The following supplement accompanies the article

Thermal adaptations of embryos of six terrestrial hermit crab species

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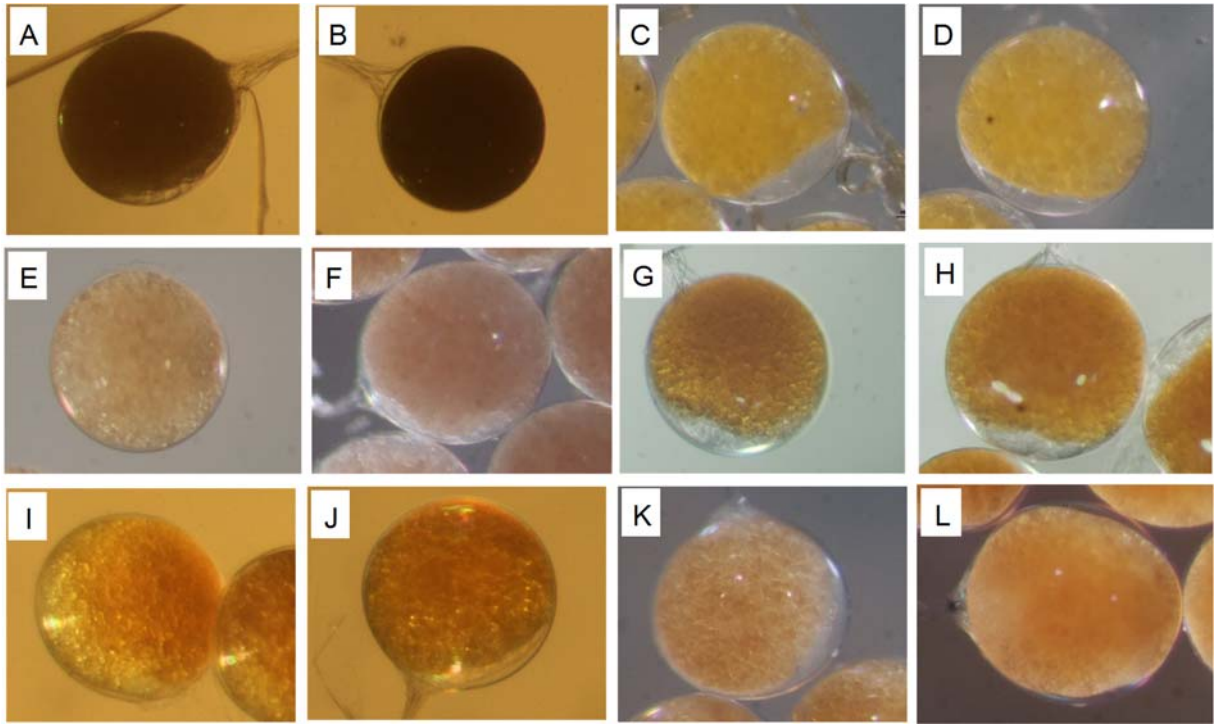

Fig. S1. Photographs showing the embryos of 6 coenobitids used for the *in vitro* culture experiments. Experiments were conducted with 2 different females of each species: (A, B) *Birgus latro*, (C, D) *Coenobita brevimanus*, (E, F) *C. cavipes*, (G, H) *C. purpureus*, (I, J) *C. rugosus*, and (K, L) *C. violascens*

