Osculum dynamics and filtration activity in small single-osculum explants of the demosponge Halichondria panicea

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INTRODUCTION

Sponges are sessile filter-feeding invertebrates that actively pump seawater through their water canal system to obtain suspended food particles (Bergquist 1978). The basic element for both water pumping and filtration is the choanocyte, with a beating flagellum to induce water flow and a collar of microvilli that sieve free-living bacteria and other particles down to ~0.1 μm for the purpose of feeding (Fjørde 1961, Bril 1973, Riisgård & Larsen 2000, Leys et al. 2011). Feeding on phytoplankton cells (>5 μm) is accomplished by phagocytosis at the sponge surface (exopinacoderm; Gaino et al. 1994) and along the inhalant canal system (Kilian 1952, Reiswig 1971a, Bergquist 1978). In demosponges, the pumping power is generated by choanocyte chambers in which 80 to 100 beating flagella impel water to enter the sponge body through numerous pores (ostia), creating flow through a complex aquiferous system composed of inhalant and exhalian canals that merge into one or more exhalant opening (oscula) on the sponge chimney or surface (Larsen & Riisgård 1994). The size (Wilkinson 1978) and density (Massaro et al. 2012) of such pumping units (i.e. choanocyte chambers) allow demosponges, such as...
Halichondria panicea, to filter a volume of water of at least 6 times their own body volume per minute (Weisz et al. 2008, Riisgård et al. 2016).

Temporal variations in filtration activity appear to be common among sponges under both laboratory and natural conditions (Frost 1978, Willenz et al. 1986, Osinga et al. 2001, Stabili et al. 2006, Riisgård et al. 2016). The intertidal sponge H. panicea, commonly found in the red algae zone of the Western Baltic Sea and the North Sea (Barthel 1986, 1988, 1989), has shown significant variations in filtration rates over a period of 6 d in the laboratory (Riisgård et al. 2016). Similar observations of variable filtration rates over a period of 6 d in the laboratory (Riisgård 1989), has shown significant variations in filtration for elucidating patterns in sponge feeding.

MATERIALS AND METHODS

Preparation and cultivation of sponge explants

Specimens of Halichondria panicea were collected in the inlet to Kerteminde Fjord, Denmark, and transported to the nearby Marine Biological Research Center in Kerteminde where sponges were processed within 24 h after collection. During this time, sponges were kept in a flow-through aquarium (30 l) with aerated seawater (~12–15°C, ~18–22 PSU). To generate explants, the upper 2.0 to 2.5 cm of a sponge chimney was cut with a razor in 2 mm thick slices, and these were further cut into ~18 mm³ pieces (= 0.018 ml × 0.07 g dry wt ml⁻¹ = 1.26 × 10⁻³ g dry wt; cf. Thomassen & Riisgård 1995) (Fig. 1A) comprising both ectsosomal (peripheral) and choanosomal (inner) parts of the donor sponge (cf. de Caralt 2003). The cuttings were placed on numbered glass slides submerged in an aquarium (30 l) with flow-through of aerated bio-filtered seawater (~15°C, ~18–22 PSU; Fig. 1B) to reduce microbial growth on cut surfaces (bacterial infection) preventing successful explant culture (Hummel et al. 1988, de Caralt et al. 2003). The seawater was pre-filtered by Mytilus edulis mussels. These mussels show retention efficiencies of up to 100% for bacteria and phytoplankton (Møhlenberg & Riisgård 1978), thus allowing the sponge explants to live under controlled food conditions. The cut surfaces healed within 5 d as seen from smoothing and rounding of the explants’ periphery (Fig. 1C). After this 5 d period, glass slides with attached explants (Fig. 1C) were placed in glass slide holders and these were transferred to a feeding tank (9 l) with continuous flow (80 to 100 ml min⁻¹) of bio-filtered seawater (~15°C, ~20 PSU). Explants were regularly fed with the alga Rhodomonas salina (about 6.3 μm diameter) added with a peristaltic pump (Ole Dich) to establish a steady-state concentration of about 4000 cells ml⁻¹ (equivalent to 5 μg chl a l⁻¹; Clausen & Riisgård 1996). These algae have previously been used in growth and filtration studies on explants of H. panicea, as well as other sponge species (Thomassen & Riisgård 1995, Osinga et al. 1999), demonstrating both ingestion and digestion of
Measurement of filtration rate

