

Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*

Maria Byrne^{1,*}, Maria Gonzalez-Bernat², Steve Doo³, Shawna Foo³, Natalie Soars³, Miles Lamare²

¹School of Medical and Biological Sciences, University of Sydney, New South Wales 2006, Australia

²Department of Marine Science, University of Otago, Dunedin, New Zealand

³School of Medical Sciences, University of Sydney, New South Wales 2006, Australia

*Email: mbyrne@anatomy.usyd.edu.au

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Supplement

Table S1. Parameters for the three water sources used for experiments with *Patiriella regularis* to the 3 day bipinnaria larva in 9 temperature-pH treatments. Mean values (\pm SE) for $p\text{CO}_2$ (μatm), Ω_{ca} and Ω_{ar} determined from average pH_{NIST} (8.16, SE = 0.01), total alkalinity (2336.6, SE = 12.01), salinity (36.3, SE = 0.6) and temperature using CO2SYS (Pierrot et al. 2006).

	pH 8.15			pH 7.8			pH 7.6		
	19°C	21°C	23°C	19°C	21°C	23°C	19°C	21°C	23°C
$p\text{CO}_2$	423 (1.81)	427 (1.84)	432 (1.87)	1058 (3.65)	1076 (3.71)	1094 (3.76)	1738 (5.77)	1772 (5.87)	1805 (5.97)
Ω_{ca}	4.3 (0.05)	4.5 (0.05)	4.8 (0.05)	2.1 (0.03)	2.3 (0.03)	2.4 (0.03)	1.4 (0.02)	1.5 (0.02)	1.6 (0.02)
Ω_{ar}	2.8 (0.04)	2.9 (0.04)	3.1 (0.04)	1.4 (0.02)	1.5 (0.02)	1.6 (0.02)	0.9 (0.01)	1.0 (0.01)	1.0 (0.01)

Table S2. Average pH_{NIST} in experiments with *Patiriella regularis* measured at 24 h and prior to water change measured in a random subsample of pots (n = 30). The start pH levels are indicated in the first row (see Table S1).

	pH 8.15			pH 7.8			pH 7.6		
	19°C	21°C	23°C	19°C	21°C	23°C	19°C	21°C	23°C
pH	8.16 (0.008)	8.14 (0.006)	8.14 (0.012)	7.79 (0.007)	7.76 (0.011)	7.73 (0.02)	7.59 (0.006)	7.58 (0.008)	7.56 (0.017)

Table S3. Mean values (\pm SE) for water parameters in long term rearing experiments with *Patiriella regularis* to Day 28 post fertilization in 4 pH levels measured at 11 time points. Parameters $p\text{CO}_2$ (μatm), Ω_{ca} and Ω_{ar} determined from measured pH_{NIST} , average total alkalinity (2288, SE=10.1) salinity (35) and temperature (15°C) using CO2SYS (Pierrot et al. 2006).

	pH 8.1	pH 7.8	pH 7.6	pH 7.0
$p\text{CO}_2$	495 (14.06)	1055.12 (33.63)	1767.87 (42.80)	7250.88 (171.02)
Ω_{ca}	3.21 (0.06)	1.75 (0.04)	1.11 (0.02)	0.29 (0.006)
Ω_{ar}	2.06 (0.04)	1.2 (0.08)	0.72 (0.02)	0.19 (0.004)

Table S4. Average pH_{NIST} ($\pm\text{SE}$) in experiments with *Patiriella regularis* to Day 24 at the start and change every 2 d measured just prior to water renewal, $n = 4$ measures per treatment on each of 11 d ($n = 40$ to 44). On Day 1 the larvae were 3.5 d post fertilization

Day	pH 8.1	pH 7.8	pH 7.6	pH 7.0
1	8.08	7.83	7.59	6.98
3	8.06 \pm 0.009	7.80 \pm 0.002	7.55 \pm 0.010	6.97 \pm 0.004
4	8.09 \pm 0.007	7.82 \pm 0.004	7.54 \pm 0.008	7.00 \pm 0.016
6	8.08 \pm 0.009	7.76 \pm 0.028	7.57 \pm 0.012	7.01 \pm 0.005
8	8.08 \pm 0.021	7.79 \pm 0.008	7.58 \pm 0.008	6.97 \pm 0.009
10	8.07 \pm 0.010	7.79 \pm 0.008	7.58 \pm 0.006	6.98 \pm 0.010
13	8.08 \pm 0.012	7.76 \pm 0.010	7.57 \pm 0.013	6.96 \pm 0.009
14	8.08 \pm 0.011	7.77 \pm 0.008	7.58 \pm 0.011	6.99 \pm 0.012
16	8.09 \pm 0.008	7.77 \pm 0.008	7.58 \pm 0.012	6.96 \pm 0.012
20	8.07 \pm 0.011	7.77 \pm 0.010	7.58 \pm 0.012	6.98 \pm 0.010
24	8.06 \pm 0.008	7.78 \pm 0.009	7.60 \pm 0.011	6.96 \pm 0.012

