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Supplementary material

Variability in egg and jelly-coat size and their contribution to target size for spermatozoa: a review for the Echinodermata

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Table S1. Two-way PERMANOVA of jelly coat (JC) hydration data for three sea urchin species measured as the percentage change across time (5, 10, 15, 30 min) compared to time zero (*n* = ~10 eggs per female per time point) for eight females

The *post hoc* pairwise comparisons for main effects and interactions were used to determine the time points when the JC was at maximum hydration. This was used to calculate the sample size of eggs available for analysis of the JC (*n*). Significant results ($P < 0.05$) indicated in bold

Source	d.f.	SS	MS	Pseudo-*F*	*P*(perm)	*Post hoc* test		JC at maximum hydration		
								Female	Time (min)	*n*
H. erythrogramma						Female		1	0[A]	50
Female	7	2180.80	311.54	3.56	**0.0014**	1 = 2 = 7 = 8 > 2 = 6 = 7 = 8 > 3 = 4 = 5 = 6 = 7 > 3 = 4 = 5		2	0[A]	50
Time	4	211.43	52.86	0.60	0.6614			3	0[A]	50
Female × Time	28	3464.40	123.73	1.41	0.0847			4	0[A]	49
Residuals	358	31359	87.594					5	0[A]	50
								6	0[A]	50
								7	0[A]	50
								8	0[A]	49
H. tuberculata						Time (Female)	Female (Time)			
Female	7	5.83E+5	83287	191.49	**< 0.001**	0(1 = 2 = 3 = 4 = 5 = 6 = 7 = 8)	1(0 = 5 = 10 = 15 = 30)	1	0[A]	50
Time	4	1.09E+5	27347	62.88	**< 0.001**	5(6 > 7 = 8 > 1 = 2 > 1 = 3 = 4 = 5)	2(0 < 5 < 10 = 15 = 30)	2	10	30
Female × Time	28	1.63E+5	5829.2	13.40	**< 0.001**	10(6 > 7 = 8 > 2 > 1 = 3 = 5 > 3 = 4 = 5)	3(0 = 5 = 10 = 15 = 30)	3	0[A]	50
Residuals	360	1.57E+5	434.93			15(6 > 7 = 8 > 2 > 1 = 5 > 3 = 4 = 5)	4(0 > 5 = 10 = 15 > 10 = 15 > 15 = 30)	4	0[A]	10
						30(6 = 7 = 8 > 2 > 1 = 5 > 3 = 5 > 4 = 5)	5(0 = 5 = 10 = 15 = 30)	5	0[A]	50
							6(0 < 5 = 10 = 15 = 30)	6	5	40
							7(0 < 5 = 10 = 15 < 10 = 15 = 30)	7	10	30
							8(0 < 5 = 10 = 15 < 10 = 15 = 30)	8	10	30
C. rodgersii						Time (Female)	Female (Time)			
Female	7	4.32E+6	6.17E+05	178.21	**< 0.001**	0(1 = 2 = 3 = 4 = 5 = 6 = 7 = 8)	1(0 < 5 = 10 < 15 = 30)	1	15	20
Time	4	4.31E+6	1.08E+06	310.80	**< 0.001**	5(8 > 1 = 5 = 6 > 4 = 6 > 2 = 3 = 4 > 7)	2(0 < 5 = 10 < 10 = 15 = 30)	2	10	30
Female × Time	28	1.52E+6	54192	15.65	**< 0.001**	10(8 > 1 = 5 > 2 = 4 = 6 > 3 = 4 > 7)	3(0 < 5 = 10 = 15 < 30)	3	30[B]	10
Residuals	360	1.25E+6	3463.7			15(1 = 8 > 5 > 2 = 6 > 2 = 4 > 3 = 4 > 7)	4(0 < 5 = 10 = 15 = 30)	4	5	40
						30(1 = 5 = 8 > 6 > 2 = 3 = 4 > 7)	5(0 < 5 < 10 = 15 < 30)	5	30[B]	10
							6(0 < 5 = 10 = 15 < 15 = 30)	6	15	20
							7(0 < 5 < 10 = 15 = 30)	7	10	30
							8(0 < 5 = 10 = 15 = 30)	8	5	40

[A]Jelly coats fully hydrated before 5 min.

[B]Jelly coats may still be hydrating.