To investigate the pumping activity of sponge explants, we repeatedly measured the filtration rate using the clearance method (cf. Riisgård et al. 2016). With 100% particle retention efficiency, the volume of water cleared of suspended algal cells (*R. salina*) per unit of time (= clearance rate) is identical to the filtration rate (= pumping rate). During a series of filtration experiments, *R. salina* cells were added to the experimental chamber with air-mixed, bio-filtered seawater (40 ml) with and without (= control) a sponge explant. For the measurements of filtration rates, we used initial concentrations of *R. salina* of ~3000 to ~6000 cells ml\(^{-1}\), corresponding to 3.8 to 7.5 µg chl a, which is within the natural range of phytoplankton biomass in shallow local coastal waters where *H. panicea* grows (Barthel 1986, 1988, Riisgård et al. 2013). The subsequent decrease of *R. salina* cell concentration was measured during the next 80 to 120 min by taking water samples (10 ml) at fixed time intervals (20 min) and measuring algal concentration with an electronic particle counter (Elzone 5380), which removed 1.5 to 2.0 ml of the sample per counting. The remainder of the sample was returned to the experimental chamber to avoid significant reduction in water volume and algal concentration due to sampling during the experiment. The filtration rate (\(FR, \text{ ml min}^{-1}\)) was determined from the exponential decrease in algal concentration (verified as a straight line on a semi-ln plot) as a function of time using Eq. (1) (Thomassen & Riisgård 1995):

\[
FR = \frac{V}{t} \times \ln\left(\frac{C_0}{C_t}\right) = V \times b
\]

where \(V\) is the volume of water in the experimental chamber in ml, and \(b\) is the slope of the regression line in a semi-ln plot for the reduction in algal concentration (\(C, \text{ cells ml}^{-1}\)) from time 0 (\(C_0\)) to time \(t\) (\(C_t\)) in min. The mean of the exponentially decreasing algal concentration during a clearance experiment of duration time \(t\) (= 80 to 120 min) was estimated as \(C_m = \exp[\ln(C_0 \times C_t)/2] = (C_0 \times C_t)^{0.5}\), where \(C_0\) and \(C_t\) are the initial and final algal concentration, respectively. To compare with the osculum cross-sectional area (OSA) of sponge explants, filtration rates were addi-
tionally estimated from the measurements of 2 sequential algal concentrations in 20 min time intervals using Eq. (1), taking into account the stepwise decrease in total water volume \( V \) in the experimental chamber due to the particle counting (1.5 to 2.0 ml removed per counting).

**Video microscopy**

The filtration experiments were combined with simultaneous top-view video stereo-microscopic observations of the osculum opening of the sponge explants. Temporal variations in OSA were determined (as described in the next subsection) at regular time intervals prior to (60 s intervals), during (~20 min intervals) and after (60 s intervals) filtration rate measurements. Top-view microscopic observations were performed using a stereo microscope (Leica M165 FC) with an integrated digital camera (Leica DFC425 C) controlled by image acquisition software (Leica Application Suite V3.8) for automated time-lapse image capture. In addition, explant pumping activity under specific conditions of the osculum opening was visualized by adding fluorescein dye (15 mg ml\(^{-1}\); Sigma-Aldrich) to the explant surface in stagnant, fully oxygenated, bio-filtered seawater (~15°C, ~20 PSU). The fluorescent dye was filled into a small glass tip (~0.24 mm diameter) attached to a micromanipulator allowing precise positioning 3 mm from the explant surface during the observation period. The fluorescein allowed visualization of incurrent and exhalant flow under blue light excitation. During pulsed fluorescein dye injections, the explant was video-recorded from both the top and side using a Leica M80 stereo microscope connected to an USB3.0 industrial camera (Imaging-source, DFK23UM021) controlled by an image acquisition software (IC Capture, 2.3).

