxia	up	D	caudal	893.14	0.113	-0.894
normoxia	up	D	mid	5446.56	0.691	0.349
normoxia	up	E	head	1091.63	0.173	-0.680
normoxia	up	E	caudal	1089.96	0.173	-0.681
normoxia	up	E	mid	4135.67	0.655	0.278
normoxia	up	F	head	7360.09	0.252	-0.472
normoxia	up	F	caudal	5906.71	0.202	-0.595
normoxia	up	F	mid	15903.6	0.545	0.079
normoxia	up	G	head	6728.2	0.223	-0.542
normoxia	up	G	caudal	4272.73	0.142	-0.783
normoxia	up	G	mid	19184.13	0.636	0.242
normoxia	up	H	head	10556.24	0.401	-0.174
normoxia	up	H	caudal	6018.42	0.229	-0.528
normoxia	up	H	mid	9747.8	0.370	-0.231
normoxia	up	I	head	11560.48	0.325	-0.318
normoxia	up	I	caudal	2981.74	0.084	-1.039
normoxia	up	I	mid	21057.28	0.592	0.161
normoxia	down	1	head	41947.31	0.615	0.204
normoxia	down	1	caudal	9971.61	0.146	-0.766
normoxia	down	1	mid	16281.03	0.239	-0.504
normoxia	up	2	head	22797.86	0.432	-0.118
normoxia	up	2	caudal	9680.54	0.184	-0.648
normoxia	up	2	mid	20243.55	0.384	-0.205
normoxia	prone	3	head	38895.85	0.425	-0.131
normoxia	prone	3	caudal	19904.57	0.218	-0.556
normoxia	prone	3	mid	32695.44	0.357	-0.255
normoxia	down	4	head	37525.79	0.538	0.066
normoxia	down	4	caudal	8515.38	0.122	-0.857
normoxia	down	4	mid	23724.17	0.340	-0.288
normoxia	down	5	head	36131.46	0.461	-0.067
normoxia	down	5	caudal	18651.19	0.238	-0.505
normoxia	down	5	mid	23519.6	0.300	-0.367
normoxia	prone	6	head	22521.03	0.397	-0.181
normoxia	prone	6	caudal	11440.29	0.202	-0.597
normoxia	prone	6	mid	22732.66	0.401	-0.174
anoxia	down	7	head	33343.53	0.562	0.109
anoxia	down	7	caudal	9786.43	0.165	-0.704
anoxia	down	7	mid	16151.28	0.272	-0.427

anoxia	up	8	head	14908.05	0.222	-0.544
anoxia	up	8	caudal	34527.88	0.514	0.025
anoxia	up	8	mid	17688.49	0.264	-0.446
anoxia	down	9	head	26715.9	0.441	-0.103
anoxia	down	9	caudal	9935.78	0.164	-0.707
anoxia	down	9	mid	23916.8	0.395	-0.185
anoxia	up	10	head	15612.73	0.248	-0.483
anoxia	up	10	caudal	21121.18	0.335	-0.298
anoxia	up	10	mid	26317.01	0.417	-0.145
anoxia	down	11	head	30058.09	0.503	0.005
anoxia	down	11	caudal	16053.76	0.269	-0.435
anoxia	down	11	mid	13661.82	0.229	-0.528
anoxia	up	14	head	14490.21	0.256	-0.464
anoxia	up	14	caudal	15634.46	0.276	-0.419
anoxia	up	14	mid	26501.49	0.468	-0.056
anoxia	down	15	head	36655.04	0.522	0.038
anoxia	down	15	caudal	11904.1	0.169	-0.690
anoxia	down	15	mid	21713.04	0.309	-0.350
anoxia	up	16	head	11865.15	0.223	-0.541
anoxia	up	16	caudal	12866.4	0.242	-0.495
anoxia	up	16	mid	28387.2	0.534	0.060
anoxia	down	17	head	35623.21	0.522	0.039
anoxia	down	17	caudal	8770.26	0.129	-0.831
anoxia	down	17	mid	23813.57	0.349	-0.270
anoxia	up	18	head	11959.28	0.179	-0.661
anoxia	up	18	caudal	27028.61	0.405	-0.168
anoxia	up	18	mid	27815.98	0.416	-0.147
anoxia	down	19	head	48323.77	0.487	-0.023
anoxia	down	19	caudal	24836.07	0.250	-0.477
anoxia	down	19	mid	26124.77	0.263	-0.447
anoxia	down	20	head	20844.68	0.344	-0.280
anoxia	down	20	caudal	15174.91	0.251	-0.475
anoxia	down	20	mid	24504.56	0.405	-0.167
normoxia	up	23	head	10059.04	0.435	-0.113
normoxia	up	23	caudal	7185.17	0.311	-0.345
normoxia	up	23	mid	5853.9	0.253	-0.469
normoxia	down	24	head	7729.12	0.303	-0.361
normoxia	down	24	caudal	3621.34	0.142	-0.781
normoxia	down	24	mid	14122.56	0.554	0.095
normoxia	prone	25	head	3975.68	0.341	-0.285

