

Supplementary material: Aragonés & Leys

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**Supplementary Table 1.** Percent error and mean percent error of measurements of Osculum Area (OSA, cm<sup>2</sup>) in a sample of 20 measurements using area function in ImageJ, and calculated from diameter dimensions.

<b>n</b>	<b>species</b>	<b>OSA calculated from diameter</b>	<b>OSA measured (Image J)</b>	<b>Percent error %</b>
1	<i>Laocetis emiliana</i>	1.55	1.54	0.9
2	<i>Laocetis emiliana</i>	1.23	1.15	6.3
3	<i>Laocetis emiliana</i>	0.95	0.93	2.1
4	<i>Laocetis emiliana</i>	0.67	0.65	2.7
5	<i>Laocetis emiliana</i>	1.09	1.02	6.7
6	<i>Laocetis emiliana</i>	0.52	0.53	1.7
7	<i>Laocetis emiliana</i>	1.21	1.15	5.0
10	<i>Haliclona permollis</i>	0.203	0.19	4.4
11	<i>Haliclona permollis</i>	0.135	0.098	27.4
12	<i>Haliclona permollis</i>	0.211	0.212	0.5
13	<i>Haliclona permollis</i>	0.053	0.05	11.3
14	<i>Haliclona permollis</i>	0.032	0.03	12.5
15	<i>Haliclona permollis</i>	0.028	0.03	2.5
16	<i>Haliclona permollis</i>	0.03	0.04	30.0
17	<i>Haliclona permollis</i>	0.014	0.01	19.9
18	<i>Haliclona permollis</i>	0.099	0.09	13.9
19	<i>Haliclona permollis</i>	0.033	0.03	14.0
20	<i>Haliclona permollis</i>	0.152	0.178	17.1
Mean Percent Error				0.7%

**Supplementary Table 2.** Measurements of 17 *Haliclona cf. permollis* patches in Bamfield, B.C., Canada showing the change in OSA/SA ratio with size and the distribution of oscula sizes.

<b>Sponge</b>	<b>length (cm)</b>	<b>Surface Area (SA, cm<sup>2</sup>)</b>	<b>Average Osculum area (xOSA, cm<sup>2</sup>)</b>	<b>Total Osculum area (tOSA, cm<sup>2</sup>)</b>	<b>xOSA:SA</b>	<b>tOSA:SA</b>
1	2.26	5.1	0.008	0.20	0.002	0.04
2	2.12	4.5	0.006	0.14	0.001	0.03
3	3.11	9.7	0.004	0.21	0.000	0.02
4	1.30	1.7	0.004	0.05	0.003	0.03
5	1.15	1.3	0.005	0.03	0.004	0.02
6	1.27	1.6	0.002	0.03	0.001	0.02
7	1.40	2.0	0.003	0.03	0.001	0.02
8	0.83	0.7	0.002	0.01	0.003	0.02
9	1.88	3.5	0.003	0.10	0.001	0.03
10	1.49	2.2	0.002	0.03	0.001	0.01
11	2.13	4.5	0.003	0.15	0.001	0.03
12	2.33	5.4	0.005	0.14	0.001	0.03
13	1.38	1.9	0.001	0.04	0.001	0.02
14	2.05	4.2	0.008	0.09	0.002	0.02
15	2.36	5.6	0.002	0.12	0.0004	0.02
16	1.59	2.5	0.003	0.07	0.001	0.03
17	2.63	6.9	0.011	0.19	0.002	0.03
Average	1.8	3.7	0.004	0.10	0.001	0.025
SE	0.15	0.57	6.48E-04	0.02	2.25E-04	1.64E-03
SD	0.6	2.4	0.003	0.07	0.001	0.007
Max	3.1	9.7	0.011	0.21	0.004	0.040
Min	0.8	0.7	0.001	0.01	0.0004	0.015
Median	1.9	3.5	0.003	0.09	0.001	0.024

