

The subtle effects of sea water acidification on the amphipod *Gammarus locusta*
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Supplementary data

Suppl. Table 1. Carbonate chemistry data for the three incubations

DIC control	DIC pH 7.8	DIC pH 7.6	ALK _{TOT} Control	ALK _{TOT} pH 7.8	ALK _{TOT} pH 7.6
2174.04	2286.38	2516.63	2770.27	2862.00	2738.72
2201.72	2255.41	2457.49	2785.96	2716.38	2786.24
2378.45	2495.02	2636.72	2947.82	2941.70	2930.79
2340.90	2497.56	2786.73	2936.65	2928.32	3088.49

Suppl. Table 2. Environmental parameters recorded for the three incubations

Measured pH			Measured temperature			Measured salinity		
CONTROL	'pH7.8'	'pH7.6'	CONTROL	'pH7.8'	'pH7.6'	CONTROL	'pH7.8'	'pH7.6'
7.95	7.73	7.51	19	19	19	34.6	34.6	35.1
8.089	7.85	7.91	19	19	19	34.8	34.6	35.6
8.019	7.821	7.457	20	20	20	34.8	34.6	34.1
7.984	7.805	7.522	19.5	19.5	19.5	35	34.6	35.4
7.96	7.81	7.469	20.3	19.6	19.5	33.9	33.9	34.2
8.008	7.822	7.493	19.8	19.5	19.3	34.2	34.1	34.8
8.015	7.834	7.503	19.9	19.4	19.5	34.6	34.2	35.1
8.081	7.895	7.504	20.1	19.5	19.6	34.1	34	34.5
8.122	7.9	7.504	19.9	19.6	19.7	34.3	34	34.5
8.059	7.925	7.554	20.5	19.5	19.8	34.1	34.1	34.2
8.067	7.907	7.512	21.3	19.8	19.8	34.3	34.3	34.6
8.12	7.812	7.507	21.2	19.8	19.8	34.3	34.1	34.4
8.131	7.812	7.449	21.2	19.8	19.8	34.3	34.1	34.4
8.192	7.856	7.435	21.5	19.6	19.9	34.6	34.2	34.5
8.07	7.807	7.44	21.5	19.6	19.9	34.1	34.1	34.1
8.127	7.813	7.411	21.3	19.7	19.9	34.1	34.1	34.2
8.09	7.79	7.5	20.5	20.2	20.6	34.4	34.2	34.6
8.048	7.792	7.39	21.7	20.3	20.5	34.3	34.3	34.5
8.109	7.807	7.485	21.2	20.3	20.5	34.5	34.4	34.7
8.123	7.812	7.475	21.5	20.3	20.5	34.6	34.5	35
8.107	7.804	7.399	21.7	20.3	20.6	34.2	34.2	34.3
8.154	7.806	7.451	20.6	20.2	20.4	34.4	34.2	34.4
8.08	7.794	7.403	19.8	20.2	20.2	34.4	34.2	34.4
8.068	7.816	7.473	20.9	20.3	20.3	34.5	34.3	34.6
8.19	7.98	7.53	22.8	20	20.8	34.7	34.4	34.6
8.25	7.87	7.54	20.6	20.2	20.5	34.6	34.4	35.1
8.19	7.84	7.48	19.6	20.2	20.6	34.7	34.4	34.6
7.95	7.794	7.51	20.7	20.3	20.8	35	34.5	34.6
8.019	7.804	7.503	19.9	20.2	20.6	35.1	34.4	34.6
8.122	7.812	7.457	19.9	20.2	20.6	34.5	34.5	34.5
8.015	7.792	7.504	20.4	20.2	20.6	34.7	34.5	34.6
8.131	7.9	7.39	18.1	20.2	20.5	34	34.6	34.8
8.048	7.834	7.403	19.8	20.1	20.6	34.7	34.6	34.6
8.067	7.821	7.44	19.8	20.1	20.5	35	34.6	34.6
8.107	7.73	7.449	19.9	20.2	20.7	34.9	34.7	34.6
8.19	7.856	7.435	20.3	20.2	20.8	35.5	34.8	34.6
8.08	7.807	7.44	20.4	19.5	20.3	35.6	35.2	34.8
7.95	7.73	7.51	20.2	20.1	20.8	35	34.8	34.6
			20.4	20.2	20.2	35.5	34.9	34.8