normoxia	prone	25	caudal	2758.5	0.237	-0.508
normoxia	prone	25	mid	4912.13	0.422	-0.137
normoxia	up	26	head	5269.98	0.379	-0.215
normoxia	up	26	caudal	4307.59	0.310	-0.348
normoxia	up	26	mid	4331.71	0.311	-0.345
normoxia	down	27	head	2722.09	0.414	-0.152
normoxia	down	27	caudal	1060.2	0.161	-0.717
normoxia	down	27	mid	2800.25	0.425	-0.131
normoxia	prone	28	head	2283.44	0.340	-0.288
normoxia	prone	28	caudal	1495.72	0.223	-0.543
normoxia	prone	28	mid	2932.94	0.437	-0.110
normoxia	down	29	head	42368.35	0.470	-0.052
normoxia	down	29	caudal	28275.48	0.314	-0.340
normoxia	down	29	mid	19510.9	0.216	-0.559
normoxia	prone	30	head	23309.89	0.483	-0.029
normoxia	prone	30	caudal	10373.45	0.215	-0.562
normoxia	prone	30	mid	14541.4	0.302	-0.365
normoxia	up	31	head	33085.08	0.414	-0.151
normoxia	up	31	caudal	20037.26	0.251	-0.475
normoxia	up	31	mid	26763.52	0.335	-0.298
normoxia	up	32	head	24836.72	0.302	-0.364
normoxia	up	32	caudal	25705.49	0.312	-0.343
normoxia	up	32	mid	31738.12	0.386	-0.202
normoxia	down	33	head	31988.39	0.549	0.086
normoxia	down	33	caudal	8585.21	0.147	-0.762
normoxia	down	33	mid	17680.66	0.304	-0.361
normoxia	down	34	head	35837.29	0.511	0.018
normoxia	down	34	caudal	15878.62	0.226	-0.534
normoxia	down	34	mid	18481.23	0.263	-0.447
normoxia	down	35	head	24554.51	0.384	-0.205
normoxia	down	35	caudal	17572.78	0.275	-0.421
normoxia	down	35	mid	21771.65	0.341	-0.287
normoxia	up	36	head	22003.61	0.346	-0.276
normoxia	up	36	caudal	15256.63	0.240	-0.500
normoxia	up	36	mid	26268.95	0.413	-0.152
normoxia	up	37	head	31852.44	0.374	-0.223
normoxia	up	37	caudal	21270.33	0.250	-0.477
normoxia	up	37	mid	32010.57	0.376	-0.220
normoxia	up	38	head	22305.46	0.180	-0.660
normoxia	up	38	caudal	66346.34	0.534	0.059

normoxia	up	38	mid	35594.39	0.286	-0.396
normoxia	prone	39	head	23654.02	0.361	-0.248
normoxia	prone	39	caudal	11494.49	0.175	-0.672
normoxia	prone	39	mid	30405.24	0.464	-0.063

Table S7.

Results of 2-way ANOVA, showing that condition (normoxia vs. anoxia) significantly affected hemolymph distribution (logit-transformed proportions for the cranial and caudal segment) within head-up animals.

Parameter	DF	SS	MS	F	P
Condition	1	0.000	0.000	0.000	1.0
Segment	1	0.1311	0.1311	2.718	0.1084
condition:segment	1	0.7974	0.7974	16.533	0.0003***
Residuals	34	1.640	0.0482		

Table S8. Heart rates of grasshoppers measured visually through the dorsal cuticle, after removing the wings, using a light microscope. Locusts were placed into one of three positions (head-up, head-down, prone) in random order. Heart rate was counted three times, 0.5, 2 and 5 min after being placed in position.

Animal	Position	Time	BPM
1	down	0.5	78.6
2	down	0.5	78.6
3	down	0.5	89.3
4	down	0.5	88.0
5	down	0.5	82.6
6	down	0.5	88.0
7	down	0.5	80.0
8	down	0.5	86.7
9	down	0.5	73.3
10	down	0.5	81.3
11	down	0.5	77.3
12	down	0.5	69.3
13	down	0.5	84.0
14	down	0.5	76.0
15	down	0.5	70.7
1	down	2	81.3
2	down	2	74.7
3	down	2	85.3
4	down	2	81.3
5	down	2	85.3
6	down	2	82.7
7	down	2	85.3
8	down	2	77.3
9	down	2	77.3
10	down	2	86.7
11	down	2	78.7
12	down	2	61.3
13	down	2	74.7
14	down	2	69.3
15	down	2	74.7
1	down	5	80.0
2	down	5	74.7
3	down	5	86.7
4	down	5	78.7

5	down	5	81.3
6	down	5	84.0
7	down	5	84.0
8	down	5	78.6
9	down	5	70.7
10	down	5	82.7
11	down	5	72.0
12	down	5	66.7
13	down	5	76.0
14	down	5	73.3
15	down	5	68.0
1	prone	0.5	81.3
2	prone	0.5	73.3
3	prone	0.5	76.0
4	prone	0.5	78.7
5	prone	0.5	76.0
6	prone	0.5	88.0
7	prone	0.5	85.3
8	prone	0.5	80.0
9	prone	0.5	76.0
10	prone	0.5	78.7
11	prone	0.5	64.0
12	prone	0.5	62.7
13	prone	0.5	77.3
14	prone	0.5	74.7
15	prone	0.5	73.3
1	prone	2	85.3
2	prone	2	70.7
3	prone	2	84.0
4	prone	2	70.7
5	prone	2	78.7
6	prone	2	88.0
7	prone	2	86.7
8	prone	2	81.3
9	prone	2	70.7
10	prone	2	81.3
11	prone	2	74.7
12	prone	2	62.7
13	prone	2	78.7

14	prone	2	76.0
15	prone	2	68.0
1	prone	5	85.3
2	prone	5	73.3
3	prone	5	85.3
4	prone	5	85.3
5	prone	5	78.7
6	prone	5	89.3
7	prone	5	86.7
8	prone	5	80.0
9	prone	5	74.7
10	prone	5	81.3
11	prone	5	72.0
12	prone	5	64.0
13	prone	5	77.3
14	prone	5	73.3
15	prone	5	70.7
1	up	0.5	80.0
2	up	0.5	77.3
3	up	0.5	89.3
4	up	0.5	80.0
5	up	0.5	76.0
6	up	0.5	89.3
7	up	0.5	84.0
8	up	0.5	81.3
9	up	0.5	70.7
10	up	0.5	84.0
11	up	0.5	74.7
12	up	0.5	68.0
13	up	0.5	82.7
14	up	0.5	73.3
15	up	0.5	70.7
1	up	2	81.3
2	up	2	73.0
3	up	2	84.0
4	up	2	80.0
5	up	2	81.3
6	up	2	84.0
7	up	2	85.3

8	up	2	80.0
9	up	2	76.0
10	up	2	89.3
11	up	2	73.3
12	up	2	66.7
13	up	2	81.3
14	up	2	82.7
15	up	2	76.0
1	up	5	81.3
2	up	5	76.0
3	up	5	87.7
4	up	5	80.0
5	up	5	76.0
6	up	5	84.0
7	up	5	85.3
8	up	5	78.7
9	up	5	74.7
10	up	5	85.3
11	up	5	73.3
12	up	5	62.0
13	up	5	80.0
14	up	5	81.3
15	up	5	74.7

Table S9. Heart rates of grasshoppers measured from x-ray video. Each row represents a different individual.

