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Oxygen dynamics and flow patterns of *Dysidea avara* (Porifera: Demospongiae)

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The present publication presents oxygen properties and pumping behaviour of *Dysidea avara*. Oxygen profiles were measured near and inside the atrial space of the osculum with a Clark-type micro-electrode. Pumping sponges had profiles with oxygen concentrations marginally lower than that of the aquarium water. In contrast, diffusive profiles, with a clear boundary layer above the sponge surface, and oxygen penetrating only 0.5 mm into the sponge tissue, were typically that of a sponge which was not pumping. Diffusive oxygen flux at the sponge surface was 4.2 µmol O_2 cm^{-2} d^{-1} and the calculated volumetric filtration rate was 0.3 cm^3 water cm^{-3} sponge min^{-1}. The oxygen concentration in the osculum was temporally fluctuating between 95 and 59% saturation at a frequency of approximately once per minute. The combination of static oxygen micro-electrode measurements and particle tracking velocimetry (PTV) allowed us to simultaneously observe fine-scale oxygen fluxes and oscular flow patterns in active sponges, even at extremely low pumping rates. Oscular oxygen concentration and flow were correlated but not always synchronous to the second. Particle tracking velocimetry was used to visualize the flow field around the sponge and to distinguish sponge-generated flow from the unidirectional current in a flow-cell.

INTRODUCTION

Sponges are filter-feeders and transport food particles, oxygen and waste products in the currents they generate and which pass through their bodies in characteristic aquiferous systems made of pores and canals. The fine spatial and temporal variability of oxygen conditions within sponges is of significant interest in sponge physiology and can be effectively measured using micro-sensors. For example, Gatti et al. (2002) used micro-optodes to study the oxygen supply in primmorphs (multi-cellular aggregates made from sponge dissociated single cells (Müller et al., 1999)) and adult specimens of *Suberites domuncula*. Oxygen micro-electrodes have also been successfully used to measure the photosynthetic activity of sponge symbionts in *Cliona nigricans* and *Cliona viridis* (Schönberg et al., 2005). Oxygen micro-electrodes have further been used to explore the nature of fine-scale oxygen dynamics in the cold water sponge *Geodia baretti* (Hoffmann et al., 2005a) and in its explants (Hoffmann et al., 2005b), where anoxic regions inside the sponge body were shown for the first time, throwing new light onto the internal micro-environments of sponges. A study of the oxygen dynamics of *Aplysina aerophoba* (Hoffmann et al., in preparation) showed that a non-pumping sponge becomes anoxic after 15 minutes, and relies on diffusional processes for oxygenation. In contrast, pumping individuals of *A. aerophoba* were subject to advectional forces, making diffusion insignificant.

Sponges may switch to an anaerobic metabolism (Grieshaber et al., 1994), thus providing a temporary niche for anaerobic micro-organisms. During such a switch, the presence and degree of activity in sponge-associated microbes are likely to be strongly controlled by oxygen. In this study, *Dysidea avara* Schmidt, 1862, a common Mediterranean species, is used as representative of a sponge with a massive growth form. We used high resolution oxygen micro-electrodes to determine fine-scale oxygen dynamics, while simultaneously visualizing overall flow patterns and quantifying sponge exhalant flow speed with particle tracking velocimetry (PTV).

MATERIALS AND METHODS

Sampling

Dysidea avara is a small sponge with massive morphology and a conulose surface which allows easy recognition of oscula (Figure 1). This species is common, easily available and has desirable morphological characteristics for micro-electrode work such as a body mostly occupied by a well developed aquiferous system and a soft spongin skeleton (Galera et al., 2000) which did not impede the experimental methods.

