

Ocean acidification but not warming alters sex determination in the Sydney rock oyster, *Saccostrea glomerata*

Laura M. Parker, Wayne A. O'Connor, Maria Byrne, Michael Dove, Ross Coleman, Hans-O Pörtner, Elliot Scanes, Patti Virtue, Mitchell Gibbs, Pauline M. Ross

Supplementary Material

Results

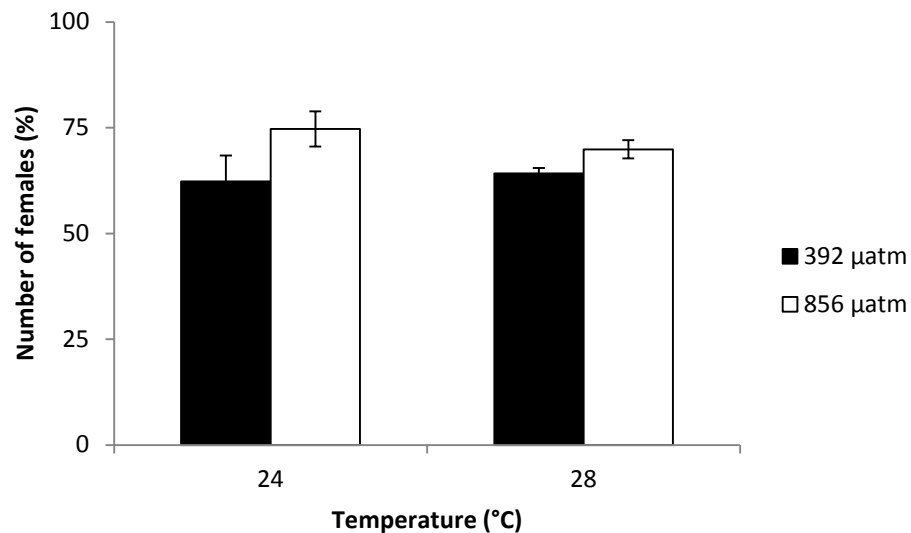


Figure S1. Percentage of *Saccostrea glomerata* adults that were female following exposure to ambient (392 µatm; 24 °C) and elevated (856 µatm; 28 °C) $p\text{CO}_2$ and temperature during reproductive conditioning, when cumulative mortalities were taken into consideration; Error bars = S. E.; $n = 3$.

Table S1. Analysis of Variance of the impact of ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature on the sex ratios of *S. glomerata* when cumulative mortalities were taken into consideration. Data used in the analysis included the percentage of females determined from histology slides combined with the cumulative mortalities that occurred during the 8 week exposure to the treatments. Since the sex of the mortalities could not be determined for every oyster any unknown sex was treated as a female for the control group and a male for all other treatment groups. $n = 3$.

Source	SS	DF	MS	F	P	F versus
Te	6.5494	1	6.5494	0.14	0.7145	RES
CO	246.7093	1	246.7093	5.41	<i>0.0484</i>	RES
TeXCO	33.5194	1	33.5194	0.74	0.4161	RES
RES	364.5859	8	45.5732			
TOTAL	651.3641	11				

SNK 392 > 856 μatm

This was a two-way analysis with $p\text{CO}_2$ and temperature being fixed and orthogonal. Data was significant if $P < 0.05$

(shown in *italics*) and indicates that sex ratios were significantly irrespective of mortality. Cochran's test = 0.6146

(not significant). SNK = Student Newman Kuels post-hoc comparisons.

Table S2. Mean seawater physicochemical conditions of the experimental seawater over the 8 week exposure period; \pm S. E.

$p\text{CO}_2$ (μatm)	Temperature (°C)	Salinity	pH_{NBS}	TA ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	Ω_{calcite}	Ω_{arag}
392 \pm 2	24.0 \pm 0.1	34.50 \pm 0.07	8.20 \pm 0.00	2348 \pm 5	2048 \pm 5	5.21 \pm 0.03	3.42 \pm 0.02
856 \pm 5	24.0 \pm 0.1	34.50 \pm 0.07	7.91 \pm 0.00	2348 \pm 5	2194 \pm 5	3.01 \pm 0.01	1.98 \pm 0.01
393 \pm 4	28.0 \pm 0.1	34.50 \pm 0.07	8.20 \pm 0.00	2348 \pm 5	2014 \pm 6	5.81 \pm 0.02	3.86 \pm 0.02
855 \pm 3	28.0 \pm 0.1	34.50 \pm 0.07	7.92 \pm 0.00	2348 \pm 5	2168 \pm 4	3.44 \pm 0.02	2.29 \pm 0.01

TA, total alkalinity; DIC, dissolved inorganic carbon.

