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Heterogeneous oxygenation resulting from active and passive flow in two Mediterranean sponges, *Dysidea avara* and *Chondrosia reniformis*

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Abstract

The oxygen dynamics and pumping behavior in *Dysidea avara* and *Chondrosia reniformis* (Porifera, Demospongiae) were investigated using oxygen microelectrodes and heated thermistor flow sensors. Both field and laboratory experiments showed the common occurrence of low oxygenation approaching anoxia in both species, lasting up to 1 h. Strong temporal and spatial heterogeneity of oxygen concentrations were observed with replicate oxygen profile series across the sponge surface, though tissue close to an osculum was generally better oxygenated than deeper in the sponge body. Because of observed lag times between a pumping event and the respective oxygenation response, the state of oxygenation of sponge tissue could only be partially attributed to its pumping activity. Ambient flow also influenced oxygenation patterns of sponges. Larger individuals possessing a functional aquiferous system regulated their pumping activity according to the ambient flow regime, whereas a small *D. avara* sponge, yet to possess its first osculum, was passively oxygenated by ambient flow and became anoxic approximately 30 min after ambient flow was stopped in its laboratory tank. These studies showed (1) sponge tissue metabolism switched frequently from aerobic to anaerobic, (2) temporally and spatially dynamic oxygen-depleted regions were commonly found within those sponges, both in captivity and in the field, and (3) tissue oxygenation was regulated both by active behavior (pumping) and passive environmental events (ambient water flow). We concluded that the metabolism of both sponge cells and sponge microbes will be influenced by the sponges' ability to control oxygen concentrations in different regions of its body at any particular time. In addition, when a sponge is actively pumping in a particular region of its body, higher oxygen concentrations will favor aerobic symbionts and aerobic metabolism, whereas when active pumping ceases, anaerobic symbionts and anaerobic tissue metabolism will be favored.

Sponges are sessile marine filter-feeders. They possess an aquiferous system (multibranching inhalant and exhalent canals) that allows them to draw oxygenated and food-laden ambient water into their bodies through thousands of micron-sized inhalant openings (ostia) and expel waste-water through fewer, larger exhalent openings (oscula). Sponge cells show a low degree of specialization and a high degree of independence so that the sponge body, in some respect, resembles a protist colony. However, sponges are without any doubt placed as true members of the Metazoa, and are considered as one of the oldest animal phyla represented by living members in both marine and aquatic fauna (Ax 1996; Müller 1998; Dunn et al. 2008). Sponges possess within their bodies populations of phylogenetically diverse yet highly sponge-specific microbes capable of a variety of metabolic processes. The densities of these internal microbial populations may reach 10^{10} microbes per gram wet weight of sponges (Hentschel et al. 2006), equaling densities found in microbial mats and biofilms. Sponge-microbe populations, however, are thought to be regulated by their metazoan host (by yet unknown mechanisms), as they are phylogenetically very different from those found in the surrounding water column (Hentschel et al. 2003; Taylor et al. 2007). The production of bioactive secondary metabolites by sponges and their associated microbes has received much attention among natural-product chemists and pharmacologists, and has found its way into diverse biotechnological applications including new medicines and efficacious cosmetic additives (Osinga et al. 2001; Proksch et al. 2002). Therefore, understanding how the behavior of sponges affects and controls their internal microbial populations is of importance for both basic and applied research questions.

Oxygen is thought to be supplied in excess to the sponge body through its pumping activity, and passively by ambient water flow (Reiswig 1974). Oxygen concentration in the sponges is the net result of water transport and oxygen consumption by the sponge and associated microbes. The oxygen concentrations in the sponge interrelate to the number and activity of sponge-associated microorganisms.

Sponges in natural environments have various pumping behaviors. Some species, such as *Mycale* sp., pump continuously, whereas others sponges, such as *Verongia gigantea*, periodically stop pumping, apparently at random intervals (Reiswig 1971), or possibly following an endogenous rhythm. Some species respond to physical disturbances. For example, *Verongia lacunosa* reduces the ventilating activity to avoid damage to its aquiferous

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system in the presence of high sediment loads in the water column (Gerrodette and Flechsig 1979). *Microciona prolifera* explants ceased pumping in response to lower salinities and their aquiferous system was reduced (Fell 1998). The ability of sponges to pump large amounts of water has led to the general assumption that permanent oxygen saturation exists within the sponge body (Pfannkuchen et al. 2009). This view is now challenged by our discoveries of tissue anoxia (Note: herein we sometimes use, in a descriptive manner, the term ''tissue'' to designate a localized grouping of sponge cells, whereas it is generally agreed that sponges do not actually possess true tissues) in several sponge species held in captivity for experimentation, e.g., the explants (small, healed pieces cut from a parent sponge used to start aquacultures) of the cold-water sponge *Geodia baretti* (Hoffmann et al. 2005*a*), *Geodia baretti* whole individuals (Hoffmann et al. 2005*b*), the Mediterranean sponges *Aplysina aerophoba* (Hoffmann et al. 2008), *Dysidea avara* (Schläppy et al. 2007), and *Chondrosia reniformis* (F. Hoffmann and M.-L. Schläppy unpubl.).

Passive advection caused by local seawater currents (ambient flow) also contribute to the oxygenation in sponge tissue (Vogel 1974, 1977). This passive flow is driven by pressure differences and will be influenced by the sponge topography and by water current velocity. Pile et al. (1997) found that *Baikalospongia bacillifera* pumped less when the ambient current was high. In the absence of active pumping or of ambient flow, diffusion is the only oxygen transport mechanism in sponge tissue (Hoffmann et al. 2005*a*). The sponge becomes anoxic if the combined demand of sponge cells and sponge-associated microbes' respiration exceeds the rate of oxygen diffusion into the tissues (Hoffmann et al. 2008).

The presence of even temporally anoxic zones within a sponge is likely to influence microbial community structures and metabolic processes in those zones because oxygen concentration is a strong regulator for microbial metabolic processes. Typically, organic matter is degraded by the energetically most favorable process—which is aerobic respiration. Anaerobic pathways like fermentation or anaerobic respiration with alternative electron acceptors such as nitrate and sulfate are generally thought to occur under anoxic conditions (Schlegel 1992). However, nitrate reducers and many sulfate reducers can also be active at low oxygen concentrations (Canfield and Marais 1991). Facultative and obligate anaerobic microbes have been found in several species of sponges, suggesting the presence of anoxic and oxygen-depleted microniches within sponges; fermenting bacteria were found in *Ceratoporella nicholsoni* (Santavy et al. 1990), sulfate-reducing bacteria in tetractinellid sponges (Schumann-Kindel et al. 1997), and anammox and denitrifying bacteria in *G. barretti* (Hoffmann et al. 2009); methanogenic euryarchaeotes may also be present in sponges (Webster et al. 2001).

Pumping activity in sponges has been described in several species in the field or in laboratory conditions, but how the temporal and spatial oxygen distribution in sponges may be influenced by active pumping and ambient flow has not yet been documented. To date, microelectrode measurements of oxygen within the sponge tissues have been restricted to laboratory experiments. In this study, we investigated the temporal and spatial oxygen dynamics and the influence of pumping behavior and ambient flow on oxygenation of *D. avara* and *Chondrosia reniformis* both in the laboratory and in the field.

Methods

Two phenomena were investigated: the temporal and spatial occurrence of oxygen-depleted microniches within the sponge tissues, and the influence of sponge pumping and ambient flow on oxygen concentrations within the target sponges. One field and three laboratory setups were used to test for the presence of the two phenomena (Fig. 1): Field setup: diver-operable microsensor setup (DOMS; Weber et al. 2007). Laboratory setups: Aquarium (125-liter) with flow-through natural seawater; flow cell (8 liters) with recirculating natural seawater; flow cell (7.5-liter) with artificial recirculating seawater.