19.9	20.1	20.2	34.4	34.1	34
20.8	20.1	20.2	34.6	34.1	34.1
21.5	20.3	20.6	34	33.9	33.9
19.1	20.1	20.2	34.1	34	34.1
17.2	20	20.1	34.1	34.1	34.2
19.8	19.9	19.9	34.2	34.1	34.1
20.1	20.1	20.2	34.3	34.1	34.2
20.2	20.1	20	32.7	32.4	32.8
19.9	20.1	20	32.9	32.5	33.1
19.8	19.9	20.1	32.4	32.4	32.4
20.1	19.9	20.1	32.5	32.4	32.6
20.4	20.1	20.1	32.8	32.4	32.8
19.8	20.1	20.2	32.6	32.4	32.6
20.1	19.9	20.2	32.8	32.5	32.8
19.9	20.1	20.1	33	32.6	32.9
20.6	19.8	20.2	32.6	32.5	32.5
21.2	19.8	20.3	32.6	32.5	32.6
19.1	19.8	20.2	32.7	32.5	32.8
18.1	19.8	20	32.8	32.6	33
18.1	19.8	20	32.4	32.4	32.4
19.6	20.3	20.2	32.6	32.4	32.6
18.3	20.2	20.1	32.8	32.5	32.7
20.2	20.2	19.9	32.8	32.9	32.8
20.1	20.1	19.8	33.2	32.7	33.3
20.5	20.3	20.1	32.9	32.7	32.9
21	20.3	20.4	33.2	32.8	33
20.1	20.1	19.9	33.5	33	33.2
19.8	20.1	20	33.3	32.9	33.1
19.9	20.2	20.1	33.7	33	33.2
20.1	20.2	19.9	33.2	32.9	33.1
20.6	20.1	20.1	33.5	33	33.6
20.5	20.3	20.3	33.1	32.9	33
20.8	20.3	20.2	33.1	32.9	33.1
20.4	20.2	20	33.2	33.1	33.3
20.8	20.2	20.5	33.2	33.1	33.2
20.5	20.1	20	33.3	33.2	33.5
20.5	20.1	20	33.2	33.2	33.2
20.5	19.8	19.8	33.7	33.2	33.3
20.8	20.2	20.2	33.2	33.1	33.2
21.3	20.2	20.3	33.2	33.1	33.2
21.3	20.2	20.3	33.2	33.1	33.2
21.3	20.2	20.3	33.2	33.1	33.2
18.5	20.1	20.1	33.5	33.2	33.6
20.5	20.2	20.2	33.6	33.2	33.2
19.5	20.1	19.9	33.7	33.3	33.6
21	21	20.5	33.7	33.3	33.6
19.9	20.1	20.1	33.5	33.4	33.7
19.1	20.1	19.8	33.4	33.6	34.3

Suppl. Table 3. Surviving amphipods during each experiment

Day	0	5	11	14
Experiment				
CC	22	22	22	22
C7.8	25	23	23	18
C7.6	22	17	16	16
DC	26	25	21	20
D7.8	26	22	19	18
D7.6	26	26	25	22

Day	0	4	11	18	25	28
Experiment						
EC	23	17	10	10	7	8
E7.8	35	28	27	24	22	22
E7.6	26	20	16	13	13	13
FC	34	33	26	22	20	18
F7.8	31	25	23	23	20	20
F7.6	34	25	23	19	16	12

Note: Batches C & D were terminated at day 14, batches E & F were terminated at day 28 (approximate time to sexually maturity)

Suppl. Table 4. Increase in amphipod weight during batch F (used in Figure 3A)

'CONTROL'		'pH 7.8'		'pH 7.6'	
Day	AFDW (g)	Day	AFDW (g)	Day	AFDW (g)
0	0.13	0	0.21	0	0.13
0	0.13	0	0.16	0	0.13
0	0.14	0	0.20	0	0.14
0	0.14	0	0.18	0	0.14
0	0.23	0	0.15	0	0.23
0	0.13	0	0.14	0	0.13
0	0.22	0	0.13	0	0.22
0	0.22	0	0.12	0	0.22
0	0.11	0	0.16	0	0.11
0	0.29	0	0.09	0	0.29
0	0.18	0	0.17	0	0.18
0	0.16	0	0.18	0	0.16
0	0.15	0	0.11	0	0.15
0	0.14	0	0.15	0	0.14
0	0.12	0	0.16	0	0.12
0	0.16	0	0.14	0	0.16
0	0.23	0	0.24	0	0.23
0	0.13	0	0.13	0	0.13
0	0.12	0	0.14	0	0.12
0	0.13	0	0.20	0	0.13
0	0.30	0	0.13	0	0.30
0	0.12	0	0.11	0	0.12
0	0.12	0	0.13	0	0.12
0	0.32	0	0.14	0	0.32
0	0.10	0	0.10	0	0.10
0	0.17	0	0.13	0	0.17
0	0.21	0	0.12	0	0.21
0	0.17	0	0.12	0	0.17
0	0.15	0	0.11	0	0.15
0	0.11	0	0.12	0	0.11
0	0.13	0	0.17	0	0.13
0	0.13	4	0.22	0	0.13
0	0.12	4	0.17	0	0.12
0	0.13	4	0.22	0	0.13

