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Exhalant jet speed of single-osculum explants of the demosponge *Halichondria panicea* and basic properties of the sponge-pump

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Abstract: Sponges are modular organisms in which each aquiferous module draws water through a canal system by means of pumping units (choanocyte chambers, CC), and the filtered water leaves the module as an exhalant jet through a single opening (osculum). A constant density of CCs in sponges would imply that the filtration rate must be proportional to the sponge volume, but it is less obvious how the osculum cross-sectional area (*OSA*) scales to sponge volume. Here, we present data obtained on single-osculum sponge explants (i.e. single aquiferous modules) of the demosponge *Halichondria panicea* to get a better understanding of the basic properties of the sponge-pump. In the experimental study of 27 explants (volume $V_s = 14$ to 1977 mm^3) osculum cross sectional area (*OSA*), exhalant jet speed (U_0) and filtration rate ($F = OSA \times U_0$) were measured. The observed scaling with size ($OSA \sim V_s^{0.66}$; $U_0 \sim OSA^{0.45}$; $F \sim OSA^{1.45}$) was found to be close to that inferred from the hypothesis of volume based CC density. Thus, the volume-specific filtration rate (= pumping rate) could be approximated as F (ml min^{-1}) $\approx 2.3V_s$ (cm^3) which is of the same order of magnitude as that of the demosponge *Haliclona urceolus*, F (ml min^{-1}) $\approx 3.5V_s$ (cm^3). This suggests that for the two sponge species CCs are very likely of similar size, with similar individual pumping rate, and of similar uniform distribution over the sponge volume. By comparing the observed increase of U_0 with increasing *OSA* to literature data on other leuconoid sponge species this revealed a power function with an identical exponent 0.45 and maximum values of $U_0 = 6$ to 8 cm s^{-1} . This indicates that U_0 of a single-osculum explant, or U_0 of an individual osculum in a multi-oscule sponge approaches an upper limit as the sponge grows, implying that a module of a multi-oscule sponge may increase only to a certain size. Time-lapse video-microscope recordings of sponge explants showed temporal variation in *OSA* during spontaneous contractions. Exposure to a neurotransmitter (GABA) as well as overloading with ink particles triggered contractions that correlated with both decreasing *OSA* and U_0 that eventually became zero. Video-microscope recordings revealed that it was contraction of the endopinacoderm lining the excurrent canals that effectively restricted or stopped the water flow.

Key words: filtration rate, osculum area, scaling, contraction, aquiferous module, video-microscope recording

1.0 Introduction

Filter-feeding benthic invertebrates need to process large amounts of water, and to prevent once filtered water from reentering the animals they discharge this water as an exhalant jet through a constricted exhalant opening (Vogel, 1994). Another function of exhalant jets may be to mix the near-bottom water because such "biomixing" provides an increased downward flux of suspended food particles (Riisgård and Larsen, 2017). Both functions depend on jet speed and distance of penetration of the jet into the surrounding water. Knowledge about jet speeds and allometry may further deepen our understanding of the principles of biological filter-pumps. For example, by scaling it can be suggested that the velocity of the exhalant jet in mussels, *Mytilus edulis*, is constant, independent of mussel size, as also found experimentally, $U_0 \approx 8 \text{ cm s}^{-1}$ for mussels of shell length $L = 16$ to 83 mm (Riisgård et al., 2011). The reasoning is this. For known filtration rate (F ; also termed volumetric flow or pumping rate) and cross-sectional area (A) of the exhalant opening (assumed to scale as $A \sim L^2$) the mean jet velocity scales as $U_0 = F/A \sim F/L^2$, but according to the allometry of the mussel $F \sim L^2$ (Riisgård et al., 2014), it follows that $U_0 \sim \text{constant}$. Although Kumala et al. (2017) recently found that $U_0 = F/OSA = \text{constant}$ (2.3 cm s^{-1}) for a limited size range of small single-osculum sponge explants, the above scaling considerations for mussels may not apply to other filter-feeding invertebrates, such as sponges.

Sponges are modular filter-feeders that consist of one or several units, known as "aquiferous modules" (Ereskovskii, 2003; Fry, 1970, 1979). An aquiferous module is a functional unit that draws ambient water through numerous inhalant openings (ostia) into an inhalant canal system by means of pumping units (choanocyte chambers, CC) that filter the water for nutrition and then rejects it via an exhalant canal system through a single exhalant opening (osculum) to the surrounding water. Reiswig (1975) assumed a more or less constant density (within a factor of about 2) of CCs in sponges (Reiswig, 1975, Table 1 therein), all delivering the same volumetric flow (F). This assumption has approximately been confirmed by measurements (Riisgård et al., 1993, their Tables 1 & 2 for *Halichondria panicea* and *Haliclona urceolus*) suggesting $F \sim L^3$ (mean of 2.7 ± 1.1 and $2.5 \pm 1.7 \text{ ml min}^{-1} (\text{ml sponge volume})^{-1}$, respectively). It is not known, however, how the osculum cross-sectional area (OSA) scales with size. In addition, contraction behaviour exhibited by sponges may obfuscate the assessment of these allometric relationships.

