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 µl of the suspension

Age (days)	*Aurelia* (µl)	*Artemia* (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 ± standard deviation (SD) and ranges are beneath the means. Mean diameter growth (±SD) and mean instantaneous growth (μ; d^{-1}) (±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 (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.)
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 %	117 ± 18 %	120 ± 20 %	127 ± 49 %	98 ± 17 %	129 ± 18 %	94 ± 32 %	72 ± 50 %	131 ± 83 %	
	μ: 15.7 ± 1.7 %									
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 %	182 ± 48 %	190 ± 40 %	198 ± 41 %	120 ± 33 %	187 ± 60 %	97 ± 32 %	86 ± 42 %	124 ± 35 %	
	μ: 13.4 ± 2.5 %									
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 %	212 ± 32 %	230 ± 33 %	223 ± 33 %	126 ± 18 %	199 ± 56 %	94 ± 33 %	116 ± 67 %	153 ± 57 %	
	μ: 11.2 ± 0.9 %									

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	12.6 ± 1.0	10.9 ± 0.7	9.1 ± 0.6	7.5 ± 0.4	2.0 ± 0.2	0.8 ± 0.2	1.4 ± 0.2	114 ± 9	87 ± 5	210.3 ± 27.6
(4, 28)	11.5 – 13.9	10.3 – 11.9	8.4 – 9.9	6.9 – 7.8	1.7 – 2.3	0.7 – 1.0	1.2 – 1.7	103 – 125	83 – 93	148 – 260
	0d: 3.7 ± 0.2	0d: 2.7 ± 0.2	0d: 2.2 ± 0.2	0d: 1.8 ± 0.2	0d: 0.8 ± 0.1	0d: 0.2 ± 0.0	0d: 0.6 ± 0.1	0d: 42.8 ± 8.3	0d: 30.4 ± 7.4	
	0d: 3.5 – 3.9	0d: 2.6 – 2.9	0d: 2.0 – 2.4	0d: 1.6 – 2.0	0d: 0.7 – 0.8	0d: 0.2 – 0.3	0d: 0.4 – 0.6	0d: 32.8 – 52.5	0d: 21.5 – 36.5	
	242 ± 42 %	301 ± 43 %	323 ± 60 %	325 ± 66 %	151 ± 39 %	240 ± 88 %	156 ± 29 %	174 ± 58 %	199 ± 75 %	
	µ: 10.5 ± 1.1 %									
42 days	12.3 ± 1.1	10.5 ± 1.1	8.6 ± 0.6	6.9 ± 0.4	2.0 ± 0.4	0.8 ± 0.2	1.3 ± 0.3	125 ± 8	89 ± 7	226.6 ± 31.6
(6, 36)	10.4 – 13.7	9.2 – 12.2	8.0 – 9.5	6.3 – 7.4	1.4 – 2.5	0.6 – 1.3	0.8 – 1.6	115 – 136	79 – 98	106 – 288
	0d: 3.4 ± 0.4	0d: 2.5 ± 0.3	0d: 1.9 ± 0.2	0d: 1.5 ± 0.2	0d: 0.8 ± 0.1	0d: 0.3 ± 0.0	0d: 0.5 ± 0.0	0d: 48.2 ± 9.7	0d: 35.1 ± 10.6	
	0d: 3.0 – 3.8	0d: 2.2 – 2.9	0d: 1.7 – 2.2	0d: 1.3 – 1.8	0d: 0.7 – 0.9	0d: 0.2 – 0.3	0d: 0.5 – 0.5	0d: 29.5 – 56.2	0d: 19.8 – 48.9	
	270 ± 51 %	327 ± 76 %	361 ± 72 %	363 ± 58 %	158 ± 52 %	237 ± 80 %	158 ± 55 %	170 ± 67 %	182 ± 118 %	
	µ: 9.3 ± 0.9 %									

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 ± standard deviation (SD) and ranges are beneath the means. Numbers of analyzed statocysts per age for LM = 4, for µ-CT = 1