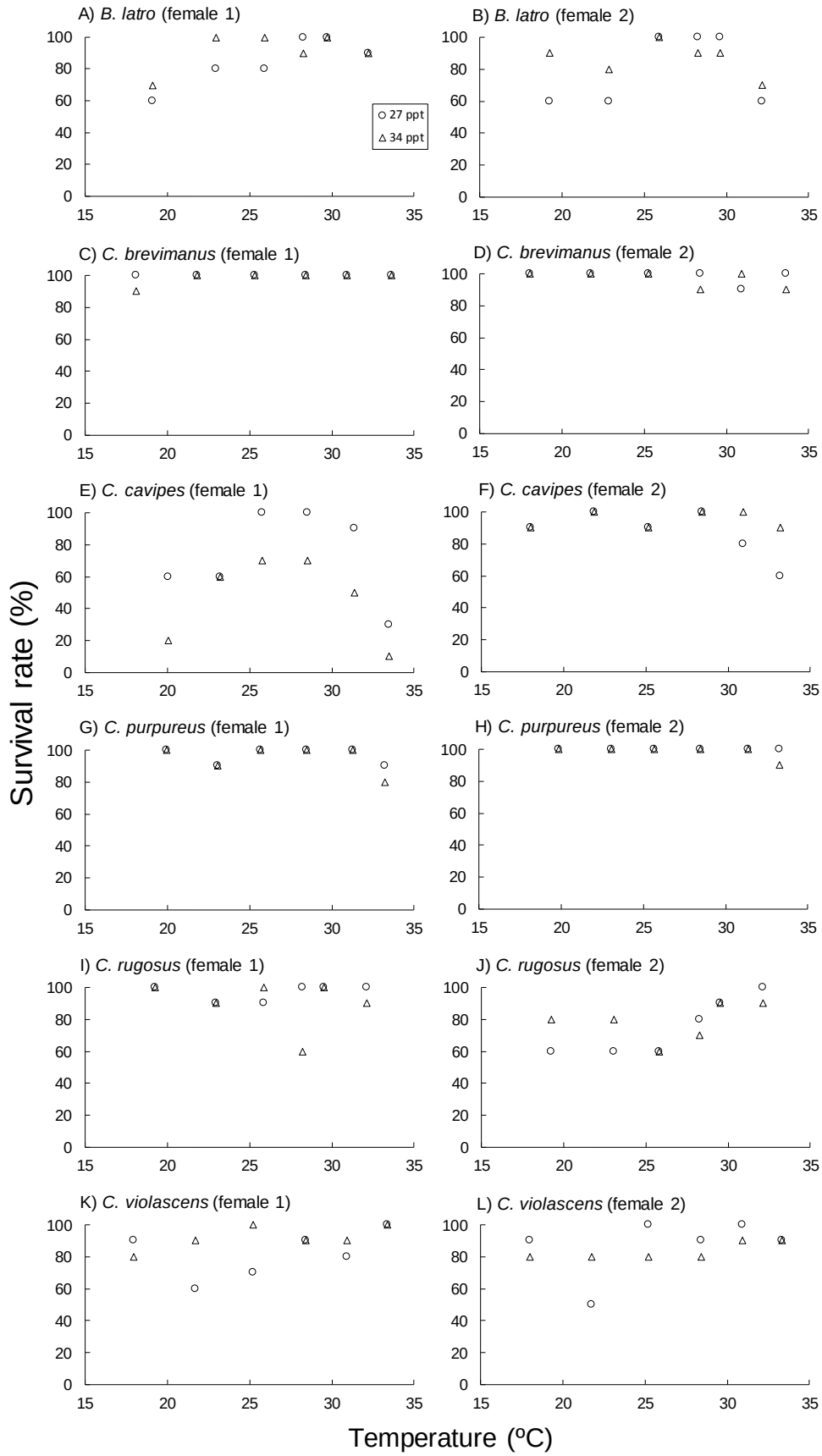

Fig. S2. Embryo survival rates of 6 coenobitids incubated *in vitro* in seawater with 2 salinity levels and 6 different temperatures. Experiments were conducted with 2 different females of each species: (A, B) *Birgus latro*, (C, D) *Coenobita brevimanus*, (E, F) *C. cavipes*, (G, H) *C. purpureus*, (I, J) *C. rugosus*, and (K, L) *C. violascens*