Although sponges lack nervous and muscle tissues, coordinated behavioral responses to mechanical and chemical stimuli are common. The epithelium-like cell layer (pinacoderm) and myocytes (Bagby, 1966; Bergquist, 1978; Elliott and Leys, 2007; Nickel, 2004; Nickel et al., 2011) are involved in contraction, diffusible chemical messengers may be involved in coordinating the contractile behavior of sponges (Elliott and Leys, 2007, 2010; Ellwanger et al., 2007), and non-motile cilia of endopinacocytes might work as water flow sensors (Hammel et al., 2012; Ludeman et al., 2014). While contraction in sponges seems to be triggered by external stimuli such as changes in water flow, particle overloading or seasonal temperature changes

(Leys, 2015; Patterson et al., 1997; Reiswig, 1971; Vethaak et al., 1982), spontaneous or cyclic contractions of oscula and pinacoderm have been shown to coincide with a decrease in filtration activity of the marine demosponge *Halichondria panicea* (Kumala et al., 2017; Riisgård et al., 2016). Under natural conditions, *H. panicea* is exposed to variable intensities of currents and particle loads (including re-suspended sediment; Barthel and Wolfrath, 1989).

In the present investigation, we studied single-osculum explants of *Halichondria panicea* experimentally. For fully expanded and actively filtering explants we determine how flow rate, exhalant jet speed and osculum cross sectional area depend on sponge size in order to explore the scaling laws of these parameters. In addition, we assessed the penetration length of the jets into the surrounding water. Our hypothesis is that both OSA and F increase with sponge size, but it is not clear if U_0 remains constant as observed in mussels. We further hypothesize that contractions, either spontaneous, or triggered by exposure to a neurotransmitter (GABA) or by overloading with inorganic (ink) particles, lead to decreases in OSA , U_0 and F to eventually become zero as a result of hydrodynamic responses of the aquiferous (canal) system. Our results provide novel insights into basic sponge pump characteristics, emphasizing the importance of contractile behavior for controlling volume flow through a sponge module.

2.0 Materials & Methods

2.1 Cultivation of sponge explants

Sponge explants were obtained from colonies of the demosponge *Halichondria panicea* in the tidal inlet of Kerteminde Fjord, Denmark. The chimneys of collected sponges were either cut in small (6 to 9 mm³) pieces without an osculum (cf. Kumala et al., 2017) or in fragments with a single osculum and were individually placed on substrate plates in flow-through aquaria with aerated bio-filtered seawater at a constant water temperature of $\approx 15^\circ\text{C}$. After attachment to substrate plates, sponge explants were further cultivated under the same conditions with regular addition of *Rhodomonas salina* algae and bacteria growing in the culture.

2.2 Fully expanded explants

Single-osculum *Halichondria panicea* explants were placed in an experimental chamber with bio-filtered seawater from flow-through cultivation tanks (15 to 22 psu, $\approx 15^\circ\text{C}$). The volume of explants (V_s , mm³) was directly measured (V_{mea}) as displaced volume of seawater when submerged in a graduated beaker or estimated as $V_{est} = \pi A^2/3h$ [Obtained from the geometry of an axisymmetric cone: $A = \frac{1}{2}hb$, so $b = 2A/h$. $V_{est} = (\pi/4)b^2(h/3)$. Eliminate b to get $V_{est} = \pi A^2/3h$] from video-microscope recordings of the side-view projected area (A , mm²), height (h , mm), baseline (b , mm) and osculum cross-sectional area (OSA , mm²) of the cone-shaped explants (Fig. 1). The flow speed of the exhalant jet near the osculum of fully expanded, actively filtering explants was estimated from the motion of ink particles smaller than 6 μm (Pelikan Scribto; dilution 2×10^5 -fold to give an estimated particle density of 10^4 ml^{-1}). High speed (60.61 fps) video recordings of the particle movement in a focal (side view) plane near the exhalant jet were analyzed by particle tracking velocimetry (PTV; cf. Riisgård et al., 2011) using the software IC

Capture. The exhalant jet speed, defined as the mean velocity U_0 at the osculum, was determined by extrapolating the velocity field to the osculum using an exponential regression curve (Fig. 1), say $U_x/U_0 = \exp(-ax)$ where U_x denotes the velocity at distance x from the osculum and a a constant. The penetration of the jet into the ambient water, expressed as the distance $x_{1/2}$ at which $U_x/U_0 = 1/2$, was calculated as $x_{1/2} = (\ln 2)/a$. In the present case, however, we do not know if the oscular jet-speed profile is flat or exhibit a bell shaped curve with highest velocity at the centre, as measured by Reiswig (1974) and Strehlow et al. (2016) in several sponge species. If so, $U_0 = U_c \times 0.9$ (profile correction factor), where U_c is the excurrent velocity at the centerline (Reiswig, 1974). Therefore, if the extrapolated velocities fell along the centerline this would mean that the present data for U_0 may be up to 10 % overestimated. But because the PTV measurements were made on particles accelerated by the jet periphery with lower velocity the error is in fact smaller.