Table S5. ANOVA of the percentage of developmental stages of *Patiriella regularis* at 4, 15 and 24 h from 9 independent populations of embryos reared in 9 temperature-pH level treatments. Temperature and pH were fixed factors. Tukey HSD post hoc test indicates treatments that differed.

Factor	MS	df	F-value	p-value	Tukey HSD
A 4 h Cleavage					
Temperature	0.552	2	13.2	< 0.001	19 < 21,23
pH	0.216	2	2.6	0.084	
Temperature x pH	0.037	4	0.9	0.480	
Residual	0.042	66			
Total		74			
B 15 h Blastulae					
Temperature	0.004	2	3.5	0.040	19=21,19=23 21>23
pH	0.008	2	6.5	0.003	8.2,7.8 > 7.6
Temperature x pH	0.001	4	0.5	0.716	
Residual	0.001	44			
Total		52			
C 24 h Gastrulae					
Temperature	0.039	2	5.0	0.011	19=21, 21=23,19>23
pH	0.270	2	34.7	< 0.001	8.2,7.8 > 7.6
Temperature x pH	0.033	4	2.1	0.096	
Residual	0.008	45			
Total		53			

Table S6. Mean percentage (\pm SE) of the developmental stages of *Patiriella regularis* in 9 temperature-pH treatments at 4 h (A), 15h (B) and 24 h (C).

A	Temperature	pH	Unfertilized	1-8 Cell	16-32 Cell	64+ Cell
	19	8.15	7.6 \pm 2.3	26.4 \pm 4.3	34.8 \pm 5.6	31.3 \pm 7.2
	19	7.8	10.0 \pm 1.0	20.0 \pm 2.6	34.6 \pm 6.4	35.5 \pm 7.1
	19	7.6	15.5 \pm 1.3	21.4 \pm 3.0	30.1 \pm 5.5	33.0 \pm 7.7
	21	8.15	6.1 \pm 1.2	14.6 \pm 4.0	29.4 \pm 6.1	49.8 \pm 9.9
	21	7.8	8.3 \pm 0.9	9.4 \pm 2.5	22.9 \pm 7.6	59.4 \pm 10.0
	21	7.6	7.1 \pm 2.2	1.7 \pm 1.0	10.7 \pm 1.4	80.5 \pm 1.9
	23	8.15	10.0 \pm 1.0	8.8 \pm 2.3	25.7 \pm 7.5	55.4 \pm 8.9
	23	7.8	12.0 \pm 2.8	14.6 \pm 4.5	12.7 \pm 2.8	60.7 \pm 5.7
	23	7.6	14.6 \pm 2.5	10.7 \pm 2.8	12.2 \pm 3.6	62.4 \pm 6.1

B	Temperature	pH	Arrested/Dead	Mid-Blastula	Late-Blastula
	19	8.15	10.9 \pm 1.9	81.1 \pm 3.1	8.1 \pm 2.1
	19	7.8	6.4 \pm 1.1	85.8 \pm 1.0	7.8 \pm 1.7
	19	7.6	10.4 \pm 0.4	84.6 \pm 0.6	5.0 \pm 1.0
	21	8.15	18.9 \pm 2.2	73.8 \pm 2.4	8.7 \pm 1.8
	21	7.8	17.0 \pm 2.0	74.9 \pm 2.4	9.8 \pm 0.8
	21	7.6	39.4 \pm 7.0	56.4 \pm 6.6	5.1 \pm 1.6
	23	8.15	24.7 \pm 3.1	68.1 \pm 3.4	7.2 \pm 1.3
	23	7.8	37.7 \pm 2.0	57.6 \pm 1.7	4.7 \pm 1.1
	23	7.6	50.6 \pm 6.2	46.1 \pm 5.4	3.3 \pm 1.1