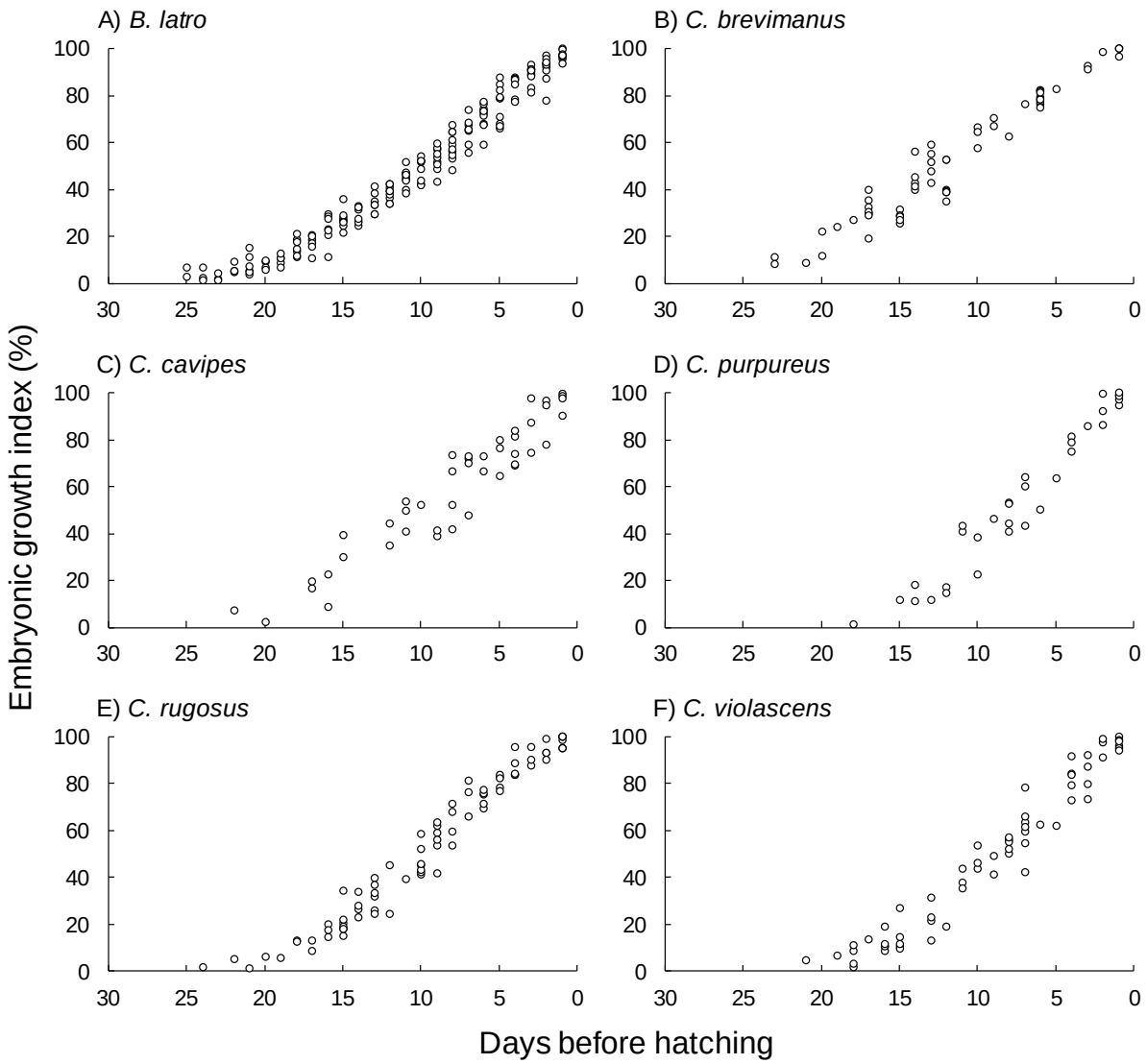

Fig. S3. Changes in the embryonic growth index (EGI) before hatching in 6 coenobitids: (A) *Birgus latro*, (B) *Coenobita brevimanus*, (C) *C. cavipes*, (D) *C. purpureus*, (E) *C. rugosus*, and (F) *C. violascens*. The EGI was measured as the relative area of the embryonic body vs. the total embryo surface in the lateral profile of the egg. See Supplementary Table S2 for the number of females and the data for each species

