Spatial and temporal anoxia in single-ostium *Halichondria panicea* demosponge explants studied with planar optodes

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Abstract

The water flow through sponges is regulated by their contractile behaviour including contraction and expansion of the aquiferous system, which leads to shifting oxygen levels in the sponge interior. Still, knowledge of spatial and temporal anoxia in sponges is lacking, but important in elucidating interactions between sponge hosts and their microbiomes. We combined 2-D luminescence lifetime imaging of oxygen with simultaneous time-lapse recordings of the sponge exhalant opening (ostium) to unveil temporal as well as spatial oxygen dynamics caused by contractile behaviour in single-ostium explants of the demosponge *Halichondria panicea*. The present study reveals an intrinsic concentric deoxygenation pattern in explants during episodes of ostium contraction generating an oxygen gradient with increasing concentrations towards the explant periphery. Four sponge explants faced 25 episodes with substantial changes in internal oxygen and anoxia which prevailed for 4.4 h of the total 92.0 h observation period. The 2-D images revealed that the total area of the explant experiencing anoxia during periods of ostium contraction–expansion varied between 0.01 and 13.22% and was on average $7.4 \pm 4.4\%$ for all sponge explants. Furthermore, oxygen respiration, as approximated by the rate of change of oxygen concentration during deoxygenation of the explant interior, was similar throughout the oxic parts of the explant base. The resolved 2-D dynamics provide an unprecedented insight into the internal O₂ distribution of sponges and complement the traditional point measurements of oxygen sensors.

Keywords Oxygen · Planar optode · Contractile behaviour · Diffusive oxygen uptake · Sponge explant · Anoxia

Introduction

Sponges are sedentary filter-feeding invertebrates that are characterized by a ‘simple’ body plan designed to obtain suspended food particles and oxygen by active water-pumping. In sponges, water pumping is accomplished by the beating flagella of choanocytes, which are arranged in choanocyte chambers to maintain unidirectional water flow through a complex internal aquiferous system composed of inhalant and exhalant canals (Fig. 1a; Kilian 1952; Fjordingstad 1961; Larsen and Riisgård 1994; Leys et al. 2011). Food particles, including bacterial and phytoplankton cells, enter the canal system through numerous pores (i.e. ostia) along the outer sponge surface (exopinacoderm). Large phytoplankton cells and other particles of $> 5 \mu\text{m}$ are phagocytosed in the inhalant water canal system, while free-living bacteria and other picoplankton cells are retained by the choanocyte collar filter (Fjordingstad 1961; Brill 1973; Leys et al. 2011). The water flow through this complex canal system oxygenates the sponge interior, in addition to the

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Fig. 1 **a** Side-view of a single-ostium sponge explant including a schematic illustration of the sponge anatomy. PC pinacoderm, OS ostia, IC incurrent canal, CH choanocyte, CC choanocyte chamber, EC excurrent canal, EX exhalant jet, OSC osculum. Green dashed line indicates the water flow through the sponge. Top-view of **b** expanded, i.e. actively pumping and **c** contracted *H. panicea* sponge explant. Uptake, internal flow and release of water is visualized by green fluorescent dye filled into a small glass tip (see also Kumala et al. 2017; Goldstein et al. 2019)

diffusive flux of oxygen across the exopinacoderm (Kumala and Canfield 2018).

Sponges possess contractile behaviour, although they lack true organs, muscles (Pavans de Cecatty 1986, 1989) and a nervous system (Jones 1962; Pavans de Cecatty 1974). Such contractile behaviour is coordinated by actin microfilaments, myocytes and actinocytes abundantly located in the pinacoderm, canal system and the exhalant opening known as the osculum (Prosser et al. 1962; Elliott and Leys 2007; Nickel et al. 2011). Contraction and expansion of the sponge body, as well as the inhalant openings (ostia) and the oscula (Prosser et al. 1962; Reiswig 1971; Gaiño et al. 1991; Nickel et al. 2006; Elliott and Leys 2007; Strehlow et al. 2016), arrest the sponge's pumping activity in regular or irregular intervals, which may further result in periodic, internal anoxia (Hoffmann et al. 2005a, 2008; Kumala and Canfield 2018; Mills and Francis et al. 2018). The diminished oxygen supply during pumping cessation may change the redox state of the sponge interior (Kumala and Canfield 2018), which is colonized by highly diverse microbial consortia (e.g. Taylor et al. 2007; Thomas et al. 2016; Moitinho-Silva et al. 2017; Lurgi et al. 2019).

