Supplement for:

Adult snow crab, *Chionoecetes opilio*, displays body-wide exoskeletal resistance to the effects of long-term ocean acidification

Tait Algayer[1], Ahmed Mahmoud[1], Sanjana Saksena[1], W. Christopher Long[2], Katherine M. Swiney[2,5], Robert J. Foy[2], Brittan V. Steffel[3], Kathryn E. Smith[4], Richard B. Aronson[3], and Gary H. Dickinson[1*]

[1]Department of Biology, The College of New Jersey, 2000 Pennington Rd., Ewing, NJ 08628, USA

[2]NOAA, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment and Conservation Engineering Division, Kodiak Laboratory, 301 Research Ct., Kodiak, AK 99615, USA

[3]Department of Ocean Engineering and Marine Sciences, Florida Institute of Technology, Melbourne, FL 32901, USA

[4]The Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK

[5]Current address: NOAA, National Marine Fisheries Service, Southwest Fisheries Science Center, Fisheries Resources Division, 8901 La Jolla Shores Dr., La Jolla, CA 92037, USA

*Corresponding author: Gary H. Dickinson, dickinga@tcnj.edu, ORCiD 0000-0003-1073-1483

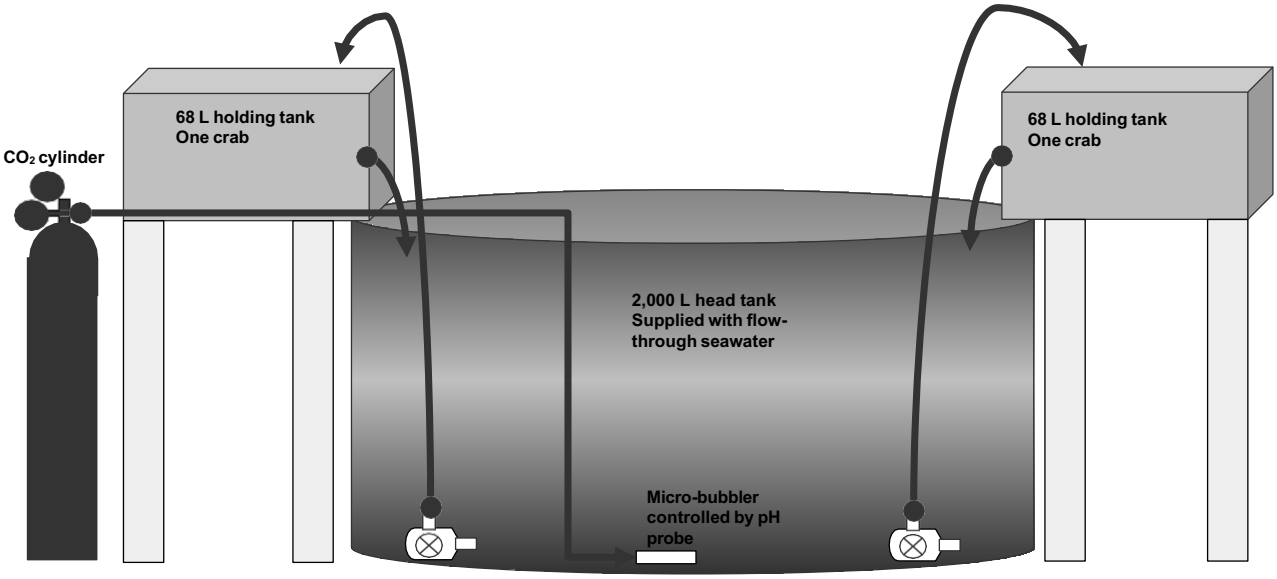

Figure S1. Illustration of holding setup for snow crab during hatching. Each crab was held in a separate 68L holding tank that received recirculating water from a larger head tank. The head tank was acidified via direct bubbling of pure CO_2 which was controlled by a Durafet III pH probe. One head tank was set up for each of three pH treatments.