**Image analysis and statistical tests**

Time-lapse images of sponge explants (top view) were analyzed in ImageJ (Version1.46r) by manually determining the OSA via pixel counts and subsequent conversion into mm\(^2\) using a reference scale bar. The relationship between OSA (determined as the mean of 2 sequential OSA measurements, one made at the beginning and one at the end of each filtration rate determination within a 20 min time interval) and filtration rate was analyzed in R version 3.2.0 (R Core Team 2015) using linear regression (LM).

**RESULTS**

**Filtration rates of single-osculum explants**

Active filter feeding of the single-osculum explants of *Halichondria panicea* was indicated by pronounced declines in the concentration of algal cells over time (Fig. 2). The mean filtration rate of all measurements was \( FR = 0.28 \pm \text{CI 95\%} 0.06 \) ml min\(^{-1}\) (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m572p117_supp/), which corresponds to a mean volume-specific filtration rate of 15 ml min\(^{-1}\) (cm\(^3\) sponge\(^{-1}\)).

The temporal variation in explant filtration rate and OSA is presented in Fig. 3 & Fig. S1 in Supplement 1, which show that the explant filtration rate follows the tendency of the OSA. In some cases, there was considerable variation in both filtration rate and OSA (e.g. Fig. 3, Experiment #9), while in other cases, there was little variation in these parameters during the recorded period (e.g. Fig. 3, Experiment #12). A plot of OSA (mm\(^2\)) versus filtration rate for all experimental results revealed a significant linear relationship (LM, \( F_{1,102} = 1469; p < 0.001; \) Fig. 4). This linear relationship indicates a constant exhalant jet velocity \( v_{\text{jet}} = FR/OSA \) = \( (1.39 \pm \text{CI 95\%} 0.08 \) cm\(^3\) min\(^{-1}\))/(10\(^{-2}\) cm\(^2\)) = 139 \pm \text{CI 95\%} 8 \) cm min\(^{-1}\) = 2.3 \pm \text{CI 95\%} 0.13 cm s\(^{-1}\). The filtration rate of small (18 mm\(^3\)) single-osculum explants (at 15°C) can hence be expressed as \( FR \) (ml s\(^{-1}\)) = \( 2.3 \pm \text{CI 95\%} 0.13 \) (cm s\(^{-1}\)) \times OSA (cm\(^2\)).

**Osculum dynamics and filtration activity**

Top-view time-lapse observations of temporal changes in OSA indicate contraction–inflation behavior of the sponge explants, with short periods of contraction and even osculum closure, followed by osculum inflation (Fig. 3B & Fig. S1B in Supplement 1). We observed a total of 5 full osculum contraction–inflation events with full osculum closure for 45 min (Experiment #7), 20 min (Experiment #8), 50 min (Experiment #10) and 31 min (Experiment...
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#13) during the filtration experiments (Fig. 3 & Fig. S1 in Supplement 1). In addition, when held in stagnant, bio-filtered seawater, we observed osculum closure of 119 min and 53 min (Fig. 5A). Osculum closure ensued independently of algal additions or experimental conditions (i.e. mixed or stagnant seawater), with a mean duration of 53 ± 35 min (n = 4 explants) throughout all experiments. Repeated osculum closure occurred in 2 explants and in time intervals of 265 min (=4.4 h; Fig. 3, Experiment #8) and 620 min (=10.3 h; Fig. 5A).