Head-up	Head-down
51	42
54	50
52	50
68	62

Table S10. Abdominal pumping rates for locusts in different orientations. Ventilation rates are abdominal pumping rates in Hz. The 1 min rates report the average rate during the first min after assuming the stated orientation, and the 5 min rates report the average during the 5 min after assuming that orientation. Animals listed in the order they were tested.

animal ID	Sex	Prior Orientation	Orientation	ventilation 1 min	ventilation rate 5 min
1	F	prone	prone	0.68	0.45
2	F	prone	head-up	0.55	0.53
3	F	prone	head-down	1.2	1.2
4	M	head-up	prone	0.6	0.65
5	M	head-up	head-up	0.69	0.6
6	M	head-up	head-down	1.01	1.02
7	F	head-down	prone	0.46	0.37
8	M	head-down	head-up	0.47	0.42
9	F	head-down	head-down	0.87	0.77
10	F	prone	prone	0.54	0.52
11	F	prone	head-up	1.16	1.57
12	M	prone	head-down	0.53	0.78
13	F	head-up	prone	0.7	0.78
14	M	head-up	head-up	0.45	0.55
15	M	head-up	head-down	0.41	0.65
16	F	head-down	prone	0.46	0.37
17	F	head-down	head-up	0.66	0.67
18	M	head-down	head-down	0.48	0.37
19	F	prone	prone	0.59	0.5
20	F	prone	head-up	0.8	0.68
21	M	prone	head-down	0.96	0.98
22	F	head-up	prone	1.02	0.93
23	F	head-up	head-up	0.88	0.83
24	F	head-up	head-down	0.92	1.22
25	M	head-down	prone	0.75	0.82
26	M	head-down	head-up	0.77	0.82
27	F	head-down	head-down	0.95	1
28	M	prone	prone	0.68	0.7
29	F	prone	head-up	0.74	0.83
30	M	prone	head-down	0.8	0.8
31	M	head-up	prone	0.8	0.72
32	F	head-up	head-up	0.96	1.02
33	M	head-up	head-down	0.93	0.8
34	M	head-down	head-up	0.85	0.78
35	F	head-down	prone	1.17	1.13

36	F	head-down	head-down	0.79	1.15
37	M	prone	prone	0.84	0.84
38	F	prone	head-up	0.59	1.02
39	F	prone	head-down	0.81	0.7
40	M	head-up	prone	0.92	0.82
41	M	head-down	head-up	0.76	0.75
42	M	head-up	head-down	0.7	0.65
43	F	head-down	prone	0.73	0.97
44	M	head-down	head-up	0.73	0.7
45	F	head-down	head-down	0.94	0.83
46	F	prone	prone	0.69	0.68
47	M	prone	head-up	0.93	0.8
48	M	prone	head-down	1.2	1.15
49	M	head-up	prone	0.84	0.83
50	M	head-up	head-up	0.9	0.93
51	M	head-up	head-down	1.06	0.93
52	F	head-down	prone	0.9	0.85
53	F	head-down	head-up	0.57	0.6
54	F	head-down	head-down	0.94	0.82
55	M	prone	prone	0.72	0.67
56	F	prone	head-up	0.96	0.9
57	F	prone	head-down	1.37	1.35
58	F	head-up	prone	0.93	0.87
59	M	head-down	head-up	0.99	0.78
60	F	head-up	head-down	0.86	0.8
61	M	head-down	prone	0.53	0.43
62	M	head-down	head-up	1.19	0.82
63	F	head-down	head-down	0.87	0.92
64	M	prone	prone	1.15	1
65	M	prone	head-up	0.51	0.5
66	F	prone	head-down	1.12	1.02
67	M	head-up	prone	0.95	1.15
68	F	head-up	head-up	0.63	0.75
69	F	head-up	head-down	0.83	0.87
70	M	head-down	prone	0.45	0.57
71	M	head-down	head-up	0.57	0.47
72	M	head-down	head-down	0.77	0.67
73	M	prone	prone	0.56	0.45
74	F	prone	head-up	0.87	0.93
75	M	prone	head-down	0.68	0.63

76	M	head-up	prone	0.95	0.98
77	F	head-up	head-up	0.59	0.48
78	M	head-up	head-down	0.58	0.52
79	M	head-down	prone	0.31	0.3
80	F	head-down	head-up	0.47	0.62
81	F	head-down	head-down	0.54	0.57
82	F	prone	prone	0.79	0.7
83	F	prone	head-up	0.84	0.83
84	M	prone	head-down	0.83	0.88
85	M	head-up	prone	0.87	0.8
86	F	head-up	head-up	1.2	1.13
87	F	head-up	head-down	1.17	1.1
88	M	head-down	prone	0.66	0.62
89	M	head-down	head-up	0.81	0.68
90	M	head-down	head-down	0.69	0.7
91	F	prone	prone	0.85	0.77
92	M	prone	head-up	0.66	0.65
93	M	prone	head-down	0.82	0.87
94	F	head-up	prone	0.58	0.63
95	F	head-up	head-up	0.66	0.58
96	M	head-up	head-down	0.98	0.98
97	M	head-down	prone	0.96	0.77
98	F	head-down	head-up	0.66	0.67
99	F	head-down	head-down	1.08	0.93

Table S11. Summary statistics for the regressions shown in Figure S4.