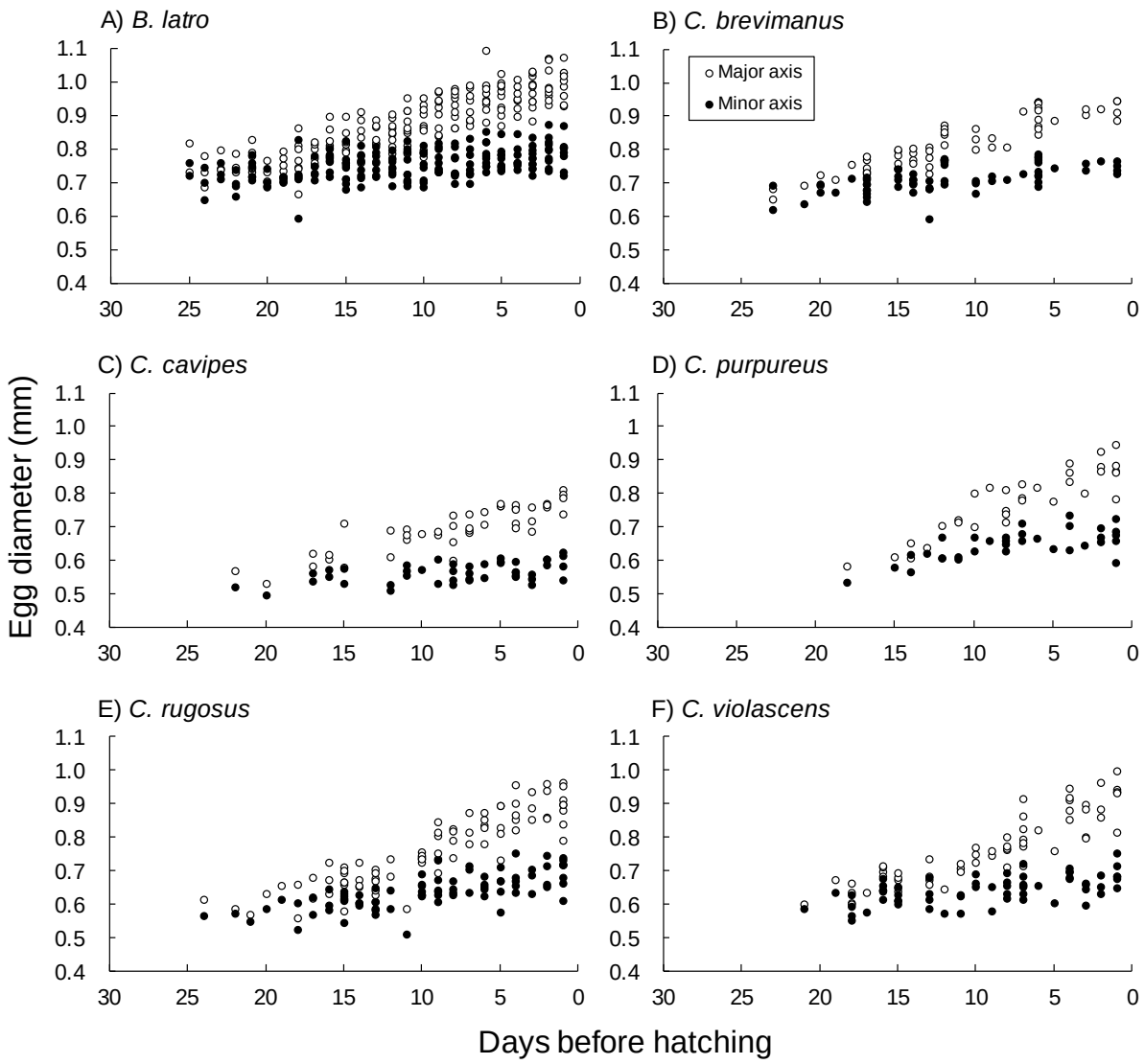

Fig. S4. Changes in egg diameter (major and minor axes) before hatching in 6 coenobitids: (A) *Birgus latro*, (B) *Coenobita brevimanus*, (C) *C. cavipes*, (D) *C. purpureus*, (E) *C. rugosus*, and (F) *C. violascens*. See Supplementary Table S2 for the number of females and the data for each species

