

The following supplement accompanies the article

Assessment of investigation techniques for scyphozoan statoliths with focus on early development of the jellyfish *Sanderia malayensis*

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Table S1. Food amounts fed to *Sanderia malayensis* specimens in different ages. *Aurelia aurita* (mashed) was provided separately to each specimen for one hour in a 10 ml plastic vessel before the specimens were transferred to separate culture flasks filled with fresh seawater containing *Artemia salina* nauplii. The number of nauplii in the applied volume of *Artemia* suspension was calculated from numbers counted in 100 μ l of the suspension

Age (days)	<i>Aurelia</i> (μ l)	<i>Artemia</i> (n)
0 – 6	100	45
7 – 13	200	60
14 – 20	300	80
21 – 27	400	100
28 – 34	500	150
35 – 41	500	300

Table S2. Sizes of body parts of *Sanderia malayensis* ephyrae and medusae at ages of 0 to 42 days and calculated percentages of individual growth from the date of detachment (0 d). Measurements are presented as mean \pm standard deviation (SD) and ranges are beneath the means. Mean diameter growth (\pm SD) and mean instantaneous growth (μ ; d^{-1}) (\pm SD) are in percentages (%). Last column shows the numbers of statoliths per statocyst for each age. D_a : adradial diameter between opposite lappet clefts, D_c : central bell diameter including the ring musculature, D_r : rhopalar diameter between opposite rhopalia tips, L_{ls} : marginal lappet length minus rhopalar lobe length (lappet stem length), L_{ml} : marginal lappet length, n_1 : number of analyzed specimens, n_2 : number of analyzed statocysts, L_{rl} : rhopalar lobe length, sd: standard deviation, sc: statocyst, sl/sc: statoliths per statocyst, D_t : total diameter between opposite lappet tips