Specimen number	Number of data points	Slope (CI) of the fit line	Intercept (CI) of the fit line	R_2
1	1205	0.83±0.12	0.36± 0.16	0.69
2	1272	0.28±0.27	1.36± 0.36	0.12
3	1962	0.58±0.08	0.53±0.11	0.59
4	1649	0.62±0.05	0.79±0.10	0.71
5	772	1.24±0.05	-0.04±0.06	0.81
6	655	0.99±0.07	-0.04±0.05	0.75
7	2952	1.12±0.04	-0.05±0.05	0.90
8	703	0.37±0.10	0.44±0.10	0.43
9	1288	3.26±1.46	-2.65±1.69	0.32

Table S12. Mean static pressure changes and calculated expected values, from figure S5.

Thorax	HO -HD	HO -HU	HD-HO	HU-HO
Specimen 1	0.12	0.23	-0.16	0.19
Specimen 2	0.44	0.37	-0.45	0.06
Specimen 3	0.19	0.46	0.44	0.37
Specimen 4	0.15	0.38	0.33	0.13
Specimen 5	-0.05	-0.35	-0.16	0.44
Specimen 6	0.06	-0.03	-0.14	0.04
Specimen 7	-0.26	0.13	0.16	0.36
Specimen 8	0.01	0.66	0.01	0.10
Specimen 9	0.03	-0.08	-0.01	0.09
Specimen 10	-0.24	-0.06	-0.20	0.02
Mean	0.05	0.17	-0.02	0.18
SE of Mean	0.07	0.10	0.08	0.05
Expected Value	0.30	0.01	-0.30	-0.01

Abdomen	HO -HD	HO -HU	HD-HO	HU-HO
Specimen 1	0.12	0.23	-0.16	0.19
Specimen 2	0.44	0.37	-0.45	0.06
Specimen 3	0.19	0.46	0.44	0.37
Specimen 4	0.15	0.38	0.33	0.13
Specimen 5	-0.05	-0.35	-0.16	0.44
Specimen 6	0.06	-0.03	-0.14	0.04
Specimen 7	-0.26	0.13	0.16	0.36
Specimen 8	0.01	0.66	0.01	0.10
Specimen 9	0.03	-0.08	-0.01	0.09
Specimen 10	-0.24	-0.06	-0.2	0.02
Mean	0.05	0.17	-0.02	0.18
SE of Mean	0.07	0.10	0.08	0.05
Expected Value	0.08	0.23	-0.08	-0.23

Fig. S1. Example image analyzed for the proportion of the image occupied by air sacs and tracheae. This is an image of a caudal region (near abdominal tip) for a head-down animal. A. Raw x-ray image. B. Air sacs traced in yellow, tracheae traced in blue, body outline traced in purple.

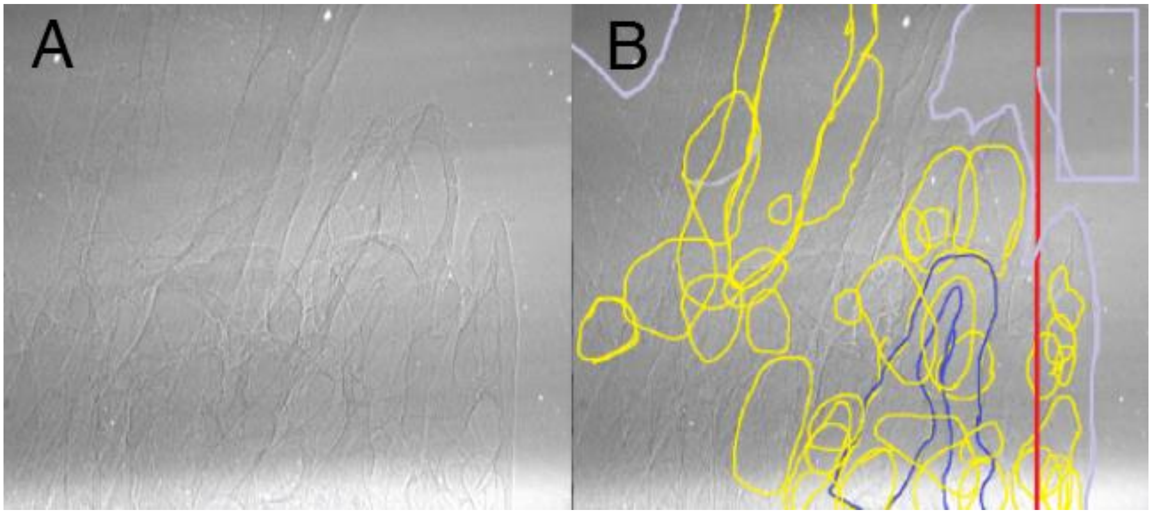


Fig. S2. Enlarged μCT images from figure 2 with tubes and automotive grease removed for image analysis (left). μCT images of the grasshopper fixed with by automotive grease (grey, right-top) and unobstructed thoracic spiracles indicated with arrows (right-bottom).