The activity and abundance of these sponge-associated microbial consortia have previously been highlighted as functionally important for sponge metabolism and ecologically vital for marine ecosystems (Southwell et al. 2008;

Engelberts et al. 2020). For instance, both the aerobic (e.g. nitrification) and anaerobic (e.g. denitrification) processes of associated microorganisms may allow for recycling of waste products within the sponge body (Hoffmann et al. 2009; Zhang et al. 2019). Alternatively, the efflux of dissolved inorganic nutrients, such as nitrate (Corredor et al. 1988; Keesing et al. 2013; Hoer et al. 2018), by both high microbial abundance (HMA) and low microbial abundance (LMA) sponges, may considerably contribute to nutrient fluxes into otherwise oligotrophic environments, such as Florida Bay (Southwell et al. 2008; Hoer et al. 2020). Because of the ability of sponges to process a volume of water of up to 35 times their own body volume per minute (Weisz et al. 2008), sponge communities are increasingly recognized as 'key engineers' for nutrient and resource cycling in reef ecosystems (De Goeij et al. 2017). Although shifting internal redox states due to sponge behaviour may greatly affect the activity of the sponge microbiome, and ultimately nutrient fluxes in marine ecosystems, knowledge of spatial as well as temporal oxygen dynamics in sponges is scarce. This scarcity of information is probably due to the limitations of conventional techniques used to record oxygen in sponges, such as oxygen microsensors (Schönberg et al. 2004).

Transparent oxygen-sensitive planar optodes, combined with luminescence lifetime imaging systems, are promising alternatives for exploring spatial, as well as temporal, oxygen dynamics in biological samples (Glud et al. 1996; Holst et al. 1998), including corals (Kühl et al. 2008), sediments (Glud et al. 2005; Kühl et al. 2007) and biofilms (Glud et al. 1998; Staal et al. 2011; Prest et al. 2012). They have also been used in complex benthic environments such as animal burrows (Volkenborn et al. 2010) and in marine plant root systems (Frederiksen and Glud 2006). We applied here, for the first time, modular luminescence lifetime imaging (Holst et al. 1998; Holst and Grunwald 2001) to determine 2-D temporal and spatial changes in internal oxygen concentration in single-ostium explants of the demosponge *Halichondria panicea*. We combined oxygen imaging with time-lapse video observations of sponge behaviour to further elucidate the relationship between internal oxygen dynamics and contraction–expansion behaviour in these sponges.

Methods

Optode preparation

The measuring principle of planar optodes is based on the dynamic quenching of luminescence of an immobilized indicator by O_2 (DeGraff and Demas 2005). We used the O_2 quenchable luminophore PtTFPP (Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin (frontiersci.com), which possess an excellent photostability and long

luminescent lifetime (Borisov and Klimant 2007). The brightness of the indicator was enhanced using Coumarin C545 (SigmaAldrich.com) as an antenna dye (Larsen et al. 2011). We prepared a sensor cocktail using PtTFPP and Coumarin C545 mixed in a 1/2% (wt/wt) ratio, along with 4% (wt/wt) polystyrene dissolved in toluene (Larsen et al. 2015). The sensing cocktail was spin-coated onto 75 × 50 × 3 mm fiber optic faceplates (Schott Std. 47A Glass) to reduce the effect of optical smearing (Fischer and Wenzhoefer 2010). The final thickness of the highly transparent O₂ sensor layer was ~2–5 µm and the sensing dye had an estimated response time of < 5 s. These planar optodes were glued with transparent silicone into the front side of a custom-built plexiglas flume (24.5 × 8.5 × 5 cm).