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Table S2. Egg and jelly-coat (JC) sizes calculated from available data for 17 echinoids, 4 asteroids and 1 holothuroid

To represent the 3-D target of the egg for sperm, surface area of the egg with and without the jelly coat was calculated. The relative size index (RSI) was calculated as the ratio between jelly-coat surface area to egg surface area to represent the increase in target area given by the jelly coat. Standard error (s.e.) was determined where available or could be calculated. (P), Planktotrophic larvae; (L), Lecithotrophic larvae. Foo (2015) and Deaker (2016) are available on request

Species	Egg diameter (µm ± s.e.)	JC thickness (µm ± s.e.)	Target size without JC (μm^2 ± s.e.)	Target size with JC (μm^2 ± s.e.)	RSI	Source
ECHINOIDS						
Arbacia punctulata (P)	69.00	28.50	14957.12	49875.92	3.33	Bolton *et al*. 2000
	74.00	30.00	17203.36	56410.44	3.28	Harvey 1956
	78.00	24.50	19113.45	50670.75	2.65	Inamdar *et al*. 2007
Centrostephanus rodgersii (P)	111 (2.21)	29.00	38707.56	89727.03	2.32	Foo 2015
	111.61 (0.25)	40.82 (0.90)	39170.48 (174.85)	119216.64 (2139.20)	3.06 (0.06)	This study
Dendraster excentricus (P)	125.00	40.00	49087.39	132025.43	2.69	Timko 1979
	128.8 (1.7)	91.55	52117.26	305619.19	5.86	Podolsky 2002
	129.00	92.00	52279.24	307778.69	5.89	Strathmann 1987
Echinometra mathei (P)	70 (1.40)	27.00	15393.80	48305.13	3.14	Foo, 2015
Echinolampas crassa (P)	220.00	143.00	152053.08	804360.82	5.29	Cram 1971
Echinarachnius parma (P)	145.00	95.00	66051.99	352565.24	5.34	Harvey 1956
Heliocidaris crassispina (P)	82.34	35.77	21301.76	74394.86	3.49	Chan, unpubl.
Heliocidaris tuberculata (P)	91 (1.32)	33.00	26015.53	77437.12	2.98	Foo 2015
	93.18 (0.23)	28.17 (0.37)	27322.77 (136.34)	70799.38 (743.89)	2.60 (0.03)	This study
Lytechinus variegatus (P)	99.4 (0.01)	47.10	31040.07	117749.91	3.79	Farley and Levitan 2001
	143.00	77.50	64242.43	278985.99	4.34	Bolton *et al*. 2000
Pseudoboletia indiana (P)	86 (2.06)	27.00	23235.22	61575.22	2.65	Foo 2015
Paracentrotus lividus (P)	100.00	40.00	31415.93	101787.60	3.24	Vogel *et al*. 1982
Pseudochinus magellanicus (P)	122 (5)	49.00	46759.47	152053.08	3.25	Marzinelli *et al*. 2008
Strongylocentrotus droebachiensis (P)	160.00	50.00	80424.77	212371.66	2.64	Bolton *et al*. 2000
Strongylocentrotus franciscanus (P)	130.00	33.00	53092.92	120687.42	2.27	Lessios 1990
Strongylocentrotus purpuratus (P)	79.00	35.00	19606.68	69746.50	3.56	Strathmann 1987

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Species	Egg diameter (µm ± s.e.)	JC thickness (µm ± s.e.)	Target size without JC (µm² ± s.e.)	Target size with JC (µm² ± s.e.)	RSI	Source
	80.00	20.00	20106.19	45238.93	2.25	Lessios 1990
Tripneustes gratilla (P)	88.10 (0.13)	38.20 (0.50)	24398.13 (70.43)	85877.30 (1057.97)	3.52 (0.04)	Deaker, unpubl.
Heliocidaris erythrogramma (L)	390 (8.03)	62.00	477836.24	829996.21	1.74	Foo 2015
	391.74 (0.82)	56.12 (0.60)	482957.37 (2017.71)	799978.33 (4061.32)	1.66 (0.01)	This study
ASTEROIDS						
Patiriella regularis (P)	172 (3.11)	16.50	92940.88	132025.43	1.42	Foo 2015
	143.5 (9.3)	8.70	64692.46	81332.10	1.26	Styan *et al*. 2005
	179.42 (1.09)	14.89 (0.21)	101429.49 (327.10)	137859.86 (420.95)	1.36 (0.01)	Deaker 2016
Acanthaster planci (P)	214.77 (0.96)	20.85 (0.29)	145134.51 (1294.35)	207025.34 (2001.11)	1.43 (0.01)	Deaker 2016
	224 (2.45)	20.00	157632.55	218956.44	1.39	Foo 2015
Meridiastra calcar (L)	425.7 (1.6)	24.30	569320.96	706734.22	1.24	Styan *et al*. 2005
	444 (15.22)	37.00	619321.01	842964.71	1.36	Foo 2015
Hippasteria spinosa (L)	1200.00	200.00	4523893.42	8042477.19	1.78	Strathmann 1987
HOLOTHUROIDS						
Cucumaria miniata (L)	520.00	35.00	849486.65	1093588.40	1.29	Strathmann 1987

