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Supplementary Materials for

Cooperation between passive and active silicon transporters clarifies the ecophysiology and evolution of biosilicification in sponges

M. Maldonado*, M. López-Acosta, L. Beazley, E. Kenchington, V. Koutsouveli, A. Riesgo

*Corresponding author. Email: maldonado@ceab.csic.es

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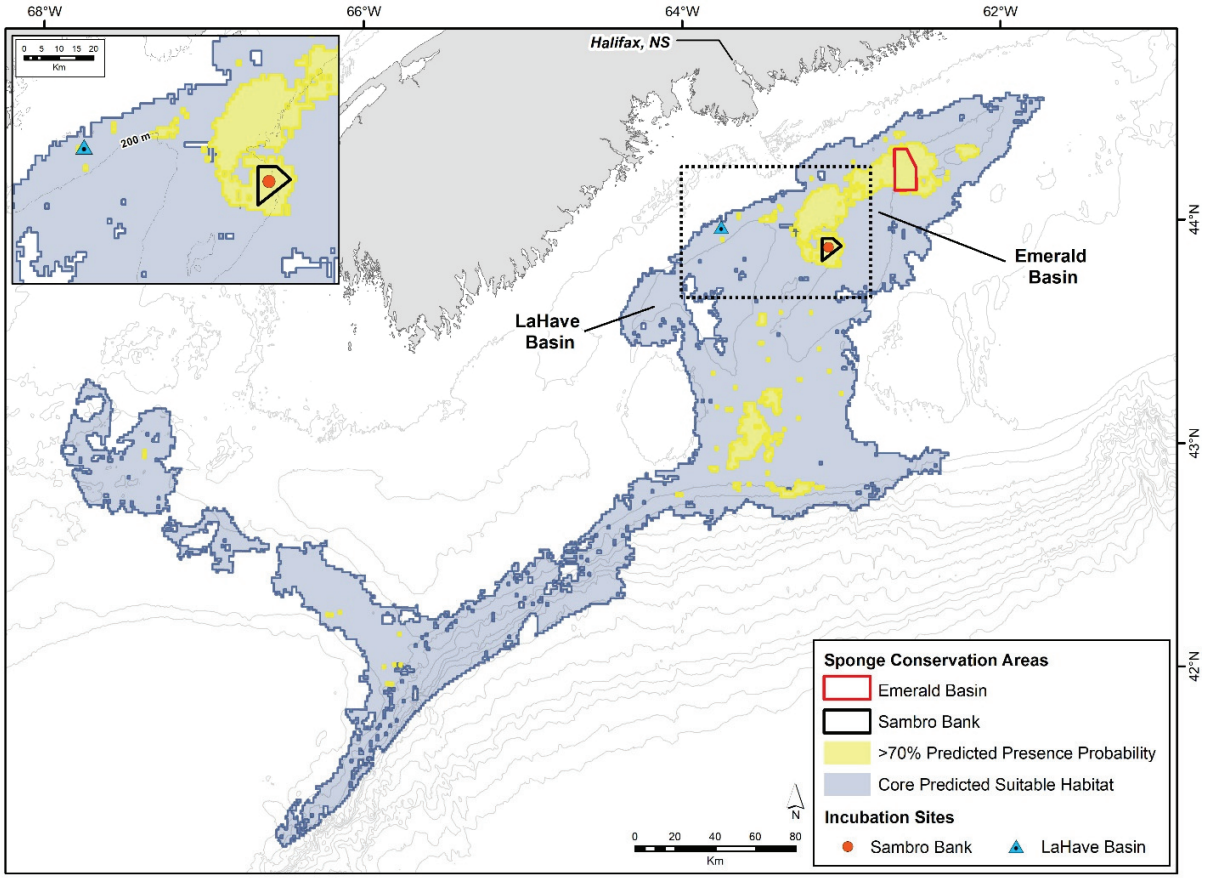

Figure S1. Map of *Vazella pourtalesii* habitat on the Scotian Shelf (Canada). It shows the extent of the suitable habitat for *Vazella pourtalesii* (in blue) and the location of the areas containing the highest sponge density (in yellow), *sensu* Beazley et al. (*27*) and the two sites where field incubations were conducted. The inset shows in greater detail the locations where *in situ* incubations and other field tasks were conducted.