The filtration (= flow) rate was determined as $F_{est} = OSA \times U_0$. The volume-specific filtration rate $F_V (= F_{est}/V_s)$ was estimated as an indicator of active filter-feeding.

2.3 Contracting explants

Exhalant jet speed U_0 and osculum cross-sectional area OSA were measured as described above, now over time during closure of the osculum of explants in bio-filtered seawater with constant mixing. This was done both during spontaneous and induced contractions after either exposure of explants to the neurotransmitter γ -aminobutyric acid (GABA, 1 mM; cf. Ellwanger et al., 2007) or after exposure to inorganic particles (Pelikan Scribtor ink; dilution 2.5×10^4 -fold, i.e. particle density $\approx 10^5 \text{ ml}^{-1}$; cf. Elliott and Leys, 2007). We comparatively estimated the response time t_c of explants, i.e. the duration of osculum closure and reduction of U_0 to zero from video recordings. Changes in in- and exhalant canal diameters d during spontaneous contraction events were measured using the software ImageJ v5.0.3.

2.4 Jet penetration and spreading. Scaling

For the axisymmetric laminar jet, starting from a flat profile U_0 and diameter D of an orifice in a plane wall, the centerline velocity (U_C) and half-width ($b_{1/2}$) of the jet far downstream, which spreads the less it penetrates into the surrounding water, may be estimated from the classical theoretical solutions (Schlichting, 1968, p. 220)

$$U_C(x)/U_0 = (3/32) \text{Re } D/x; \quad b_{1/2}/D = (5.945/\text{Re}) x/D; \quad \text{Re} = U_0 D/\nu, \quad (1)$$

where Re denotes the Reynolds number, ν ($1.274 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$; 15 °C, 20 psu; Riisgård and Larsen, 2009, Eq. (1) therein) the kinematic viscosity of seawater, and x the distance from the osculum. For a given sponge explant of exhalant jet velocity U_0 and osculum diameter D , the above scaling leads to a penetration of the jet (i.e. how slowly the center velocity U_C decreases with increasing distance x from the osculum) and spreading (i.e. half-width $b_{1/2}$ of the jet), given by

$$U_c(x)/U_0 \approx U_0 \times D^2/x; \quad b_{1/2}/D \approx x/(U_0 \times D^2). \quad (2)$$

For the scaling of other parameters, for fully expanded and filtering sponges, we first recall the observation (see Introduction) that choanocyte pump density, hence flow rate F , seems to scale directly with sponge volume, i.e. $F \sim V_s \sim L^3$, where L denotes a length scale of the sponge. Assuming the osculum diameter to scale as $OSA \sim L^2$, and using the continuity relation, $F = OSA \times U_0$, we arrive at the scaling relations

$$F \sim L^3 \sim V_s; \quad F \sim OSA^{3/2}; \quad U_0 = F/OSA \sim OSA^{1/2}, \quad (3)$$

which suggest that U_0 is not constant as found for mussels.

For contracting and closing explants, say for OSA decreasing to zero linearly over time $t = 0$ to t_c as $OSA/OSA_{\text{start}} \sim (1 - t/t_c)$, we should expect, from Eq. (3), that jet velocity would decrease as

$$U_0/U_{0,\text{start}} \sim (1 - t/t_c)^{1/2}, \quad (4)$$

i.e. slowly at start but faster near full closure.

2.5 Statistical analyses

Statistical data analyses were performed in R version 3.1.3 (R Core Team 2015). Linear models (LM) were parameterized to extrapolate the velocity field of moving particles to the osculum ($x = 0$) using exponential regression, to test for differences between estimated and measured sponge explant volumes (V_s) and for fitting power functions to the relationships between osculum cross-sectional area (OSA) and V_s , jet speed (U_0) and OSA , filtration rate (F) and OSA , or the distance ($x_{1/2}$), where the center-line velocity of the exhalant jet becomes $0.5U_0$, and the osculum diameter (D). Model assumptions were met without the need for fitting generalized linear models. Non-linear, asymptotic regression models (NLM) from R package ‘nlstools’ (Baty and Delignette-Muller, 2015) were used to obtain comparative least-squares estimates of the parameter U_0 as a function of OSA .