As indicated from simultaneous measurements of OSA and filtration rate, osculum contraction coincided with substantial change in the filtration activity of sponge explants (Fig. 4). We also explored the relationship between osculum closure and filtration activity directly through the addition of fluorescent dye. In these experiments, we observed an increase in exhalant jet height with increasing OSA during osculum inflation (Fig. 5). Explants with wide-open osculum (Fig. 6A) were actively pumping water, as demonstrated by both the uptake and release of fluorescent dye (Figs. 5 & 6C), but no water flow in the channel system was observed in explants during periods of osculum closure (Fig. 6B,D). Pumping activity resumed approximately at the same time as the osculum opened. Occasionally, we observed inflation of the body cavity in proximity of the osculum with simultaneous compression of the explant apical pinacoderm (i.e. outer surface) during osculum closure (see Video 1 at www.int-res.com/articles/suppl/m572p117_supp/). Thus, it seems likely that cessation of the pumping and filtration activity of explants at least sometimes concurred with contractions of inhalant openings (ostia) and water canals during periods of osculum closure.

DISCUSSION

Relationship between osculum dynamics and filtration rate

Filtration experiments combined with time-lapse video observations of the osculum revealed that the filtration rate (FR) of the single-osculum explants of Halichondria panicea correlates with the osculum cross-sectional area (OSA), as expressed by an increase in filtration rate with increasing OSA (Figs. 4 & 5). Thus, changes in OSA explain >90% of the variations in the measured filtration rates (Figs. 3 & 4), as also expressed in exhalant jet height (Fig. 5). The linear relationship between FR and OSA (Fig. 4) implies that exhalant jet speed is constant.
The observed constant exhalant jet velocity at varying osculum diameter \( D \) leads to a penetration of the jet (i.e. how the center velocity decreases with distance \( x \) from the osculum) that increases as \( D \) increases. Because an axisymmetric, laminar jet penetration varies as \( D^2/x \) (Schlichting 1968, p. 220), this phenomenon appears to be confirmed by Fig. 5B, which shows increased penetration moving from State 2 to 3. Further, the observation that filtration rate \( FR \) is proportional to the osculum cross-sectional area \( OSA \propto D^2 \) suggests a ‘contraction parameter’ \( FR/FR_{\text{max}} = (D/D_{\text{max}})^2 \) that can vary from maximum open state (=1) to fully closed state (=0). If reduction of \( OSA \) is correlated with a contraction of sponge structure so that canals with diameters \( d \) contract in the same manner, i.e. \((d/d_0)^2 \propto (D/D_0)^2\), this may explain the observation. Thus, a significant contribution to the frictional pressure drop comes from the
pressure drop of laminar flow in the canal system, which is proportional to $FR \times L/d^2$ (Eq. 19 in Riisgård & Larsen 1995), where $L$ is the canal length. If the choanocyte pumps were autonomous, delivering a constant pressure that equals the frictional pressure drop in canals, then $FR \propto d^2$, which supports our observations if a given contraction of $D$ gives rise to a proportional contraction of $d$. This possibility needs to be experimentally verified and can of course be criticized, e.g. does a contraction involve the whole sponge, are the choanocyte pumps autonomous, and are there other contributions to pressure drop? Hopefully the present observations may inspire future studies to address such basic questions.

Our findings suggest that temporal variability in the filtration rate of $H. \text{panicea}$ (Olesen & Weeks 1994, Riisgård et al. 2016), and possibly other sponge species (Frost 1978, Willenz et al. 1986, Osinga et al. 2001, Stabili et al. 2006), is associated with contraction-inflation behavior, including oscula dynamics. Contractions of the apical pinacoderm and inflation of the explant body observed during osculum closure (Video 1) indicate activity of the choanocyte pumps and resemble contraction-inflation behavior observed in other sponges (Nickel 2004, Nickel et al. 2006, Elliott & Leys 2007). This observation emphasizes that oscula dynamics are closely related to contractile behaviors of sponges (Reiswig 1971b, Gaino et al. 1991, Nickel et al. 2004, Leys & Meech 2006, Nickel et al. 2006, 2011, Elliott & Leys 2007), which are modulated by both external (e.g. Elliott & Leys 2007) and internal (Reiswig 1971b) stimuli. The present results further suggest that cessation of the filtration activity of explants with closed osculum may be associated with contractions of inhalant openings (ostia) and water canals (Parker 1910, Gaino et al. 1991, Nickel...
et al. 2006, Elliott & Leys 2007), including choanocyte chambers (Elliott & Leys 2007).

