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Re-plumbing in a Mediterranean sponge

D. Mendola^{1,2,*}, J. G. M. van den Boogaart²,
J. L. van Leeuwen² and R. H. Wijffels¹

¹Food and Bioprocess Engineering, and ²Experimental Zoology, Wageningen University, PO Box 8129, 6700 EV, Wageningen, The Netherlands

*Author for correspondence (dominick.mendola@wur.nl).

Observations are reported for *Dysidea avara* sponges where once functioning oscula (outlets) are converted through internal re-plumbing into functioning oversized ostia (OSO; inlets). Flow tank studies employed high-speed photography and particle tracking of laser-illuminated 0.5–6.0 µm diameter glass beads to trace particles streaming into OSO. A fluorescein dye/glass bead uptake experiment showed that an oversized ostium was connected through internal structures to the lone osculum. Beginning 30 s after uptake and continuing over a 20 min period, dye streamed from the osculum, but no beads emerged. Scanning electron microscopy revealed that beads were deposited only on the inhalant side of particle filtering choanocyte chambers and not on the exhalant side, suggesting that internal re-plumbing had occurred. Functioning OSO were also found on freshly collected specimens in the field, making it highly unlikely that formation of OSO was only an artefact of sponges being held in a laboratory tank.

Keywords: sponges; oversized ostia; re-plumbing

1. INTRODUCTION

Sponges filter bacteria, microalgae and other small particles from ambient water as food. Water is drawn in by flagellar motion through thousands of 5–50 µm ostia (size is species dependent). Inflow passes through inhalant canals to particle filtering choanocyte chambers, and then through exhalant canals to millimetre-sized (or larger) surface openings called oscula (Bergquist 1978).

Dysidea avara (Schmidt) is a spongin-supported demosponge (order: Dictyoceratida) found in low flow cryptic habitats of the rocky sublittoral of the middle and western Mediterranean basins (Uriz *et al.* 1992). Normal-sized ostia range from 27 to 31 µm in diameter grouped into clusters of 15–20. Cluster groups are located between dermal reticulations radiating from the tips of conules formed by uplifting of the surface membrane by major skeletal fibres (Teragawa 1985). Oscula ranging from 1 to 10 mm in diameter are generally found on elevated and conulated protuberances, and have raised cylindrical membranes that open/close to control outflow rate (figure 3 in the electronic supplementary material).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2007.0357> or via <http://www.journals.royalsoc.ac.uk>.

Remodelling in sponges is the process by which individuals change body forms (including the positions of ostia and oscula) in response to changes in environmental factors (Simpson 1984). Here, we report evidence that *D. avara* is able to convert oscula into functioning oversized ostia (OSO; 1.5–2.5 mm in diameter) by internal re-plumbing, i.e. old exhalant canals are converted to new inhalant canals and vice versa.

2. MATERIAL AND METHODS

Nine small-sized (15–50 cm³) *D. avara* specimens were collected at 9–12 m depth on pieces of native rock from the L'Escala region in northeastern Spain. Specimens were transported to The Netherlands and maintained in a 550 l aquarium with biofiltration. Over the entire three months period of this study, sponges were fed two times a day with marine broths made from fish and shrimps, and/or manufactured shellfish diets (INVE, Belgium CAR-1), and three to four times a week with cultured microalgae (*Phaeodactylum tricornutum*, *Nannochloropsis* sp.); initial concentrations were 2×10^{-5} to 3×10^{-5} particles ml⁻¹.