The presence of near-anoxic and fully anoxic microniches was measured with oxygen microsensors both in the field and in the laboratory. These measurements were made either in an osculum or in the sponge tissue nearby an osculum. The influence of sponge pumping rates (i.e., the speed at which the water exited the sponges' osculum for a given duration) on local tissue oxygenation was measured in a laboratory combination experiment using an oxygen microsensor and a heated thermistor flow sensor concurrently. To measure the influence of ambient flow on sponge tissue oxygenation, a flow cell with controllable flow speeds was used in combination with the oxygen microsensor and the heated thermistor flow sensor.

Use of microsensors—Clark-type oxygen electrodes (Revsbech 1989) with a 20-μm tip diameter were made at the Max-Planck-Institute for Marine Microbiology, Bremen, Germany and calibrated with a two-point calibration using oxygen-saturated seawater and oxygen-depleted natural seawater made anoxic by the addition (to saturation) of an oxygen scavenger, sodium sulfite. Care was taken to obtain the zero value as quickly as possible as a longer exposure to the sulfite solution can affect the sensor performance.

For laboratory measurements the sensors were mounted on a motorized, computer-controlled micromanipulator (Fig. 2). In practice, the oxygen microsensor penetrated the surface of *D. avara* (Schmidt, 1862, Demospongiae) (Fig. 3) easily, but the cortex of *C. reniformis* (Nardo, 1847, Demospongiae) (Fig. 4) had to occasionally be prepierced before a microsensor could be inserted. In these cases, a hypodermic needle (0.90 $\times$ 40 mm) was used to perforate the first 2–3 mm of the sponge surface and the microsensor measurement was carried out directly after this perforation. The effect of the procedure must have been minimal as no change in pumping behavior was observed.

When measuring the temporal occurrences of anoxic niches, the oxygen microsensors were left static either in the tissue or in the osculum. When measuring the spatial occurrence of oxygen-depleted microniches replicate profiles were conducted always in the same manner: the motor

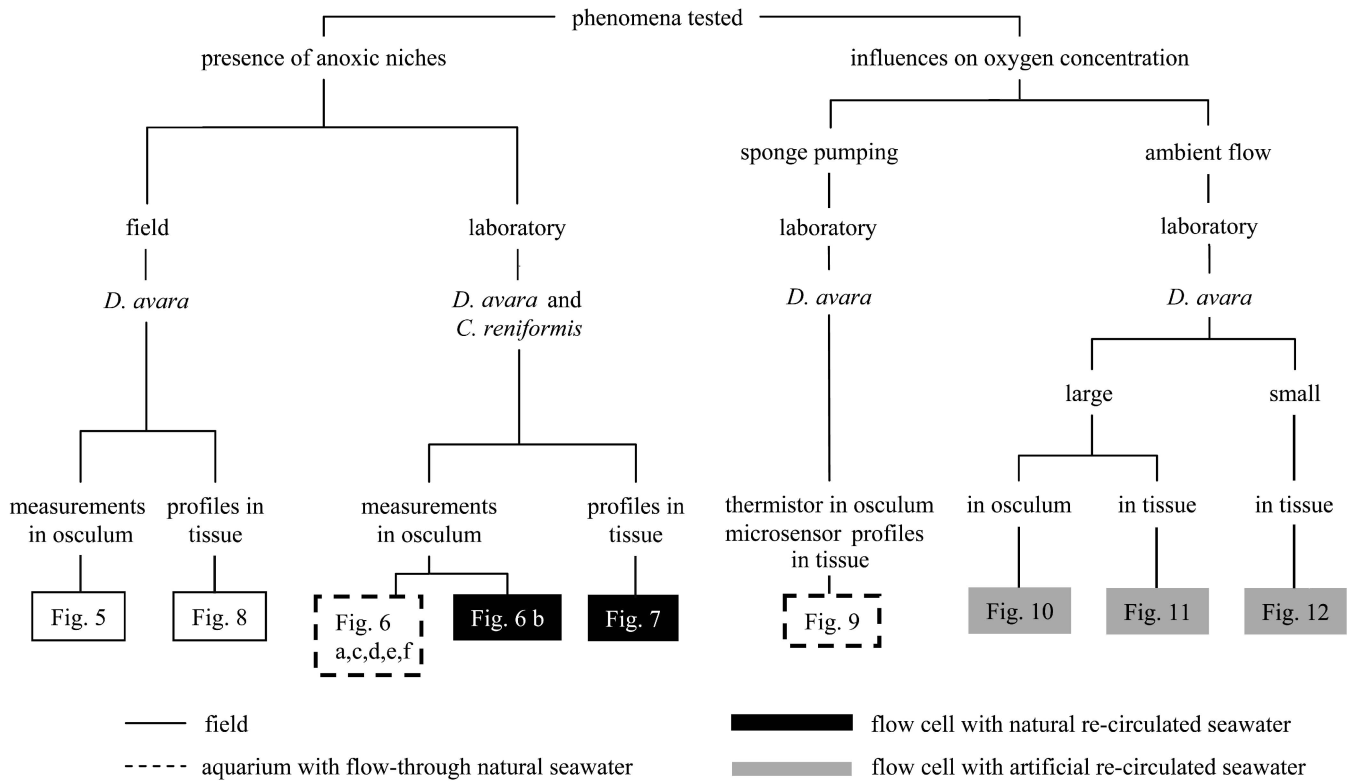

Fig. 1. Schematic representation of the experiments conducted to test the presence of micro-oxic and anoxic niches in sponges and the influence of sponge pumping and ambient flow on sponge oxygenation.

was programmed to lower the microsensor while it measured oxygen concentrations, first through the water column, then nearing the sponge surface, and finally within the sponge itself, and this at several locations on the sponge body. Oxygen measurements were recorded once per second but only the mean value, averaged for every minute, was used.

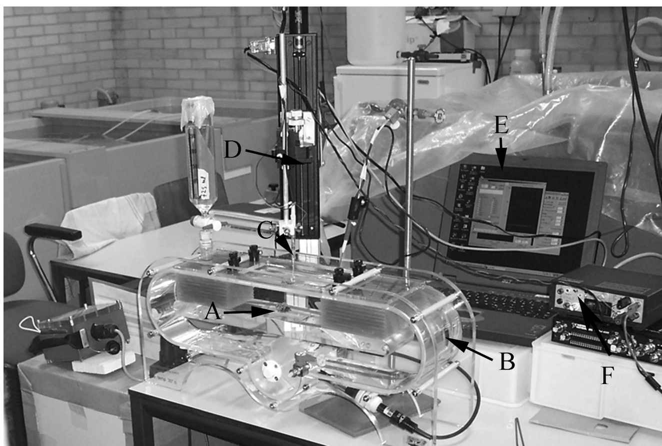

Fig. 2. Microsensor and flow-cell setup, showing a specimen of (A) *Dysidea avara* (B) in the flow cell and (C) an oxygen microsensor controlled by (D) a micromanipulator connected to (E) a computer and (F) a picoampere meter indicating oxygen concentrations measured by the microsensor.