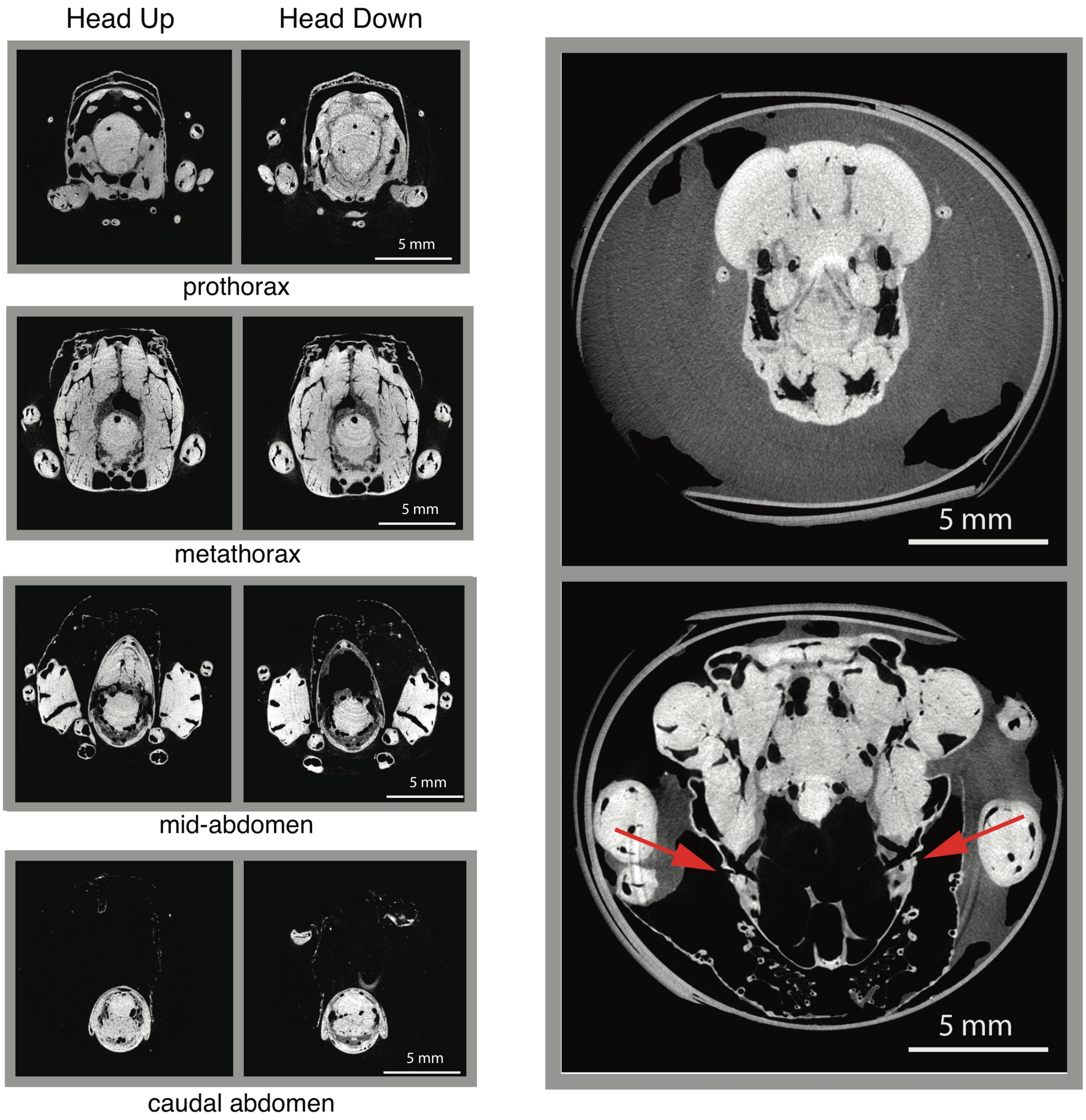


Fig. S3. Output of heart rate counter tool used to analyze synchrotron x-ray video of the tracheae surrounding the locust heart. Each peak indicates a movement of the cardiac tracheae.