3.0 Results

For fully expanded and actively pumping explants, the experimental data for a range of osculum cross-sectional areas ($OSA = 0.1$ to 3.14 mm^2 ; $n = 27$) show a mean volume specific filtration rate of $F_v = 3.46 \pm 3.25 \text{ ml min}^{-1} \text{ cm}^{-3}$ (Table 1). Deviation between estimated and measured explant volumes ($n = 6$; Table 1) was negligible (LM, $t_{0.3, 10} = 0.13$, $P = 0.996$). Obtained power-law regression relations for OSA versus volume V_s , jet speed U_0 versus OSA , and pumping rate F_{est} versus OSA , respectively, are in good agreement with the theoretical scaling parameters (Table 2). The OSA increased significantly as a function of increasing sponge volume (V_s) as expressed by a power exponent 0.66 per cm^3 volume increase (Fig. 2; LM, $t_{0.24, 25} = 7.2$, $P = 1.6 \times 10^{-7}$). The exhalant jet speed (U_0) increased significantly with increasing OSA as indicated by a

power function with the exponent 0.45 (Fig. 3; LM, $t_{0.16, 25} = 5.8$, $P = 5.4 \times 10^{-6}$). For the investigated range of *OSAs* (Table 1), U_0 reached a maximum value of 5.3 cm s^{-1} (Fig. 3). Note that parameters F , *OSA* and U_0 are not independent but related by continuity of flow, $F = OSA \times U_0$ ($\sim OSA^{1+0.45}$), where the estimated filtration rate (F_{est}) increased with increasing *OSA* (Fig. 4) according to a power function with the exponent 1.45 (LM, $t_{0.16, 25} = 18.6$, $P = 3.6 \times 10^{-16}$). Figure 5 shows how the distance ($x_{1/2}$) where center velocity of jet has decreased to half its initial value ($U_C = 0.5U_0$) increases with increasing osculum diameter (D) as indicated by a power function with the exponent 1.48 (LM, $t_{0.23, 25} = 6.6$, $P = 6.5 \times 10^{-7}$). The dependence on jet penetration ($x_{1/2}$) on jet velocity (not shown) appears to show somewhat more scatter, but is approximately $x_{1/2} \sim U_0^{0.99}$ (LM, $t_{0.30, 25} = 4.1$, $P = 3.6 \times 10^{-4}$).

The experimentally measured decrease of jet velocity with distance from osculum (Fig. 1) follow an exponential relationship rather than the theoretical $1/x$ suggested by Eq. (2). The jet penetration length expressed by $x_{1/2}$ is relatively long, dependet on the size of the sponge explant (Table 1). [Data on half-width of jet $b_{1/2}$ is missing?]

Long-term time-lapse video recordings of an initially fully expanded explant (ID #7, Table 1; Video 1 in the Supplement) revealed repeated spontaneous contractions over time, despite otherwise undisturbed conditions. The different stages of a spontaneous contraction in the same single-osculum explant (Fig. 6) start from normal flow, visualized with fluorescein dye (Fig. 6A), through expanded excurrent canals (Fig. 6B). As the osculum closes within a response time of $t_c = 38 \pm 8 \text{ min}$ (Figs. 6C-D & 7A), the endopinacoderm lining constricts the excurrent canals by up to $87.1 \pm 2.7 \%$ (Fig. 7B, arrows) which may effectively restrict or stop the water flow. Exposure to GABA (1 mM) triggered contractions in selected *H. panicea* explants ($n = 6$; cf. Table 1) and they were correlated with decreases in both *OSA* and exhalant jet speed U_0 to eventually become zero within a mean response time $t_c = 23 \pm 7 \text{ min}$ (Figs. 8 & 9). Besides chemical stimulation by the addition of GABA (Video 2 in the Supplement), overloading with inorganic ink particles (particle density $\approx 10^5 \text{ ml}^{-1}$) was found to trigger contractions with subsequent cessation of the pumping rate in within $t_c \approx 50 \text{ min}$ (Video 3 in the Supplement).

4.0 Discussion

The data for expanded and actively pumping explants in Table 1 are ordered in terms of increasing osculum area *OSA* and show a corresponding increase in jet velocity U_0 and the related filtration rate F (Figs. 3 & 4). The observed trend of an increase in exhalant jet speed U_0 with increasing osculum cross-sectional area *OSA* (Fig. 3) in *Halichondria panicea* explants extends to larger sponges with identical power exponent of 0.45 as appears from a comparison to literature values (Fig. 10; LM, $t_{0.17, 31} = 7.6$, $P = 1.6 \times 10^{-8}$). For various sponge species with *OSAs* in the range 0.1 to 7 mm^2 , U_0 seems to reach a maximum of $U_0 = 6$ to 8 cm s^{-1} (Table 3; Reiswig, 1974; Strehlow et al., 2016).