Use of thermistors—A heated thermistor (temperature-sensitive electrical resistor) and recorder were built at the Max-Planck-Institute for Marine Microbiology according to LaBarbera and Vogel (1976). The thermistor sensor design allowed measurement of the speed of water flow exiting the sponges' oscula. The nondirectional flow probe combined a 1-mm-diameter glass-bead-covered thermistor and an additional temperature compensation thermistor sealed onto the distal end of a 2-mm-diameter stainless steel tube in which the electrical connection led to the recorder

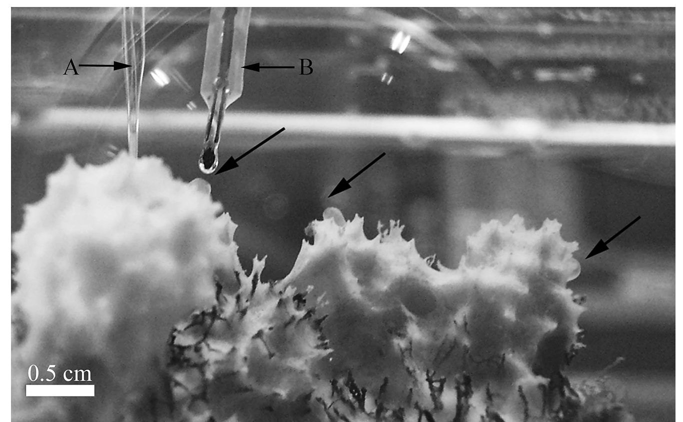

Fig. 3. *Dysidea avara* with (A) oxygen microsensor in its body and (B) a hot bead thermistor above an osculum. The unlabeled arrows point to the multiple sponge oscula.

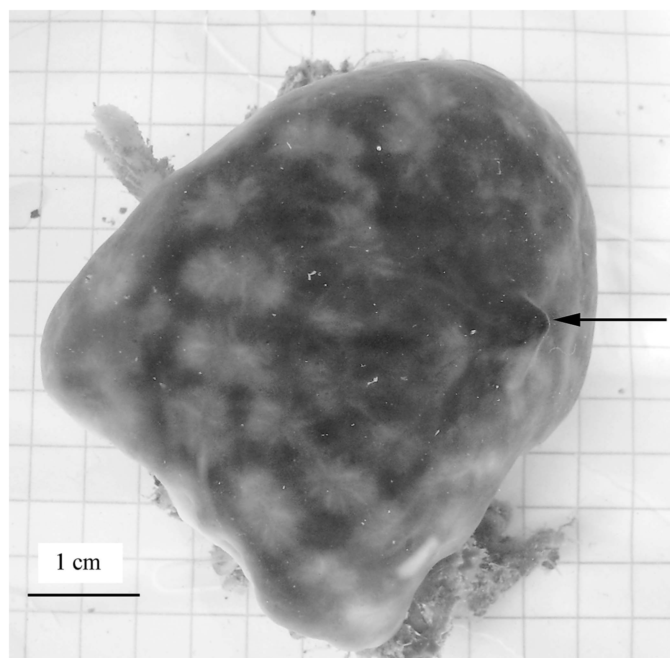

Fig. 4. *Chondrosia reniformis* specimen with one (semiclosed) osculum (arrow).

(*see* diagram, LaBarbera and Vogel 1976). The apparatus functions according to the following principle: the bead is heated electrically, and the heat is removed in proportion to the water flow rate created by the sponge. The electrical current needed to keep the bead heated is measured by the electronic circuitry, stored on a computer file, and later corrected with respect to the calibration. To calibrate the thermistor, a cylindrical polyvinyl chloride (PVC) block with a 10-cm-deep circular groove (30-cm long) was made to rotate at a known speed with a motor. The groove was filled with seawater and the thermistor was held stationary inside the groove while the block rotated and the electrical output recorded. Error was estimated to range between 2% and 15%.

In the case of the laboratory measurements, the flow sensor was used to measure the speed of water exiting a sponge osculum by accurately placing the heated thermistor bead above and within approximately 1–2 mm of the rim of the osculum. The flow sensor was also utilized to set the background flow speed in the flow cell by regulating the current to the motor operating the flow-cell paddlewheel while reading the water flow-speed output of the heated thermistor sensor.

Experimental setups—Field oxygen measurements: To perform underwater measurements of oxygen in *D. avara*, self-contained underwater breathing apparatus (SCUBA) diving and a DOMS were used (Weber et al. 2007). For microsensor tissue oxygen measurements made in the field in March 2006 (Cala Montgó, Catalan coast, Spain, Mediterranean Sea, 42°06.863′N, 03°10.116′E) four *D. avara* ranging from 4.7- to 14.3-m depths were used. The prevailing currents at the site were oscillating at 0.20–0.33 Hz and never stagnant, with the range of time-averaged proximal flow speeds (i.e., speeds measured within 2 cm of the sponges and 2 cm of the native substratum) of 0.16–0.40 cm s^{-1}. The sponges were all located either at the base of a submerged rock shelf or at the entrance of a rock cave; all were in a low light regime (Mendola et al. 2008). When investigating the temporal occurrence of near-anoxic or fully anoxic microniches, static oxygen measurements were conducted within the oscula of test specimen (*D. avara*). The oxygen microelectrode was lowered 3 mm inside the target osculum and left overnight. When investigating the spatial occurrences of such niches, profiles were carried out by moving the microsensor from first above the sponge surface, then into the tissue while continuously recording oxygen concentrations. A total of 36 oxygen profiles in 200-μm steps was made with three replicate profiles at three different locations on the sponge, namely, away (2 cm), midway (1 cm), and close (0.1 cm) to an arbitrarily chosen osculum. The results from two individuals are presented here.

Aquarium (125-liter) with flow-through natural seawater: Five specimen of each species, *D. avara* and *C. reniformis*, were collected by SCUBA diving at 20-m depth on a benthic community off the Montgri region of Spain (Catalan coast; NW Mediterranean Sea, 42°3′N, 3°13′E). Whole specimens were removed together with a piece of native rock substratum on which they were attached, and then cleaned of other sessile organisms and any macro-epibionts. The flow regime at the site (although not measured) was likely turbulent or oscillating and never stagnant. Sponges were transported (within 1 h) in large tubs of natural seawater kept at the original temperature by a cooling system. Before any measurements, the sponges remained 3 weeks at the Experimental Aquaria Zone of the Marine Science Institute, Barcelona, Spain, in 125-liter flow-through open-system tanks with natural seawater at 20°C with natural photoperiod. We estimated the renewal rate for new seawater at 100% exchange every 15 min. The sponges did not receive additional food (i.e., any nourishment came from filtration of natural food particles present in the water exchanged into the holding tank).

Flow cell (8-liter) with recirculating natural seawater: Four *D. avara* and four *C. reniformis* specimens were collected using SCUBA diving at 10–15-m depth in Cala Montgó in March 2006. The specimens were brought directly from the collection site to the laboratory (distance: 400 m) in 1-liter jars containing in situ seawater and acclimatized for temperature before being introduced in the experimental flow cell with recirculated natural seawater from the collection site. The flow-cell water temperature was maintained close to the temperature of the sampling site (13°C) and unidirectional flow was produced using a standard aquarium pump. The natural seawater was used without adding any other chemicals and the total volume of water was fully replaced each hour. Dissolved oxygen within the flow cell was maintained close to saturation levels using air stones. The test sponges were not fed during the experiments. Indirect low-level lighting was used during the experiments but available light is not expected to have affected the oxygen measurements since none of the target sponges contained photosynthetic symbionts.