Several specimens of *D. avara* ranging from 3 to 20 cm^3 were collected in 10–15 m water depth in Muntanya de Montgó, Punta del Romani at Cala Illa Mateua in the township of l'Escala, Girona, Spain (42°06.863'N

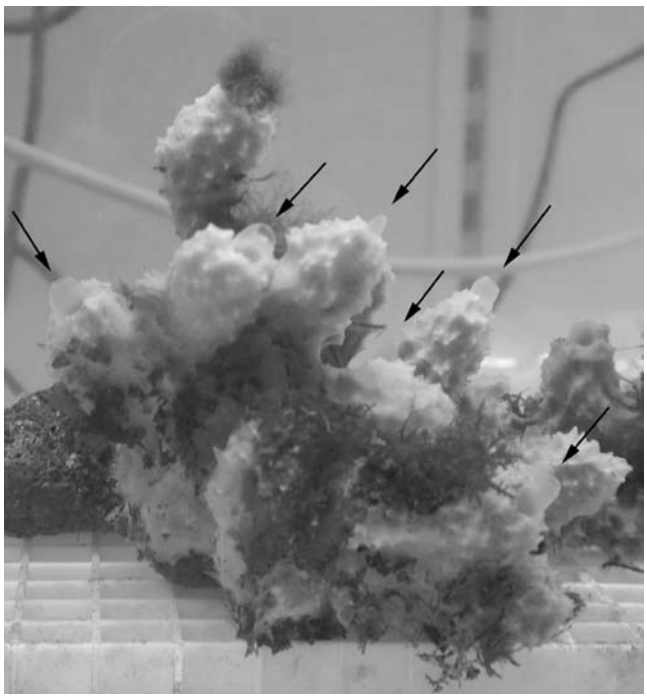

Figure 1. *Dysida avara* specimen in aquarium showing the prominent oscules (arrows).

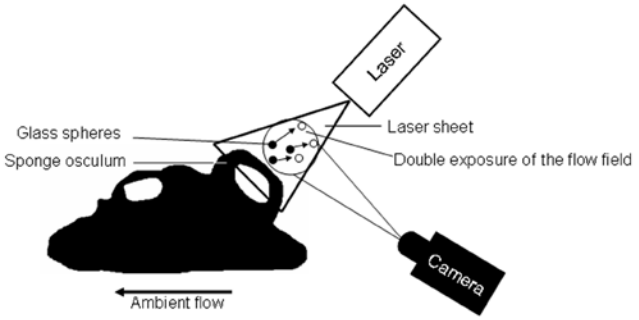

Figure 2. Sketch showing the location of the laser sheet in relation to the camera and the sponge. The distance between the spheres at time 1 (dark spheres) and at time 2 (white spheres) were calculated from two pictures taken from the interrogation area.

03°10.116'E), by SCUBA diving, at an ambient temperature of 20°C and an ambient salinity of 37.4 parts per thousand (ppt). Sponges were kept in an 85-l aquarium for one month before experiments commenced at the University of Wageningen (the Netherlands). The holding aquarium was filled with 50% natural seawater from the sampling site and 50% artificial seawater made by mixing 37 g/l into reverse osmosis water using Reef Crystals salts (Aquarium Systems, Sarrebourg, France). After the initial filling of the aquarium, 5% was changed per week with the artificial seawater (first drain, then re-fill). Over time, the natural seawater was nearly completely diluted out of the system. The total re-circulating volume was approximately 150 l. Temperature and salinity were adjusted to the ambient conditions at the sampling location: temperature was maintained at 20°C (±0.3°C), and salinity at 37.4 ppt (±0.2–0.8 ppt). Aeration was provided through air stones, airlifts and continuous water recirculation driven by a pump. The oxygen in the water within the aquarium was always maintained at close to air saturation levels. Sponges were fed twice daily with marine broths made from fish and shrimps, and/or manufactured shellfish diets (INVE, Belgium CAR-1), and three to four times weekly with cultured *Phaeodactylum tricornutum*, *Nannochloropsis* sp. (2–3×10^5 particles/ml).

Oxygen micro-electrode measurements

A Clark-type oxygen electrode (Revsbech, 1989) with a 20 µm tip diameter was made at the Max Planck Institute for Marine Microbiology, Bremen, in Germany and used for all measurements in the present study. A two point calibration was made using oxygen saturated seawater from the holding tank and anoxic seawater made by addition of an oxygen scavenger (sodium sulphite). Care was taken to obtain the zero value as quickly as possible so as to avoid 'poisoning' of the sensor by a protracted stay in the sodium sulphite solution.