Table S3. Summary of likelihood ratio tests of multinomial models for percentage of *Saccostrea glomerata* adults at each gonadal stage following exposure to the ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature treatments during reproductive conditioning. Gonadal samples taken at 0, 3, 5, 7 and 8 weeks; n = 3.

Factor	Likelihood ratio statistic	<i>P</i> (Chi ²)
Week x Temp x CO2	8.4	0.9
Week x Temp	24.3	0.059*
Week x CO2	10.2	0.8
Temp x CO2	11.2	0.047*
Week	112	<0.0001**
Temp	16.9	0.004**
CO2	36.1	<0.0001**

Likelihood ratio tests were done using the “ANOVA” function (STATS package; R software) to compare multinomial logistic regression models.

Dataset supporting the article

Table 1. Raw data for the analysis of the percentage of *Saccostrea glomerata* adults that were female following exposure to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning.

		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 1	6	1	6	0	5	2	6	0
Week 5	Replicate 1	5	2	4	3	4	3	5	2
Week 7	Replicate 1	6	1	3	4	6	1	5	2
Week 8	Replicate 1	3	3	5	2	5	2	4	3
	Sum	20	7	18	9	20	8	20	7
	Total oysters sampled per treatment	27	27	27	27	28	28	27	27
	Percentage	74.07407	25.92593	66.66667	33.33333	71.42857	28.57143	74.07407	25.92593
		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 2	4	2	6	1	5	2	6	0
Week 5	Replicate 2	3	4	5	1	5	2	6	1
Week 7	Replicate 2	6	1	7	0	5	2	6	0
Week 8	Replicate 2	3	4	5	2	5	2	4	3
	Sum	16	11	23	4	20	8	22	4
	Total oysters	27	27	27	27	28	28	26	26

	sampled per treatment								
	Percentage	59.25926	40.74074	85.18519	14.81481	71.42857	28.57143	84.61538	15.38462
		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 3	3	3	6	1	4	3	4	2
Week 5	Replicate 3	5	2	5	2	5	2	5	2
Week 7	Replicate 3	4	3	5	0	5	2	6	0
Week 8	Replicate 3	2	5	5	2	5	2	4	3
	Sum	14	13	21	5	19	9	19	7
	Total oysters sampled per treatment	27	27	26	26	28	28	26	26
	Percentage	51.85185	48.14815	80.76923	19.23077	67.85714	32.14286	73.07692	26.92308

Table 2. Raw data for the analysis of the stage of gametogenesis of *Saccostrea glomerata* adults following exposure to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning.

Week 0												
Gonadal stage	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C								
G1	14.29	0.00	28.57	14.29								
G2	0.00	0.00	0.00	0.00								
G3	0.00	0.00	0.00	0.00								
G4	0.00	0.00	0.00	0.00								
G5	0.00	0.00	0.00	0.00								
R	85.71	100.00	71.43	85.71								
Week 3												
Gonadal stage	Ambient $p\text{CO}_2$; 24 °C			Elevated $p\text{CO}_2$; 24 °C			Ambient $p\text{CO}_2$; 28 °C			Elevated $p\text{CO}_2$; 28 °C		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
G1	0.00	28.57	14.29	28.57	28.57	0.00	0.00	0.00	0.00	14.29	0.00	0.00
G2	28.57	28.57	28.57	42.86	57.14	57.14	28.57	57.14	57.14	57.14	42.86	50.00
G3	57.14	28.57	57.14	14.29	14.29	28.57	42.86	28.57	28.57	14.29	57.14	50.00
G4	14.29	0.00	0.00	0.00	0.00	14.29	28.57	14.29	14.29	0.00	0.00	0.00
G5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R	0.00	14.29	0.00	14.29	0.00	0.00	0.00	0.00	0.00	14.29	0.00	0.00
Week 5												
G1	0.00	14.29	0.00	0.00	14.29	0.00	0.00	0.00	14.29	14.29	0.00	14.29
G2	42.86	28.57	71.43	42.86	57.14	85.71	14.29	14.29	0.00	28.57	14.29	14.29
G3	57.14	57.14	28.57	42.86	14.29	14.29	28.57	14.29	42.86	42.86	85.71	57.14
G4	0.00	0.00	0.00	14.29	0.00	0.00	57.14	71.43	42.86	14.29	0.00	14.29