Flow cell (7.5-liter) with artificial recirculating seawater: One month before experiments commenced at Wageningen University (the Netherlands) nine specimens (15–80 cm^3 vol.) of *D. avara* were collected at 10–15-m depth in Cala Montgó, Spain, and transported by car (8 h) in 15-liter refrigerated containers with filters, then subsequently kept in a closed system, a recirculating 85-liter fiberglass aquarium with redundant biofiltration modules (total recirculating volume was approximately 150 liters). The aquarium contained a mixture of natural and artificial seawater made by mixing 37 g L^{-1} into reverse osmosis water using Reef Crystals salts (by Aquarium Systems). After the initial filling of the aquarium, 5% were changed per week with the artificial seawater and therefore over time, the natural seawater was effectively completely diluted out of the system. Temperature and salinity were kept as close as possible to those measured at the collection site with an aquarium heater/cooler unit and reverse-osmosis water addition (i.e., 20°C [± 0.3°C] and 37.4 [± 0.2–0.8]). Dissolved oxygen within the sponge-holding aquarium was continually maintained at close-to-saturation levels using air stones and a system constantly recirculating seawater. Sponges were fed twice daily with marine broths made from fish and shrimps, or manufactured shellfish diets (INVE, Belgium CAR-1), and three to four times weekly with cultured *Phaeodactylum tricornutum* and *Nannochloropsis* sp. (2–3 $\times$ 10^{-5} particles L^{-1} final concentration in tank at onset of feeding). The light in the laboratory was set to 8 h of daylight per day and the air temperature controlled by an air conditioner. Light levels in the holding tank were purposefully kept low, ca. 30–50 $\mu E\ m^{-2}\ s^{-1}$ using a translucent plastic tank cover. A 7.5-liter Perspex plastic flow cell (Fig. 2B) was specially built for experiments with live sponges. It could produce laminar flow within its working section (18 $\times$ 12 $\times$ 6 cm) in the velocity range of 0–13 cm s^{-1} as unidirectional constant flow. Light levels in the flow cell were slightly higher than in the holding tank (ca. 100 $\mu E\ m^{-2}\ s^{-1}$) so that precision work with placement of the microsensor could be carried out. Individuals were transferred to the flow cell for microsensor work and acclimatized for approximately 1 h before the measurements started. The water in the setup was changed completely with each new sponge used. In this setup, the effects of ambient flow on large and small sponges were investigated. A large specimen was used to investigate the oxygen content of oscular water with and without ambient flow. A different large specimen was used to observe the effect of ambient flow on the oxygenation of the sponge tissues, and a small individual without an osculum (and presumably without a fully functional aquiferous system) was used to test the effect of varying background flow on tissue oxygenation.

Study species—*D. avara* (Schmidt 1862) is a fist-sized sponge with well-defined conuli and interdispersed oscula (exhalent openings) (Fig. 3). This species is a common Mediterranean sponge (Uriz et al. 1992) and easily obtainable from shallow water of 4–40 m. It has desirable morphological characteristics for microelectrode work, such as a body made of a soft spongin skeleton (Galera et al. 2000) that which facilitates easy penetration of the fine microelectrode. This species is of commercial interest because of its ability to produce avarol and anti-inflammatory compounds. *C. reniformis* (Nardo, 1847) is a Mediterranean sponge commonly found in shallow waters of 0.5–40 m (Wilkinson and Vacelet 1979) and has a cushion-like appearance, with a smooth body surface and few oscula (Fig. 4). *C. reniformis* lacks spicules but has high amounts of collagen fibers (Boury-Esnault 2002), which make the species of interest for commercial production of collagen for cosmetics or as a nanoparticle carrier (Swatschek et al. 2002).

Results

Oxygen depletion—Field measurements of oxygen dynamics within the outflowing water from the oscula of two of four *D. avara* specimens studied produced periods of oxygen depletion (i.e., where oxygen levels could not be distinguished from zero with our sensors and under the experimental conditions < 2 μmol L^{-1}, hereinafter defined as anoxia) of 1 h (Fig. 5A) and of 6 min (Fig. 5B). Before and after these anoxic events, the water exiting those oscula approached oxygen saturation at 200–250 μmol L^{-1} (Fig. 5 A,B). For two other *D. avara* specimens in the field, oxygen in water streams exiting the oscula fluctuated between 120 and 225 μmol L^{-1} and never approached anoxia (Fig. 5 C,D).

Under laboratory conditions, oxygen depletion (< 40 μmol L^{-1}) was found in 4 of 12 specimens of *D. avara* and in 2 of 10 specimens of *C. reniformis* under a variety of conditions. In *D. avara*, oxygen depletion was observed for 6-min (Fig. 6A) and 41-min periods (Fig. 6B). In another two specimens, rapidly decreasing oxygen concentrations were followed immediately by rapidly increasing oxygen concentrations (Fig. 6C,D). These ephemeral low-oxygen events occurred several times during a period of 7 h (Fig. 6C) and 16 h (Fig. 6D). In *C. reniformis* those events were observed for 1 h and for 42 min (Fig. 6E,F). The remainder of the time the oxygen content of the water exiting *C. reniformis'* oscula remained stable and in the range of 150–200 μmol L^{-1}. Oxygen depletion occurred at random times intervals after insertion of the microsensors (i.e., 1 min to 6 h).

When profiling into *C. reniformis* (Fig. 7A,B) and *D. avara* (Fig. 7C,D) in aquaria a clear differentiation was observed between well-aerated (Fig. 7A,C) and poorly aerated (Fig. 7B,D) individuals. Poorly aerated sponges showed a steep reduction in oxygen concentration as the sensor approached the sponge's surface, forming a boundary layer of 0.5–1-mm thickness, and a continued decrease in oxygen down to 1 and 2 mm into each sponge's body (Fig. 7B,D). The shape of the profiles, with the steepest gradients at the sponge surface, indicates that diffusion was the dominant transport mechanism inside the sponge tissue at the time of the measurements. Conversely, well-aerated sponges (Fig. 7A,C) displayed no boundary layer, and the sponge tissue down to 1 mm into the sponge exhibited oxygen concentrations close to those of the ambient water.

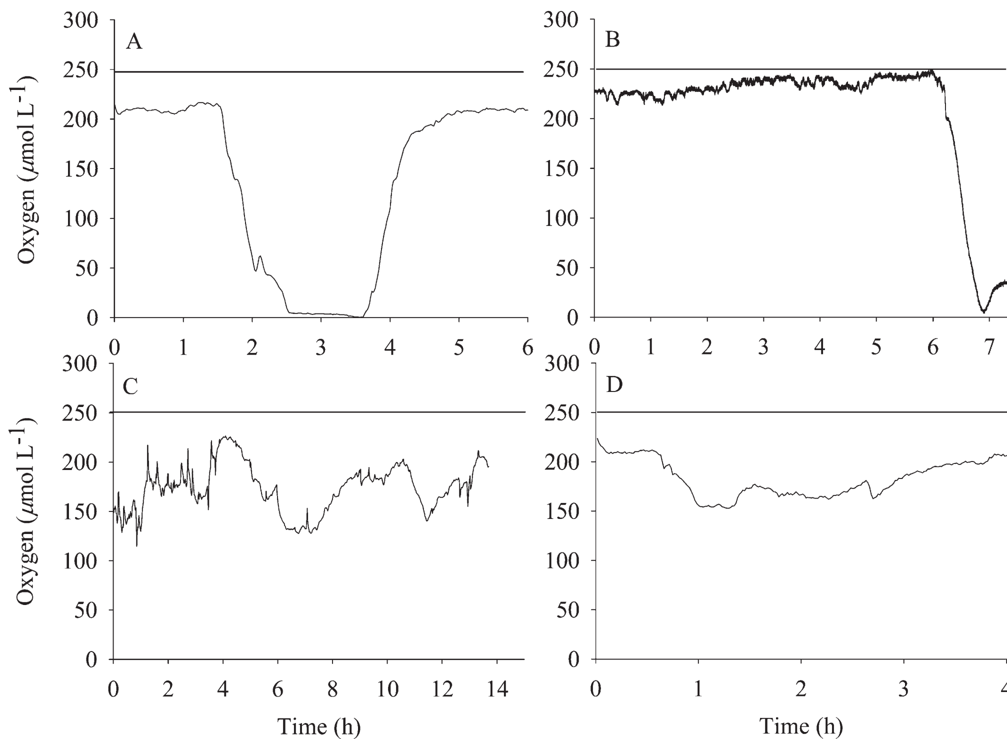

Fig. 5. Field long-term oxygen microelectrode measurements in the oscula of four different *Dysidea avara* individuals. The upper horizontal lines indicate oxygen concentration in the seawater. (A) Showing anoxia for 1 h and 4 min, (B) showing anoxia for 6 min, and (C, D) showing fluctuating oxygen concentrations but without anoxia.