Inflow/outflow velocities, flow rates and local background velocities (LBV) were derived using particle tracking velocimetry (PTV) in a 7.5 l flow tank (figure 4 in the electronic supplementary material). A mixture of 0.5–6.0 µm in diameter hollow glass beads (J. J. Bos, NL) was seeded onto the flow tank (approx. 100 000 particles ml⁻¹) to reflect laser light and trace flow streamlines entering OSO and exiting oscula. A laser light sheet (0.5 mm width) was projected inside the flow tank from an Excel 2300 laser. High-speed photography (50–500 fps) employed a Redlake digital camera, Nikon 105/1 : 2.8 lens and 50 mm extension ring. Images were captured onto a PC using Redlake's Midas Player and analysed using MATLAB subroutines. We estimated the accuracy of determination of outflow rates at ±8% and inflow rates at ±14% (see methods addendum in the electronic supplementary material). Flow tank experiments in Spain were conducted in an indoor laboratory 150 m from the collection site. Local seawater and ambient conditions were used.

To trace inflow and outflow of specimens and test for particle retention, a fluorescein dye (Aldrich F2456, 95%) and glass bead uptake experiment was performed. A 15 cm³ specimen with one osculum and three OSO was selected (DA-7, table 1 in the electronic supplementary material). The tip of a 0.9 mm bore hypodermic needle was positioned close to OSO-1. Glass beads were mixed in a fluorescein dye/seawater solution (approx. 1×10^6 beads ml⁻¹) and loaded into a syringe attached to the needle. Beginning at t_0 , and spaced 2–3 min apart, three small increments of dye/bead suspension were carefully expelled from the needle directly in front of OSO-1. A video camera and laser (and normal light) were used to visualize and record movies of the fluorescein dye/glass beads being sucked into the OSO and exiting the osculum.

Specimen fixation and SEM methods followed Johnston & Hildemann (1982). After fixation, the entire specimen was frozen in 100% ethanol/liquid nitrogen and cryofractured. The first fracture was on the centreline of the filmed OSO-1 structure and in-line with the osculum, and the second by splitting one of the first halves on the centreline of one of the two remaining OSO. One part from the second fracture broke into two, which resulted in four pieces. While still in 100% ethanol, excess material was trimmed away. The four pieces were dried in a critical point dryer (Balzers) and then glued onto sample holders using conductive carbon cement. Each sample was sputter coated with 20 nm platinum and viewed using a field emission scanning electron microscope at 4 kV (JEOL 6300 F, Tokyo, Japan).

3. RESULTS

(a) Morphological changes and flow rates

Within days of introduction into the 550 l aquarium, six of nine specimens resorbed most of their oscular membranes but not all of them. For three sponges where oscular membranes had been resorbed, 1.5–2.5 mm circular or oval holes remained. Pinacoderm grew to cover some holes, and in other cases holes remained uncovered. These three sponges maintained some holes for their entire three months in the aquarium. Within days to weeks, new oscula developed elsewhere on their body surfaces.

After four weeks in the 550 l aquarium, flow tank studies were conducted with the three specimens with