The observed relationship between filtration rate and OSA (Fig. 4) indicates that changes in OSA must be closely correlated with the water-pumping activity and resistance to flow due to contractions within the sponge, ultimately resulting in the cessation of filtration activity during periods of complete osculum closure (Fig. 6). Numerous sponges from different habitats show cessation in pumping activity in regular as well as irregular intervals ranging from minutes to several days (Reiswig 1971b, Patterson et al. 1997, Tompkins-MacDonald & Leys 2008, McMurray et al. 2014). Pumping ceased in 4.4 to 10.3 h intervals in 2 of the explants of H. panicea, which lies within the same time range determined in Verongia gigantea (Reiswig 1971b). These vase-shaped sponges periodically interrupt water pumping by contracting their exhalant canals independent of environmental conditions, suggesting that such cyclic contractions may be part of an intrinsically generated rhythm (Reiswig 1971b). Periods of cessation in the in situ water-pumping activity averaging 42 min in V. gigantea (Reiswig 1971b) are comparable to those found in explants of H. panicea in the present study.

Osculum contractions subsequent to decreasing exhalant jet speed in the demosponge species Tethya crypta (Reiswig 1971b) and Cliona orientalis (Strehlow et al. 2016) further suggest that osculum dynamics follow changes in choanocyte flagellar beating activity (Reiswig 1971b). Variations in OSA as a response to changes in the activity of choanocyte pumps may enable sponges to maintain a constant velocity of the exhalant jet efficiently removing the excurrent water (Bidder 1923, Reiswig 1971b). Non-motile, sensory cilia on epithelial cells lining the inside of oscula of various demosponges and other sensory-cell-type candidates in the water canal system (e.g. ciliated endopinacocytes; Nickel 2010 and references therein) reveal the ability of sponges to sense changes in internal water flow (Ludeman et al. 2014). Cascading stimulus propagation from sensory cells towards contractile elements, including actinocytes or actin microfilaments (Elliott & Leys 2007), via chemical messenger-based systems may further be involved in coordinating whole-animal responses (Ludeman et al. 2014). The cellular origin and pathway of the contractile behavior, nevertheless, remains rather unclear; however, growing single-osculum explants under coverslips in ‘sandwich’ preparations

Fig. 6. Halichondria panicea. (A,B) Video-microscope observation of osculum and (C,D) top-view images of the exhalant current visualized with fluorescein dye (arrow) in a sponge explant with open (A, C) and closed (B, D) osculum. T: glass tip filled with fluorescein dye. Scale bars: 0.5 mm
Rhodomonas salina was not related to increasing amounts of ingested cells and subsequent digestive processes over the observation period. Overall, we observed no obvious environmental drivers for osculum behavior.

Why should a sponge alter (under constant environmental conditions) its pumping activity, and even cease pumping altogether (e.g. Tompkins-MacDonald & Leys 2008, McMurray et al. 2014)? It has been shown that cessation of the water pumping activity (Reiswig 1971b, Nickel 2004, Tompkins-MacDonald & Leys 2008) results in temporal oxygen depletion in the sponge body (Hoffmann et al. 2005, 2008, Schläppy et al. 2007, 2010a), which usually serves as habitat for some highly diverse microbial consortia (Taylor et al. 2007, Thomas et al. 2016). The demosponge Aplysina aerophoba developed anoxic conditions in the sponge body within 15 min of pumping cessation (Hoffmann et al. 2008). Pumping cessation may thus allow sponges to regulate their internal oxygen concentration to control the activity of aerobic and anaerobic microbes inhabiting the sponge host (Hoffmann et al. 2005, 2009, Schläppy et al. 2010b, Hentschel et al. 2012). In this way, sponges may benefit from their associated microbial symbionts (Hoffmann et al. 2005, 2008, Schläppy et al. 2010b), which considerably contribute to the sponge host metabolism (Weisz et al. 2007, Maldonado et al. 2012 and references therein). Therefore, and consistent with the suggestions of Hoffmann et al. (2005), H. panicea may perhaps cease its pumping activity to establish internal body anoxia to maintain its endosymbiotic community (Althoff et al. 1998, Wichels et al. 2006, Schneemann et al. 2010). Bacteria isolated from H. panicea are potential sources of neuro-active compounds (Perovic et al. 1998), suggesting that sponge-associated microbes also play a functional role in coordinating contractile behavior of the sponge host (Meech 2008, Leys 2015).