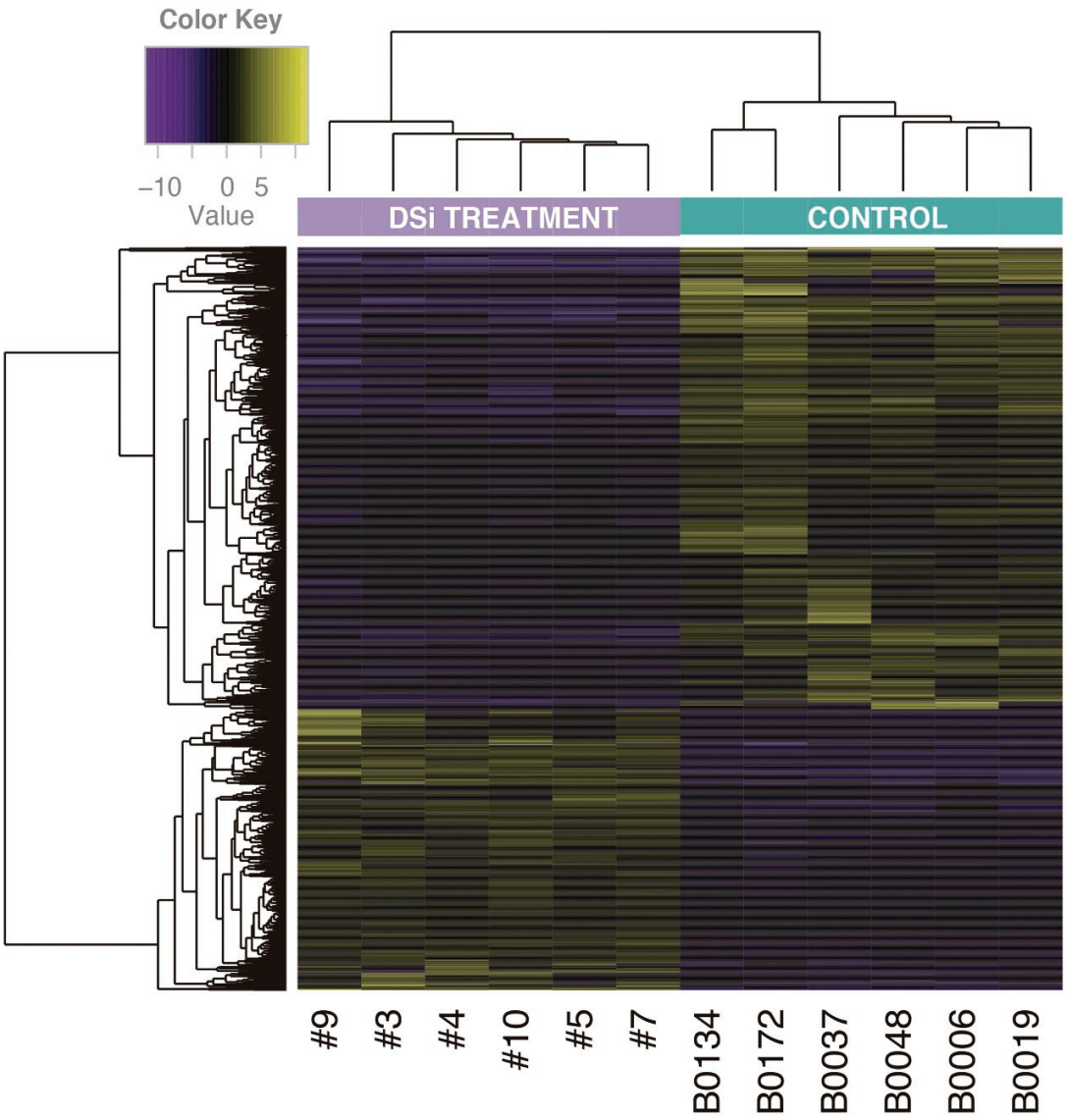

Figure S2. Heatmap of differential gene expression. Differentially expressed genes obtained in DSi-enriched vs. control individuals of *Vazella pourtalesii* are shown. The clustering of samples (upper tree) and genes (left tree) was obtained with UPGMA.