	Number		Length (µm)		Width (µm)		Area (µm^2)		Ratio	
Age (d)	LM	µ-CT	LM	µ-CT	LM	µ-CT	LM	µ-CT	LM	µ-CT
0	28.5 ± 3.1 24 – 30	41	10.5 ± 3.0 3.4 – 18.6	7.9 ± 2.7 2.8 – 12.1	3 ± 1.7 0.3 – 7.4	5.3 ± 2.0 1.4 – 9.0	32.3 ± 21.2 1.9 – 89.1	45.5 ± 27.7 3.8 – 99.0	5.1 ± 4.2 0.6 – 24.1	1.5 ± 0.4 0.4 – 2.4
14	65.8 ± 11.1 50 – 74	68	11.4 ± 2.7 3.9 – 18.6	13.2 ± 3.4 3.8 – 18.0	8.6 ± 3.8 0.7 – 16.0	11 ± 3.7 1.9 – 15.7	101.8 ± 53.4 2.7 – 204.6	156.5 ± 73.4 7.3 – 263.2	1.7 ± 1.2 0.6 – 9.4	1.3 ± 0.3 1.0 – 2.6
42	229.5 ± 11.0 217 – 241	194	12.5 ± 3.8 1.5 – 27.9	15.2 ± 3.3 4.6 – 27.9	7.1 ± 4.3 0.4 – 23.2	11.7 ± 3.2 3.0 – 19.3	91.8 ± 62.3 1.5 – 278.7	183.6 ± 75.0 13.9 – 508.4	2.9 ± 3.0 0.3 – 24.3	1.4 ± 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 ± standard deviation (SD). Con: applied calcein concentration (µMol L^{-1}), n_1: number of measured diameters per statolith, n_2: number of measured side face increments per statolith

Con	No	Length (µm)	Diam (µm) $n_1 = 6$	Inc (µm) $n_2 = 12$
100	1	10.6	16.1 ± 0.7	2.5 ± 0.3
100	2	11.4	16.7 ± 0.4	3.8 ± 0.3
100	3	8.2	14.1 ±0.6	1.7 ± 0.1
100	4	11.5	14.1 ± 0.3	3.1 ± 0.1
100	5	9.7	15 ± 0.5	4.4 ± 0.3
200	6	10.3	15.2 ± 0.4	2.6 ± 0.2
200	7	11.6	15.7 ± 0.1	3.1 ± 0.2
200	8	9	12.8 ± 0.4	3.5 ± 0.2
200	9	10.5	13.7 ± 0.8	2.7 ± 0.3
200	10	12.5	14.7 ± 0.5	3.4 ± 0.2
200	11	6.7	10.4 ± 0.6	2 ± 0.2
200	12	10.1	15.7 ± 1.4	3.1 ± 0.4
200	13	10.3	11.9 ± 0.5	2.1 ± 0.3
200	14	17.9	12.2 ± 0.4	3 ± 0.2
200	15	12	14.1 ± 0.5	2.9 ± 0.6
200	16	11.5	12 ± 1.2	2.1 ± 0.2
200	17	18.1	12.5 ± 0.4	2.5 ± 0.2
200	18	8.4	11.9 ± 0.4	1.8 ± 0.2

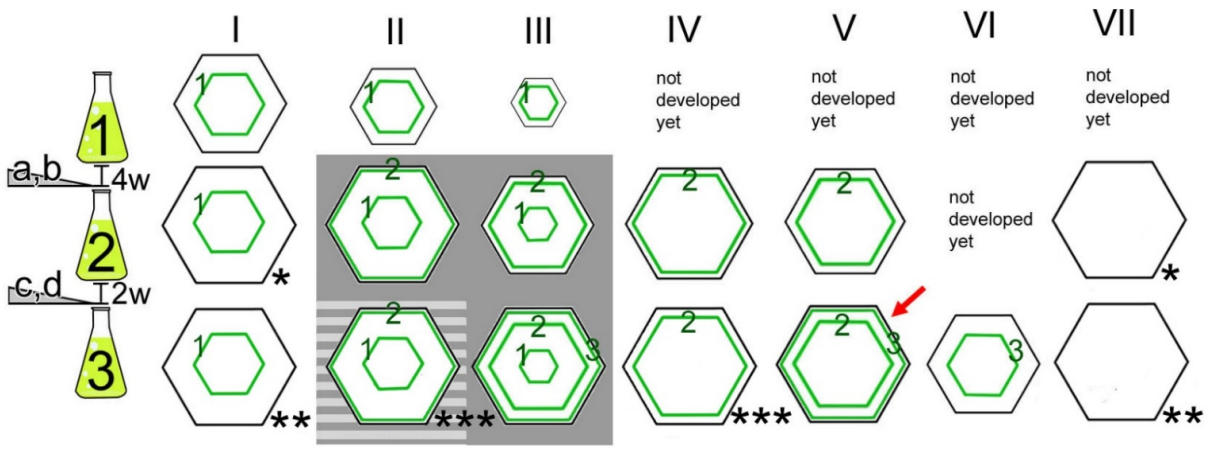

Fig. S1. Theoretical scenarios (I – VII) of statolith labeling after one to three incubations with the fluorescent marker calcein. The bottles (left column) represent the calcein solutions used for the incubations of *Sanderia malayensis* specimens (a – d). Grey background: statoliths not found in samples, 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