Oxygen profiles

A total of 51 oxygen profiles in 0.2 mm increments was made from nine sponges (three replicate profiles per location and some individuals were measured at more than one location). The profiles of four individuals were selected for their representativeness of a range of *D. avara* tissue oxygenation. Profiles started at 2–1.5 mm above the sponge surface down to 2–3 mm depth in the sponge body. Before profiling commenced, a sponge specimen was transferred from the holding tank to the experimental 8-l flow-cell. Unidirectional flow was produced through a standard aquarium pump which recirculated the seawater originating from the sponge holding tank. The water in the flow-cell was aerated with an air stone, kept at 20°C through periodical water replacement and no food was added. The micro-electrode was attached to a computer-controlled micro-manipulator and lowered three times within 20 min at the same location in the sponge body without delay between single profiles. The sensor penetrated the sponge easily so no pre-piercing of the body was necessary as is sometimes required in other sponge species (Schönberg et al., 2004). There were no recognizable signs of stress in the experimental animals. From diffusive oxygen profiles over the sponge surface, oxygen fluxes were calculated based on Fick's 1st law of diffusion.

Static oxygen measurement

Another individual than those used for profiles was selected for continuous oxygen concentration measurements at a fixed location into the atrial space of an osculum. To assess the nature of sponge tissue oxygenation over time, oxygen concentration was recorded every second for 20 min. The oxygen micro-electrode was inserted 2 mm into an open osculum and was left in the atrium without ambient water flow, under stable salinity and temperature conditions.

Particle tracking velocimetry

Particle tracking velocimetry was carried out for 148 s at the same time as the static oxygen measurement (and on the same sponge) to enable quantitative estimation of oscular

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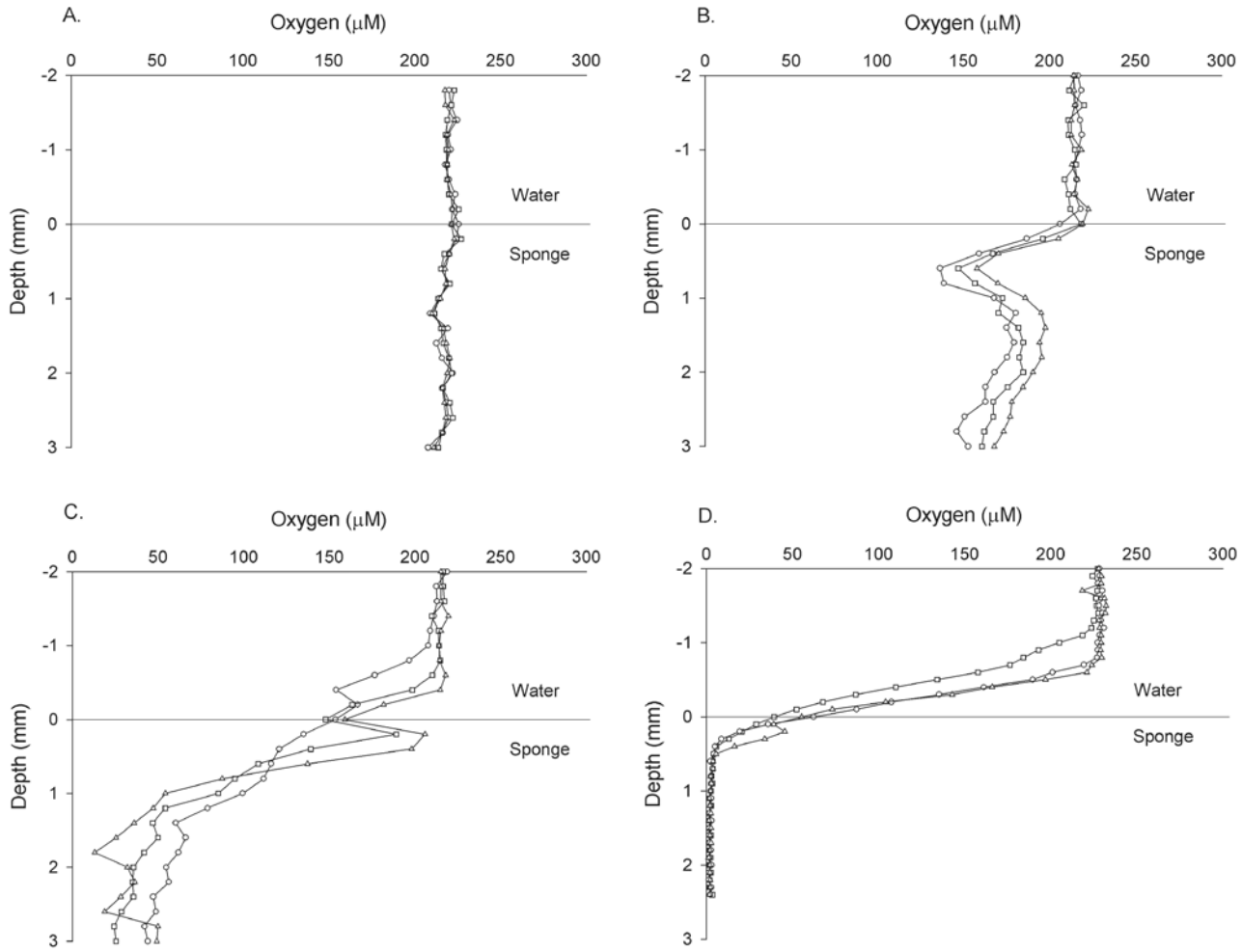