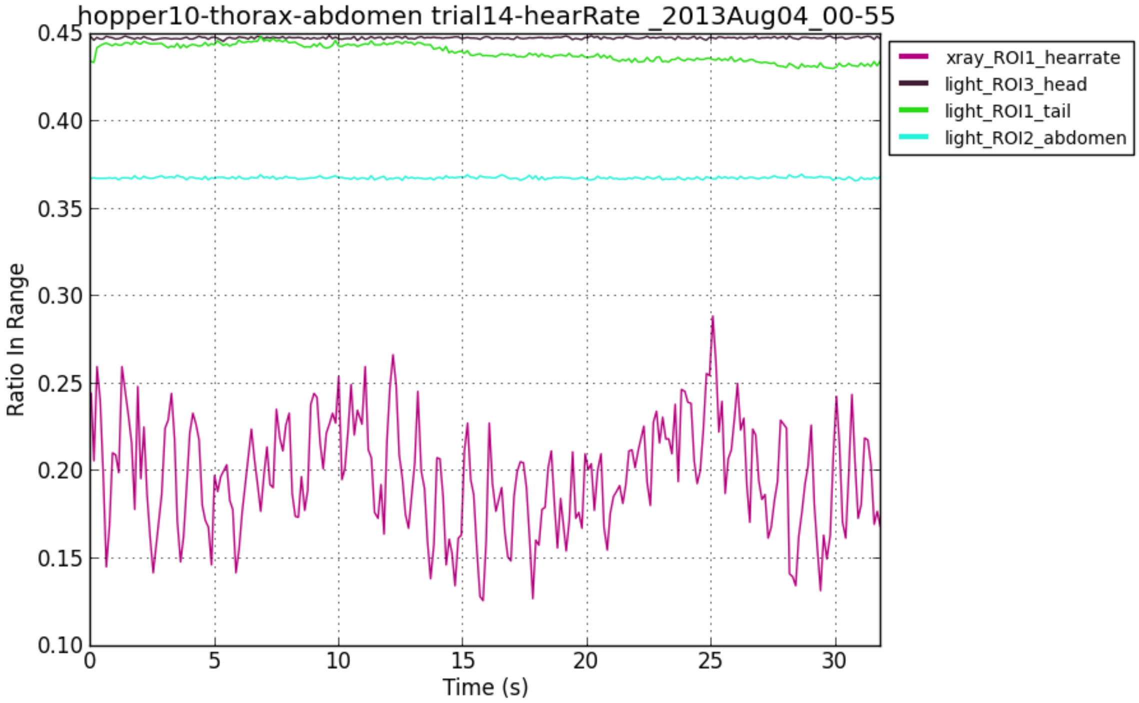
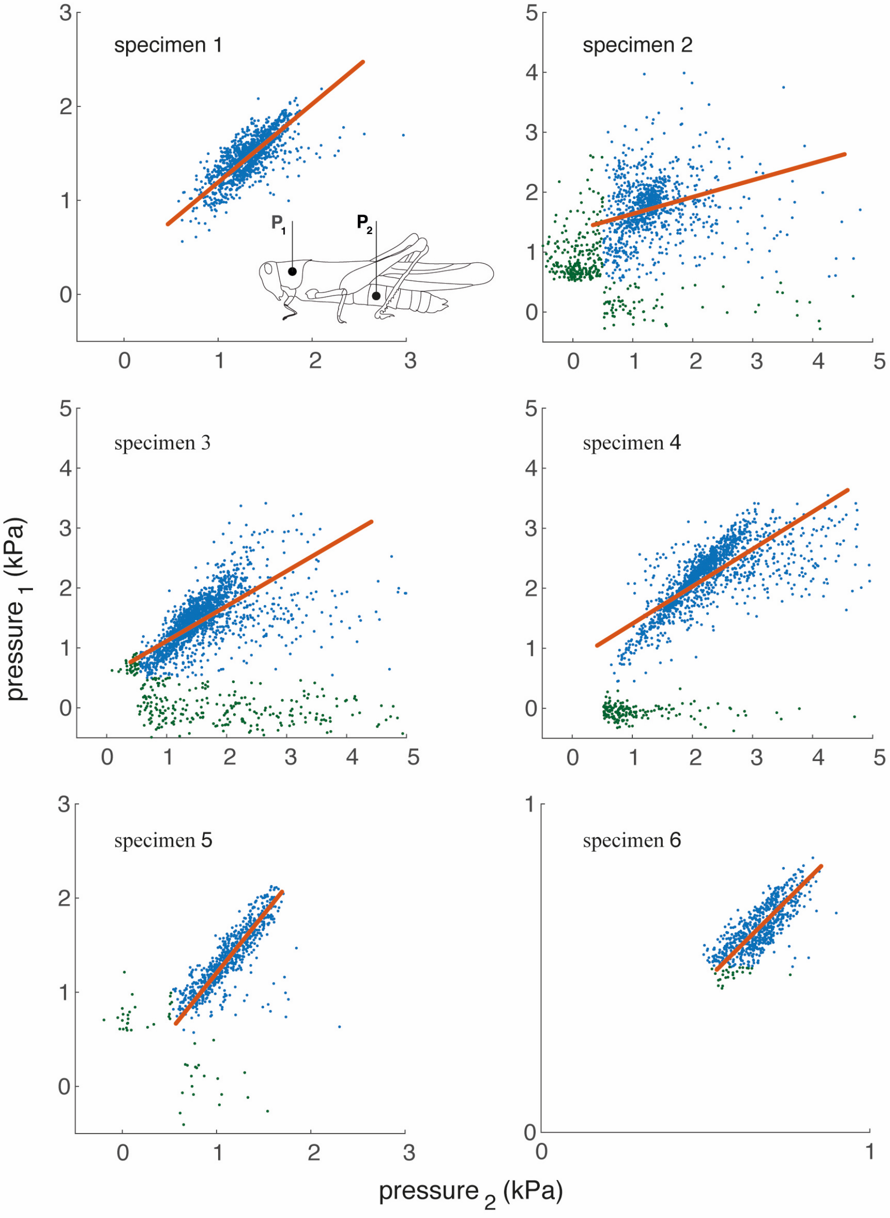


Fig. S4. Hemolymph pressure plots for each individual specimen. Each data point represents the maximum pressure for pressure pulsations measured at two locations in the body (points 1 and 2). The red line represents a fit using the total least square method as calculated in (1). Slopes, intercepts, and confidence limits for these plots are in Table S11. In this fit, only larger pressure pulsations are included; specifically, points in which $P_1 > 0.5$ and $P_2 > 0.5\ kPa$ (blue dots) due to concerns that low pressures could result from occlusion of the sensor tip. The green dots represent points that do not meet these criteria. A previous study determined that a slow coagulation of hemolymph around the sensor tips resulted in an error of 5% (1). Therefore, if the calculated slope range is beyond 1.0±0.05, we considered that $P_1 \neq P_2$ with a confidence of 95%. Using this definition, the data show that the pressure pulses in the thorax and abdomen are significantly different in all specimens except one animal (#6).