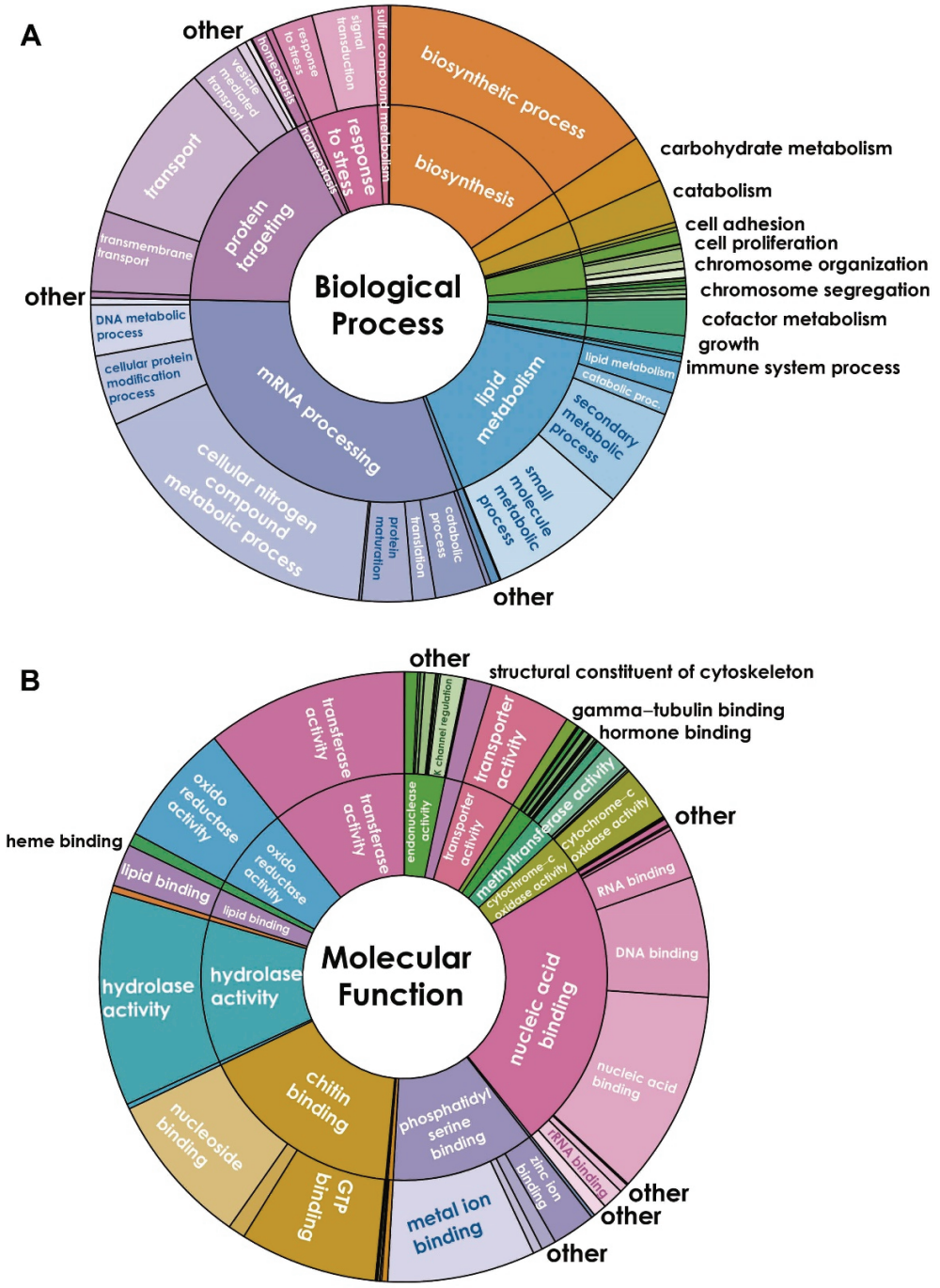

Figure S3. Circos plots of the gene ontology (GO) terms. Sets of classes for the differentially upregulated genes in DSi-enriched individuals are given for the parent categories “Biological Process” and “Molecular Function”. Note importance of increase in lysosome-related cellular component genes given link between proteases/subcellular vesicles and sponge silicification.