G5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R	0.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Week 7												
G1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G2	0.00	28.57	0.00	28.57	57.14	28.57	28.57	14.29	14.29	14.29	14.29	28.57
G3	71.43	42.86	71.43	28.57	42.86	42.86	28.57	14.29	42.86	71.43	71.43	57.14
G4	28.57	28.57	28.57	42.86	0.00	0.00	42.86	71.43	28.57	14.29	14.29	14.29
G5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00
R	0.00	0.00	0.00	0.00	0.00	28.57	0.00	0.00	0.00	0.00	0.00	0.00
Week 8												
G1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G2	0.00	0.00	0.00	14.29	28.57	28.57	0.00	0.00	0.00	14.29	0.00	14.29
G3	14.29	28.57	0.00	28.57	14.29	42.86	28.57	0.00	28.57	42.86	57.14	57.14
G4	85.71	71.43	100.00	57.14	57.14	28.57	71.43	85.71	71.43	42.86	42.86	28.57
G5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00

Table 3. Raw data for the analysis of the area of the gonad of *Saccostrea glomerata* adults following exposure to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	37.42	24.36	39.65	33.84
Rep 2	38.12	20.55	38.84	35.94
Rep 3	38.25	28.19	37.70	31.19
Mean	37.93	24.37	38.73	33.66
Var	0.20	14.56	0.96	5.68
S.E.	0.26	2.20	0.57	1.38

Table 4. Raw data for the analysis of the percentage of *Saccostrea glomerata* adults that spawned following exposure to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	80	45	90	20
Rep 2	85	35	70	60
Rep 3	70	15	65	50
Mean	78.33	31.67	75.00	43.33
Var	58.33	233.33	175.00	433.33
S.E.	4.41	8.82	7.64	12.02

Table 5. Raw data for the analysis of the total lipid content per egg from *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	6.17	4.13	4.77	1.39
Rep 2	8.22	7.33	3.80	3.34
Rep 3	6.00	6.96	4.87	3.54
Mean	6.79	6.14	4.48	2.76
Var	1.53	3.05	0.35	1.42
S.E.	0.72	1.01	0.34	0.69

Table 6. Raw data for the analysis of the size of eggs from *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	49.57	50.34	49.01	47.44
Rep 2	50.14	49.08	47.99	47.59
Rep 3	50.92	49.71	47.18	47.30
Mean	50.21	49.71	48.06	47.44
Var	0.46	0.40	0.84	0.02
S.E.	0.39	0.45	0.53	0.10

Table 7. Raw data for the analysis of the total spawned lipid content of eggs from *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	50.13	15.49	49.63	12.20
Rep 2	61.41	12.31	45.05	30.65

Rep 3	24.02	19.19	71.34	32.26
Mean	45.19	15.66	55.34	25.03
Var	367.79	11.85	197.20	124.20
S.E.	11.07	1.99	8.11	6.43

Table 8. Raw data for the analysis of the spawned fecundity of female *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C ($\times 10^6$)	Elevated $p\text{CO}_2$; 24 °C ($\times 10^6$)	Ambient $p\text{CO}_2$; 28 °C ($\times 10^6$)	Elevated $p\text{CO}_2$; 28 °C ($\times 10^6$)
Rep 1	8.13	3.75	10.39	8.80
Rep 2	7.47	1.68	11.86	9.16
Rep 3	4.01	2.76	14.66	9.12
Mean	6.53	2.71	12.30	9.03
Var	4906007.23	1068760.44	4699374.59	40015.71
S.E.	1.28	0.73	1.25	0.14