The present observed relationship between sponge osculum dynamics and filtration further indicates that asynchronous closure of oscula, as observed in sponges with numerous oscula (Parker 1910, Pfannkuchen et al. 2009, Riisgård et al. 2016), may result in a spatially and temporally heterogeneous supply of oxygen (Schläppy et al. 2010a, Lavy et al. 2016), as well as of food particles.

Filtration of single-osculum explants

The mean filtration rate of a ‘standard’ explant was 0.28 ± CI95% 0.06 ml min⁻¹ (Fig. 2 & Table S1 in Supplement 1), corresponding to a mean volume-specific filtration rate of 15 ml min⁻¹ (cm³ sponge)⁻¹ which is 2.5 times higher than the volume-specific filtration rate recently determined in a much larger specimen of H. panicea with numerous oscula (Riisgård et al. 1993, 2016, Thomassen & Riisgård 1995). Riisgård et al. (2016) observed highly variable filtration rates of H. panicea as a consequence of changing flow-through that was found to be correlated with asynchronous closure of 2 (up to 9) adjacent oscula so that the measured filtration rate reflects a mean value for a sponge with multiple open and closed oscula. In sponges, oscula are associated with aquiferous modules (Fry 1979, but see Ereskovskii 2003), and their number, including new inhalant and exhalant canal systems, increases with sponge growth (Bergquist 1978). Pumping rates of a sponge individual with numerous exhalant openings, such as the freshwater sponge Baikalospongia bacillifera, are highly variable, but exhalant flow velocities are maintained within the range of 1 cm s⁻¹ among oscula (Patterson et al. 1997). Hartman & Reiswig (1973) suggested that coordinated and asynchronous closure of oscula allows multiple-oscula sponges to direct the water current through the more complex but continuous aquiferous system. This may enable intertidal and shallow-water sponge species, such as H. panicea, to continue water pumping and filtration, albeit at reduced rates, during periods of ebbing tides when parts of the sponge body are exposed to air (Hartman & Reiswig 1973).

The present study reveals volume-specific filtration rates of single-osculum explants that are comparable to but within the upper range of those measured in large, multi-oscula sponges (Reiswig 1974, 1975, 1981, Riisgård et al. 1993, Thomassen & Riisgård 1995, but see Weisz et al. 2008). Filtration rates of
sponges with numerous oscula are further strongly influenced by coordinated and asynchronous contractile behaviors (Parker 1910, Pfannkuchen et al. 2009, Riisgård et al. 2016). Using single-osculum explants in the present experimental setup (see Fig. S2 in Supplement 1), we were nevertheless able to determine the relationship between osculum opening dynamics and the filtration activity of small individuals of *H. panicea*.

**CONCLUSIONS**

The present study shows that small single-osculum explants are well-suited for exploring the relationship between sponge physiology and behavior, which is more difficult to assess in larger sponges with numerous oscula. We found a clear relationship between osculum opening degree and filtration rate in the explants. From this relationship, we calculated that explants maintained a constant jet speed of 2.3 ± C195%, 0.13 cm s⁻¹, which probably reflects some basic features of the sponge pump to be addressed in future studies. The present findings show that oscula dynamics can explain commonly observed temporal variability in sponge filtration rates, and further may be associated with contraction−inflation events of the sponge body and canal system. In the present study, osculum dynamics of *H. panicea* were apparently not triggered by environmental factors and may, therefore, have been driven by internally generated stimuli.

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**LITERATURE CITED**


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