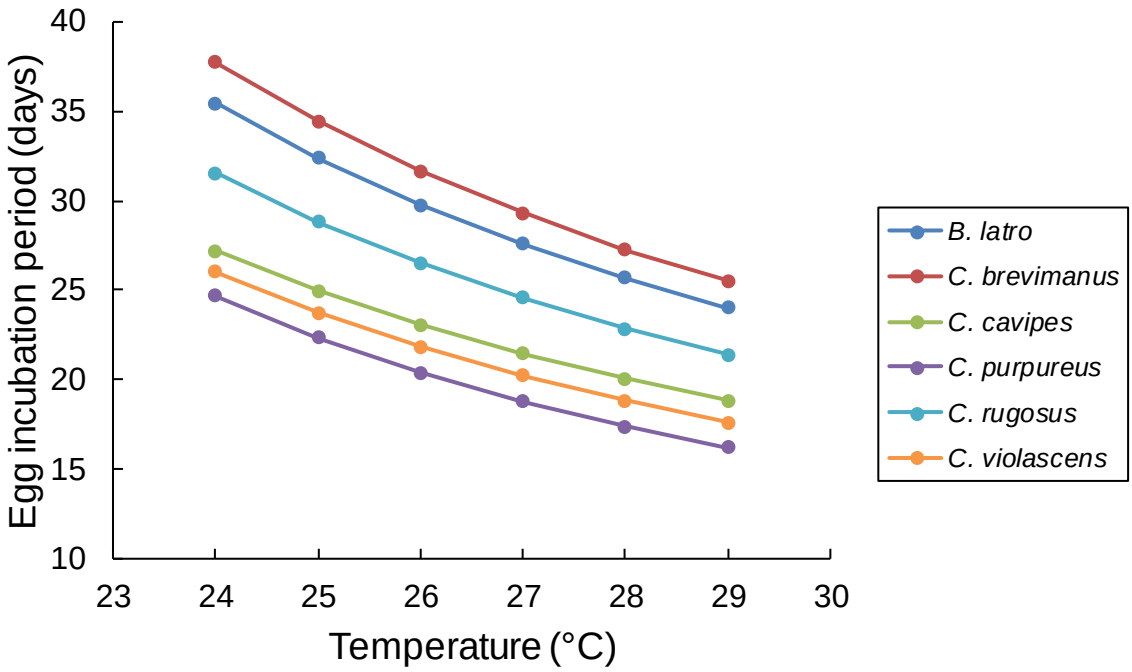

Fig. S5. Egg incubation periods from the appearance of the embryonic body to hatching at 24–29°C during the main reproductive season of coenobitid species by calculating as: partial effective cumulative temperature from the appearance of the embryonic body to hatching/(mean temperature – lower threshold temperature)