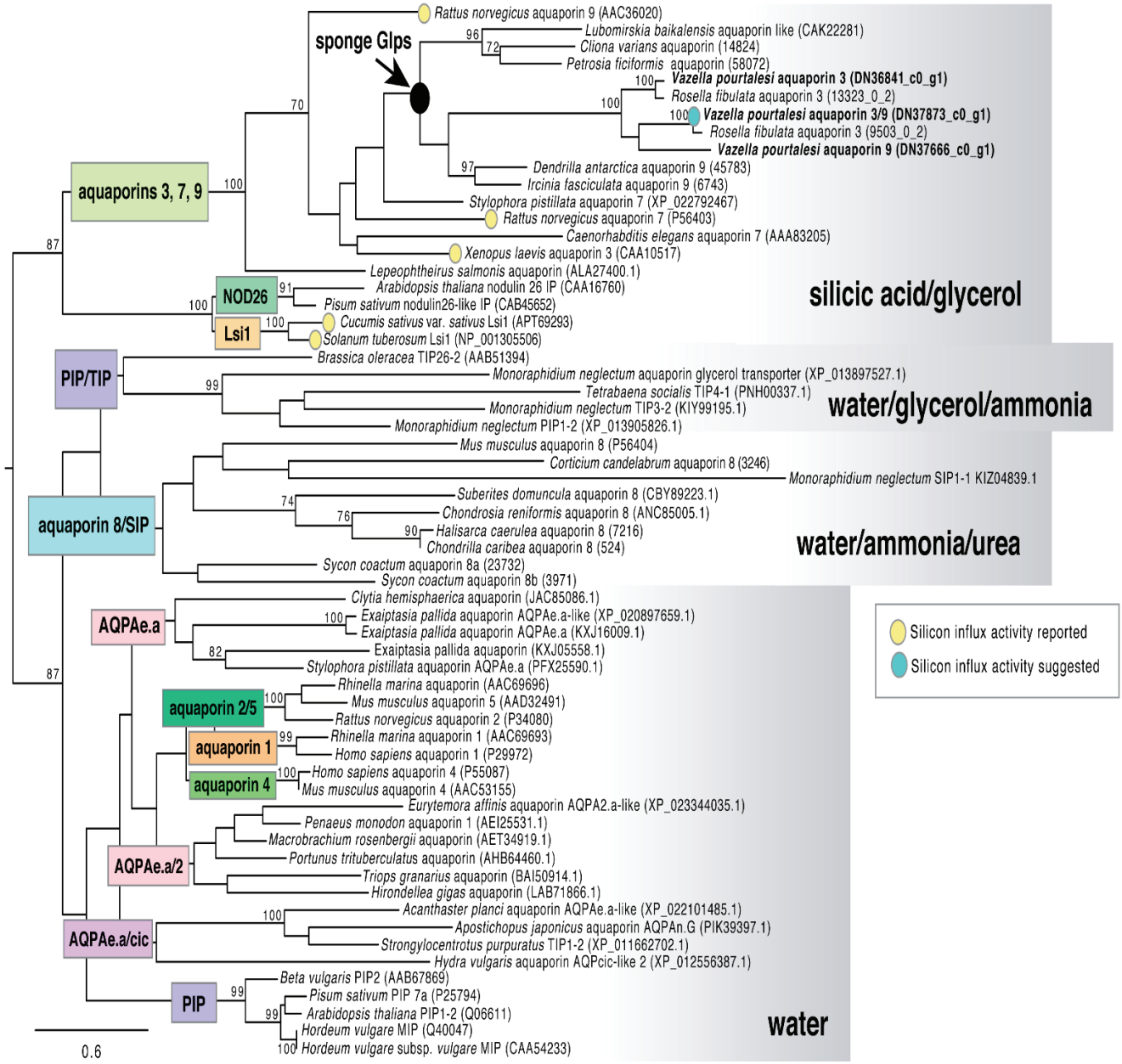

Figure S4. Phylogenetic hypothesis of the aquaporin protein family obtained with Maximum Likelihood in RaxML. Only bootstrap values over 70 are shown on the nodes. Accession numbers and contig names are shown in brackets.

Table S1. Normalized DSi consumption rates. The consumption achieved by each of the 11 assayed individuals of *V. pourtalesii* during the laboratory incubations at the various DSi concentrations (10 to 250 µM DSi) was normalized as µmol Si per mL of sponge body and hour.