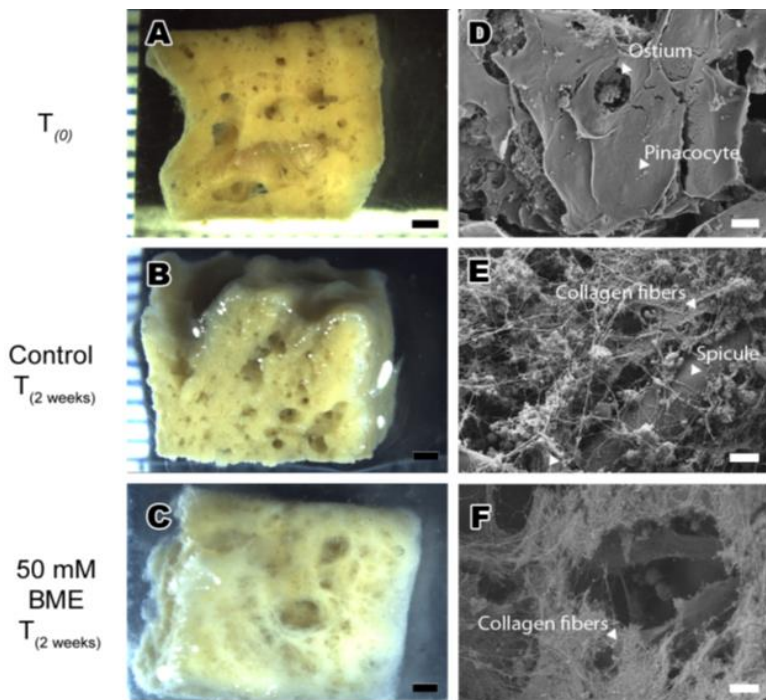
**Supplementary Table 3.** Correlation of length to average osculum area (xOSA) and osculum to surface area (OSASA) in *Haliclona cf permollis*.

<b>Grouping category</b>	<b>equation</b>	<b>R<sup>2</sup></b>	<b>p</b>	<b>slope</b>	<b>intercept</b>	<b>Slope 95% bootstrapped CI N=1999</b>	<b>Intercept 95% bootstrapped CI N=1999</b>
length to xOSA	Y=-0.004+0.004X	0.25	0.04	0.004	-0.0039	0.002, 0.007	-0.007, 0.0001
length to OSASA	Y=0.003+0.012X	0.11	0.19	0.012	0.003	0.004, 0.03	-0.04, 0.016

### Supplementary Figure 1. Experimental decay experiment in *Neopetrosia problematica*.

To determine whether reducing conditions alone can significantly slow down decay to potentially allow for tissue-detailed preservation in a fossil sponge, tissue was allowed to decay for 15 days under 'standard' reducing conditions. Living pieces of sponge were placed in seawater with and without  $\beta$ -ME in a closed vial at room temperature. Following the methods of Raff et al. (2006),  $\beta$ -ME was used *in lieu* of  $H_2S$  that would normally be present in natural settings. The treatments were 5 mm<sup>2</sup> pieces of sponge in 20 mL vials at three concentrations of  $\beta$ -ME (50, 100 and 150 mM) and one vial with only filtered sea water.

After two weeks samples were fixed for SEM following previously described protocols (Leys et al. 2018). After fixation, sponge tissues were rinsed gently, twice, for 10 minutes each in distilled water, and dehydrated to 70% ethanol and transported to the University of Alberta. Fragments generated in this way were transferred in 100% ethanol to a Bal-Tec 030 critical point drier. After drying, samples were mounted on aluminum stubs using nail polish, coated with gold, and viewed in a Zeiss Sigma 300 Field Emission scanning electron microscope.