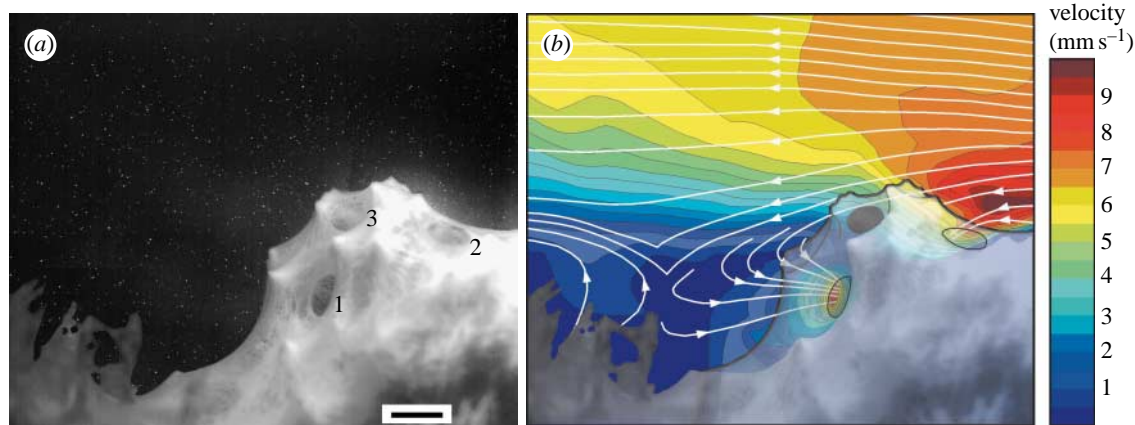


Figure 1. (a) Normal light photograph of *D. avara* sponge showing three OSO (1–3) superimposed onto a high-speed video image showing laser-illuminated glass beads in the background. (b) Computed speed distributions (colours) and streamlines (white) around sponge which is superimposed semi-transparently onto calculated speed distributions. Inflow is visible only for ostia 1 and 2, given that ostium 3 was not illuminated by the laser light sheet. Scale bar, 2 mm (movie in the electronic supplementary material shows beads entering OSO).

large holes on their surfaces. A total of 49 inflow (into OSO) and 93 outflow (from oscula) PTV experiments were performed (table 1 in the electronic supplementary material). For one sponge with residual holes (sponge DA-7), three holes had been converted into functioning OSO, i.e. beads were seen being drawn into the OSO. Figure 1a shows the three OSO on the surface of DA-7. Figure 1b shows PTV-derived streamlines and flow speed distributions around this sponge and into the OSO. Spatially averaged input velocities were $7.2 \pm 2.7 \text{ mm s}^{-1}$ for OSO-1 and $10.7 \pm 1.5 \text{ mm s}^{-1}$ for OSO-2 (see details of velocity and flow rate calculations in the electronic supplementary material). LBV upstream of the sponge averaged 7 mm s^{-1} , whereas velocities in the lee of the protuberance with the OSO were reduced by morphology-induced drag to $1\text{--}4 \text{ mm s}^{-1}$ (figure 1b).

Calculated spatially averaged inflow rates were $18.3 \text{ mm}^3 \text{ s}^{-1}$ for OSO-1 and $19.4 \text{ mm}^3 \text{ s}^{-1}$ for OSO-2. Beads had been seen entering OSO-3; therefore, it was assigned the average inflow rate of the two filmed ostia. Accordingly the combined inflow rate of the three OSO was estimated at $55\text{--}60 \text{ mm}^3 \text{ s}^{-1}$. Less than an hour earlier, we had measured the outflow rate for the osculum located on the same protuberance at $108.4 \text{ mm}^3 \text{ s}^{-1}$. We assumed that local ostia served the local osculum; therefore, the three OSO contributed approximately 50% of the oscular outflow. Concurrently, the sponge possessed two oscula, the second was on the other protuberance. Its outflow rate was $96.5 \text{ mm}^3 \text{ s}^{-1}$. Therefore, the three OSO contributed approximately 28% of the total flow rate through the sponge. In keeping with the principle of continuity, the remaining 72% was provided by fields of normal-sized ostia.

Large surface holes were observed on many *D. avara* sponges *in situ* in Spain. Of 12 specimens collected for flow tank studies, 7 were pumping sufficiently to allow recording oscular outflow movies. Two of the seven were checked for OSO and inflow of particles. On one sponge (DA-R, table 1 in the electronic supplementary material), we found and filmed one functioning OSO. Calculated spatially

averaged inflow velocity was $1.7 \pm 0.4 \text{ mm s}^{-1}$, average inflow rate $5.7 \text{ mm}^3 \text{ s}^{-1}$ and LBV 0.5 mm s^{-1} .

(b) *Fluorescein dye/glass bead uptake experiment*

Three releases of fluorescein dye/bead solution (each approx. $25 \mu\text{l}$) were performed over a 10 min period. Less than 30 s elapsed between the first release and when the dye began to exit the osculum, indicating a functional connection to OSO-1. We estimated that 10% of each release ($2.5 \mu\text{l}$) escaped the suction of OSO-1. These portions were carried downstream by the background flow (LBV = 2.9 mm s^{-1}) and diluted in the 7.5 l of $0.2 \mu\text{m}$ filtered seawater in the flow tank (dilution: approx. 1 bead ml^{-1}). Given this high dilution factor, it is not surprising that neither the dye nor the beads were observed being sucked into normal-sized ostia (figure 2a).