	Si uptake (µmol Si mL^{-1} h^{-1})						
sponge	**10 µM**	**30 µM**	**60 µM**	**100 µM**	**150 µM**	**200 µM**	**250 µM**
1	0.009	0.022	0.053	0.070	0.076	0.082	0.076
2	0.001	0.008	0.024	0.033	0.021	0.027	0.031
3	0.012	0.017	0.060	0.051	0.049	0.050	0.036
4	0.010	0.025	0.053	0.067	0.061	0.065	0.060
5	0.020	0.041	0.079	0.108	0.112	0.127	0.119
6	0.011	0.038	0.055	0.112	0.110	0.107	0.113
7	0.019	0.049	0.075	0.062	0.080	0.080	0.092
8	0.016	0.060	0.078	0.088	0.109	0.113	0.112
9	0.028	0.067	0.174	0.187	0.169	0.081	0.077
10	0.010	0.054	0.051	0.195	0.195	0.138	0.138
11	0.016	0.016	0.040	0.092	0.092	0.053	0.059

Table S2. Raw data of the DSi consumption kinetics. Sumary of data showing the experimental DSi concentrations (µM) at the begining (t_0) and the end (tf) of each DSi step in each of the incubation containers for each sponge individual (ind. 1 to 11) and the controls (C1 to C3) during the kinetic experiment. The size (mL) of theassayed sponge is also given.

	Step 1: 10 µM			Step 2: 30 µM			Step 3: 60 µM			Step 4: 100 µM			Step 5: 150 µM			Step 6: 200 µM			Step 7: 250 µM			Sponge
Ind.	[DSi] µM		Time	[DSi] µM		Time	[DSi] µM		Time	[DSi] µM		Time	[DSi] µM		Time	[DSi] µM		Time	[DSi] µM		Time	size
#	t_0	t_f	(h)	t_0	t_f	(h)	t_0	t_f	(h)	t_0	t_f	(h)	t_0	t_f	(h)	t_0	t_f	(h)	t_0	t_f	(h)	(mL)
1	12.6	11.5	23.1	29.9	28.0	23.1	59.0	54.3	23.2	96.3	90.8	24.1	146.2	140.3	23.3	198.6	188.8	23.2	243.0	233.2	23.8	65.2
2	12.0	11.8	23.1	29.4	27.9	23.1	58.8	54.2	23.2	90.7	85.1	24.1	142.9	140.1	23.3	195.6	188.1	23.2	234.5	225.6	23.8	144.3
3	12.6	10.6	23.1	28.9	26.6	23.1	60.3	51.5	23.2	94.2	87.6	24.1	141.9	136.1	23.3	196.0	186.6	23.3	235.5	227.5	23.8	104.8
4	12.3	10.6	23.1	30.4	26.6	23.1	60.0	52.0	23.2	93.1	83.7	24.1	142.1	134.1	23.3	189.3	177.2	23.3	227.8	215.8	23.8	108.4
5	12.6	11.0	23.1	29.9	27.1	23.1	59.4	54.0	23.2	92.5	85.8	24.1	139.6	133.0	23.3	194.8	183.7	23.3	236.0	224.8	23.8	50.8
6	12.6	11.5	23.1	30.8	27.9	23.1	58.0	53.8	23.2	92.8	84.6	24.1	140.7	133.1	23.3	190.9	180.2	23.3	231.9	220.0	23.8	58.0
7	12.5	10.0	23.1	31.6	25.7	23.1	58.6	49.7	23.2	94.4	87.9	24.1	141.3	132.8	23.3	185.0	173.1	23.3	235.1	221.0	23.9	86.8
8	12.5	10.9	23.1	30.4	24.9	23.1	60.4	53.4	23.2	92.6	85.5	24.1	140.1	131.4	23.3	187.7	175.2	23.3	231.3	218.2	23.9	65.2
9	12.6	11.0	23.1	31.0	27.7	23.1	57.6	48.8	23.2	94.4	85.7	24.1	142.0	134.6	23.3	192.2	186.1	23.3	239.7	233.3	23.9	36.5
10	12.3	11.3	23.1	31.2	26.4	23.1	60.5	56.1	23.2	91.0	73.6	24.1	140.3	123.5	23.3	186.9	172.1	23.3	240.1	224.5	23.9	65.2
11	12.6	11.5	23.2	30.9	30.1	23.1	56.7	54.6	23.2	90.8	86.3	24.1	140.3	136.0	23.3	193.3	188.1	23.3	219.2	213.1	23.9	43.6
C1	12.2	12.5	23.1	28.6	30.1	23.1	57.0	57.2	23.2	88.2	92.4	24.1	144.7	143.2	23.3	198.8	194.4	23.2	220.3	219.7	23.8	—
C2	12.2	12.0	23.1	31.3	30.4	23.1	62.0	62.2	23.2	95.6	95.5	24.1	137.8	142.0	23.3	185.5	183.7	23.3	225.7	218.8	23.8	—
C3	12.1	11.7	23.1	31.2	31.0	23.1	57.8	58.5	23.3	89.1	89.5	24.1	141.8	143.6	23.3	187.1	187.7	23.3	216.8	217.3	23.9	—

Table S3. Raw data of the *in situ* incubations. DSi concentrations (µM) in the incubation chambers containing either a sponge on its rock (ind. 1 to 4) or only a rock (control chamber= C) at the beginning and the end of the incubation period (Time in hours). The size of the sponge is given in mL.