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Table S3. PERMANOVA of the relative size index (RSI) of the jelly-coat data with female nested within each sea urchin species

The RSI was calculated as the ratio between jelly-coat surface area to egg surface area to represent the increase in target area given by the jelly coat. *H. ery*, *H. erythrogramma*; *H. tub*, *H. tuberculata*; *C. rod*, *C. rodgersii*. Estimates of components of variation: species = 0.6175, Female (species) = 0.21883. Significant results are displayed in bold (P < 0.05)

Source	d.f.	SS	MS	Pseudo-F	P(perm)	*Post hoc*
Species	2	307.51	153.75	23.39	**<0.001**	Species
Female (Species)	21	169	8.05	152.46	**<0.001**	*H. ery* < *H. tub* = *C. rod*
Residuals	864	45.607	0.05			
						Species (female)
						H. ery (4 < 3 < 7 < 5 < 6 < 8 < 2 < 1)
						H. tub (5 < 7 = 8 < 1 = 3 < 2 < 4 < 6)
						C. rod (7 < 1 = 4 = 6 < 2 = 6 < 3 = 5 = 8)

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Table S4. The correlation between egg diameter and jelly-coat thickness for each individual (within-spawn) and across the total population of females was calculated using Pearson's r for normally distributed data or Kendall's τ for non-normal data

Average values for each female was used in the correlation analysis of egg diameter and jelly-coat thickness across a species (among-female, n = 8). The number of eggs at the time points where the jelly coat had reached maximum hydration was used as the data for the correlation analysis (see Table S1). Significant results are displayed in bold ($P < 0.05$)

Female	*H. erythrogramma*		*H. tuberculata*		*C. rodgersii*	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
All	τ = –0.429	0.179	τ = 0.092	**0.020**	τ = 0.00	1.00
1	r = 0.209	0.146	τ = 0.032	0.744	τ = –0.08	0.63
2	r = 0.005	0.973	τ = –0.184	0.153	τ = 0.07	0.60
3	τ = –0.213	**0.029**	τ = –0.005	0.972	τ = –0.11	0.73
4	τ = 0.007	0.952	τ = 0.333	0.216	τ = –0.26	**0.02**
5	τ = –0.014	0.887	τ = 0.117	0.232	τ = –0.11	0.73
6	τ = –0.149	0.126	τ = 0.113	0.313	τ = 0.02	0.92
7	τ = –0.102	0.296	r = 0.305	0.101	τ = –0.13	0.32
8	τ = –0.143	0.151	τ = 0.347	**0.007**	τ = –0.07	0.52

Table S5. The range and percentage difference in egg diameter and jelly-coat thickness reported in different studies of the same species

Sources are: 1, Harvey 1956; 2, Bolton *et al*. 2000; 3, Inamdar *et al*. 2007; 4, Foo 2015; 5, this study; 6, Strathmann 1987; 7, Timko 1979; 8, Podolsky 2002; 9, Farley and Levitan 2001; 10, Lessios 1990; 11, Styan *et al*. 2005. Foo (2015) is available on request

Species	Range egg diameter (μm)	Percentage difference	Range JC thickness (μm)	Percentage difference	Source
Arbacia punctulata	69.00–78.00	12.24	24.50–30.00	20.18	1,2,3
Centrostephanus rodgersii	111.00–112.23	0.54	29.00–39.66	33.87	4,5
Dendraster excentricus	125.00–129.00	3.15	40.00–92.00	78.79	6,7,8
Heliocidaris tuberculata	91.00–93.54	2.36	28.71–33.00	15.78	4,5
Lytechinus variegatus	99.40–143.00	35.97	47.10–77.50	48.80	2,9
Strongylocentrotus purpuratus	79.00–80.00	1.26	20.00–35.00	54.55	6,10
Heliocidaris erythrogramma	390.00–391.74	0.45	56.12–62.00	9.95	4,5
Patiriella regularis	143.50–179.42	22.25	8.70–16.50	61.90	4,5,11
Acanthaster planci	214.77–224.00	4.21	20.00–20.85	4.16	4,5
Meridiastra calcar	425.70–444.00	4.21	24.30–37.00	41.44	4,5,11

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Table S6. Data presented in Marshall *et al*. (2008) of the coefficient of variation (CV, %) in the egg size of marine invertebrates with planktotrophic (P), lecithotrophic (L) or direct developing (D) larvae measured as either egg volume or egg diameter