The spatially averaged inflow velocity was $1.3 \pm 0.2 \text{ mm s}^{-1}$ and inflow rate was $5.5 \text{ mm}^3 \text{ s}^{-1}$. Approximately 20 min into the experiment, the camera was focused onto outflow from the osculum. Two 10 s movies were recorded: one using laser illumination and the other using normal light. No glass beads were filmed within the green dye plume exiting the osculum. This indicated that the OSO was connected via internal structures to the only osculum, and glass beads were being completely retained within the sponge. Since after 20 min no glass beads had been seen exiting the osculum, the experiment was terminated and the sponge fixed for SEM.

(c) *SEM images*

We made an oblique cut across the inhalant canal leading directly from OSO-1 down into the sponge. Although no SEM pictures were made of the entire canal, a few glass beads were seen and imaged. Glass beads were deposited on internal structures of only those pieces produced from cryofracturing across OSO-1 (where beads were administered) and then only on the prosopyle (inlet) side of the choanocyte chambers and not on the apopyle (outlet) side. This suggests that the sponge re-plumbed some of its

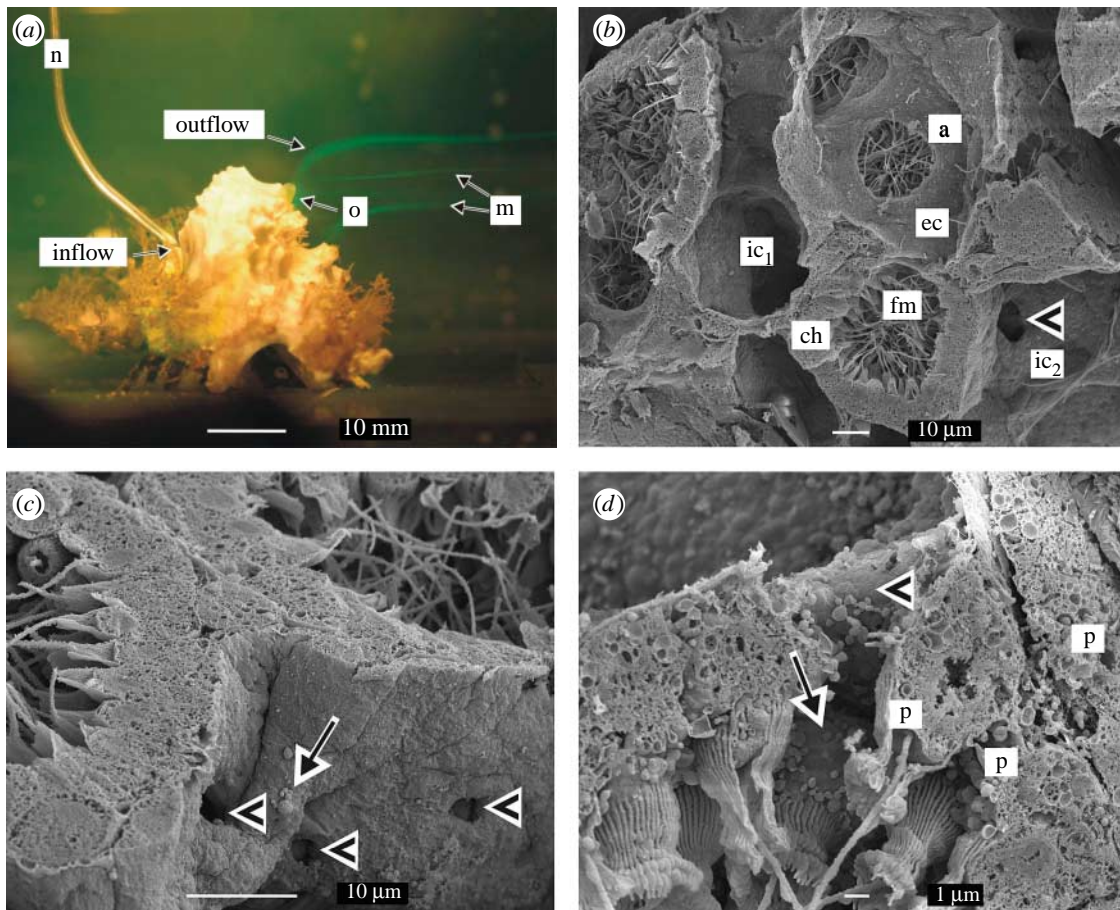


Figure 2. (a) Fluorescein dye experiment. Normal light digital photograph of sponge DA-7 as fluorescein dye began to exit the osculum. (b) SEM digital image of internal anatomy of sponge DA-7. (c) Inhalant canal and prosopyles (inlets to choanocyte chambers); see enlarged image of beads in the electronic supplementary material [figure 2c](#). (d) Glass beads deposited on inhalant side of choanocyte chamber. *Note.* Partially torn prosopyle (artefact of cryofracture) with glass beads deposited onto the prosopyle wall and at the base (inhalant side) of choanocyte collars; a, apophyle (exit of choanocyte chamber); ch, typical cryofractured choanocyte chamber showing mass of tangled flagella; ec, exhalant canal; fm, flagellar mass; ic₁, inhalant canal; ic₂, inhalant canal leading to prosopyle; m, streams of dye which escaped the suction of the ostium; n, hypodermic needle; o, osculum; p, phagosomes with ingested glass beads; arrow heads, prosopyle openings that lead into the choanocyte chambers; arrows, glass beads.

inhalant canals to connect with OSO-1 and at least some of its choanocyte chambers ([figure 2b–d](#); arrows). No glass beads were observed within the masses of tangled flagella of any of the seven choanocyte chambers imaged ([figure 2b](#)).