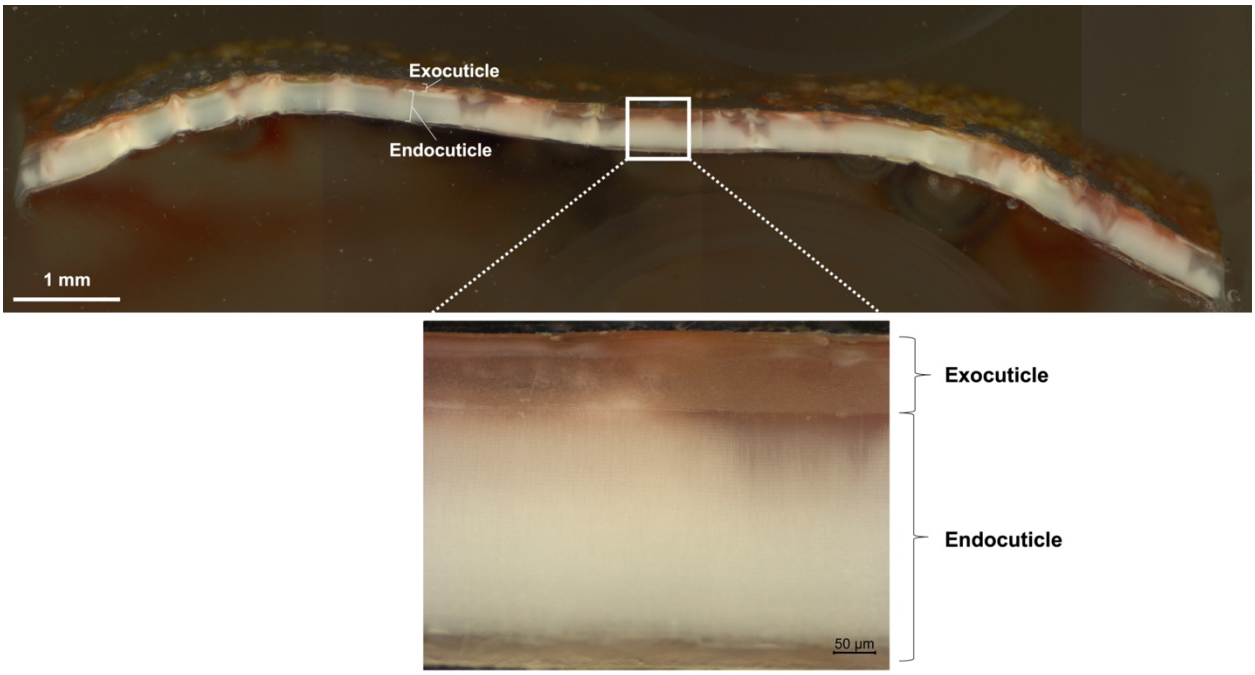

Figure S2. *Chinoecetes opilio* embedded and polished carapace cross-section showing distinction between exo- and endocuticle layers. The exocuticle is characterized by a higher density of Bouligand layers (thinner individual layers), which results in a distinct difference in coloration under darkfield illumination.

Table S1. Sample sizes for structural, mechanical, and chemical assessments.

	Carapace	Right Claw	Left Claw	Claws Combined	Right Leg	Left Leg	Legs Combined
pH 8.1	4	1	3	4	1	2	3
pH 7.8	12	12	10	22	8	8	16
pH 7.5	7	5	6	11	3	5	8

Table S2. PERMANOVA analysis of structural, mechanical, and chemical properties of the snow crab exoskeleton from animals held at three different pH levels for two years.

	df	Sums Of Squares	Mean Squares	F	R^2	p
pH Treatment	2	13.45	6.725	1.8296	0.01738	0.07532
Body region	4	447.03	111.758	30.4045	0.57756	0.00001
pH Treatment x Body region	8	35.48	4.436	1.2067	0.04585	0.21077
Crab ID (pH Treatment)	20	86.89	4.345	1.182	0.11227	0.1787
Residuals	52	191.14	3.676	0.24695		
Total	86	774	1			

Table S3. SIMPER analysis of structural, mechanical, and chemical properties of the snow crab exoskeleton showing which factors contributed most to the differences among body regions.