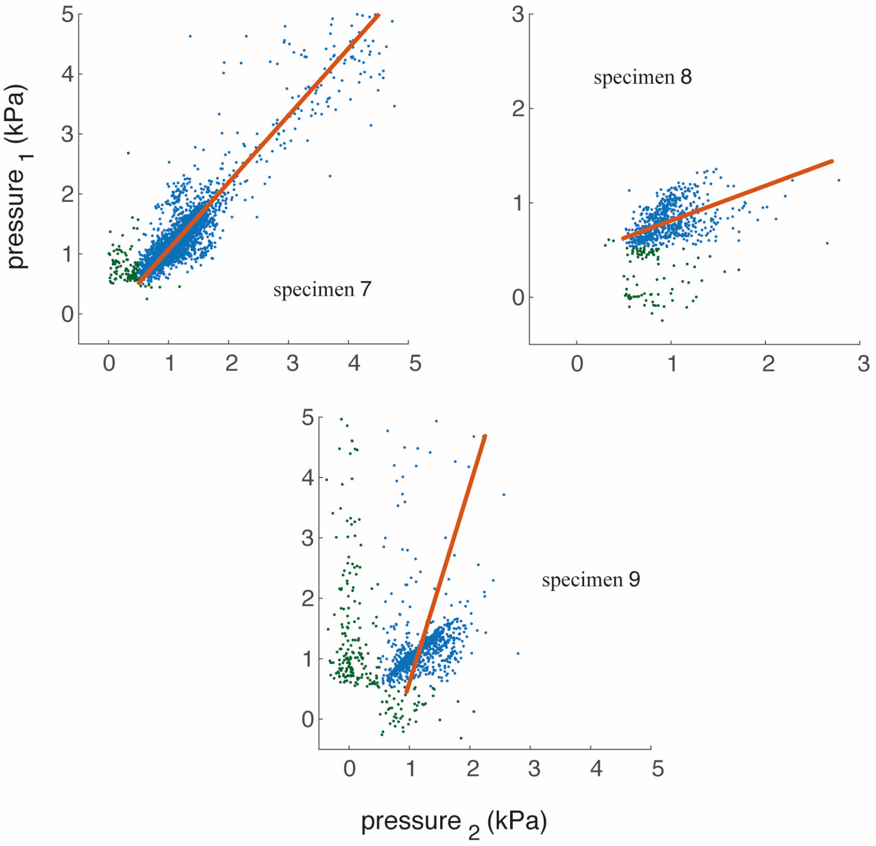

pressure 1 (kPa)
specimen 7
specimen 8
specimen 9
pressure 2 (kPa)
0
1
2
3
4
5

Figure S5. Theoretical consideration of hemolymph pressures in *Schistocerca americana*. Pressures were experimentally measured at two locations (i): in the thorax (P_1) and the abdomen (P_2), separated by a distance of approximately 1.5 cm. In the schematic illustrations (ii-v), the hemocoel is considered as a fluidic container. In (ii), pressures at the thorax and abdomen are determined by hydrostatic pressures, set by the different heights (h_1, h_2) of the fluidic column above each location. Compared to (ii), the magnitude of h_2 would decrease if the hemocoel were functionally compartmentalized, represented in (iii) by a barrier (gray circle); h_1 would remain the same. If the cylinder were reoriented to the horizontal position, both h_1 and h_2 would change. Because we found no effect of body orientation on pressures, we conclude that hydrostatic pressures are relatively unimportant. Hemolymph flows at either location (speeds u_1 and u_2, iv) would add a dynamic pressure contribution. However, as discussed in the main body, our data demonstrate that the dynamic pressure contribution must be negligible. Finally, pressure differences between P_1 and P_2 could be due to losses due to viscous shear as hemolymph is pushed through confined spaces separating the thorax and abdomen. As discussed in the main body by analyzing a connecting pipe (vi), this effect is plausible but unlikely.

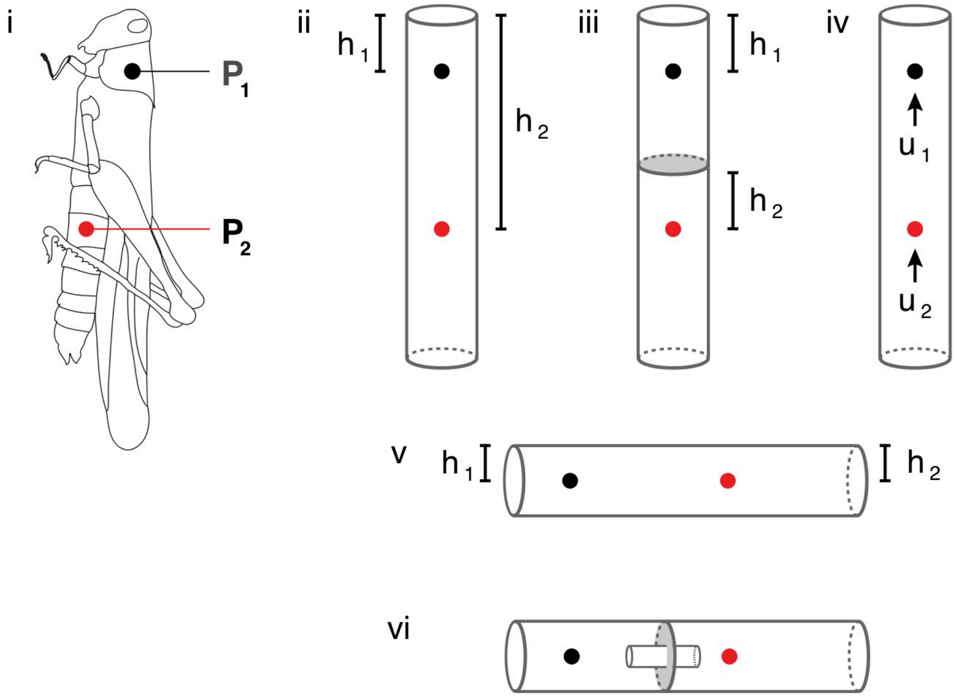


Fig. S6. Locusts were able to regulate thorax and abdominal hemolymph pressures at statistically constant levels during changes in body orientation (Paired t tests, all P's > 0.05). Red circles indicate predicted changes in pressure due to passive effects of gravity. Results and predicted values can be found in supplemental table S11.