Age	td (mm)	D_r (mm)	D_a (mm)	D_c (mm)	L_{ml} (mm)	L_{ls} (mm)	L_{rl} (mm)	sc length (μ m)	sc width (μ m)	sl/sc (no.)
0 days	3.8 ± 0.2	2.8 ± 0.2	2.3 ± 0.2	1.8 ± 0.1	0.8 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	44 ± 4	35 ± 6	31.3 ± 7.4
(6, 36)	3.6 – 4.1	2.6 – 3.0	2.1 – 2.6	1.7 – 1.9	0.7 – 0.8	0.2 – 0.3	0.4 – 0.5	39 – 51	27 – 46	15 – 55
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7 days	5.1 ± 0.6	3.9 ± 0.4	3.0 ± 0.5	2.5 ± 0.6	1.1 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	46 ± 12	43 ± 6	38.6 ± 7.2
(3, 24)	4.5 – 5.6	3.3 – 4.3	2.3 – 3.3	1.9 – 3.0	0.9 – 1.3	0.3 – 0.5	0.5 – 0.7	33 – 56	36 – 47	21 – 50
	0d: 3.4 ± 0.1	0d: 2.5 ± 0.1	0d: 2.0 ± 0.1	0d: 1.8 ± 0.1	0d: 0.8 ± 0.0	0d: 0.3 ± 0.1	0d: 0.5 ± 0.0	0d: 39.4 ± 6.1	0d: 36.8 ± 4.6	
	0d: 3.4 – 3.6	0d: 2.3 – 2.6	0d: 1.9 – 2.1	0d: 1.7 – 1.9	0d: 0.8 – 0.8	0d: 0.2 – 0.3	0d: 0.4 – 0.5	0d: 32.9 – 44.9	0d: 32.2 – 41.5	
	49 ± 15 % μ : 17.0 ± 3.5 %	52 ± 22 %	51 ± 32 %	44 ± 30 %	37 ± 15 %	63 ± 24 %	15 ± 13 %	0 ± 22 %	18 ± 28 %	
14 days	7.3 ± 0.3	5.4 ± 0.2	4.3 ± 0.2	3.4 ± 0.4	1.5 ± 0.1	0.5 ± 0.0	0.9 ± 0.1	59 ± 4	65 ± 10	67.5 ± 8.0
(3, 24)	7.0 – 7.5	5.2 – 5.5	4.1 – 4.5	2.9 – 3.7	1.5 – 1.6	0.5 – 0.5	0.8 – 1.0	54 – 62	54 – 74	46 – 86
	0d: 3.5 ± 0.2	0d: 2.5 ± 0.1	0d: 1.9 ± 0.1	0d: 1.5 ± 0.2	0d: 0.8 ± 0.0	0d: 0.2 ± 0.0	0d: 0.5 ± 0.0	0d: 36.0 ± 7.9	0d: 29.4 ± 6.8	
	0d: 3.4 – 3.7	0d: 2.3 – 2.6	0d: 1.9 – 2.1	0d: 1.4 – 1.7	0d: 0.8 – 0.8	0d: 0.2 – 0.2	0d: 0.4 – 0.5	0d: 27.0 – 41.6	0d: 22.7 – 36.4	
	109 ± 17 % μ : 15.7 ± 1.7 %	117 ± 18 %	120 ± 20 %	127 ± 49 %	98 ± 17 %	129 ± 18 %	94 ± 32 %	72 ± 50 %	131 ± 83 %	
21 days	8.9 ± 1.2	7.1 ± 1.0	5.8 ± 0.8	4.8 ± 0.6	1.7 ± 0.2	0.6 ± 0.1	1.0 ± 0.1	71 ± 7	72 ± 5	88.1 ± 10.2
(6, 36)	7.2 – 10.0	5.7 – 8.0	4.7 – 6.5	3.9 – 5.5	1.3 – 1.9	0.4 – 0.7	0.9 – 1.3	61 – 78	67 – 78	54 – 109
	0d: 3.5 ± 0.3	0d: 2.5 ± 0.2	0d: 2.0 ± 0.1	0d: 1.6 ± 0.1	0d: 0.8 ± 0.0	0d: 0.2 ± 0.0	0d: 0.5 ± 0.1	0d: 39.4 ± 5.8	0d: 33.1 ± 6.5	
	0d: 3.1 – 3.8	0d: 2.2 – 2.9	0d: 1.8 – 2.2	0d: 1.4 – 1.8	0d: 0.7 – 0.9	0d: 0.2 – 0.3	0d: 0.5 – 0.6	0d: 30.4 – 45.8	0d: 27.0 – 45.2	
	158 ± 42 % μ : 13.4 ± 2.5 %	182 ± 48 %	190 ± 40 %	198 ± 41 %	120 ± 33 %	187 ± 60 %	97 ± 32 %	86 ± 42 %	124 ± 35 %	
28 days	10.1 ± 0.8	8.2 ± 0.7	6.7 ± 0.5	5.4 ± 0.3	1.8 ± 0.2	0.7 ± 0.1	1.0 ± 0.1	82 ± 9	75 ± 6	110.9 ± 15.7
(6, 36)	8.8 – 11.3	7.3 – 9.1	5.9 – 7.3	5.1 – 5.8	1.6 – 2.2	0.6 – 0.9	0.8 – 1.1	73 – 98	70 – 86	84 – 157
	0d: 3.5 ± 0.1	0d: 2.6 ± 0.1	0d: 2.1 ± 0.1	0d: 1.7 ± 0.1	0d: 0.8 ± 0.1	0d: 0.2 ± 0.0	0d: 0.5 ± 0.1	0d: 39.8 ± 8.0	0d: 30.3 ± 4.7	
	0d: 3.5 – 3.7	0d: 2.5 – 2.7	0d: 1.9 – 2.2	0d: 1.5 – 1.8	0d: 0.7 – 0.9	0d: 0.2 – 0.3	0d: 0.5 – 0.6	0d: 29.4 – 50.1	0d: 24.4 – 35.8	
	184 ± 24 % μ : 11.2 ± 0.9 %	212 ± 32 %	230 ± 33 %	223 ± 33 %	126 ± 18 %	199 ± 56 %	94 ± 33 %	116 ± 67 %	153 ± 57 %	