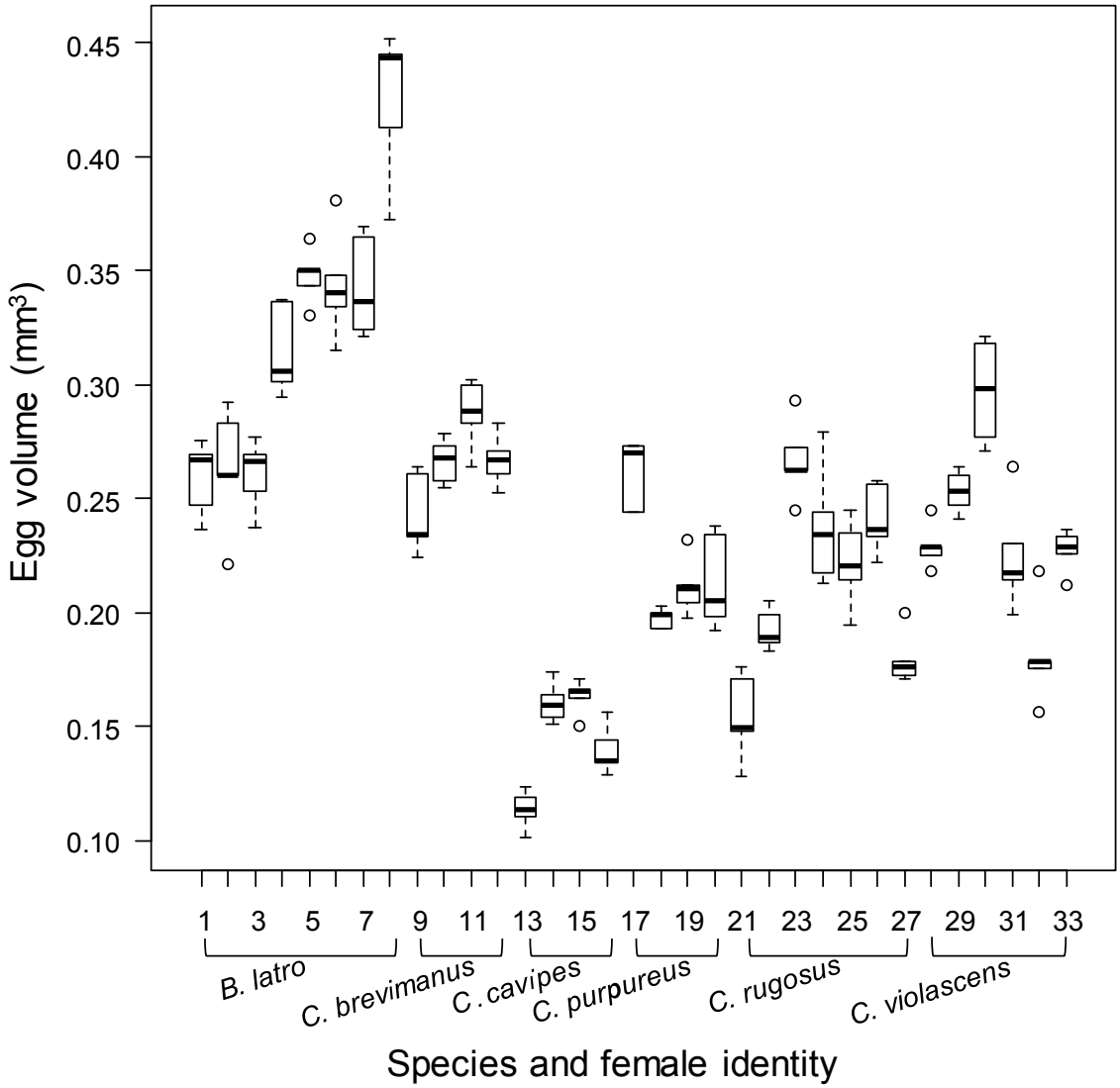

Fig. S6. Box plots of the volume of eggs (N = 5) within 1 day of hatching for different females in 6 coenobitids, *Birgus latro*, *Coenobita brevimanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens*

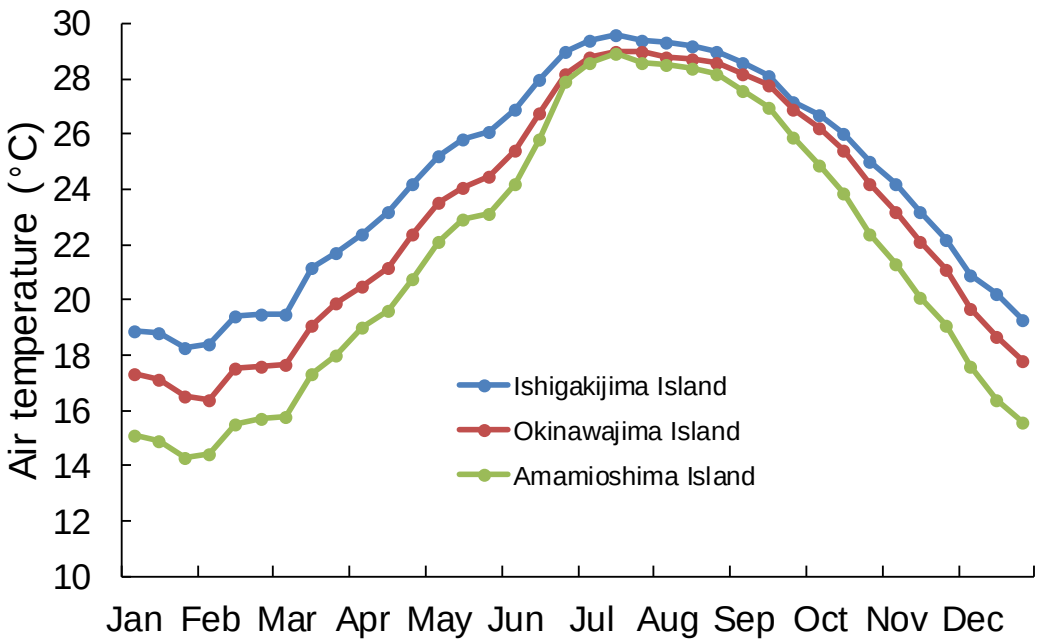

Fig. S7. Seasonal air temperature fluctuations at 3 locations in the Ryukyu Archipelago region. Monthly (10-day interval) mean temperatures (1981–2010) are shown for Ishigakijima Island (24°20′N, 124°9′E), Okinawajima Island (26°12′N, 127°41′E), and Amamioshima Island (28°22′N, 129°29′E)