At 3-mm tissue depth, oxygen saturation reached approximately 200 μmol L^{-1} in both species (Fig. 7A,C).

Spatial oxygen heterogeneity—Spatially heterogeneous oxygenation patterns were found in field measurements made on four *D. avara* individuals (only two shown here, Fig. 8). Replicate profiles on the same sponge at the same location showed some variability in the oxygen concentrations within the 3–6 min required to measure three replicate profiles (Fig. 8A,B). Nonetheless, a pattern emerged from the data that showed that in proximity to an osculum the tissue was better oxygenated (Fig. 8C,F) than in areas farther away from the same osculum (Fig. 8A,D).

Oxygenation and sponge pumping activity—Under laboratory conditions, the high temporal and spatial variability in oxygen concentrations was even more pronounced than in the field (Fig. 9). Particularly near the osculum, variability was strong (Fig. 9C,F), whereas in areas relatively more remote from an osculum more reproducible oxygen profiles were measured (Fig. 9A,D). High oscular outflow speed (ca. 0.6 mm s^{-1}) did not always correlate with profiles of well-oxygenated sponge tissue (Fig. 9A,E). Likewise, at low oscular outflow speeds the tissue oxygen profiles were not always approaching anoxia (Fig. 9B,F).

Minimum oxygen concentrations (< 20 μmol L^{-1}) were observed 1 cm (i.e., mid-distance) from the osculum (Fig. 9B,E). At this location a steep diffusion boundary layer was occasionally observed (Fig. 9B). In some cases, a delay occurred between the oscular outflow speed and the oxygenation (Fig. 9B,F). When the first profile mid-distance from an osculum was made (Fig. 9B), the sponge had already stopped pumping (oscular outflow 0 mm s^{-1}), but we could still measure oxygen in the tissues for the length of time required to carry out all three profiles (approx. 6 min). In another case, the oscular outflow had returned to high while measuring the first two profiles (Fig. 9F), which still showed poorly oxygenated sponge tissue. The effect of this renewed strong pumping activity only became evident 2 min later, however, when measuring the last profile, which showed highly oxygenated sponge tissue close to the pumping osculum. We conclude that nonpumping eventually leads to oxygen depletion and pumping to oxygenation in sponge tissue; however, a lag time of several minutes can occur before the effect of changed pumping activity on tissue oxygenation becomes evident.

Oxygenation and ambient flow—Flow-cell experiments using sponges with many oscula (and a presumably a fully functional aquiferous system) revealed that these sponges modulated their pumping activity according to ambient flow. Exposed to unidirectional low-speed flow, the average oxygen content of the sponge oscular water was 5 μmol L^{-1} ($\pm$ 0.37 standard error of the mean) below the seawater concentration (227 μmol L^{-1}). Approximately once every 2 min, stronger deoxygenated water (150 μmol L^{-1}) exited the osculum over a period of approximately 30 s (*see* Fig. 10). When ambient flow in the flow cell was shut off

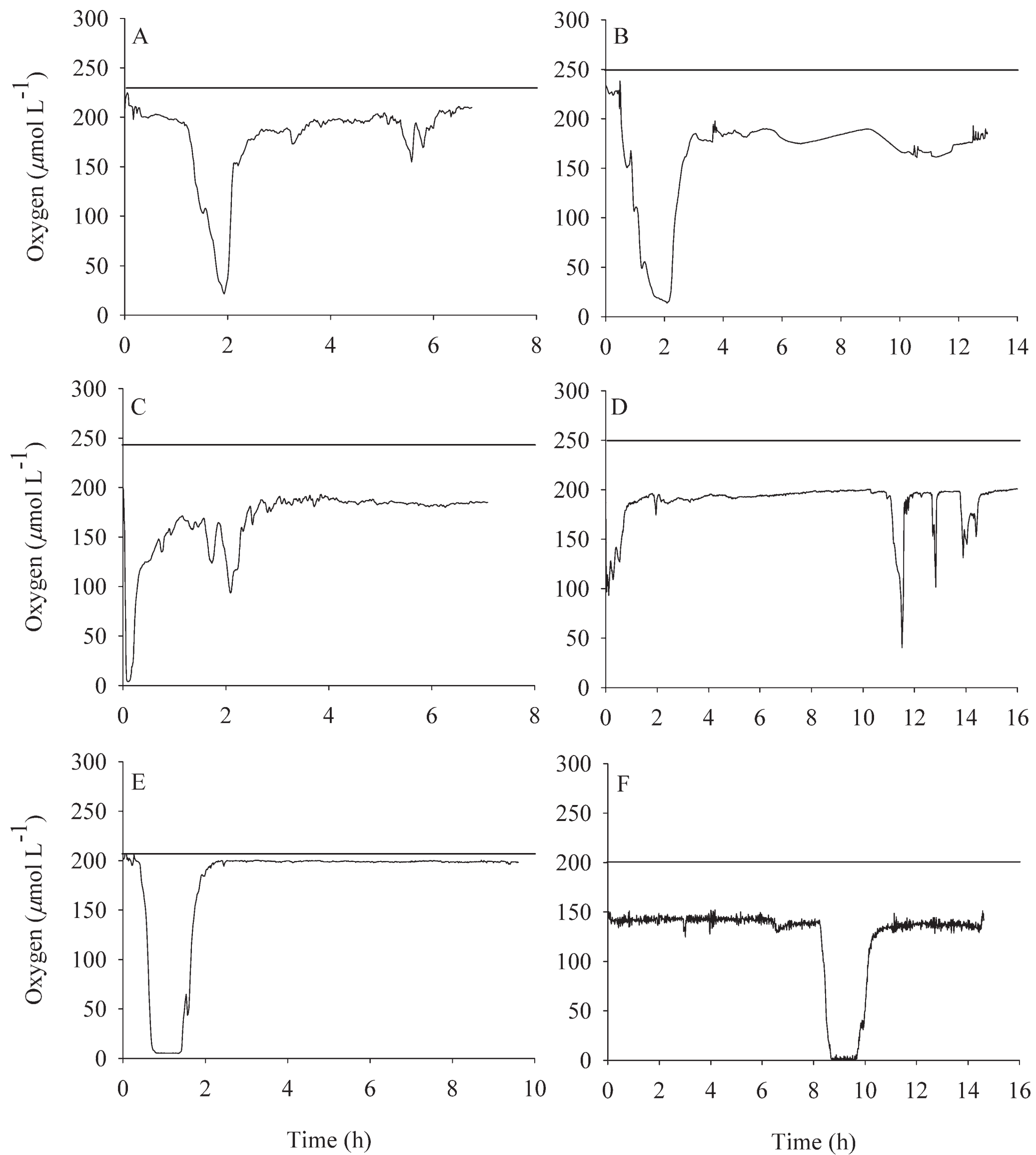

Fig. 6. Laboratory long-term oxygen microelectrode measurement in the osculum of (A, B, C, D) *Dysidea avara* and (E, F) *Chondrosia reniformis*. The upper horizontal lines indicate oxygen concentration in the seawater. (A, B) *D. avara* showing conditions close to anoxia for 6 and 41 min, respectively, (B, C) *D. avara* showing repeatedly fluctuating oxygen conditions approaching anoxia, and (E, F) in two freshly sampled *C. reniformis* individuals, showing anoxia for 60 and 42 min, respectively.