Preparation and cultivation of sponge explants

Single-osculum explants of the demosponge *Halichondria panicea* were obtained from cuttings of specimens harvested from Kertinge Nor on the island of Fyn in Denmark. The sponge cuttings (~100 mm³) were placed on the planar optodes after submerging the flume in an aquarium (30 L) with a constant flow of well-aerated bio-filtered (*Mytilus edulis*) seawater (~15 °C, salinity of ~18–22). These cuttings attached onto the planar optodes within 3–6 days and developed an osculum in the subsequent 5–10 days, initiating regeneration of the water canal system and thus, active filter-feeding. These single-osculum explants were regularly (i.e. 1–2 times day⁻¹) fed with lab-cultured *Rhodomonas salina* algal cells (~5000 cells mL⁻¹) prior to and during the experiment. Experiments were performed on explants ($n=4$) that showed pumping activity, as verified by the uptake and release of a fluorescent dye (Fig. 1b, c). Further details on the preparation and cultivation of sponge cuttings are found in Kumala et al. (2017).

Lifetime imaging system and image calibration

We applied modular luminescence lifetime imaging (Holst et al. 1998; Holst and Grunwald 2001) to determine oxygen concentrations as measured by the planar optodes at the base of the single-osculum explants (Fig. S1). The luminescent lifetime (τ) of the O₂ quenchable luminophore was inferred from bottom-view luminescence intensity images acquired within two well-defined time frames on the luminescent decay curve (i.e., after light excitation of the sensor; cf. Staal et al. 2011). Images were recorded with a fast gateable, Peltier-cooled charge-coupled device (CCD) 12-bit camera (SensiCam, PCO.de) equipped with a 560 nm long-pass filter (UQGoptics.com). Excitation light was delivered by five blue high-power LEDs (LXHL-LR3C, Luxeon.com). Image acquisition and light-pulse excitation were synchronized through a custom-made triggerbox controlled by the

software Look@Molli (Holst and Grunwald 2001). Images were recorded using a 16-image average and 1 × 1 binning to increase the signal-to-noise ratio. Images were further processed in ImageJ (Version 1.47 g).

Recorded images were calibrated using the modified Stern–Volmer equation (Eq. 1; Klimant et al. 1995).

$$\tau/\tau_0 = [(\alpha + (1 - \alpha)(1/1 + K_{sv} \times C)] \quad (1)$$

where τ_0 and τ are the luminescent lifetimes at anoxia and in the presence of any given O₂ concentration C , respectively. K_{sv} is the quenching coefficient and α represents the nonquenchable fraction of the signal. Both K_{sv} and α were estimated by non-linear curve fitting the inverse of the Stern–Volmer (τ_0/τ) plotted as a function of O₂ levels (Staal et al. 2011), using the average lifetime from three randomly selected areas (each containing ~625 pixels) in images recorded at known oxygen levels (~98%, ~70%, ~54%, ~34%, ~16%, ~7%, ~3%, 0% air saturation).

The maximum theoretical spatial resolution achieved with our imaging system was ~100 µm × 100 µm. The detection limit of the optode, determined as three times the standard deviation of the oxygen level measured for an area of 1 cm² at anoxia, amounted to 1.1% air saturation, which we define as anoxia.

We combined luminescence lifetime imaging of oxygen with simultaneous time-lapse recordings of the explant osculum. Temporal variations in the osculum cross-sectional area (OSA , mm²), length (l , mm) and the diameter (d , mm) of the osculum tube were assessed from top-view time-lapse images (Fig. S1) captured by a digital camera (Canon EOS 1000D) equipped with a macro-lens (Sigma 50 mm F2.8 EX DG Macro). The camera was synchronized with external blue LED light, and both the camera and light were triggered via a control unit (LED trigger light; Fish ‘n’ chips, Germany). Top-view images were recorded every ~60–300 s using the image acquisition software Look@RGB (<http://www.fish-n-chips.de/Look@RGB/publish.htm>).

Experimental setup

Experiments were performed with single-osculum explants in fully oxygenated (0.2 µm) filtered seawater (salinity of 20) with *Rhodomonas salina* algal cells added twice per day (Fig. S1). Recirculation and flow of water were provided by an aquarium pump in a 2L beaker placed below the excurrent opening of the flume to collect the outflowing water (Fig. S1). A temperature controller maintained a stable water temperature ($T = 15.1 \pm 0.2$ °C) throughout the experiment. Experiments were performed in darkness to prevent potential impacts of sponge-associated photosynthetic cyanobacteria and algae (Knobloch et al. 2019) on O₂ measurements. For

calibration of O_2 images, the oxygen concentration was regulated with a gas mixer (Brooks Instrument, Model 0154) and monitored using a FireSting O_2 optical oxygen meter (Pyro Science, Germany) connected to a computer with Pyro Oxygen Logger[®] software. Oxygen readings were corrected for changes in temperature by the software using an external temperature sensor placed in the flume water.