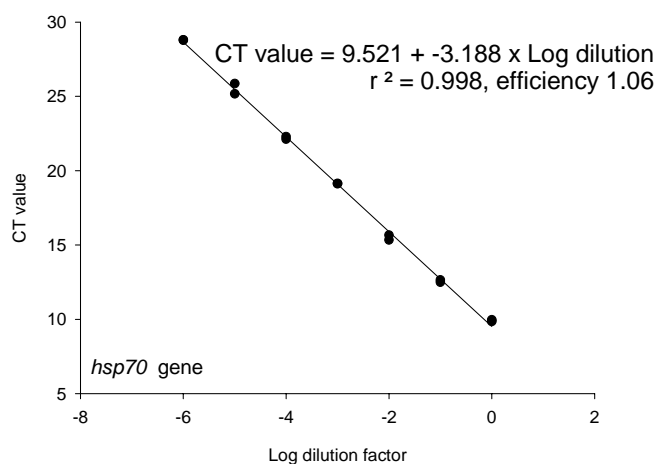
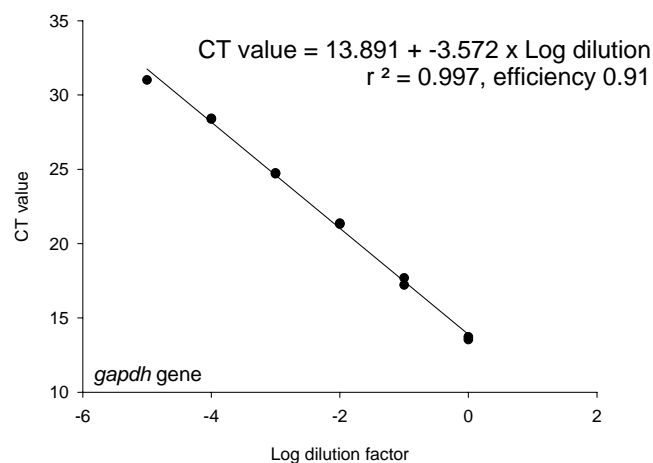
4	0.24	4	0.20	4	0.25
4	0.17	4	0.25	4	0.18
4	0.21	4	0.30	4	0.23
4	0.29	4	0.30	4	0.17
4	0.32	4	0.21	4	0.15
4	0.19	4	0.19	4	0.20
4	0.31	4	0.30	4	0.43
4	0.24	4	0.18	4	0.19
4	0.20	4	0.27	4	0.17
4	0.31	4	0.21	4	0.29
4	0.16	4	0.16	4	0.19
4	0.20	4	0.15	4	0.26
4	0.25	4	0.21	4	0.26
4	0.15	4	0.17	4	0.47
4	0.27	4	0.24	4	0.16
4	0.37	4	0.19	4	0.17
4	0.18	4	0.13	4	0.31
4	0.23	4	0.17	4	0.34
4	0.17	4	0.33	4	0.23
4	0.32	4	0.20	4	0.14
4	0.23	4	0.16	4	0.21
4	0.22	4	0.20	4	0.23
4	0.19	11	0.58	4	0.24
4	0.17	11	0.64	4	0.18
4	0.17	11	0.72	4	0.30
4	0.12	11	0.60	11	0.46
4	0.19	11	0.67	11	0.46
4	0.17	11	0.85	11	0.22
4	0.22	11	0.91	11	0.45
4	0.17	11	0.54	11	0.38
4	0.16	11	0.60	11	0.37
4	0.16	11	0.60	11	0.33
4	0.18	11	0.56	11	0.49
11	0.53	11	0.58	11	0.41
11	0.61	11	0.37	11	0.37
11	0.47	11	0.36	11	0.88
11	0.31	11	0.60	11	0.86
11	0.30	11	0.64	11	0.45
11	0.32	11	0.90	11	0.85
11	0.38	11	0.95	11	0.45
11	0.22	11	1.12	11	0.63
11	0.27	11	1.33	11	0.46
11	0.27	11	0.66	11	0.45
11	0.36	11	0.47	11	0.75
11	0.36	18	1.29	11	0.56
11	0.29	18	1.21	11	0.63
11	0.25	18	1.11	11	0.83
11	0.31	18	1.29	11	0.91
11	0.39	18	0.98	18	0.90
11	0.66	18	1.23	18	0.93
11	0.64	18	1.07	18	1.14
11	0.29	18	1.39	18	0.90
11	0.43	18	2.07	18	1.40
11	0.31	18	1.44	18	1.25
11	0.45	18	1.75	18	1.09
11	0.41	18	1.39	18	1.35
11	0.77	18	1.60	18	0.81
11	0.41	18	1.62	18	0.66
11	0.33	18	1.46	18	0.43
18	0.54	18	0.88	18	0.91
18	0.57	18	1.25	18	1.03
18	0.78	18	1.09	18	1.71
18	0.39	18	1.23	18	0.80
18	0.61	18	1.54	18	0.67
18	0.51	18	1.44	18	0.86
18	0.88	18	2.34	18	1.40
18	0.64	18	1.50	18	0.66