(i.e., zero background flow), the frequency of pulses of deoxygenated water exiting the osculum increased to approximately 1 per minute, and the oxygen content of the sponge oscular water fluctuated in peaks between 105 and 218 μmol L^{-1} (i.e., 9–122 μmol L^{-1} below seawater) (Fig. 10). Thus absence of ambient flow decreased the oxygen supply in the sponge. When the flow velocity in the flow cell was again increased from 0 to 2 cm s^{-1} (in 0.5 cm s^{-1} increments), the sponge's oxygenation increased only marginally (Fig. 11). With again decreasing ambient flow from 2 cm s^{-1} to 0 in 0.5 cm s^{-1} increments, oxygenation in the sponge decreased instantaneously from 200 μmol L^{-1} to approximately 100 μmol L^{-1}. However, before ambient flow was stopped (i.e., when ambient flow was at 1cm s^{-1}) sponge pumping resumed, causing the oxygenation within the sponge tissue to increase to approximately 200 μmol L^{-1} (Fig. 11).

When a small individual lacking an osculum (and presumably a functional aquiferous system) was left in stagnant water, its tissues became repeatedly anoxic within 30 min. When flow was re-established, the tissue oxygenation recovered in 15 min to steady-state concentrations (50–100 μmol L^{-1}); however, various ambient flow levels above 0.9 cm s^{-1} did not further increase the oxygen concentration in the tissue (Fig. 12).

Discussion

Oxygen depletion—Field and laboratory static oxygen measurements in *D. avara* and *C. reniformis* specimens

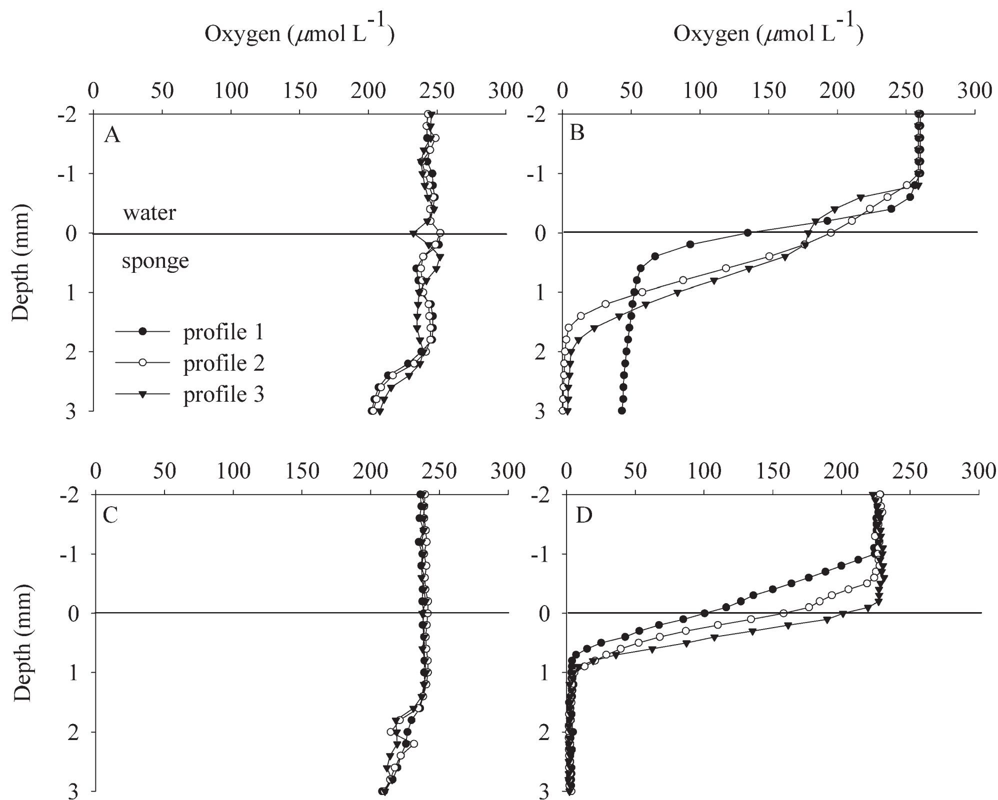

Fig. 7. Oxygen profiles (replicated three times) in the body of *Chondrosia reniformis* (A) when the sponge was well oxygenated and (B) when the sponge was anoxic. In the body of *Dysidea avara* (C) when it was well oxygenated and (D) when the sponge was anoxic. The shape of the profiles is indicative that diffusion was the sole transport mechanism in anoxic sponges (B, D). Well-aerated sponges (A, C) displayed no diffusive boundary layer.

revealed that those sponges underwent periods of low tissue oxygenation. Low oxygenation was not a consequence of potentially unfavorable sponge laboratory conditions, as *D. avara* showed the same pattern of oxygen dynamics and oxygen distributions in both undisturbed field conditions and in the laboratory (Figs. 5, 6). The onset of oxygen depletion was not induced by the sensor measurements, but occurred at random times after insertion of the sensor (Figs. 5, 6). Times of low oxygenation appear to be common and part of the sponge's normal functioning. For these reasons, we feel confident that our results represent a realistic picture of the occurrence of near-anoxia in both target species.

Anoxia in sponges has been observed previously in a variety of species kept in captivity (Hoffmann et al. 2005*b*, 2008; Schläppy et al. 2007), but before this work no field data were available. Our data show that oxygenation is spatially and temporally heterogeneous across the sponge surface and that tissue oxygen concentrations are highest near an osculum, with more remote areas not as well oxygenated and relying on diffusion. Pumping is the main oxygenation process and seems to be alternately active in different zones of a sponge. It is therefore possible that a given set of water inlets (ostia), inhalant canals, choanocyte particle-filtering chambers, and exhalent canals leading to a single osculum function as an independent and complete autonomous unit (i.e., module), and that older, larger sponges would then be made up of several such functioning modules to form the whole ''colonial'' organism. Observations made in the field (D.M. unpubl.) and in the laboratory (M.-L.S. unpubl.) showed that one osculum can be entirely inactive while the neighboring osculum has a high oscular outflow speed, supporting the concept of modular functioning units in *D. avara*. Our data further indicate that oxic and anoxic zones can be present at various locations on the sponge and that the change from oxic to anoxic conditions occurs abruptly and not gradually. This means that within the deeper layers in sponges a highly dynamic oxygen regime is present. Bacterial populations existing in such conditions will need to be adapted to such a variable oxic environment.

Variable oxygenation in space and time—Highly variable tissue oxygenation patterns were observed in space and time regardless of how close the oxygen measurement was made to an osculum (Figs. 8, 9). Oxygenation being a function of the distance to a target osculum was observed in the field (Fig. 8) but less so in the laboratory (Fig. 9). Contrasting our expectations, oxygen concentrations were not lowest at largest distance from the osculum in *D. avara* (Fig. 9). If *D. avara* really has a ''modular'' oxygenation system, then it is possible that our measurements were made