Image and statistical analysis

Top-view time-lapse images were analyzed in ImageJ (Version 1.47 g). The osculum cross-sectional area (OSA), length (l) and diameter (d) of the osculum tube were manually determined via pixel counts and subsequent conversion into mm^2 using a reference scale bar. The OSA and d were measured at the tip and at center of the osculum tube, respectively. The l was determined as the distance between tip and base of the oscular tube, while $l=0$ mm represents a fully retracted osculum.

We manually defined the area of the explant base (A , mm^2) grown on planar optodes in bottom-view images and converted pixel counts into mm^2 using a reference scale bar. Bottom-view image sequences were transformed to a 32-bit greyscale format to convert each pixel's brightness/luminescence signal into an oxygen measurement using the calibration described above. The mean O_2 within A of all explants was autonomously measured for each image of the image sequence using the 'multiple measure' function in the ROI manager in ImageJ. Minimum and maximum threshold values were adjusted to determine the (cumulative) relative area of the sponge in various ranges of oxygen levels, i.e. 100 to 80%, < 80 to 50%, < 50 to 10%, < 10 to 1% and < 1 to 0% air saturation. To characterize oxygen dynamics during contraction–expansion events, we calculated the rate of change in oxygen levels (% air saturation min^{-1}) by subtracting the oxygen value ($[O_2]$) from pixels in subsequent images acquired at time t , i.e. $[O_2]_t - [O_2]_{t+1}$, divided by the time difference between images. We determined the mean and the maximal rate of change in oxygen levels during both explant contraction (i.e. O_2 decrease) and expansion (i.e. O_2 increase).

Statistical analyses were performed in R, version 3.2.0 (R Core Team 2015). We tested for differences in time intervals, i.e. time until subsequent deoxygenation/oxygenation events, and their duration, between the sponge individuals ($ID1$ – $ID4$) using a generalized linear model (GLM) parameterized with gamma error structure in package lme 4.

Fig. 2 Mean O_2 concentration as a function of time simultaneously measured in the ambient seawater and at the base of single-osculum explants ($ID1$ – $ID4$) on transparent oxygen optodes in a flume with temperature-controlled, oxygenated seawater (15.1 °C, salinity of 20). Legend is shown

Results

Temporal O_2 and osculum dynamics

Oxygen measured at the base of single-osculum *Halichondria panicea* explants ($n=4$, $ID1$ – $ID4$) approached the levels in the ambient seawater for most, i.e. $78 \pm 3\%$ of the total observation period of 92 h (Fig. 2). However, we also observed 25 events with substantial changes in internal O_2 in regular as well as irregular intervals (Fig. 2). These oxygen decreases and increases varied in both extent and duration between events and explants (Fig. 2).

We found no significant difference between the four explants in deoxygenation/oxygenation event duration (GLM, $P=0.981$) and event time intervals (GLM, $P=0.291$) (Tables 1 and S1). We observed, however, occasional apparently synchronized timing in events including the event #1 in sponge explants $ID1$ and $ID2$ and the events #6 in $ID2$ and $ID3$ with #5 in $ID4$.

Simultaneous recordings of temporal variations in O_2 concentration and sponge osculum dynamics documented that changes in O_2 were accompanied by contraction–expansion dynamics in the cross-sectional area (OSA), length (l) and the diameter (d) of the osculum (Fig. 3a). Typically, the contraction–expansion event began with constriction of OSA and d , followed by contraction of the oscular tube (l) and subsequent osculum closure, where $OSA=0$ mm^2 . Osculum closure coincided with a rapid decrease in mean O_2 concentrations measured at the explant base (Fig. 3b). During inflation of the oscular tube, osculum l and d measurements exceeded those both prior to as well as post osculum closure,

while the osculum opening remained closed (Fig. 3). Only osculum expansion, i.e. $OSA > 0 \text{ mm}^2$, was followed by an immediate oxygenation of the explant base, as expressed by an increase in mean O_2 until reaching initial levels (Fig. 3b).