Species	Type	Metric	CV (%) within-spawn	CV (%) among-females	Source in Marshall *et al*. (2008)
Crepidula adunca	D	Diameter	6.04	25.71	Collin (2000)
Parvulastra parvivipara	D	Diameter	5.92	7.16	M. Byrne (unpubl. data)
Echinaster modestus	D	Diameter	12.23	16.21	Turner and Lawrence (1979)
Alderia modesta	L	Volume	9.78	12.37	Krug (1998)
Lottia pelta	L	Diameter	8.6	2.4	Hadfield and Strathmann (1996)
Diadora aspersa	L	Diameter	12.9	4.9	Hadfield and Strathmann (1996)
Bugula neritina	L	Diameter	6.5	6.9	D. Marshall (unpubl. data)
Meridiastra occidens	L	Diameter	4.24	4.42	M. Byrne (unpubl. data)
Meridiastra calcar	L	Diameter	3.85	3.87	M. Byrne (unpubl. data)
Meridiastra gunnii	L	Diameter	4.14	5.07	M. Byrne (unpubl. data)
Echinaster modestus	L	Diameter	10.39	8.27	Turner and Lawrence (1979)
Uniophora granifera	L	Diameter	7.86	6.3	D. Marshall (unpubl. data)
Clypeaster rosaceus	L	Diameter	1.67	2.74	Emlet (1986)
Pyura stolonifera	L	Diameter	7.9	9.18	Marshall *et al*. (2000)
Pyura fissa	L	Diameter	4.89	5.21	Marshall and Keough (2003)
Styela plicata	L	Diameter	3.9	7.9	Marshall and Keough (2003)
Ciona intestinalis	L	Diameter	4.5	5.17	Marshall and Keough (2003)
Galeolaria caespitosa	P	Diameter	11.56	1.99	Marshall and Keough (unpubl. data)
Dendraster exentricus	P	Diameter	3.5	3.5	Podolsky (2002)
Alderia modesta	P	Volume	13.7	11.75	Krug (1998)
Asterias forbesi	P	Volume	22.91	16.31	Turner and Lawrence (1979)
Luidia clathrata	P	Volume	15.53	8.52	Turner and Lawrence (1979)
Encope aberrans	P	Volume	15.26	11.17	Turner and Lawrence (1979)
Lytechinus variegatus	P	Volume	10.01	9.05	Turner and Lawrence (1979)
Strongylocentrotus droebachiensis	P	Volume	9.04	4.96	Turner and Lawrence (1979)

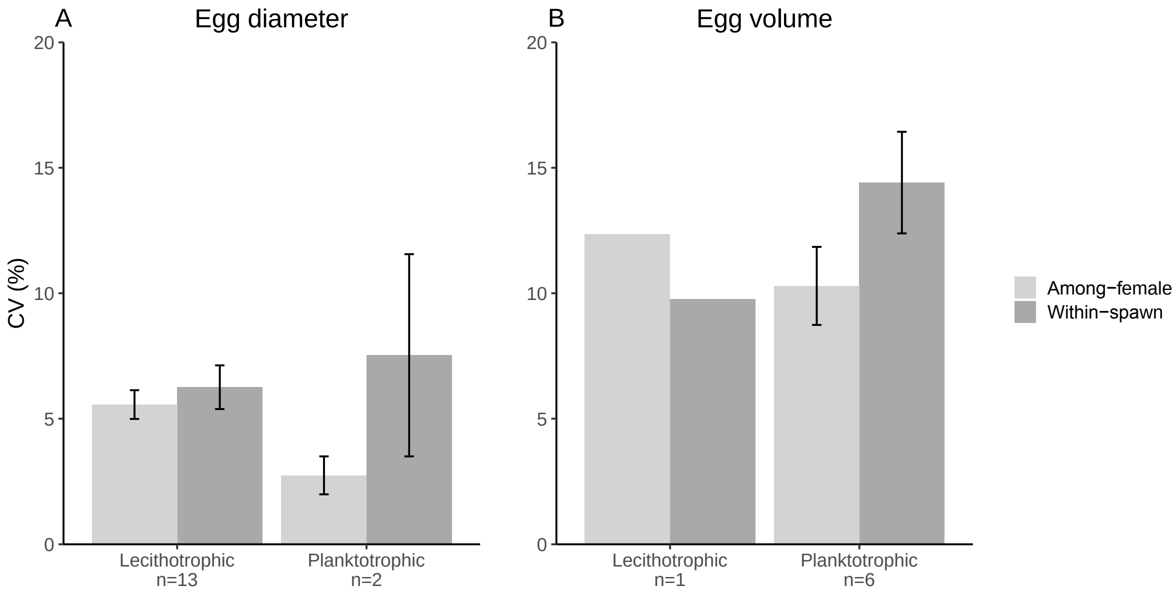

Fig. S1. The mean coefficient of variation (CV, % ± s.e.) of the eggs of marine invertebrates with planktotrophic and lecithotrophic larvae in the data from Marshall *et al*. (2008) measured by either (A) diameter or (B) volume.

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