Figure 3. Oxygen profiles above and within *Dysidea avara*. (A) Oxygen profile of a pumping specimen, showing oxygen-saturated water above and within the sponge; (B) oxygen profile of a mildly pumping specimen; (C) oxygen profile of a weakly pumping specimen; and (D) oxygen profile of a non-pumping *D. avara*, showing oxygen-saturated water above the sponge surface, a strong and gradual decrease in the diffusive boundary layer, and very low oxygen concentrations within the sponge tissue, reaching almost anoxic conditions at 0.5 mm. Circles represent the first profile (of the set of three), squares the second and triangles the third. Each profile is a unique data set showing oxygen saturation at a particular location within the sponge at a particular second. The error bars are not shown as they would only represent the accuracy of the micro-electrode measurement.

flow speed by following the trajectory of individual particles in the water over time. The particles used were neutrally buoyant hollow glass spheres of 10 μm diameter. The flow-cell water was periodically spiked with fresh seeding particles because as the sponge pumped, it retained a portion of the spheres, thus gradually decreasing their density in the flow-cell.

During PTV, the particles were illuminated with a diode laser (Lasiris LAS-670-30) equipped with line generating optics (LAS-1 line-20 TS). The 0.2 mm wide sheet of light was aligned perpendicular to the sponge surface, crossing the centre of the sponge osculum, and being oriented in parallel to the flow-cell unidirectional flow (Figure 2). The particles moving in the path of the laser sheet were recorded with a charge-coupled device (CCD) camera (Sony XCD-X710) at 7 frames per second. An interrogation area was chosen adjacent to the osculum out of which particles were tracked over several picture frames to depict the spheres' movement near the sponge's surface and out of the exhalant current. Their velocity was determined by measuring the distance between the same particles on subsequent images over 148 s. Simultaneously with PTV, oxygen concentrations were measured by placing the micro-electrode in the atrium of the sponge osculum, as close as possible to the laser sheet. Moreover, once the static oxygen measurement was completed, the flow-cell flow was restored and PTV was used on a different sponge to enable qualitative visualization of two-dimensional sponge and ambient flow fields. From these images a mean oscular flow velocity was calculated from the six sets of seven spheres (over 1 s) closest to the osculum.

RESULTS

Four selected oxygen profiles describe a variety of tissue oxygenation in *Dysidea avara* (Figure 3A–D). The oxygen concentration in the body of four *D. avara* specimens ranged from fully oxic (Figure 3A) through intermediate

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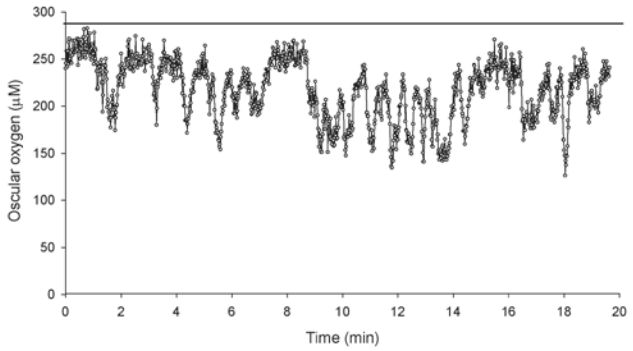

Figure 4. Continuous oxygen micro-sensor measurement in an osculum of *Dysidea avara* over a time span of 20 min, showing temporal fluctuations and where each data point represent the oxygen saturation at that particular second. The concentration of oxygen in the overlaying water is shown as a straight line.

