Supplementary Material

Using the *F/R*-ratio for an evaluation of the demosponge *Halichondria panicea* to nourish solely on phytoplankton versus free-living bacteria in the sea

HANS ULRIK RIISGÅRD¹, LARS KUMALA^{1,2,3} & KATERINA CHARITONIDOU¹

¹Marine Biological Research Centre, University of Southern Denmark, Kerteminde, Denmark

²Max-Planck Odense Center on the Biodemography of Aging & Department of Biology, University of Southern Denmark, Odense M, Denmark

³Max Planck Institute for Demographic Research, Rostock, Germany

Variations in the filtration rate of *Halichondria panicea* during a 49 day experiment (Series #1, Fig. 3) were systematically investigated in a follow-up study by performing repeated measurements of the filtration rate combined with simultaneous video-microscope observation of an osculum in various sponge explants (Series #2, Figs. 4 & 5 and Figs. S1 to S3 & S6, Tables 1 & S1). To evaluate osculum dynamics within an individual sponge colony possessing several oscula, area variations in two adjacent oscula were compared over an extended period of time (Figs. S4 & S6).

Table S1. *Halichondria panicea*. Clearance experiments with various sponge colonies (Series #2). Experimental series (Exp.), sponge identification number (*ID*), number of oscula (*N*), volume (*V_{sp}*), dry weight (*DW*, 24h 100 °C) and ash free dry weight (*AFDW*, 6h 500 °C) of sponge, time, volume of experimental chamber (*V*), initial (*C_o*), final (*C_t*) and mean algal concentration [*C_m* = (*C_o* × *C_t*)^{0.5}] during the experiment, slope of regression line (*b*) for ln(*C*) versus time, and estimated filtration rate (*F* = *b* × *V*, cf. Eq.2). The temperature during the clearance experiments was 8 to 15 °C. n.d. = not determined.

Exp.	<i>ID</i>	<i>N</i>	<i>V_{sp}</i> (ml)	<i>DW</i> (g)	<i>AFDW</i> (g)	Time (min)	<i>V</i> (ml)	<i>C_o</i> (cells ml ⁻¹)	<i>C_t</i> (cells ml ⁻¹)	<i>C_m</i> (cells ml ⁻¹)	<i>b</i> (min ⁻¹)	<i>F</i> (ml min ⁻¹)
1	3	9	9	0.66	0.46	0-40	550	3388	2137	2691	-0.0112	6.2
						50-130	550	3001	2250	2598	-0.0041	2.3
						150-230	550	3794	2508	3084	-0.0052	2.9
						250-330	550	3858	2154	2883	-0.0070	3.9
2	3	9	9	0.66	0.46	53-133	550	3969	1468	2414	-0.0120	6.6
						161-241	550	3524	1725	2466	-0.0087	4.8
						261-341	550	3322	1466	2207	-0.0103	5.7
3	5	1	1	0.07	n.d.	97-137	480	5621	4925	5262	-0.0030	1.4
						157-237	480	4764	4159	4451	-0.0016	0.8
						257-337	480	4228	3440	3814	-0.0016	0.8
						1251-1379	440	3521	1735	2472	-0.0076	3.3
4	20	4	12.5	0.50	0.50	1399-1479	440	3713	2651	3137	-0.0042	1.8
						1799-1879	440	3329	2249	2736	-0.0050	2.2
						1899-1939	440	3987	3061	3493	-0.0066	2.9
						26-86	560	3104	2814	2955	-0.0024	1.3
20	4	12.5	0.50	0.50	0.50	176-236	570	3990	3154	3547	-0.0037	2.1
						251-311	570	3414	2737	3057	-0.0039	2.2
						492-552	570	4098	3261	3656	-0.0033	1.9
						1561-1621	570	3402	2848	3113	-0.0028	1.6
1893-1953	570	3402	2848	3113	-0.0028	1757-1817	570	3792	3177	3471	-0.0030	1.7
						1893-1953	570	3630	2118	2773	-0.0078	4.4

Figure S1. *Halichondria panicea*. Repeated measurement of filtration rate of various sponge colonies (Table S1) with simultaneous video observation of an osculum (Fig. S2 and Fig. S3). Black arrows indicate addition of *Rhodomonas salina* cells and the beginning of a new clearance experiment. Linear regression lines and equations are shown.

Figure S2. *Halichondria panicea*. (Upper panel) Video-observation of osculum cross sectional area (*OSA*), and (lower panel) simultaneous measurement of filtration rate (*F*) of sponge colony in observation tank (Exp. 2 & 3, Table S1). To measure the filtration rate using the clearance method, the through flow of water was stopped (arrow) so that the exponential reduction of algal cells could be followed (Fig. S1), and afterwards the water flow to the observation tank was re-established (punctured arrow). The mean filtration rate for all the measurements in Exp. 2 & 3 (Table S1) was $F = 5.7 \text{ ml} \pm 0.9 \text{ ml min}^{-1}$ (coefficient of variation = $cv = 0.16$) and $F = 1.9 \text{ ml} \pm 1 \text{ ml min}^{-1}$ ($cv = 0.52$), respectively.

Fig. S3. *Halichondria panicea*. (Upper panel) Video-microscope observation of osculum cross sectional area (OSA), and (lower panel) simultaneous measurement of filtration rate (F) of sponge colony in observation tank (Exp. 4, Table S1). To measure the filtration rate using the clearance method, the through flow of water was stopped (arrow) so that the exponential reduction of algal cells could be followed (Fig. S1), and afterwards the water flow to the observation tank was re-established (punctured arrow). The mean filtration rate for all the measurements in Exp. 4 (Table S1) was $F = 2.2 \text{ ml} \pm 1 \text{ ml min}^{-1}$ ($cv = 0.47$).

Fig. S4. *Halichondria panicea*. Video-microscope observation of osculum area variations in two oscules (upper & lower panel) within an individual sponge colony possessing several exhalant apertures ($ID = 3$, Table S1). For each osculum the state of expansion (shown as dots) is shown relative to 1 (opened), where 0 describes the fully closed state of the osculum. The maximum osculum cross sectional area observed during the experimental period was 2.7 mm^2 (upper panel) and 1.6 mm^2 (lower panel), respectively.

Fig. S5. *Halichondria panicea*. Video-microscope observation of osculum area variations in two oscula (upper & lower panel) within an individual sponge colony possessing several exhalant apertures ($ID = 20$, Table S1). For each osculum the state of expansion (shown as dots) is shown relative to 1 (opened), where 0 describes the fully closed state of the osculum. The maximum osculum cross sectional area observed during the experimental period was 1.5 mm^2 (upper panel) and 0.3 mm^2 (lower panel), respectively.

Fig. S6. *Halichondria panicea*. (A) Video time-lapse recording through stereo-microscope for observation of changes in osculum cross-sectional area during filtration rate measurement. (B) Open osculum. (C) Closed osculum (scale bar: 5 mm).