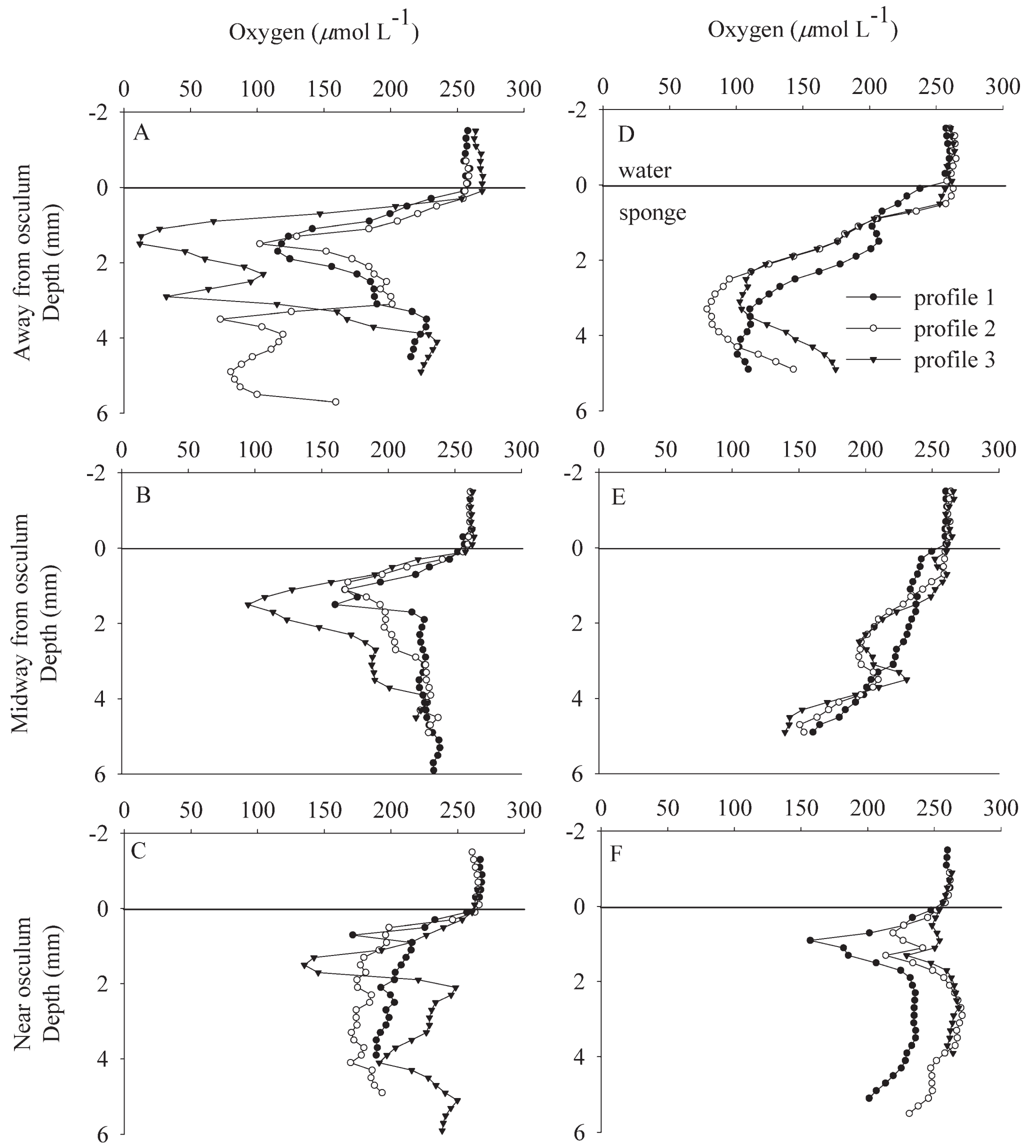

Fig. 8. Field profiles in the tissue of two *D. avara* individuals (individual 1 = A, B, C; individual 2 = D, E, F), (A, D) away from (2 cm); (B, E) midway from (1 cm); and (C, F) near (0.1 cm) an arbitrarily chosen osculum. Tissue in the proximity of an osculum tends to be better oxygenated than farther away from the osculum.

in different functional compartments and were variable for this reason (e.g., it is not unlikely that neighboring functional units would be intimately intertwined within the matrix of the sponge tissue of an adult sponge).

Oscular outflow speed measured in the laboratory was not always related to how well the sponge was oxygenated. The presence of a lag time between pumping and oxygenation would help explain why pumping and oxygenation do not always match. In *A. aerophoba*, the situation is very different, and Hoffman et al. (2008) showed a clear relationship between sponge tissue oxygenation and pumping activity in this species. However, *A. aerophoba* has a simple chimney-like architecture unlike *D. avara*, which is more massive, often with many oscula, making it difficult to predict the architecture of the canal system. The tissue of *D. avara* sampled with the oxygen electrode may only have been partially linked to the target osculum where the exhalent flow was measured.

Oxygenation and ambient flow—Another factor influencing oxygenation patterns in sponges was the level of ambient flow and sponge pumping behavior. Larger specimens in this study showed the ability to modify their pumping behavior according to the presence or absence of ambient flow (Fig. 10) and according to ambient flow speed (Fig. 11). This ability to change behavior implies that the sponges were able to detect the presence or absence of ambient flow and adjusted their pumping accordingly, although we have no direct evidence as to the method of detection. Furthermore, the sponges reacted rapidly (within minutes) to a change of ambient flow magnitude by changing their pumping activities. When exposed to varying regimes of