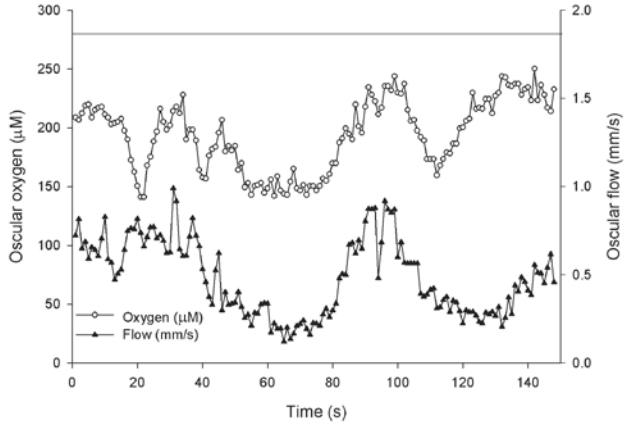

Figure 5. Oxygen and flow velocity measured simultaneously in the atrium of an osculum of *Dysidea avara*. Internal oxygen concentration and oscular flow velocity were occasionally decoupled, but mostly synchronous and approximately in accordance with one another (regression with N=148, r^2=0.17, df =1 (degrees of freedom), F=0.0001, P=0.05). The concentration of oxygen in the overlaying water is shown as a straight line.

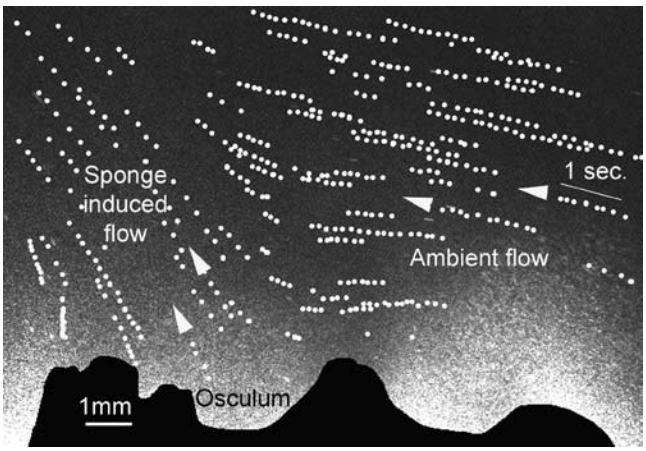

Figure 6. Multiple image overlay showing typical particle velocimetry above the surface of a *Dysidea avara* specimen and near the sponge's osculum, over the duration of several seconds. Ambient flow is almost horizontal from right to left while sponge induced flow is more vertical. Seven spheres in a line =1 s.

stages (Figure 3B&C) to anoxic (Figure 3D). One sponge had well-oxygenated tissue (Figure 3A) and, at 3 mm tissue depth, oxygen concentrations still reached 99.4% of the fully saturated flow-cell water. The oxygen content in this sponge remained stable over the 20 min required to carry out the measurements, and yielded three nearly identical profiles (Figure 3A).

In two other individuals, intermediate oxygen concentrations were present, and changed over the 20 min required to make the profiles. The oxygen concentration in the second individual increased over time, but retained the same characteristics, namely a sharp decrease of oxygen concentration just below the sponge surface down to 1 mm depth, followed by oxygen concentration approaching saturation at 2 and 3 cm depth (Figure 3B). The oxygen profile in the third individual showed a mixture of increasing oxygen concentration over time in the first 1 mm of sponge tissue, whereas oxygen concentration in the tissue below 2 mm decreased, approaching anoxia, over the course or the 20 min required to make the profile (Figure 3C).