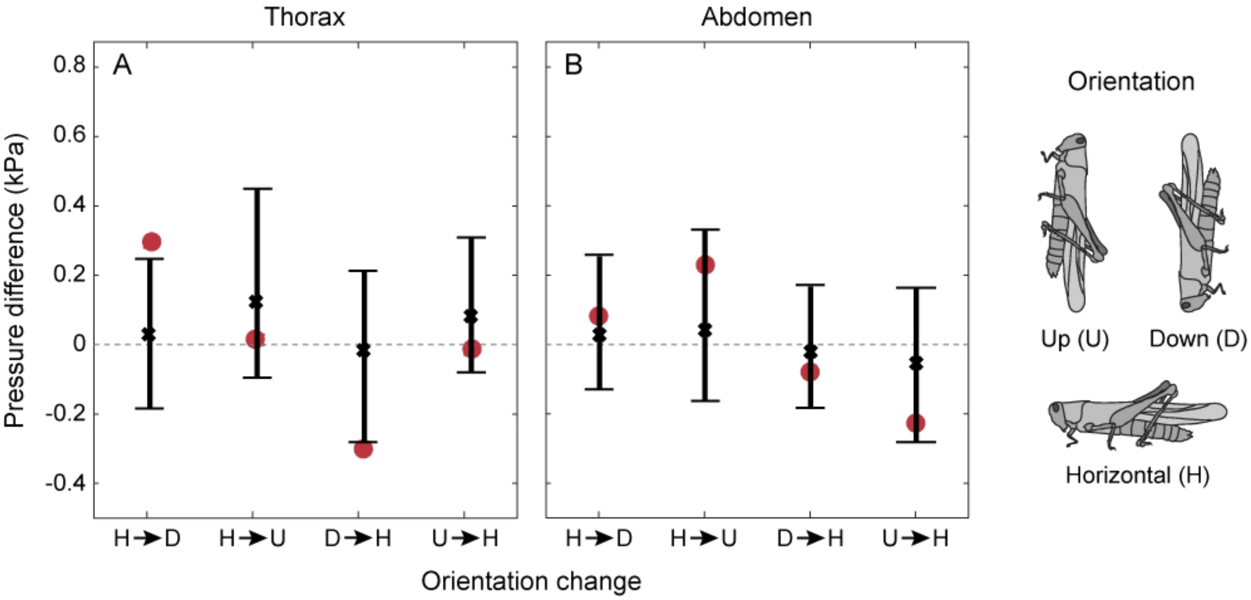
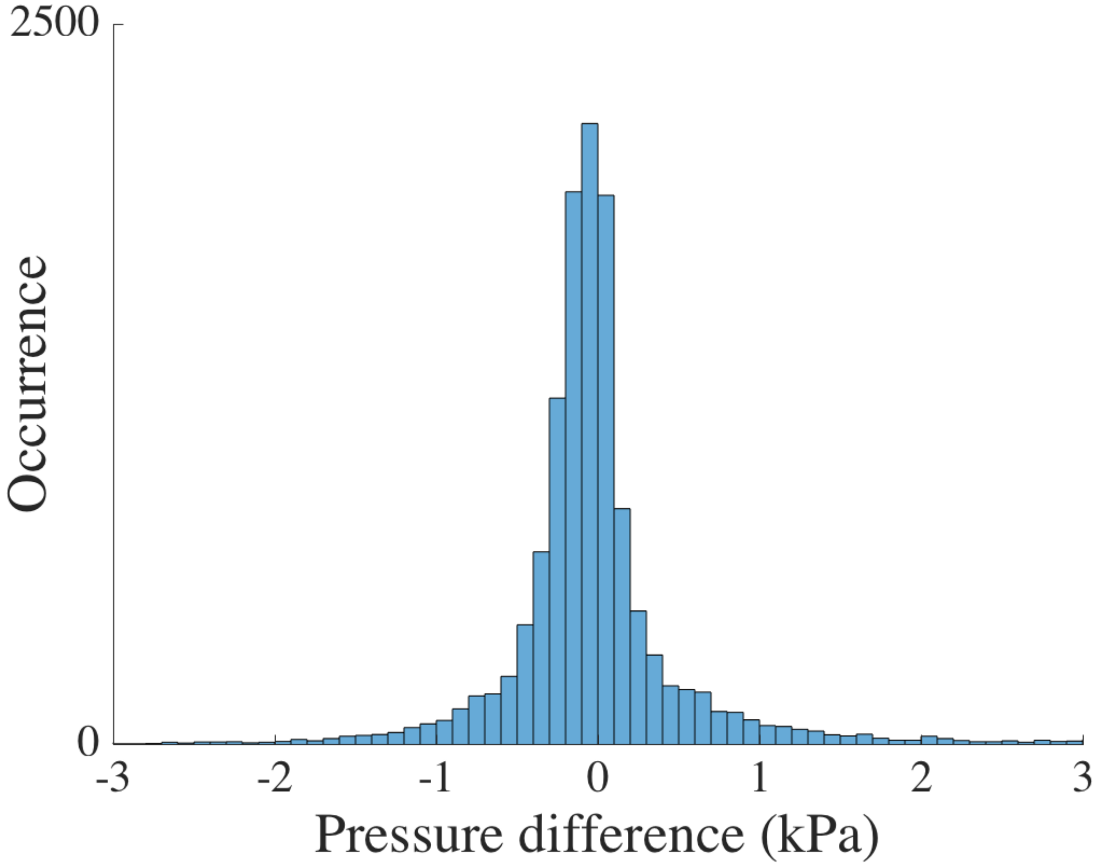


Fig. S7. Distribution plot of pressure differences between thorax and abdomen, including all data. Negative values indicate that pressures in the thorax were higher than in the abdomen, and positive values the reverse. The average absolute value pressure difference was 350 pascals (standard deviation = 527 pascals, mean pulse duration = 1.33 s, standard deviation = 0.69 s).



Supplementary movies S1. Left: Light video of grasshopper mounted head-down with automated ventilation counter. Right: Simultaneous x-ray imaging of air sacs inside locust.

Supplementary movies S2. Left: X-ray video of cardiac tracheae of *S. americana*, with overlaying pulse-counter. Right: Output of heart rate counter.

References:

1. H. Pendar, J. Aviles, K. Adjerid, C. Schoenewald, J. J. Socha, Functional compartmentalization in the hemocoel of insects. *Scientific Reports* **9**, 6075 (2019).