Table 9. Raw data for the analysis of shell length of *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	51.71	51.75	48.30	47.12
Rep 2	52.10	51.87	49.07	45.39
Rep 3	52.91	51.94	51.12	47.77
Mean	52.24	51.85	49.50	46.76
Var	0.38	0.01	2.12	1.52
S.E.	0.36	0.05	0.84	0.71

Table 10. Raw data for the analysis of the extracellular pH (pH_e) of *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	7.44	7.03	7.43	6.93
Rep 2	7.49	6.92	7.41	7.06
Rep 3	7.49	7.10	7.42	7.02
Mean	7.47	7.01	7.42	7.00
Var	0.00	0.01	0.00	0.00
S.E.	0.02	0.05	0.01	0.04

Table 11. Raw data for the analysis of cumulative mortality of *Saccostrea glomerata* adults exposed to ambient (392 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature for 8 weeks during reproductive conditioning. The mean value for each tank was used as the observation, with a resulting sample size of $n = 3$.

	Ambient $p\text{CO}_2$; 24 °C	Elevated $p\text{CO}_2$; 24 °C	Ambient $p\text{CO}_2$; 28 °C	Elevated $p\text{CO}_2$; 28 °C
Rep 1	0.00	2.56	5.13	5.13
Rep 2	0.00	2.56	2.56	3.85
Rep 3	1.28	3.85	2.56	5.13
Mean	0.43	2.99	3.42	4.70
Var	0.55	0.55	2.19	0.55
S.E.	0.43	0.43	0.85	0.43

Table 12. Raw data for the analysis of the percentage of *Saccostrea glomerata* adults that were female following exposure to ambient (395 μatm ; 24 °C) and elevated (856 μatm ; 28 °C) $p\text{CO}_2$ and temperature during reproductive conditioning, when cumulative mortalities were taken into consideration.

		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 1	6.00	1.00	6.00	0.00	5.00	6.00	6.00	3.00
Week 5	Replicate 1	5.00	2.00	6.00	3.00	4.00	3.00	6.00	2.00
Week 7	Replicate 1	6.00	1.00	3.00	4.00	6.00	1.00	5.00	2.00
Week 8	Replicate 1	3.00	3.00	5.00	2.00	5.00	2.00	4.00	3.00
	Sum	20.00	7.00	20.00	9.00	20.00	12.00	21.00	10.00
	Total	27.00	27.00	29.00	29.00	32.00	32.00	31.00	31.00
	Percentage	74.07	25.93	68.97	31.03	62.50	37.50	67.74	32.26
		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 2	4.00	2.00	6.00	1.00	5.00	4.00	6.00	4.00
Week 5	Replicate 2	3.00	4.00	6.00	2.00	5.00	2.00	6.00	1.00
Week 7	Replicate 2	6.00	1.00	7.00	0.00	5.00	2.00	7.00	0.00
Week 8	Replicate 2	3.00	4.00	5.00	2.00	5.00	2.00	4.00	3.00
	Sum	16.00	11.00	24.00	5.00	20.00	10.00	23.00	8.00
	Total	27.00	27.00	29.00	29.00	30.00	30.00	31.00	31.00
	Percentage	59.26	40.74	82.76	17.24	66.67	33.33	74.19	25.81

		Ambient $p\text{CO}_2$; 24 °C		Elevated $p\text{CO}_2$; 24 °C		Ambient $p\text{CO}_2$; 28 °C		Elevated $p\text{CO}_2$; 28 °C	
		Female	Male	Female	Male	Female	Male	Female	Male
Week 3	Replicate 3	4.00	3.00	6.00	4.00	4.00	5.00	4.00	5.00
Week 5	Replicate 3	5.00	2.00	5.00	2.00	5.00	2.00	6.00	2.00
Week 7	Replicate 3	4.00	3.00	5.00	0.00	5.00	2.00	7.00	0.00
Week 8	Replicate 3	2.00	5.00	5.00	2.00	5.00	2.00	4.00	3.00
	Sum	15.00	13.00	21.00	8.00	19.00	11.00	21.00	10.00
	Total	28.00	28.00	29.00	29.00	30.00	30.00	31.00	31.00
	Percentage	53.57	46.43	72.41	27.59	63.33	36.67	67.74	32.26