Table S1 Six temperature levels (mean ± standard deviation) for *in vitro* incubation of embryos in 6 coenobitid species in the genera *Birgus* and *Coenobita*

Species	Female no.	Temperature (°C)					
B. latro	1	19.1 ± 0.6	23.0 ± 0.5	25.9 ± 0.3	28.3 ± 0.1	29.7 ± 0.3	32.2 ± 0.5
	2	19.3 ± 0.5	22.9 ± 0.5	25.9 ± 0.3	28.2 ± 0.1	29.6 ± 0.3	32.2 ± 0.5
C. brevimanus	1	18.1 ± 0.3	21.8 ± 0.2	25.3 ± 0.2	28.4 ± 0.2	30.9 ± 0.2	33.6 ± 0.5
	2	18.0 ± 0.3	21.8 ± 0.2	25.3 ± 0.2	28.4 ± 0.2	30.9 ± 0.2	33.6 ± 0.5
C. cavipes	1	20.0 ± 0.3	23.2 ± 0.3	25.8 ± 0.2	28.5 ± 0.2	31.3 ± 0.3	33.5 ± 0.3
	2	18.0 ± 0.3	21.9 ± 0.3	25.2 ± 0.2	28.4 ± 0.2	30.9 ± 0.2	33.2 ± 0.5
C. purpureus	1	19.9 ± 0.3	23.0 ± 0.3	25.7 ± 0.2	28.5 ± 0.2	31.3 ± 0.3	33.2 ± 0.3
	2	19.9 ± 0.3	23.1 ± 0.3	25.7 ± 0.2	28.5 ± 0.2	31.4 ± 0.2	33.3 ± 0.2
C. rugosus	1	19.3 ± 0.5	23.0 ± 0.4	25.8 ± 0.3	28.2 ± 0.1	29.5 ± 0.2	32.1 ± 0.3
	2	19.2 ± 0.5	23.1 ± 0.4	25.8 ± 0.3	28.2 ± 0.1	29.5 ± 0.3	32.1 ± 0.3
C. violascens	1	17.9 ± 0.3	21.7 ± 0.2	25.2 ± 0.2	28.4 ± 0.2	30.9 ± 0.2	33.4 ± 0.6
	2	18.0 ± 0.3	21.7 ± 0.2	25.2 ± 0.2	28.4 ± 0.2	30.9 ± 0.2	33.3 ± 0.6

Table S2 Number of estimates of embryonic growth index and effective cumulative temperature required for partial embryonic development until hatching for different females in 6 coenobitid species in the genera *Birgus* and *Coenobita*

Year	Number of estimates											
	Female no.	*B. latro*	Female no.	*C. brevimanus*	Female no.	*C. cavipes*	Female no.	*C. purpureus*	Female no.	*C. rugosus*	Female no.	*C. violascens*
2005	1	24										
	2	21										
	3	22										
	4	24										
2006	5	1										
2007	6	1										
	7	2										
	8	1										
2008			1	2	1	2			1	1		
			2	2					2	1		
			3	1								
2009	9	1			2	1	1	2	3	1		
	10	2			3	2						
	11	1			4	1						
	12	1										
2010	13	5	4	2	5	2	2	1			1	2
	14	4			6	1					2	1
	15	5			7	2					3	1
	16	2			8	2					4	2
					9	2					5	1
2011					10	3	3	1	4	2		
							4	1	5	2		
									6	2		
									7	2		
2012	17	1	5	3			5	2	8	3	6	2
	18	2	6	3			6	2	9	3	7	3
	19	2	7	3					10	3	8	3
			8	3					11	2	9	2
			9	3					12	2	10	3
									13	1	11	3
											12	3
											13	2
											14	3
											15	2
2013	20	6	10	3	11	4	7	4	14	3	16	4
	21	6	11	3	12	3			15	3	17	3
			12	4					16	2	18	4
			13	3					17	3		
			14	5					18	2		
									19	3		
									20	3		
									21	2		
									22	3		
2014	22	16	15	1	13	2	8	3	23	2	19	7
	23	14	16	6	14	2	9	4	24	3	20	6
	24	16	17	3	15	2	10	2	25	2	21	1
			18	1	16	2	11	4	26	3	22	4
			19	2	17	1			27	4		
			20	5	18	6			28	1		
			21	3	19	4			29	2		
			22	1					30	1		
									31	1		
									32	8		
									33	1		
									34	1		
									35	1		
									36	3		
2015							12	1				
							13	1				
							14	1				
							15	1				
							16	1				
							17	1				
							18	1				
Total		180		62		44		33		82		62