18	1.48	25	1.79	25	1.62
18	0.69	25	1.62	25	1.21
18	0.78	25	1.94	25	1.18
18	0.64	25	1.37	25	1.31
18	0.85	25	2.21	25	1.71
18	0.58	25	1.23	25	1.90
18	0.85	25	1.90	25	1.35
18	0.72	25	1.54	25	1.18
18	0.49	25	1.98	25	1.66
18	0.98	25	1.69	25	0.67
18	0.75	25	1.88	25	1.64
18	0.69	25	2.12	25	2.12
18	1.12	25	2.75	25	1.73
18	0.88	25	2.48	25	2.39
25	1.46	25	1.79	25	2.18
25	1.00	25	2.05	25	1.88
25	1.05	25	1.33	28	1.54
25	1.07	25	1.85	28	1.90
25	1.03	25	1.83	28	1.50
25	1.02	28	1.33	28	2.03
25	0.70	28	2.27	28	2.27
25	0.91	28	2.16	28	2.77
25	1.05	28	2.27	28	1.88
25	1.31	28	2.27	28	1.29
25	0.86	28	2.21	28	1.31
25	1.31	28	2.67	28	1.31
25	2.41	28	1.79	28	2.30
25	1.09	28	1.90	28	0.69
25	1.56	28	1.31		
25	1.75	28	1.83		
25	0.57	28	2.01		
25	1.25	28	2.12		
25	1.46	28	2.37		
25	0.83	28	1.60		
28	1.00	28	2.16		
28	1.00	28	2.07		
28	1.60	28	2.85		
28	1.42	28	1.88		
28	0.95	28	1.81		
28	2.01				
28	1.42				
28	1.58				
28	0.96				
28	1.07				
28	1.66				
28	0.64				
28	2.48				
28	1.09				
28	1.02				
28	1.60				
28	0.98				
28	1.14				

Note, data from other batches available from senior author on request.

Suppl. Table 5. Amphipod morphometries used for Fig. 3 (B)

Length (mm)	Dry weight (mg)	Ash wt (mg)	AFDW (mg)
6.7	1.51	0.72	0.79
6.5	0.93	0.49	0.44
7.1	1.09	0.40	0.69
8.4	1.74	0.72	1.02
10.1	1.68	0.66	1.02
8	2.66	1.40	1.26
5.3	1.14	0.42	0.72
6.1	0.62	0.23	0.39
6.3	0.63	0.19	0.44
9.5	0.51	0.20	0.31
10.9	1.73	1.13	0.6
7.4	2.04	1.12	0.92
11.4	1.33	0.73	0.6
15	4.06	1.68	2.38
13.4	6.19	3.15	3.04
9.9	6.45	2.68	3.77
10.1	1.94	0.78	1.16
8	1.64	0.57	1.07
12.7	3.92	1.86	2.06
9.2	1.71	0.51	1.2



Suppl. Fig. 2. Real-time PCR standard curves for optimized protocols, data used for Table 1.