The hypothesis of a more or less constant density of choanocyte chambers in sponges, all delivering the same volume flow, is supported by the power law of Fig. 3 ($U_0 \sim OSA^{0.45}$) that, along with continuity of flow ($F = OSA \times U_0$), leads to $F \sim OSA \times OSA^{0.45} = OSA^{1.45} \sim L^{2.9} \sim V_s$.

Although a forced linear regression of the data of Fig. 2 (i.e. a power-law exponent of 1.0 instead of $0.66 = 2/3$ found by free regression) would lead to a higher R^2 -value, it contradicts dimensional scaling, hence not considered likely. Values of the volume-specific filtration rate F_V ($= F/V_s$) for individual explants listed in Table 1 show large variations and an arithmetic mean of F_V (ml min^{-1}) $\approx 3.46 \pm 3.25 V_s$ (cm^3) which may be explained by strong temporal dynamics in the expansion state of explants (Video 1 in Supplement). A more objective correlation-smoothed mean value is obtained from the relations $OSA = 1.31 V_s^{0.66}$ (Fig. 2) and $F = 1.55 OSA^{1.45}$ (Fig. 4), which after elimination of OSA gives F (ml min^{-1}) $\approx 2.3 V_s$ (cm^3) and is in good agreement with previous findings (Larsen and Riisgård, 1994; Riisgård et al., 1993). This relation may be compared to data for *Haliclona urceolus* (Larsen and Riisgård, 1994, Table 1 therein), giving a filtration rate $F = 6 \text{ ml min}^{-1}$ for a sponge volume $V_s [= 3.2 \times \pi(0.86^2 - 0.23^2)/4] = 1.73 \text{ cm}^3$, or F (ml min^{-1}) $\approx 3.5 V_s$ (cm^3). The two constants (2.3 and 3.5) must represent the number of choanocyte chambers (CC) per unit volume times the average filtration rate of one CC . It is remarkable that the volume-specific filtration rate is of the same order of magnitude for the two sponge species as it likely suggests choanocyte chambers of similar size, with similar individual filtration rate, and of similar uniform distribution over the sponge volume. This also supports the hypothesis of volumetric scaling (cf. Table 2).

Turning to the question of penetration and spreading of the exhalant jet into the surrounding water, the decrease of jet velocity with distance from osculum (x ; Fig. 1) appears to follow an exponential relationship rather than $1/x$ as suggested by Eq. (2). Also, our data show that $x_{1/2} \sim D^{1.48}$ (Fig. 5) rather than $x_{1/2} \sim U_0 D^2$ (Eq. 2) or $x_{1/2} \sim D^3$ for $U_0 \sim OSA^{0.45} \sim D$ (Table 2). One reason is that the boundary layer assumption ($Re \gg 1$) implicit in the solutions of Eq. (1) is not satisfied for the low Reynolds number at hand (mean of 16.8 for the 27 sponge explants), or that the initial velocity profile is not flat (but see Lee et al., 1997) or that the fictive origin of the solution is upstream of $x = 0$ (but see Revuelta et al., 2002). However, it is noted that the jet penetration is considerable. For the small explant ID #1 of height $h = 3.8 \text{ mm}$, for example, $x_{1/2} = 2.3 \text{ mm}$ (Table 1) but organized flow due to the jet reaches much further out into the otherwise quiescent water (see Fig. 1), so that sponges avoid re-circulation of already filtered water (Bidder, 1923; Riisgård and Larsen, 2017).

For the experiments on contracting explants in response to exposure to GABA, OSA typically decreases nearly linearly to zero within the mean response time t_c . The corresponding exhalant jet velocity (Fig. 9) shows more scatter and a tendency to slowly decrease at the start and accelerate towards the end, as suggested by Eq. (4). This is particularly evident for ID #16 (Fig. 9B). As known from an experiment on the back-pressure characteristics of sponges (Riisgård et al., 1993), the choanocyte pumps continue to operate as net flow is reduced to zero, at which time pumps deliver a pressure more than 3 times as high as at their normal operating pump pressure. Internal leakage within the pumps makes this possible. As osculum and canals begin to contract, the choanocytes merely raise their delivered pressure in an attempt to maintain flow, so that jet velocity would decrease slowly at start, as suggested by Eq. (4). Therefore, even during complete osculum closure, the choanocyte pumps may continue to operate as leaky peristaltic

pumps (Larsen & Riisgård, 1994). The rate of osculum expansion after a period of closure appears to be as high as the rate of decrease toward closure (Fig. 7A) which indicates that accompanying changes in exhalant jet velocity are reversible.