In the fourth specimen, the water above the sponge was fully oxygen-saturated but oxygen concentrations then strongly and steadily decreased in the upper 0.5 mm above the sponge surface, showing the presence of a boundary layer. Oxygen concentrations continued to decrease with distance from the sponge surface and reached almost anoxic conditions in ≥0.5–2 mm tissue depth (Figure 3D). In the hypothetical case of a *D. avara* specimen being anoxic for 24 h, this would translate into an oxygen flux over the sponge surface of 4.22 µmol O_2 cm^{-2} d^{-1}, and an oxygen consumption rate of 84 µmol cm^{-3} d^{-1}. The pumped volume, which would be necessary to balance this respiration rate to prevent tissue anoxia, was calculated to be 373 cm^3 water cm^{-3} sponge d^{-1} (0.26 cm^3 water cm^{-3} sponge min^{-1}).

Static oxygen measurements in the oscular atrial space of the first sponge showed additional temporal variation in oxygen concentrations. When measured over 20 min without ambient flow, the atrial oxygen concentration oscillated between near fully oxygenated with 269 µmol l^{-1} ±1.3 SE (95% saturated) to lower concentrations of 167 µmol l^{-1} oxygen ±3.7 SE (59% saturated), while the oxygen in the ambient water remained at 283 µmol l^{-1} (Figure 4). De-oxygenated water was expelled from the sponge osculum approximately 25 times in 20 min. Those flushing events occurred over durations of 4–28 seconds (average 13 ±1 SE; see troughs on Figure 4), indicating a pumping rhythm interrupted by resting phases.

Simultaneous oxygen micro-electrode and PTV measurements showed a correlation between internal oxygen concentrations and oscular flow velocity, although those variables were not always synchronous to the second (Figure 5). The relationship between sponge pumping activity (particle speed mm s^{-1}) and oxygen concentration (µM) in the sponge tissue was statistically significant (regression with N=148, r^2=0.17, df=1, F=0.0001, P=0.05). Flow velocity out of the osculum was between 0.12 and 0.99 mm s^{-1} with an average of 0.48 mm^{-1} (±0.04 SE).

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Particle tracking velocimetry allowed the two-dimensional visualization of sponge oscular and ambient flow in a *D. avara* specimen kept in a flow-cell with unidirectional flow. Glass spheres carried in the cell's flow were deflected by the sponge-induced exhalant current, which changed the direction of the sphere movement from horizontal and parallel to the sponge surface to nearly vertical (Figure 6). The calculated oscular flow speed for this specimen was 1.4 cm s^{-1} (±0.2 SE).

DISCUSSION

Four sets of oxygen profiles illustrated a range of oxygen concentrations in *Dysidea avara*. The profiles of the well-oxygenated *D. avara* specimen were characteristic of a pumping sponge (Hoffmann et al., in preparation; Hoffmann et al., in press), as the oxygen within the tissue was only marginally lower than that of the aquarium water (Figure 3A). This shows that the water passed through the sponge rapidly, that the residence time was short, and that the water was only slightly oxygen depleted through the respiration of sponge cells and sponge-associated microbes.

In two intermediate sets of profiles (Figure 3B&C) sponge tissue oxygenation lay between fully oxygenated and anoxic and showed some temporal variations. Sometimes, the sponge increased its pumping during the measurement of replicate profiles (Figure 3B). At other times, it switched from opening-up to shutting-down during profile measurements (Figure 3C).

In contrast, the anoxic specimen of *D. avara* was undoubtedly not pumping, because the profiles displayed a pronounced decrease in oxygen concentration from the fully oxygenated aquarium water to the sponge surface, and into the tissue (Figure 3D). Only the uppermost 0.5 mm of sponge tissue remained oxygenated, while the rest was anoxic. The presence of an unmistakable boundary layer also showed that the sponge tissue oxygenation was solely diffusional and typical for diffusively determined profiles (Hoffmann et al., 2005a; Hoffmann et al., in preparation). In that specimen, the interfacial flux was in the range of those found in other studies on non-pumping sponges, for example by Hoffmann et al. (in preparation) in *Aplysina aerophoba* which had an average surface flux of 4.6 μmol O_2 cm^{-2} d^{-1}. *Geodia barretti* explants showed a surface flux of 2.3 μmol O_2 cm^{-2} d^{-1} (Hoffmann et al., 2005b). The fact that the oxygen surface flux in the two Mediterranean sponges (*D. avara* and *A. aerophoba*) is higher (4.2 and 4.6 μmol O_2 cm^{-2} d^{-1}, respectively) than in the cold water sponge, *G. barretti*, may be explained by the water temperature in which the experiments were carried out, namely 20°C for *D. avara*, 18°C for *A. aerophoba* and 10°C for *G. barretti*.