Table S3 Parameter estimates for heat summation theory equations describing the relationship between mean temperature (T, ºC) and days required until the appearane of eye-pigmentation of embryos (D, days) incubated in seawater medium at 2 salinity levels in 6 coenobitid species in the genera *Birgus* and *Coenobita*: $D = a/(T - b)$, where a and b are parameters and b is so-called "lower threshold temperarure (LTT)" for embryonic development. N: number of observations; SE: standard error

				Parameter *a*				Parameter *b* (LTT)			
Species	Female no.	Salinity	N	Estimate	SE	*t* value	*P*	Estimate	SE	*t* value	*P*
B. latro	1	27	51	165.2	10.4	15.9	< 0.001	13.3	0.5	27.1	< 0.001
		34	55	158.6	9.1	17.4	< 0.001	13.8	0.4	34.8	< 0.001
	2	27	48	233.7	9.0	26.0	< 0.001	13.3	0.3	43.3	< 0.001
		34	52	228.8	10.4	21.9	< 0.001	13.5	0.3	40.4	< 0.001
C. brevimanus	1	27	60	45.1	2.2	20.5	< 0.001	13.8	0.3	53.8	< 0.001
		34	59	53.3	2.6	20.3	< 0.001	12.9	0.3	40.7	< 0.001
	2	27	59	57.5	1.9	30.8	< 0.001	13.9	0.2	86.8	< 0.001
		34	58	60.9	2.6	23.8	< 0.001	13.9	0.2	66.5	< 0.001
C. cavipes	1	27	45	284.1	7.0	40.7	< 0.001	13.1	0.2	57.8	< 0.001
		34	28	300.9	13.8	21.8	< 0.001	12.6	0.5	25.9	< 0.001
	2	27	52	135.3	3.3	40.5	< 0.001	12.5	0.2	75.3	< 0.001
		34	57	137.9	3.1	44.3	< 0.001	12.4	0.2	78.3	< 0.001
C. purpureus	1	27	58	95.4	5.0	19.1	< 0.001	14.8	0.3	45.3	< 0.001
		34	57	80.3	3.4	23.7	< 0.001	15.0	0.3	58.5	< 0.001
	2	27	60	87.2	4.2	20.6	< 0.001	14.4	0.3	42.4	< 0.001
		34	59	83.9	4.2	20.1	< 0.001	13.6	0.4	34.9	< 0.001
C. rugosus	1	27	58	152.9	6.2	24.6	< 0.001	13.7	0.3	47.7	< 0.001
		34	54	173.3	8.6	20.1	< 0.001	13.3	0.4	36.1	< 0.001
	2	27	45	126.3	6.5	19.4	< 0.001	14.1	0.3	41.5	< 0.001
		34	47	151.5	8.4	18.1	< 0.001	12.8	0.4	28.4	< 0.001
C. violascens	1	27	49	90.6	2.1	43.9	< 0.001	13.5	0.1	114.6	< 0.001
		34	55	91.4	1.9	48.0	< 0.001	13.6	0.1	122.1	< 0.001
	2	27	52	104.6	2.2	46.7	< 0.001	13.7	0.1	125.9	< 0.001
		34	50	105.4	2.3	46.1	< 0.001	13.6	0.1	117.4	< 0.001

Table S4 Results of a nested ANOVA for evaluating interspecific and intraspecific variations of egg volume of 6 coenobitid species, in which the species is the independent factor and the female is the nested factor (6 species × 4–8 females per species × 5 eggs per female). η^2: proportion of between-group SS in total SS

Source of variation	DF	SS	MS	*F*	p	η^2
Species	5	0.48315	0.09663	313.177	< 0.001	0.646
Female (species)	27	0.22438	0.00831	26.934	< 0.001	0.300
Residuals	132	0.04073	0.000309			
Total	164	0.74826				