The duration of osculum closure was in a similar range among spontaneous and GABA- or particle-triggered contractions in explants (Videos 1 to 3 in Supplement). It is seen that the mean autonomous closing time of $t_c = 38 \pm 8$ min during spontaneous contractions (Fig. 7) is somewhat longer than the GABA-triggered response time of $t_c = 23 \pm 7$ (Fig. 9) which may be due to an applied concentration of the neurotransmitter (1 mM) above physiological levels (Elliott and Leys, 2010; Ellwanger et al., 2007). The observed osculum response times are comparable with the duration of spontaneous ($t_c \approx 23$ min; Nickel, 2004, Table 1 therein) and chemically induced choanosome contractions in *Tethya wilhelma* ($t_c \approx 15$ min after exposure to 1 mM GABA; Ellwanger et al., 2007, their Fig. 5). The response time after exposure to inorganic particles (dilution 2.5×10^4 -fold) was somewhat extended to $t_c \approx 50$ min compared to spontaneous contractions. This is in agreement with previous observations that overloading with inorganic particles slows down the contraction cycle in the freshwater sponge *Ephydatia muelleri* ($t_c \approx 17$ min after exposure to 4×10^3 -fold diluted ink particles; Elliott and Leys, 2007) which uses coordinated peristaltic waves to expel water and waste material from its aquiferous system. Our findings suggest contractile behavior as an important mechanism to avoid overloading of the sponge pump with inorganic particles, while cellular transport seems to regulate the removal of particles from the aquiferous system of *H. panicea* (Video 3 in Supplement). While sponges seem to use their pinacoderm for mediating contractions via sensory systems, signaling pathways and actinocytes as effectors (Leys, 2015; Nickel et al., 2011), it remains to determine the mechanisms that control contractions. A volumetric contraction of an explant would likely reduce diameters of the canal system and hence lower the filtration rate, but this in itself would not stop the choanocyte pumps.

As previously elaborated by Kumala et al. (2017), several previous observations suggest various forms of sensing control of flow and contraction, including the regulation of the choanocyte pumps. Kumala et al. (2017) found the relationship $F \text{ (ml min}^{-1}\text{)} = 1.39 \times OSA \text{ (mm}^2\text{)}$ to be a good approximation for single-osculum *H. panicea* explants with $OSA < 0.5 \text{ mm}^2$, which does not essentially contradict the present data (Fig. 4). Their data did not justify other regression analysis but the linear one for the limited size range investigated.

For the data shown in Figs. 3 & 10, an exponential regression curve of form $U_0 = U_{\max}[1 - \exp(-OSA/OSA_0)]$ has also been considered, leading to asymptotic maximal values $U_{\max} \approx 4.1 \text{ mm s}^{-1}$ (NLM, $t_{0.77, 25} = 7.2$, $P = 1.6 \times 10^{-7}$) and $\approx 6.3 \text{ mm s}^{-1}$ (NLM, $t_{1.22, 32} = 7.6$, $P = 1.3 \times 10^{-8}$), respectively. This may indicate that the exhalant jet speed of a single-osculum sponge explant, or the jet speed from an individual osculum in a multi-oscula sponge, approaches a certain upper limit as the sponge grows, and further that an aquiferous module of a multi-oscula sponge supplying a specific osculum with water may increase to a certain size, after which the sponge pump may become insufficient for the increasing system head loss, so that instead a new small module with an initially small *OSA* is formed. We offer the following possible explanation based

on the earlier mentioned indication that the volume-specific density of choanocyte chambers (CC) is approximately constant among sponges. This would imply that the fraction of total volume occupied by CCs would be independent of size, $V_{s,CC} \sim c_1 V_s$ where c_1 is a constant <1 . The number of canals (at least the smaller branching ones) servicing CCs is proportional to their number, hence the canal volume is proportional to V_s . In addition, the volume taken up by the larger inhalant and exhalant canals is a summation over their cross-sectional areas πr_i^2 and lengths L_i , ($\sim \sum \pi r_i^2 L_i \sim V_s^{1/3}$) based on the assumption that the distribution of radii r over canal sections is approximately independent of sponge size. Therefore the fraction of volume occupied by canals would depend on sponge volume as $V_{s,canals} \sim V_s \times V_s^{1/3} \sim V_s^{4/3}$, hence an increasing part of the sponge volume, which suggests that single-osculum aquiferous modules may be size-limited. The increase in frictional head loss in canals as their lengths increases (as $L \sim V_s^{1/3}$) would tend to lower the filtration rate even though CC-pumps can deliver the associated pressure rise. On the other hand, if a sponge develops a large atrium its total volume may still continue to increase since not all canals must merge into a single large exhalant canal terminating at the osculum, but then many large canals can terminate at the atrium. While our findings emphasize the importance of contractile behavior for the pumping activity of the leuconoid demosponge *H. panicea*, future studies on the comparative functional morphology of sponges at different levels of organization (ascon, sycon, or leucon; cf. Ereskovskii, 2003) may shed further light on purpose and function of the 'sponge neural toolkit' (Leys, 2015), including sensory cells, conduction pathways and signaling molecules. Finally, it is of interest to test if our hypothesis of near constant volume-specific filtration rate and possibly also density of choanocyte chambers would apply to other species of leuconoid sponges.