Ind.	[DSi] µM		Time	Sponge
#	t_0	t_f	(h)	size (mL)
1	15.3	12.4	19.2	492.4
2	14.7	13.8	18.9	64.5
3	15.0	9.2	21.3	126.4
4	16.1	4.0	28.7	323.4
C	16.1	17.4	22.0	—

Table S4. Natural DSi concentrations available to the sponge aggregations. Summary of average (AVRG ± SD), minimum (MIN) and maximum (MAX) DSi concentration values (µM), and number of observations (*N*) recorded in bottom water (100-275 m) on the Central Scotian Shelf (43-45 °N - 62-64 °W) during a long term monitoring from the early 1970s to the mid-1990s. Data are extracted from Table 5b in Petrie et al. (*29*).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
AVRG	15.69	14.61	14.26	15.17	13.44	16.58	16.67	23.30	15.63	16.25	17.41	11.92
SD	3.61	3.67	2.20	3.68	3.29	5.21	6.66	8.30	2.16	4.01	6.98	2.93
MIN	11.52	9.00	11.39	7.84	7.37	5.89	9.00	12.03	11.10	10.40	8.55	6.02
MAX	21.33	24.47	18.97	23.75	19.99	26.10	35.71	44.31	19.38	25.01	35.48	13.80
N	15	17	13	45	27	24	30	19	21	35	27	6

Table S5. **Transcriptome basics.** Collection details for the individuals (Label) used in the DSi experiment and assembly metrics for the de novo assembled reference transcriptome of *Vazella pourtalesii*.

Label	Dsi treatment	N paired reads	N surviving pairs	Features of the de novo assembly	
B0019	Control	10,908,636	10,212,704	N genes	133,604
B0048	Control	14,794,817	13,854,082	N transcripts	172,460
B0037	Control	19,337,215	18,066,736	% GC	40.57
B0172	Control	21,401,051	19,994,268	Median contig length	328
B0134	Control	22,624,379	21,155,221	N50	1,282
B0006	Control	22,287,708	20,871,341	Mb assembled	119.58
9	Dsi enriched	14,137,746	10,699,324	Av. Contig length	693.38
10	Dsi enriched	11,386,305	9,536,103	N Blast hits refseq	60,077
3	Dsi enriched	10,549,969	8,584,178	N Blast hits swissprot	45,338
5	Dsi enriched	13,018,935	9,682,295	N transcripts with GO term	30,208
4	Dsi enriched	32,486,567	23,452,447	N BUSCO (metazoans)	854 (87.3%)
7	Dsi enriched	9,382,027	7,172,217	N BUSCO (eukaryotes)	288 (95.1%)

Caption to Data File S1. Raw transcriptome data. Excel file containing the list of differentially expressed genes in DSi-enriched individuals (up-and downregulated), logFC (log2 fold change), logCPM (log2 counts per million, normalized for library sizes), p-value and corrected FDR p-value, their expression values and annotations.

Caption to Movie S1. Sponge collecting. Accelerated video showing the collection of an individual of *Vazella pourtalesii* from the aggregation by using the manipulating arm of the ROV ROPOS. Note how the sponge was always handled by holding the rock that served as substrate rather than by touching the sponge itself. This procedure ensured that every sponge collected for incubation, either *in situ* or laboratory incubation, was not damaged.

Caption to Movie S2. *In situ* sponge incubation. Accelerated video showing the placing of a sponge on the floor piece of a chamber, the subsequent sealing of the incubation unit, its deployment on the bottom of the sponge ground, and the triggering of the collecting bottle that stores the initial seawater sample of the incubation. Note how the sponge was always handled by holding the rock that served as substrate rather than by touching the sponge itself. This procedure ensured that every sponge collected for incubation, either *in situ* or laboratory incubation, was not damaged.