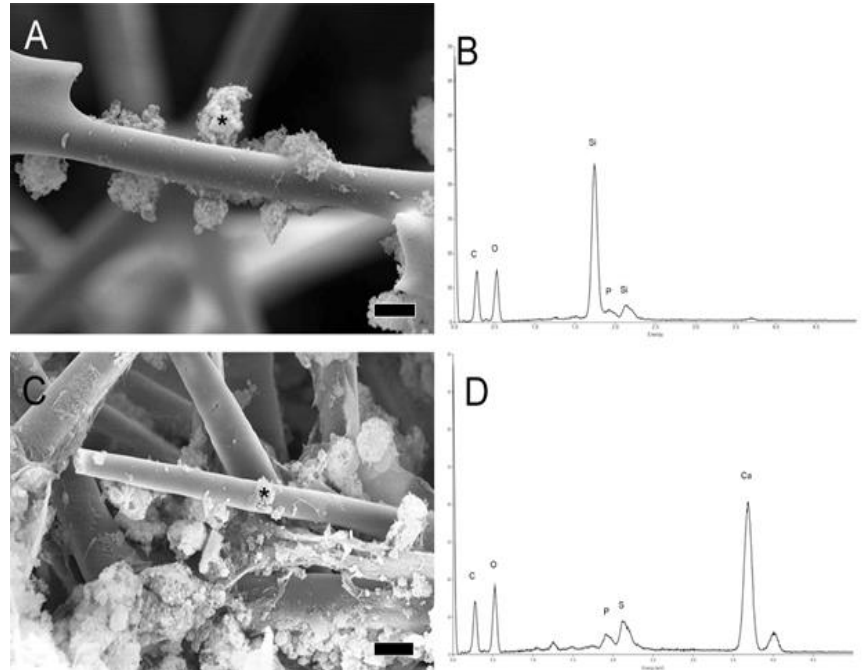
Comparison of fixed tissue at  $T_0$  to decaying tissue after two weeks ( $T_{2weeks}$ ) under control conditions (i.e. filtered seawater and no treatment) and 50 mM  $\beta$ -ME.

(A-C) Light micrographs of tissue. Internal structure is lost as internal canals lose definite boundaries, but the skeleton maintains its gross shape: scale bar 1 cm. (D-F) Scanning electron micrographs comparing regular tissue to decayed tissue. (D) Fixed regular tissue shows poorly preserved pinacocytes and ostia; scale bar 10  $\mu$ m. (E). Decayed tissue after two weeks in plain seawater. Collagen fibers and spicules are present: scale bar 5  $\mu$ m. (F) After two weeks in 50 mM  $\beta$ -ME treatment, collagen fibers

networks outlining the decayed tissue are better preserved; scale bar 5  $\mu$ m.

**Supplementary Figure 2.** To examine the potential for mineralization of sponge tissues under conditions of sea water saturated with silicate, sulfate and phosphate, living sponge tissues were treated with  $\beta$ -ME together with a) sodium metasilicate ( $\text{Na}_2\text{SiO}_3$ ), b) sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) and c) sodium phosphate ( $\text{Na}_3\text{PO}_4$ ) (Sigma-Aldrich, Ont. Canada), left undisturbed for two weeks in sealed vials. Controls consisted of sponge tissue left in 50 mM  $\beta$ -ME with no added salt i.e. the only different between experimental and control groups is the mineral salt added.

Crystal formation and attachment to *Neopetrosia problematica* spicules after 15 days of decay. A) 100 mM  $\beta$ -ME and a silicate solution treatment with attached crystals, and Energy Dispersive X-ray Spectroscopy (EDX) spectrum was obtained (\*) B) EDX shows a silicate predominance, and carbon and oxygen. C) Control of decayed tissue in 100 mM  $\beta$ -ME. Crystals were recovered and D) EDX shows a predominance of calcium. Scale bars: 5  $\mu\text{m}$ .