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Supplementary data

Supplementary video material to this article can be found online at xxx.

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Table 1. *Halichondria panicea*. Characteristics of single-ostium sponge explants. *OSA*: ostium cross-sectional area; *A*: projected area; *h*: height; $V_{est} [= \pi A^2/3h]$: estimated volume; V_{mea} : measured volume; U_0 : exhalant jet speed at distance $x = 0$ mm from ostium (cf. Fig. 1); $x_{1/2}$: distance where center velocity of jet $U_C = 0.5U_0$; $F_{est} [= OSA \times U_0]$: estimated filtration rate; $F_V [= F_{est}/V_{est}$, or when possible $= F_{est}/V_{mea}]$: volume-specific filtration rate.

<i>ID</i> #	<i>OSA</i> (mm ²)	<i>A</i> (mm ²)	<i>h</i> (mm)	V_{est} (mm ³)	V_{mea} (mm ³)	U_0 (mm s ⁻¹)	$x_{1/2}$ (mm)	F_{est} (ml min ⁻¹)	F_V (ml min ⁻¹ [cm ³ sponge] ⁻¹)
1	0.10	10.8	3.8	32	-	6.6	2.3	0.04	1.23
2	0.14	6.5	3.2	14	-	5.9	2.2	0.05	3.64
3	0.15	30.6	7.0	139	-	6.6	2.3	0.06	0.43
4	0.17	54.6	12.0	259	-	10.7	3.1	0.11	0.42
5	0.20	14.3	4.4	49	-	13.7	2.4	0.16	3.39
6	0.20	34.5	7.8	159	-	14.0	5.1	0.17	1.05
7	0.24	25.9	4.3	164	-	23.4	5.3	0.34	2.05
8	0.25	22.4	5.5	95	-	30.8	3.7	0.46	4.89
9	0.28	38.5	9.2	169	-	14.9	6.4	0.25	1.48
10	0.28	-	-	-	132	21.1	7.1	0.35	2.69
11	0.35	51.3	14.5	191	136	24.4	6.9	0.51	3.77
12	0.37	52.9	10.9	268	-	18.7	6.8	0.41	1.54
13	0.37	42.4	7.4	256	-	10.8	6.5	0.24	0.94
14	0.48	53.5	10.4	289	-	11.0	2.5	0.32	1.10
15	0.59	66.2	6.0	766	811	17.1	41.0	0.60	0.74
16	0.60	33.9	8.0	151	-	25.9	9.0	0.93	6.16
17	0.61	44.0	9.8	207	-	15.2	7.8	0.56	2.69
18	0.68	-	-	-	183	25.2	14.8	1.03	5.64
19	0.68	65.5	12.8	351	346	33.9	5.9	1.38	4.00
20	0.89	36.9	11.1	128	95	27.8	5.0	1.48	15.63
21	1.01	100.4	13.0	810	838	24.4	19.3	1.48	1.77
22	1.13	-	-	-	528	23.9	36.7	1.62	3.07
23	1.13	-	-	-	411	39.5	17.4	2.68	6.51
24	1.69	66.1	13.1	347	374	34.4	10.3	3.49	9.33
25	2.36	-	-	-	1591	20.2	25.5	2.86	1.80
26	2.54	-	-	-	1899	31.5	17.9	4.81	2.53
27	3.14	-	-	-	1977	53.0	24.7	9.98	5.05
Mean $\pm$ S.D.									3.46 $\pm$ 3.25

438 **Table 2.** Theoretical and observed scaling of sponge volume V_s , osculum cross-sectional area
 439 OSA , exhalant jet speed U_0 and filtration rate F .

Figure	Theoretical scaling	Observed scaling
2	$OSA \sim V_s^{2/3}$	$OSA \sim V_s^{0.66}$
3	$U_0 \sim OSA^{1/2}$	$U_0 \sim OSA^{0.45}$
4	$F \sim OSA^{3/2}$	$F_{est} \sim OSA^{1.45}$

440 **Table 3.** Measured exhalant jet speed (U_0), osculum diameter (D), osculum cross-sectional area
 441 (OSA), Reynolds number ($Re = U_0 D/\nu$), and sample size (n) of various sponge species.