Spatial O_2 dynamics

We observed localized oxygen depletion expanding through the explant area over time during osculum closure generating concentric bands of oxygen isopleths, with anoxia (i.e. $< 1\%$ AS) in the middle (Figs. 4, 5). Overall, an oxygen gradient was established with increasing oxygen levels towards the explant edge/circumference. This situation persisted as long as the osculum opening remained contracted (Table 1, Fig. 5). The mean fraction of the explant A 's experiencing anoxia during periods of osculum contraction–expansion varied between 0.01% ($ID2$) and 13.2% ($ID3$) and was on average $7.4 \pm 4.4\%$ for all sponge explants (Table 1, Fig. 4).

The duration of sponge anoxia ranged from 0.08 to 4.42 h ($ID4$) and was on average 2.0 h.

Oxygenation of the explant base area began with increasing oxygen concentrations at the explant periphery, followed by differential oxygenation of the explant interior, possibly following the water canal system, at least in some cases (e.g. event #6 in sponge $ID3$, Fig. 6). We estimated the mean and maximum rate of change in oxygen levels from successive oxygen images during all contraction–expansion events (#2–#6). An example for sponge explant $ID3$ is depicted in Figs. 6, 7. The mean rate of change at the base of this explant ranged from 0, at the explant periphery, to $4.4\% \text{ AS min}^{-1}$ and $3.3\% \text{ AS min}^{-1}$ at the explant interior during contractions and expansions, respectively (Fig. 6). The maximum rates of change in oxygen, as observed within the inner parts of the explant base during the respective contraction–expansion events, ranged from 10.4 to $11.9\% \text{ AS min}^{-1}$ during contractions, and from 7.0 to $10.6\% \text{ AS min}^{-1}$ during

Table 1 Events with substantial changes in O_2 (#1 to #7, Figs. 2, 4) measured at the base of single-osculum *H. panicea* explants ($ID1$ to $ID4$) grown on oxygen-sensitive planar optodes in a flume with $0.2 \mu\text{m}$ filtered seawater and added *R. salina* algae (Fig. S1)

ID	Event #	t (h)	$\Delta t_{\text{interval}}$ (h)	Δt (h)	t_{anox} (h)	Δt_{anox} (h)	A_{anox} (%)
$ID1$	1	4.42	–	4.66	4.92	3.60	10.43
	2	20.66	16.25	3.08	21.00	2.17	11.49
	3	32.58	11.92	4.58	33.08	2.75	10.92
	4	37.50	4.92	2.33	38.92	0.16	0.02
	5	57.25	19.75	4.50	57.58	2.08	11.39
	6	62.83	5.58	1.75	–	–	–
	7	89.27	26.44	2.47	–	–	–
$ID2$	1	3.25	–	2.42	4.66	0.08	0.01
	2	13.58	10.33	5.33	14.00	3.00	3.25
	3	36.08	22.50	2.33	36.58	1.33	8.98
	4	54.33	18.25	1.92	54.75	0.50	3.07
	5	64.58	10.25	2.42	65.17	1.42	5.97
	6	76.19	11.61	3.77	76.41	2.70	3.80
	7	89.47	13.28	2.58	89.77	1.50	5.07
$ID3$	1	0 ^a	–	1.92	0 ^a	1.42	10.76
	2	6.00	6.00	2.17	6.25	1.25	12.91
	3	16.50	10.50	4.25	17.17	3.00	13.22
	4	33.25	16.75	4.25	33.58	3.50	12.68
	5	52.75	19.50	4.00	53.17	3.33	11.83
	6	75.89	23.14	2.42	76.11	1.83	9.57
$ID4$	1	0	–	3.00	0.25	2.17	6.16
	2	14.50	14.50	2.50	15.33	0.08	0.07
	3	27.83	13.33	3.08	28.17	2.33	5.89
	4	48.75	20.92	5.17	49.00	4.42	9.59
	5	76.72	27.97	2.68	77.14	1.35	4.12
Mean			15.41	3.18		2.00	7.44
($\pm$ SD)			(± 6.61)	(± 1.10)		(± 1.19)	(± 4.39)

t initial time of changes in mean O_2 , $\Delta t_{\text{interval}}$ time interval until subsequent change in mean O_2 , Δt duration of changes in mean O_2 , t_{anox} initial time of anoxia (thus, $OSA = 0 \text{ mm}^2$, cf. Figure 3), Δt_{anox} duration of anoxia, A_{anox} mean fraction of sponge area exhibiting anoxia