Age (n ₁ , n ₂)	td (mm)	D _r (mm)	D _a (mm)	D _c (mm)	L _{ml} (mm)	L _{ls} (mm)	L _{rl} (mm)	sc length (μm)	sc width (μm)	sl/sc (no.)
35 days (4, 28)	12.6 ± 1.0 11.5 – 13.9 0d: 3.7 ± 0.2 0d: 3.5 – 3.9 242 ± 42 % μ: 10.5 ± 1.1 %	10.9 ± 0.7 10.3 – 11.9 0d: 2.7 ± 0.2 0d: 2.6 – 2.9 301 ± 43 %	9.1 ± 0.6 8.4 – 9.9 0d: 2.2 ± 0.2 0d: 2.0 – 2.4 323 ± 60 %	7.5 ± 0.4 6.9 – 7.8 0d: 1.8 ± 0.2 0d: 1.6 – 2.0 325 ± 66 %	2.0 ± 0.2 1.7 – 2.3 0d: 0.8 ± 0.1 0d: 0.7 – 0.8 151 ± 39 %	0.8 ± 0.2 0.7 – 1.0 0d: 0.2 ± 0.0 0d: 0.2 – 0.3 240 ± 88 %	1.4 ± 0.2 1.2 – 1.7 0d: 0.6 ± 0.1 0d: 0.4 – 0.6 156 ± 29 %	114 ± 9 103 – 125 0d: 42.8 ± 8.3 0d: 32.8 – 52.5 174 ± 58 %	87 ± 5 83 – 93 0d: 30.4 ± 7.4 0d: 21.5 – 36.5 199 ± 75 %	210.3 ± 27.6 148 – 260
42 days (6, 36)	12.3 ± 1.1 10.4 – 13.7 0d: 3.4 ± 0.4 0d: 3.0 – 3.8 270 ± 51 % μ: 9.3 ± 0.9 %	10.5 ± 1.1 9.2 – 12.2 0d: 2.5 ± 0.3 0d: 2.2 – 2.9 327 ± 76 %	8.6 ± 0.6 8.0 – 9.5 0d: 1.9 ± 0.2 0d: 1.7 – 2.2 361 ± 72 %	6.9 ± 0.4 6.3 – 7.4 0d: 1.5 ± 0.2 0d: 1.3 – 1.8 363 ± 58 %	2.0 ± 0.4 1.4 – 2.5 0d: 0.8 ± 0.1 0d: 0.7 – 0.9 158 ± 52 %	0.8 ± 0.2 0.6 – 1.3 0d: 0.3 ± 0.0 0d: 0.2 – 0.3 237 ± 80 %	1.3 ± 0.3 0.8 – 1.6 0d: 0.5 ± 0.0 0d: 0.5 – 0.5 158 ± 55 %	125 ± 8 115 – 136 0d: 48.2 ± 9.7 0d: 29.5 – 56.2 170 ± 67 %	89 ± 7 79 – 98 0d: 35.1 ± 10.6 0d: 19.8 – 48.9 182 ± 118 %	226.6 ± 31.6 106 – 288

Table S3. Statoliths per statocyst and statolith measurements (length, width, area, ratio) based on light microscopic image analyses (LM) and micro-computed tomographic label analyses (μ -CT) on statocysts of the same *Sanderia malayensis* specimens at three ages. Numbers are presented as mean \pm standard deviation (SD) and ranges are beneath the means. Numbers of analyzed statocysts per age for LM = 4, for μ -CT = 1

Age (d)	Number		Length (μ m)		Width (μ m)		Area (μ m ²)		Ratio	
	LM	μ -CT	LM	μ -CT	LM	μ -CT	LM	μ -CT	LM	μ -CT
0	28.5 \pm 3.1 24 – 30	41	10.5 \pm 3.0 3.4 – 18.6	7.9 \pm 2.7 2.8 – 12.1	3 \pm 1.7 0.3 – 7.4	5.3 \pm 2.0 1.4 – 9.0	32.3 \pm 21.2 1.9 – 89.1	45.5 \pm 27.7 3.8 – 99.0	5.1 \pm 4.2 0.6 – 24.1	1.5 \pm 0.4 0.4 – 2.4
14	65.8 \pm 11.1 50 – 74	68	11.4 \pm 2.7 3.9 – 18.6	13.2 \pm 3.4 3.8 – 18.0	8.6 \pm 3.8 0.7 – 16.0	11 \pm 3.7 1.9 – 15.7	101.8 \pm 53.4 2.7 – 204.6	156.5 \pm 73.4 7.3 – 263.2	1.7 \pm 1.2 0.6 – 9.4	1.3 \pm 0.3 1.0 – 2.6
42	229.5 \pm 11.0 217 – 241	194	12.5 \pm 3.8 1.5 – 27.9	15.2 \pm 3.3 4.6 – 27.9	7.1 \pm 4.3 0.4 – 23.2	11.7 \pm 3.2 3.0 – 19.3	91.8 \pm 62.3 1.5 – 278.7	183.6 \pm 75.0 13.9 – 508.4	2.9 \pm 3.0 0.3 – 24.3	1.4 \pm 0.4 1.0 – 3.9