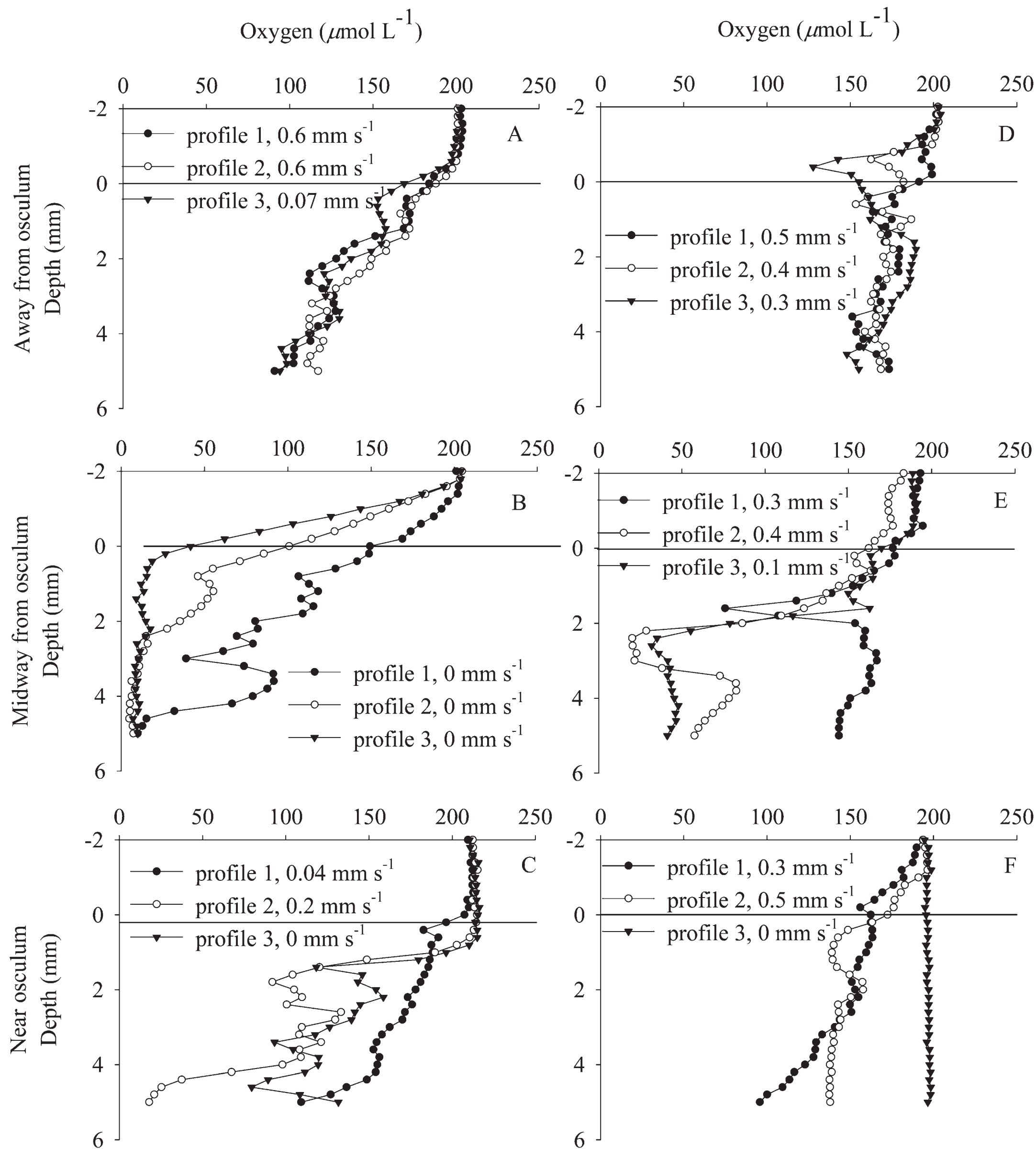

Fig. 9. Laboratory profiles in the tissue of two *Dysidea avara* individuals (individual 1 = A, B, C; individual 2 = D, E, F). (A, D) Away from, (B, E) midway from, and (C, F) near an osculum. The values in the legend represent the oscular flow measured at the osculum closest to the microelectrode.

ambient flow, the oxygenation within the body of a *D. avara* specimen fluctuated little (approx. 180–200 μmol L^{-1}), as long as the ambient flow was above 1 cm s^{-1} (Fig. 11). However, when ambient flow was stopped, the sponge maintained oxygen concentration above 100 μmol L^{-1} within its tissues and did not become anoxic, suggesting a compensatory mechanism through increased pumping.

A small *D. avara* individual that lacked an osculum (and thus probably a functional aquiferous system) exhibited much greater dependence on ambient flow. When ambient flow was stopped, an immediate decrease of oxygen occurred within the sponge as it was unable to compensate by pumping. Cellular respiration in this nonosculated individual, together with the respiration of any sponge-associated microbes living at the location of measurement, must have collectively exceeded the supply of oxygen through diffusion, causing oxygen depletion within 39 min. Varying levels of ambient flow did not substantially influence the maximum concentration of oxygen content within the sponge tissue (Fig. 12), suggesting that, above a certain minimum ambient flow, the benefits of increased flow are not proportional. Our results support Vogel's findings (1974, 1977) that sponges can take advantage of ambient flow. Although the sponges we studied are unlikely to be subjected to stagnant waters in their natural environment, these results point to the importance of ambient flow for small sponges, for example explants (small sponge pieces) often used as starter stock for sponge aquacultures (Osinga et al. 1999).

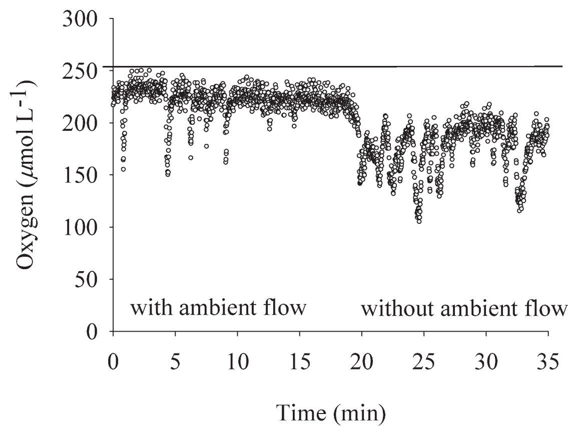

Fig. 10. Oxygen microelectrode measurement in the osculum of *Dysidea avara* in a flow cell with recirculating seawater, with the presence and absence of ambient flow. The upper horizontal line indicates oxygen concentration in the seawater. The absence of ambient flow influenced sponge activity.

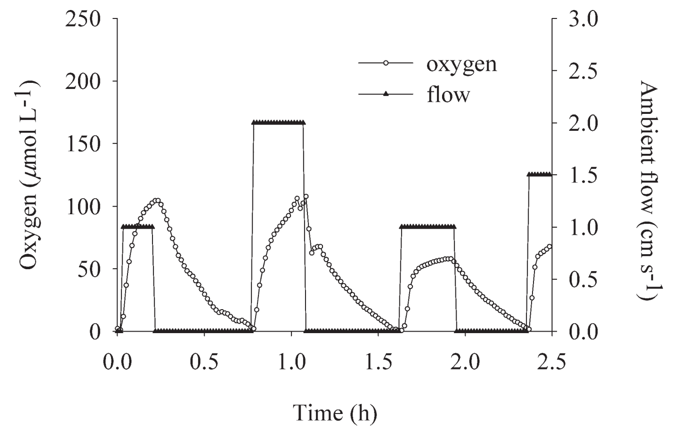

Fig. 12. Oxygen microelectrode measurement in the sponge's tissue in relation to ambient flow velocities in a small *Dysidea avara* without oscula. The upper horizontal line indicates oxygen concentration in the seawater. Decrease of ambient flow decreased sponge tissue oxygenation (passive response).

Consequences of sponge tissue anoxia—The oxygen-depleted niches within the sponges allow the presence and activity of obligate anaerobic or facultative anaerobic microorganisms in the sponge tissue. Such microbes and related metabolic processes have been demonstrated in several species of sponges such as *Rhopaloeides odorabile* (Webster et al. 2001) and *G. baretti* (Hoffmann et al. 2005*b*, 2009). Recent description of denitrification rates in *C. reniformis* and *D. avara* shows the potential for anaerobic microbial processes to occur in these species (Schläppy et al. 2010). Moreover, the sponge-associated microbes must be able to tolerate variable oxygen concentrations and must be active even under rapidly changing oxygen conditions.

Because of the presence of varying internal oxygen conditions within its body, a sponge may be able to maintain a diverse population of associated microbes that can consume sponge metabolic waste products, such as ammonium, via combined nitrification and denitrification (Hoffmann et al. 2009; Schläppy et al. 2010). Alternatively, the sponge-associated anaerobic microbes may simply colonize these very open organisms, and survive in the sponges because of the periodic shutting-down of pumping. Such a commensalistic association may be only of advantage to the microbes, without affecting the sponge. Our data show that sponges can potentially harbor an enormous variety of possible microbial processes because of the high variability in oxygen concentrations. To assess whether there are metabolic interactions between sponges and sponge microbes, the controls of the sponges over their microbial inhabitants need to be understood. For example, the mechanisms triggering pumping need to be fully unraveled. Moreover, methods need to be developed to establish whether an exchange of metabolites occurs between sponges and their microbial inhabitants.

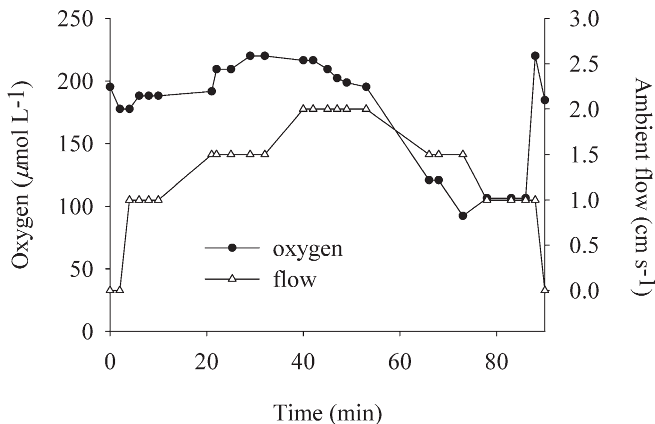

Fig. 11. Oxygen microelectrode measurement in the sponge's tissue in relation to ambient flow velocities in a large *Dysidea avara* with multiple oscula. Decrease of ambient flow increased sponge tissue oxygenation (active response).

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