Species	U_0 (mm s ⁻¹)	D (mm)	OSA (mm ²)	Re	n (ind.)	Reference
<i>Mycale</i> sp.	79	2.1	3.42	130	13	Reiswig (1974)
<i>Verongia gigantea</i>	128	1.0	0.78	100	11	Reiswig (1974)
<i>Tethya crypta</i>	173	1.2	1.04	157	8	Reiswig (1974)
<i>Haliclona permollis</i>	51	2.8	6.16	113	2	Reiswig (1975)
<i>Haliclona urceolus</i>	56	1.5	1.77	66	8	Riisgård et al. (1993)
<i>Baikalospongia bacillifera</i>	33	3.0	6.84	77	2	Patterson et al. (1997)
<i>Cliona orientalis</i>	61	2.1	3.60	103	2	Strehlow et al. (2016)
<i>Halichondria panicea</i>	23	0.7	0.38	13	7	Kumala et al. (2017)
<i>Halichondria panicea</i>	22	1.0	0.76	17	27	This study

Figure 1. *Halichondria panicea*. Flow pattern and speed of tracer particles accelerated by the (particle-free) exhalant jet of a single-osculum sponge explant (ID #2, Table 1). Ink particles (2×10^5 -fold dilution) were traced over 10- (●) or 50-frame (×) intervals (Δt ; from 60.61 fps video recordings) to obtain shown particle speeds (mm s^{-1}). Projected area A , baseline b (assumed equal to diameter of base area) and height h were used to calculate the explant volume. Insert: Particle speed U versus distance x from osculum in one particle trace (PT; maximum observed speed $U_{\max} = 4.6 \text{ mm s}^{-1}$). The exponential regression function $U_c/U_0 = \exp(-ax)$ (LM, $t_{0.04, 23} = -23.2$, $P < 2 \times 10^{-16}$) indicates an exhalant jet speed $U_0 = 5.9 \text{ mm s}^{-1}$ at $x = 0 \text{ mm}$ for $a = 0.32 \text{ s}^{-1}$, and $x_{1/2} = (\ln 2)/a = 2.2 \text{ mm}$. Scale bar: 1 mm.

Figure 2. *Halichondria panicea*. Osculum cross-sectional area (OSA) as a function of sponge explant volume (V_s).

Figure 3. *Halichondria panicea*. Exhalant jet speed (U_0) of single-osculum explants as a function of the osculum cross-sectional area (OSA).

Figure 4. *Halichondria panicea*. Estimated filtration rate ($F_{est} = OSA \times U_0$) as a function of osculum cross-sectional area (OSA).

Figure 5. *Halichondria panicea*. Distance ($x_{1/2}$) where center velocity of jet $U_C = 0.5U_0$ as a function of osculum diameter (D).

Figure 6. *Halichondria panicea*. (A) Close-up of a single-osculum explant (ID #7, Table 1) showing flow through the excurrent canals (*ec*) and osculum (*osc*) after uptake of green fluorescent dye from the ambient seawater via incurrent canals (*ic*; visible from top view, diameter $\sim 200 \mu\text{m}$). (B-D) Different stages of a spontaneous contraction in the explant during long-term observation (Video 1 in Supplement) including (B) expanded osculum and excurrent canals (measured canal diameters $d1$ - $d3$ are indicated by broken lines) and (C, D) contraction of the osculum and endopinacoderm lining the excurrent canals (arrows). Scale bars: 1 mm.

Figure 7. *Halichondria panicea*. Spontaneous contractions over time of a single-osculum explant (ID #7; cf. Fig. 6 & Video 1 in Supplement), expressed by (A) repeated osculum closure (contraction-expansion events 1-3, arrows) and (B) associated constriction of excurrent canals (arrows; cf. *d1-d3*, Fig. 6B).

Figure 8. *Halichondria panicea*. Flow pattern and speeds of tracer particles accelerated by the (particle-free) exhalant jet of an explant during osculum closure (at $t = 0, 4, 8, 15, 20, 25, 30$ min; Fig. 9, ID #1) triggered by addition of GABA (1 mM; at $t = 0$). Ink particles (2×10^5 -fold dilution) were traced over 10- (●), 50- (+), 100- (□), or 200-frame (○) intervals (Δt ; from 60.61 fps video recordings). The maximum observed particle speed (U_{max} , mm s⁻¹) is indicated for each instantaneous cross-sectional area (OSA, mm²). Scale bar: 1 mm.

Figure 9. *Halichondria panicea*. **(A)** Osculum cross-sectional area (OSA) and **(B)** exhalant jet speed (U_0) of selected single-osculum explants (ID#, Table 1) after addition of the neurotransmitter GABA (1 mM) at $t = 0$. The response time t_c for GABA-triggered reductions in the exhalant jet speed U_0 to zero is indicated by vertical broken lines.

487
 488 **Figure 10.** Exhalant jet speed measured in various leucon-type sponge species by different
 489 authors (Tables 1 & 3).