Table S4. Statolith lengths, diameters (Diam) and increments (Inc) of *Sanderia malayensis* statoliths measured on calcein labeled crystals. Numbers are presented as mean \pm standard deviation (SD). Con: applied calcein concentration (μ Mol L⁻¹), n₁: number of measured diameters per statolith, n₂: number of measured side face increments per statolith

Con	No	Length (μ m)	Diam (μ m) n ₁ = 6	Inc (μ m) n ₂ = 12
100	1	10.6	16.1 \pm 0.7	2.5 \pm 0.3
100	2	11.4	16.7 \pm 0.4	3.8 \pm 0.3
100	3	8.2	14.1 \pm 0.6	1.7 \pm 0.1
100	4	11.5	14.1 \pm 0.3	3.1 \pm 0.1
100	5	9.7	15 \pm 0.5	4.4 \pm 0.3
200	6	10.3	15.2 \pm 0.4	2.6 \pm 0.2
200	7	11.6	15.7 \pm 0.1	3.1 \pm 0.2
200	8	9	12.8 \pm 0.4	3.5 \pm 0.2
200	9	10.5	13.7 \pm 0.8	2.7 \pm 0.3
200	10	12.5	14.7 \pm 0.5	3.4 \pm 0.2
200	11	6.7	10.4 \pm 0.6	2 \pm 0.2
200	12	10.1	15.7 \pm 1.4	3.1 \pm 0.4
200	13	10.3	11.9 \pm 0.5	2.1 \pm 0.3
200	14	17.9	12.2 \pm 0.4	3 \pm 0.2
200	15	12	14.1 \pm 0.5	2.9 \pm 0.6
200	16	11.5	12 \pm 1.2	2.1 \pm 0.2
200	17	18.1	12.5 \pm 0.4	2.5 \pm 0.2
200	18	8.4	11.9 \pm 0.4	1.8 \pm 0.2

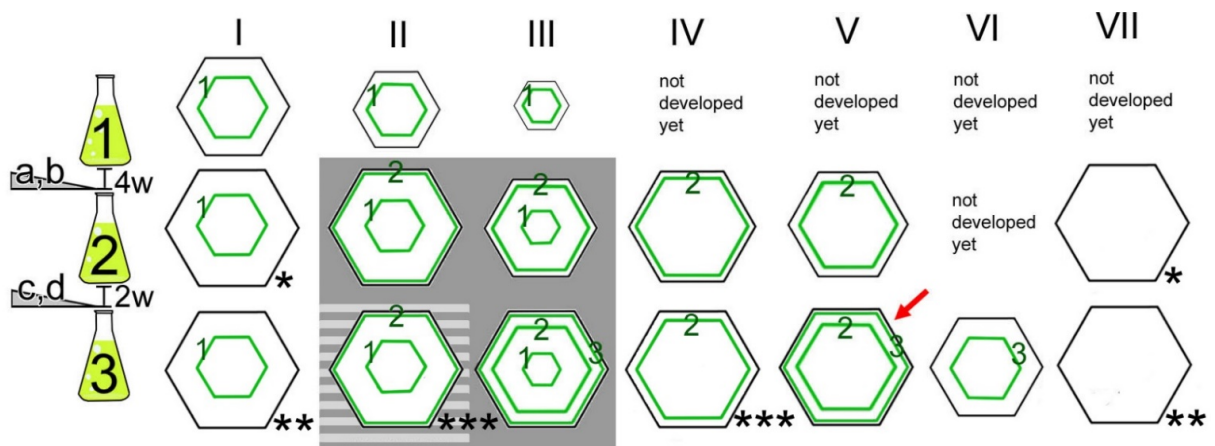


Fig. S1. Theoretical scenarios (I – VII) of statolith labeling after one to three incubations with the fluorescent marker calcein. The bottles (left column) represent the calcein solutions used for the incubations of *Sanderia malayensis* specimens (a – d). Grey background: statoliths not found in samples, stripes: statoliths probably not found in samples. Red arrow: statoliths selected for measurements of statolith increment, w: weeks between incubations, *: growth stopped before 2nd incubation, **: growth stopped before 2nd and 3rd incubation, ***: growth stopped before 3rd incubation