Suppl. Table 6. Gene expression data from heat shock experiment (used for Fig. 4)

<i>hsp70</i> gene		<i>gapdh</i> gene	
Control	Shocked	Control	Shocked
2.8163E+06	6.6869E+09	3.6103E+07	7.6401E+07
6.4669E+05	3.8537E+09	2.3657E+07	1.0565E+07
1.5222E+06	7.0657E+09	3.2789E+07	2.2288E+07
4.0855E+05	5.8584E+09	1.3333E+07	6.3348E+07
6.0370E+06	1.9746E+09	1.0838E+07	1.1374E+07
1.5334E+06	3.7421E+09	1.2280E+07	2.1542E+07
1.4353E+06	3.7697E+09	1.5631E+07	2.1118E+07
8.2135E+05	1.0551E+09	2.0729E+07	1.2677E+07
2.4813E+06	1.9040E+09	1.7165E+07	5.3952E+06
8.0638E+05	7.3993E+08	3.8570E+07	4.7863E+06

Suppl. Table 7. Gene expression data from 14-day and 28-day incubations (batches C & D and E &F, respectively). Used in Table 2.

<i>hsp70</i> gene Log10 (copies/ug total RNA)											
@ 14 days						@ 28 days					
CC	C7.8	C7.6	DC	D7.8	D7.6	EC	E7.8	E7.6	FC	F7.8	F7.6
5.3604	5.8587	6.6282	5.6888	5.4871	7.0841	5.8134	5.7997	5.8220	5.7658	5.8413	6.1345
5.9184	5.9702	6.1626	5.5615	6.0173	6.7289	5.9209	6.1697	5.9221	6.5646	6.2082	6.0237
5.8783	6.0019	6.1327	5.9309	6.4052	5.8008	5.3834	5.8519	5.7725	5.8159	5.8312	6.3025
5.4990	5.7067	6.1263	6.2284	5.9972	5.9785	5.8620	6.1650	5.7206	6.5082	6.1855	6.1697
5.5842	5.9972	6.4504	6.6687	6.3950	5.9925	5.3641	6.6859	6.2922	6.2003	6.1581	6.5505
5.5007	6.0268	5.7925	5.9121	6.5480	6.2698	7.2880	6.6057	6.3097	6.7790	7.0114	6.0177
5.5542	5.8621	6.4342	7.0833	6.4099	6.2286	5.5727	6.1379	5.6727	5.3708	4.9376	7.0385
5.5207	6.1206	6.1632	5.9049	4.7176	6.1921	5.8864	5.1387	5.9348	6.3981	6.2273	6.4049

<i>gapdh</i> gene Log10 (copies/ug total RNA)											
@ 14 days						@ 28 days					
CC	C7.8	C7.6	DC	D7.8	D7.6	EC	E7.8	E7.6	FC	F7.8	F7.6
6.8486	7.1576	7.7469	7.5141	7.0860	7.8273	7.4977	7.1065	7.5533	7.4541	7.6079	7.5467
7.0593	7.5754	7.5028	7.5395	7.5392	7.4382	7.3166	7.2119	7.2476	7.1405	7.2975	7.7011
6.8748	7.3508	7.4729	7.0083	6.8914	7.1413	6.7831	7.2784	7.4412	7.2572	6.7066	7.4770
6.5407	7.2638	7.5274	7.4208	7.3588	7.9470	7.4413	7.3563	7.6719	7.2633	6.8326	7.7583
7.0693	6.9015	7.7598	7.4414	6.8391	8.1054	7.5125	7.7481	7.2808	6.8595	6.9425	7.2762
6.9982	7.3435	7.8215	7.0889	7.2268	7.2453	7.1725	7.1195	7.0316	6.7307	6.7480	7.5082
6.9870	6.6675	7.3903	7.4297	6.7571	7.4100	7.2932	6.8244	7.5766	7.0687	6.8999	7.0297
6.6367	7.2512	7.8167	7.5794	6.6730	7.3955	7.2881	7.4373	7.4980	6.8887	6.7003	7.4481

Suppl. Table 8. Summary of nested ANOVA comparing pH, time (Ti, nested in pH) and batch (Ba, nested in pH and Time) for the *hsp70* gene expression data. Analysis conducted using GMAV5 (Underwood, 1997).

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	<i>P</i>
pH	1.0585	2	1.0585	3.72	0.154
Ti(pH)	0.4267	3	0.4267	0.41	0.7514
Ba(pHxTi)	2.0776	6	2.0776	1.8	0.1084
Residual	16.14	84	16.14		
Total	19.7029	95			