Groups: Carapace & Claw **Average squared distance = 20.80**						
	Group: Carapace	**Group: Claw**				
Variable	**Av. Value**	**Av. Value**	**Av.Sq.Dist**	**Sq.Dist/SD**	**Contrib%**	**Cum.%**
Exo Thickness	0.751	-0.648	3.83	0.88	18.39	18.39
Endo Bouligand	-0.98	0.623	3.27	0.97	15.74	34.13
Endo Hardness	-0.714	0.612	2.78	0.64	13.39	47.52
Exo Hardness	-1.06	0.316	2.76	1.07	13.25	60.77
% Ca	-0.405	1.06	2.28	1.93	10.97	71.74
% Mg	0.288	-1.03	1.88	2.04	9.02	80.76
Endo Thickness	-0.00603	0.801	1.45	0.64	6.96	87.72
% Sr	-0.173	0.43	1.44	0.77	6.92	94.64
Total Thickness	0.181	0.684	1.11	0.6	5.36	100
Groups: Carapace & Leg. **Average squared distance = 15.47**						
	Group: Carapace	**Group Leg:**				
Variable	**Av. Value**	**Av. Value**	**Av.Sq.Dist**	**Sq.Dist/SD**	**Contrib%**	**Cum.%**
Exo Hardness	-1.06	0.473	3.4	1.13	21.97	21.97
Total Thickness	0.181	-1.09	2.65	0.95	17.13	39.1
% Sr	-0.173	-0.442	2.05	0.61	13.24	52.34
Endo Thickness	-0.00603	-1.09	1.8	1.07	11.61	63.95
Exo Thickness	0.751	0.249	1.77	0.55	11.43	75.38
Endo Bouligand	-0.98	-0.0194	1.3	0.84	8.38	83.75
% Mg	0.288	1.16	0.964	1.22	6.23	89.98
Endo Hardness	-0.714	-0.23	0.89	0.79	5.75	95.74
% Ca	-0.405	-1.11	0.659	1.2	4.26	100

Groups: Claw & Leg Average squared distance = 29.49						
	Group: Claw	Group: Leg				
Variable	**Av. Value**	**Av. Value**	**Av.Sq.Dist**	**Sq.Dist/SD**	**Contrib%**	**Cum.%**
% Ca	1.06	-1.11	5	2.76	16.97	16.97
% Mg	-1.03	1.16	4.96	2.4	16.82	33.79
Endo Thickness	0.801	-1.09	4.63	1.16	15.71	49.5
Total Thickness	0.684	-1.09	4.41	1.04	14.94	64.44
% Sr	0.43	-0.442	3.21	0.64	10.88	75.32
Exo Thickness	-0.648	0.249	2.07	0.87	7.02	82.34
Endo Hardness	0.612	-0.23	1.88	0.56	6.39	88.73
Endo Bouligand	0.623	-0.0194	1.86	0.71	6.32	95.05
Exo Hardness	0.316	0.473	1.46	0.82	4.95	100

Table S4. Univariate GLM analysis of structural, mechanical, and chemical properties of the snow crab exoskeleton from animals held at three different pH levels for two years.

Exocuticle microhardness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Corrected Model	6636.290	28	237.01	2.29	0.004	0.525
Intercept	72785.39	1	72785.39	703.274	<0.0005	0.924
pH	167.434	2	83.717	0.809	0.45	0.027
Body region	3020.165	2	1510.082	14.591	<0.0005	0.335
pH(CrabID)	1095.487	20	54.774	0.529	0.942	0.154
pH * Body region	210.063	4	52.516	0.507	0.73	0.034
Error	6002.712	58	103.495			
Total	163985.264	87				
Corrected Total	12639.002	86				
Endocuticle microhardness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	6452.427	28	230.444	2.366	0.003	0.533
Intercept	54476.197	1	54476.197	559.325	<0.0005	0.906
pH	20.728	2	10.364	0.106	0.899	0.004
Body region	3606.689	2	1803.344	18.516	<0.0005	0.39
pH(CrabID)	1941.079	20	97.054	0.996	0.48	0.256
pH * Body region	754.418	4	188.605	1.936	0.116	0.118
Error	5648.983	58	97.396			
Total	121626.769	87				
Corrected Total	12101.41	86				