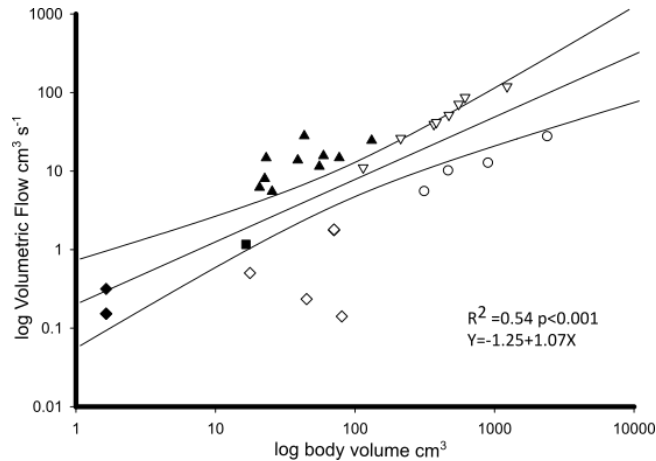


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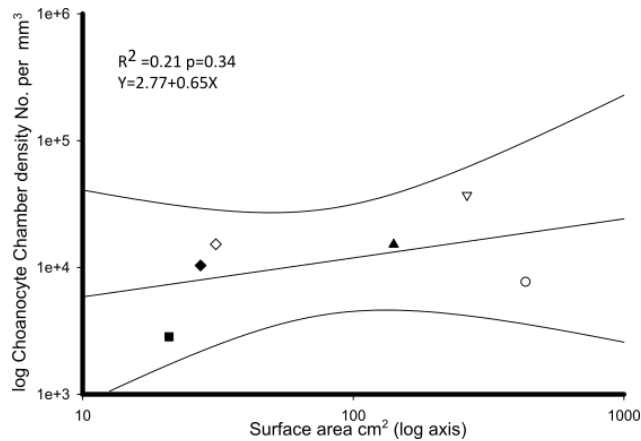
## References

- Leys SP, Kahn AS, Fang JKH, Kutti T, Bannister RJ (2018) Phagocytosis of microbial symbionts balances the carbon and nitrogen budget for the deep-water boreal sponge *Geodia barretti*. 63:187-202
- Raff EC, Villinski JT, Turner FR, Donoghue PCJ, Raff RA (2006) Experimental taphonomy shows the feasibility of fossil embryos. 103:5846-5851 doi:10.1073/pnas.0601536103

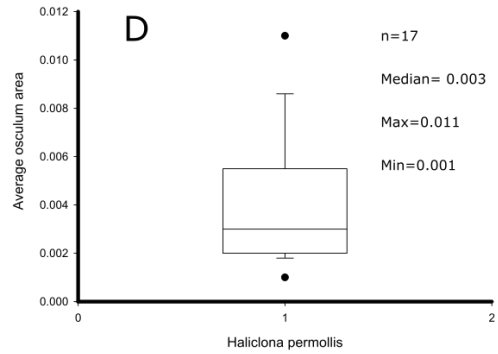
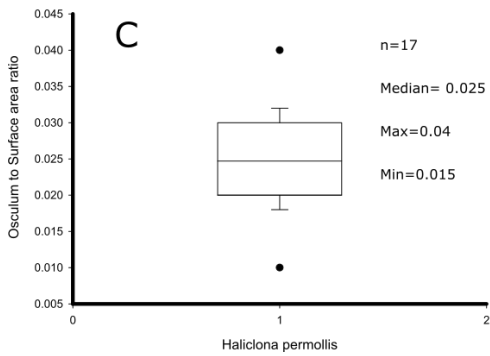
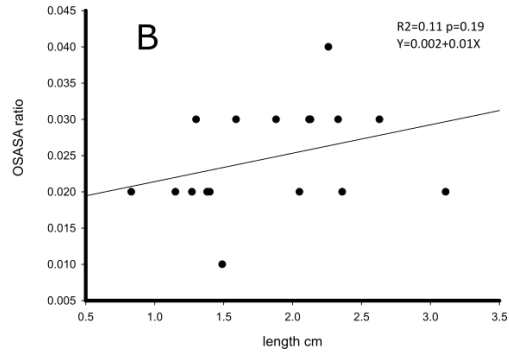
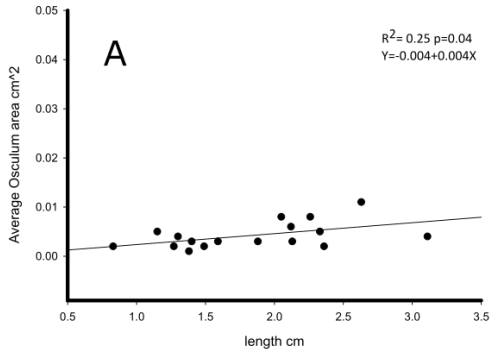
**Supplementary Figure 3.** Scatterplot showing volumetric flow rate  $Q$  and sponge volume for extant species of demosponges: *Cliona delitrix* (inverted open triangle), *Geodia barretti* (open circle), *Callyspongia vaginalis* (filled triangle), *Haliclona mollis* (filled square), *Neopetrosia problematica* (filled diamond), *Tethya californiana* (open diamond) ( $R^2=0.54$   $p<0.001$ ).