^aMinimum value

Fig. 3 **a** Example of temporal changes in osculum length (l , mm), osculum diameter (d , mm), osculum cross-sectional area (OSA , mm²) and **b** mean O₂ level (black line) and cumulative relative area under various oxygen levels (see legend) in a single-osculum explant ($ID3$) during a contraction–expansion event (#5). Dashed line indicates period of osculum closure (i.e. $OSA = 0$ mm²)

expansions (Fig. 7). Rates of change were not uniform but increased towards the sponge interior (Figs. 6, 7).

Fig. 4 Cumulative relative area of the base of single-osculum explants ($ID1$ – $ID4$) in various ranges of oxygen levels (100 to 80%, <80 to 50%, <50 to 10%, <10 to 1% and <1 to 0% air saturation) measured as a function of time. Legend is shown in the lower panel

Diffusive Oxygen Uptake (DOU)

To provide a first-order comparison to previous assessments of the O₂ consumption rates of sponge explants, we derived the diffusive oxygen uptake (DOU) from concentration profiles extracted from steady-state O₂ images of fully contracted explants. For this, we determined the oxygen concentrations along a transect line crossing the sponge area to establish the oxygen gradient (Fig. 5). We then use Fick's first law of diffusion (Jørgensen and Revsbech 1985): $DOU = \phi \times D_s \times \partial C / \partial z$, where ϕ represents the sponge porosity and D_s is the molecular diffusion coefficient of O₂ in the sponge corrected for the tortuosity imposed by the sponge spicules where $D_s = \phi^2 \times D$ (Ullman and Aller 1982). In making this tortuosity correction, we assume that sponge spicules impose a restriction to diffusion similar to sediment particles. D is the molecular diffusion coefficient for O₂ at the given temperature and salinity (i.e. 1.76×10^{-5} cm² s⁻¹; Himmelblau 1964; Broecker and Peng 1974) and $\partial C / \partial z$ represents the O₂ gradient. We estimated the sponge porosity from the water content in a *H. panicea* sponge divided by its volume, using the conversion factor from Thomassen and Riisgård (1995): sponge dry weight (DW , g) = $0.07 V_{\text{sponge}}$. If we assume that most of the DW is due to spicules, which are composed of amorphous opal (with a density of ~ 2), then the volume of the DW is $0.07/2 = 0.035$ cm³ (cm⁻³)_{sponge}. Thus, the porosity of our *H. panicea* sponge explants was estimated to be 0.965.

Fig. 5 **a** Oxygen distribution at the base of a single-ostium explant (*ID3*) during a contraction–expansion cycle (#6) in fully oxygenated (100% air saturation) ambient seawater (salinity of 20) with *R. salina* algal cells. Air saturation level is denoted by colour. Black line indicates the contour area (i.e. base) of the sponge grown on the oxygen-sensitive planar optode. **b** Corresponding oxygen levels (% air saturation) along a transect (grey dashed line; **a**) crossing the sponge base (black dashed line; middle panel). Symbols represent individual pixels with a spatial resolution of $100 \times 100 \mu\text{m}$

Fig. 6 Mean rate of change in oxygen levels (% AS min^{-1}) at the base of a single-ostium explant (*ID3*) during contraction (upper panel)–expansion (lower panel) cycles (#2 to #6) in fully oxygenated seawater (salinity of 20) with added *R. salina* algal cells. Air saturation level is denoted by colour. White line indicates the contour area (i.e. base) of the sponge grown on the oxygen-sensitive planar optode. Pixels contain mean value over all images during contraction–expansion events (#2 to #6, Fig. 2)

Fig. 7 Maximum rate of change in oxygen levels (% AS min^{-1}) at the base of a single-ostium explant (*ID3*) during contraction (upper panel)–expansion (lower panel) cycles (#2 to #6) in fully oxygenated seawater (salinity of 20) with added *R. salina* algal cells. Air saturation level is denoted by colour. White line indicates the contour area (i.e. base) of the sponge grown on the oxygen-sensitive planar optode. Pixels contain maximal value over all images during contraction–expansion events (#2 to #6, Fig. 2)