Total thickness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	924004.921	28	33000.176	5.527	<0.0005	0.727
Intercept	9703227.81	1	9703227.81	1625.007	<0.0005	0.966
pH	7324.812	2	3662.406	0.613	0.545	0.021
Body region	374793.069	2	187396.535	31.383	<0.0005	0.52
pH(CrabID)	134015.589	20	6700.779	1.122	0.354	0.279
pH * Body region	37166.148	4	9291.537	1.556	0.198	0.097
Error	346329.158	58	5971.192			
Total	19974173.3	87				
Corrected Total	1270334.08	86				

Exocuticle thickness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	35323.560	28	1261.556	2.119	0.008	0.506
Intercept	196531.007	1	196531.007	330.079	<0.0005	0.851
pH	557.388	2	278.694	0.468	0.629	0.016
Body region	13576.384	2	6788.192	11.401	<0.0005	0.282
pH(CrabID)	7048.162	20	352.408	0.592	0.903	0.17
pH * Body region	2641.887	4	660.472	1.109	0.361	0.071
Error	34533.55	58	595.406			
Total	401037.072	87				
Corrected Total	69857.11	86				

Endocuticle thickness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	1075522.943	28	38411.534	6.93	<0.0005	0.77
Intercept	7121864.67	1	7121864.67	1284.802	<0.0005	0.957
pH	12297.15	2	6148.575	1.109	0.337	0.037
Body region	470731.124	2	235365.562	42.461	<0.0005	0.594
pH(CrabID)	120375.848	20	6018.792	1.086	0.388	0.272
pH * Body region	29712.172	4	7428.043	1.34	0.266	0.085
Error	321503.456	58	5543.163			
Total	15420023.2	87				
Corrected Total	1397026.4	86				

Endocuticle Bouligand thickness						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	322.517	28	11.518	3.059	<0.0005	0.596
Intercept	1665.856	1	1665.856	442.427	<0.0005	0.884
pH	7.998	2	3.999	1.062	0.352	0.035
Body region	189.729	2	94.865	25.195	<0.0005	0.465
pH(CrabID)	63.48	20	3.174	0.843	0.654	0.225
pH * Body region	11.955	4	2.989	0.794	0.534	0.052
Error	218.385	58	3.765			
Total	3817.446	87				
Corrected Total	540.903	86				

Calcium content						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	2967.906	28	105.997	46.75	<0.0005	0.958
Intercept	25379.833	1	25379.833	11193.91	<0.0005	0.995
pH	29.097	2	14.548	6.417	0.003	0.181
Body region	1678.777	2	839.388	370.217	<0.0005	0.927
pH(CrabID)	75.512	20	3.776	1.665	0.068	0.365
pH * Body region	7.039	4	1.76	0.776	0.545	0.051
Error	131.503	58	2.267			
Total	53901.932	87				
Corrected Total	3099.409	86				

Magnesium content						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	9.373	28	0.335	39.488	<0.0005	0.95
Intercept	36.166	1	36.166	4266.238	<0.0005	0.987
pH	0.084	2	0.042	4.967	0.01	0.146
Body region	5.281	2	2.641	311.496	<0.0005	0.915
pH(CrabID)	0.387	20	0.019	2.282	0.008	0.44
pH * Body region	0.059	4	0.015	1.751	0.151	0.108
Error	0.492	58	0.008			
Total	70.108	87				
Corrected Total	9.865	86				

Strontium content						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**p**	**Partial Eta Squared**
Corrected Model	.035	28	0.001	2.982	<0.0005	0.59
Intercept	6.523	1	6.523	15562.934	<0.0005	0.996
pH	0.002	2	0.001	2.717	0.075	0.086
Body region	0.005	2	0.003	6.039	0.004	0.172
pH(CrabID)	0.02	20	0.001	2.393	0.005	0.452
pH * Body region	0.001	4	0	0.485	0.746	0.032
Error	0.024	58	0			
Total	12.242	87				
Corrected Total	0.059	86				

Table S5. Maximum percent difference between ambient and reduced pH (pH 7.8 or 7.5) conditions for exoskeleton properties in which a significant effect of pH was not detected.

Exoskeleton Property	**Percent Difference**
Exocuticle microhardness	6.8
Endocuticle microhardness	3.1
Total thickness	7.8
Exocuticle thickness	6.8
Endocuticle thickness	10.1
Endocuticle Bouligand thickness	8.6
Strontium content	5.7