4. DISCUSSION

Remodelling in sponges (i.e. subtle or radical changes in the body form) is a well-studied phenomenon triggered by environmental changes including temperature, salinity, other stressors or age and reproductive imperative ([Van de Vyver & Willenz 1975](#); [Fell *et al.* 1989](#); [Cerrano *et al.* 2001](#)). We subjected *D. avara* sponges to the cumulative stress of collection, long distance transportation and introduction to an artificial environment. Shortly thereafter, they underwent external and internal remodelling and produced functioning OSO from resorbed oscula. We also showed the existence of functioning OSO in freshly collected individuals making it highly unlikely that such structures developed purely as a consequence of collection, transport and culture stress. We hypothesize that such structures are formed opportunistically in

response to environmental triggers as a mechanism for increasing food particle ingestion rates.

Based on the results of our fluorescein dye/glass bead uptake experiment, we concluded that it was highly improbable that more than a few of the glass beads deposited within the choanocyte chambers and inhalant canals imaged entered the sponge through normal-sized ostia, given the extremely low density of stray beads in the tank coupled with the short time period of the experiment, and the fact that only choanocyte chambers in-line with the osculum and OSO were imaged. Glass beads were also seen within phagosomes of choanocytes ([figure 2d](#); p) indicating that ingestion had occurred during the 20 min experiment (supported by the findings of [Turon *et al.* \(1997\)](#) who fed latex beads to *D. avara*, and [Leys & Eerkes-Medrano \(2006\)](#) who fed latex beads to a syconoid calcareous sponge).

Although we cannot fully exclude that some of our quantifications of inflow and outflow might have been influenced by friction boundary layers (FBLs) generated in the flow tank, calculations showed that all velocity measurements were made well outside the calculated FBLs.

This work opens the door for more thorough follow-on studies designed to identify the exact triggers for OSO formation and test their quantitative effect on filtration rate in *D. avara*, and possibly other sponges as well.

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