The four sets of oxygen profiles in *D. avara* show that oxygenation patterns close to the sponge surface can either be the product of advection or diffusion. Similar profiles have been made in other species of sponges (Hoffmann et al., 2005b; Hoffmann et al., in preparation). This suggests that oxygenation near the surface may be more a reflection of both the animal's pumping behaviour and cell and associated-microbes respiration than of different morphology, size, or body density.

The estimated volumetric pumping rate of 0.26 cm^3 water cm^{-3} sponge min^{-1} which is necessary to maintain sufficient oxygen levels in *D. avara*'s body compares well to similar findings in *A. aerophoba*, where the minimum volumetric pumping rate was 0.2 cm^3 water cm^{-3} sponge min^{-1} (Hoffmann et al., in preparation). Thus, only low pumping rates are needed to maintain adequate oxygen supply in the upper layer of the sponge body. It is therefore unlikely that oxygenation is the main reason for the sponge pumping behaviour. Obtaining food through filtration or the need for dilution of internal waste products is more likely to be the reason for pumping unless the sponge has an intrinsic rhythm (e.g. *Tethya crypta*, Reisweig, 1974). Pumping behaviour for other reasons than acquiring oxygen is also demonstrated in the static oxygen measurement. In stagnant water, the sponge pumped approximately once per minute (Figure 4) although the water inside the sponge was far from anoxic and contained at least 59% of oxygen compared to saturated water. This sponge may have been trying to feed (although no food was added in the flow-cell) or to dilute its metabolic products through pumping.

Oxygen concentration in the osculum was partially correlated to flow velocity (measured through PTV), which explained 17% of the oxygen variability (Figure 5). The oscular flow velocity of our *D. avara* specimen was extremely low (0.48 mm s^{-1}). Whether the insertion of the micro-electrode, the handling of the experimental sponge or the prolonged stay in a holding aquarium may be responsible for such a low pumping activity is difficult to ascertain, but we have no evidence that the sponge was stressed. Although the oscular flow calculated from the specimen used for flow field images (Figure 6) was higher (1.4 cm s^{-1}), the values for *D. avara* in this study are much lower than the reported oscular velocity of other sponges that were studied *in situ*. For example, the Jamaican sponges *Mycale* sp., *Verongia gigantea* and *Tethya crypta* generated an average exhalant current velocity of 7.9, 12.8 and 17.3 cm s^{-1}, respectively (Reiswig, 1974). Pile et al. (1996) found that boreal *Mycale lingua* in the Gulf of Maine reached pumping velocities of 14 cm s^{-1}. Vogel (1977) reports pumping rates of 7.5 to 22 cm s^{-1} in several Caribbean sponge species studied in the field.

The differences in oscular velocity between *D. avara* and those sponges may be explained by the sponge captivity, by the amount of food in the water or by intrinsic rhythms. The triggers responsible for the onset or discontinuation of pumping activity are still poorly understood and are likely to vary with species, environmental conditions, sponge morphology and oscula size. Oscular flow speeds have also been reported to vary within (Savarese et al., 1997) and among (Gerodette & Flechsig, 1979) individual sponges and between locations (Pile et al., 1996).

The various oxygen profiles, the static oxygen measurements and oscular flow velocities of this study show that the study sponges had means of finely regulating their pumping activity which was, in turn, partially reflected in the oxygen concentrations in the sponge. The effects of the most likely triggers for pumping activity (feeding, dilution of waste products and intrinsic rhythms) on oxygen concentrations within this species remain to be explored. Particle tracking velocimetry enabled the direct measurement of oscular

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flow in the aquarium even at very low speed. Coupled with microsensor measurements, the non-invasive PTV could be used to further explore the effect of food availability on flow and oxygenation of sponges in captivity. In this respect, our data represent a stimulating starting point for further investigations in the physiology of sponges in *ex-situ* situations.

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