C	Temperature	pH	Arrested/Dead	Early Gastrula	Late Gastrula
	19	8.15	35.2 \pm 1.2	64.8 \pm 1.2	0 \pm 0
	19	7.8	40.7 \pm 1.5	58.2 \pm 1.4	1.1 \pm 1.1
	19	7.6	62.8 \pm 4.0	37.2 \pm 4.0	0 \pm 0
	21	8.15	41.2 \pm 3.1	21.8 \pm 6.0	37.0 \pm 5.8
	21	7.8	43.9 \pm 1.1	21.1 \pm 8.1	35.0 \pm 7.6
	21	7.6	62.6 \pm 5.4	22.2 \pm 7.7	15.2 \pm 4.9
	23	8.15	49.0 \pm 2.8	19.1 \pm 2.9	31.9 \pm 1.8
	23	7.8	52.0 \pm 3.4	12.1 \pm 2.4	35.8 \pm 4.4
	23	7.6	60.5 \pm 3.4	15.4 \pm 1.5	24.1 \pm 3.8

Table S7. ANOVA results of the percentage mortality of *Patiriella regularis* at 15 and 24 h from 9 independent populations of embryos reared in 9 temperature-pH level treatments. Temperature and pH were fixed factors. Tukey HSD post hoc test indicates treatments that differed. For the interaction, underlined treatments did not differ.

Factor	MS	df	F-value	p-value	Tukey HSD
A 15 h Mortality					
Temperature	1.553	2	84.0	< 0.001	19 < 21 < 23
pH	0.264	2	14.2	< 0.001	8.2,7.8 < 7.6
Temperature x pH	0.080	4	4.3	0.005	
Residual		45			
Total	0.019	53			
Tukey HSD:					
Temperature x pH	<u>19,7.8</u>	<u>19,8.2</u>	<u>19,7.6</u>	21,7.8	21,8.2
				23,8.2	21,7.6
					23,7.8
					23,7.6
B 24 h Mortality					
Temperature	0.025	2	6.3	0.004	19=21,21=23,19 < 23
pH	0.141	2	35.3	< 0.001	8.2,7.8 < 7.6
Temperature x pH	0.010	4	2.4	0.061	
Residual	0.004	45			
Total		53			

Table S8. ANOVA of the percentage of normal of *Patiriella regularis* larvae on Day 3 and the mean length of larvae in treatments from 7 independent populations of embryos reared in 9 temperature-pH level treatments. Temperature and pH were fixed factors. Tukey HSD post hoc test indicates treatments that differed.

Factor	MS	df	F-value	p-value	Tukey HSD
A Normal larvae					
Temperature	0.129	2	6.2	< 0.007	19=21,21=23,19 > 23
pH	0.012	2	0.6	0.556	
Temperature x pH	0.228	4	1.1	0.383	
Residual	0.115	24			
Total		32			
B Larval length					
Temperature	8672.184	2	6.9	0.002	19=21,21=23 19 > 23
pH	389.91	2	0.3	0.735	
Temperature x pH	232.49	4	0.2	0.94	
Residual	0.001	54			
Total		62			

Table S9. Repeated Measures ANOVA: A Mean density of *Patiriella regularis* larvae on Days 4, 8, 11, 16, 20, 24 and 28 post fertilization from 4 independent populations reared in 4 pH treatments. B. Mean length of larvae on Days 6, 10, 16 and 24 (n = 12). pH and time were fixed factors. Departures from the assumption of sphericity were subject to Greenhouse-Geisser correction.

Source	MS	df	F-value	p-value
A Larval density				
pH	152.73	3	407.3	<0.001
Error	0.375	12		
Time	59.44	6	118.89	<0.001
pH × Time	6.72	18	13.44	<0.001
Error	0.5	72		
Mauchly criterion = 0.044, df = 20, p = 0.06				
B Larval length				
pH	221855.16	3	20.35	<0.001
Error	10900.61	40		
Time	114258.73	3	12.51	<0.001
pH × Time	30477.38	9	3.34	0.001
Error	9134.16	120		
Mauchly criterion = 0.86, df = 5, p = 0.32				

Table S10. ANOVA of the density of *Patiriella regularis* larvae from 4 independent populations reared in 4 pH treatments on Day 11 when the treatments diverged. pH was the fixed factor. Tukey HSD post hoc test indicates treatments that differed.

Factor	MS	df	F-value	p-value	Tukey HSD
pH	42.833	3	60.47	< 0.0001	8.1, 7.8 > 7.6 > 7.0
Residual	0.708	12			
Total		15			