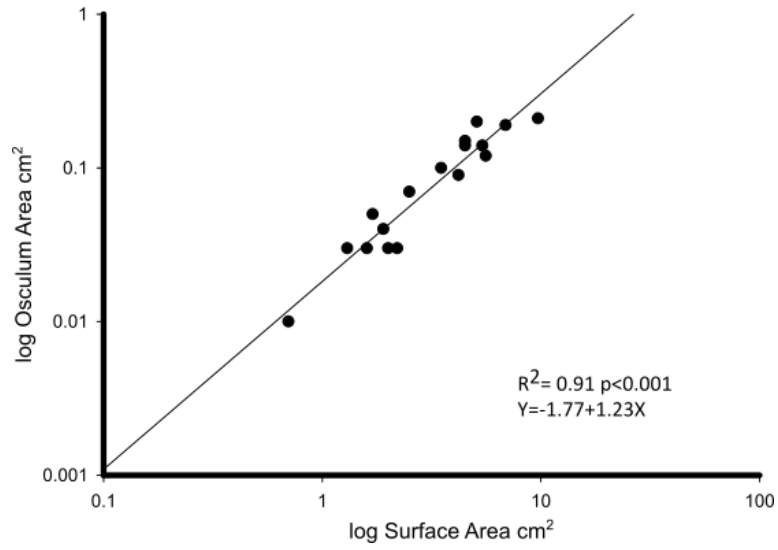
**Supplementary Figure 4.** Correlation of surface area to choanocyte chamber density demosponges *Cliona delitrix* (inverted open triangle), *Geodia barretti* (open circle), *Callyspongia vaginalis* (filled triangle), *Haliclona mollis* (filled square), *Neopetrosia problematica* (filled diamond), *Tethya californiana* (open diamond). We found that chamber density did not vary significantly with surface area ( $R^2=0.21$   $p=0.34$ ).



**Supplementary Figure 5.** Change in the oscula dimensions with respect to length in *Haliclona permollis*. A) Average osculum area does not increase with size, ( $R^2=0.25$   $p=0.04$ ) B) The osculum to surface area ratio does not increase with size in this species ( $R^2=0.11$   $p=0.19$ ) C) Median osculum to surface area ratio is  $0.025\pm 0.007$ SD D) The average osculum area is  $0.003\pm 0.003$ SD  $\text{cm}^2$ .



**Supplementary Figure 6.** Scatterplot and correlation of surface area (SA) to osculum area (OSA) in log scale of *Haliclona permollis* ( $R^2=0.91$   $p<0.001$ ).





**Supplementary Figure 7.** Scatterplot and correlation of surface area (SA) to osculum area (OSA) in log scale of fossil (black,  $Y=0.6X-1.3$ ) and modern (red,  $Y=1.8X-2.1$ ) *Calcarea* ( $\chi^2=0.35$ ;  $p=0.5$ ).