The mean *DOU* ($\pm$ SD) during the recorded 23 periods of osculum closure with anoxia amounted to $1.38 \pm 0.37 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ d}^{-1}$. Our oxygen measurements were made at the base of the sponge explant, and if we assume a similar O_2 penetration depth and oxygen gradients from the explant surface into its interior for all of the explant body, we can estimate the total diffusion mediated O_2 consumption rate of the contracted sponge explant. Thus, for our cone-shaped explants with a mean outer respiratory surface area of $1.4 \pm 0.6 \text{ cm}^2$, we estimate an integrated *DOU* of $1.9 \pm 0.8 \mu\text{mol O}_2 \text{ d}^{-1}$.

Discussion

Oxygen dynamics in sponges studied by planar optodes

This is, to our knowledge, the first record of internal imaging of oxygen dynamics in sponges. Previous studies on demosponges, such as *Aplysina aerophoba* and *Dysidea avara*, applied oxygen microsenors for oxygen profiling above and within the sponge tissue to monitor internal and diffusive oxygen fluxes (Hoffmann et al. 2005b, 2008; Schläppy et al. 2007; 2010a; Lavy et al. 2016). While microsenors can indeed record oxygen gradients into the sponge body, they lack 2-D resolution, and temporal dynamics are difficult to record. Furthermore, intrusion of the microsenor may also lead to localized contraction of the water canal system (cf. Prosser et al. 1962; Elliott and Leys 2007), ultimately leading to changes in internal flow regimes which may cause variations in the measured oxygen values (Schoenberg et al. 2004; Schläppy et al. 2007). Planar oxygen-sensitive optodes thereby offer a non-intrusive alternative allowing for both temporal as well spatial oxygen measurements to unravel oxygen dynamics, as previously shown in complex biological samples, such as corals (Kühl et al. 2008), or in benthic environments, such as animal burrows (Wenzhöfer and Glud 2004; Volkenborn et al. 2010).

The observed contraction dynamics in sponges of the present study were similar in duration and periodicity to previous time-lapse observations on *H. panicea* explants (Kumala et al. 2017; Kumala and Canfield 2018) and other in situ demosponge species, such as *Verongia gigantea* (Reiswig 1971). Each episode of osculum closure and contraction in our explants was associated with deoxygenation of the explant body (Figs. 2, 3, 5) as was predicted from respiration rate measurements during periods of pumping cessation by Kumala and Canfield (2018). The current study demonstrates that isopleths of oxygen concentration were approximately concentric around the explant interior and only the innermost part of the sponge explant experienced complete anoxia during periods of osculum closure.

The area (and presumably also volume) of the explant experiencing anoxia ($\text{O}_2 < 1\%$ PAL) was relatively small during osculum contraction–expansion ($7.4 \pm 4.4\%$ for all our sponge explants; Table 1, Fig. 4). In contrast, the rates of oxygen respiration, as approximated by the rate of change of oxygen concentration during sponge deoxygenation (Figs. 6, 7) were relatively similar through most of the explant base area. To fully quantify the O_2 consumption rates and dynamics of the contracted explants, we would need to resolve the 3-D O_2 distribution and diffusion geometry of the explants (Meysman et al. 2010). Our simplified approach for assessing the O_2 consumption from planar (i.e. flat plane) images that only cover the base of the explant neglects potential geometric (i.e. curvature) effects on diffusive fluxes at the external exopinacodermal sponge–seawater interface. Furthermore, we assume that our estimate of the O_2 penetration depth and consumption derived at the explant base is representative of the entire explant. The patterns of O_2 distribution observed at the base of our explants appear to be similar to those measured through the outer surfaces of non-pumping demosponges investigated using O_2 microsenors (Fig. 5; Schläppy et al. 2007, 2010a; Hoffmann et al. 2008). Also, our estimate of the integrated diffusive oxygen uptake rate of $1.9 \pm 0.8 \mu\text{mol O}_2 \text{ d}^{-1}$ across the entire sponge explant surface is in accordance with the oxygen consumption rate of $1.1 \pm 0.3 \mu\text{mol O}_2 \text{ d}^{-1}$ measured during incubation experiments with (non-pumping) *H. panicea* explants of comparable size (Kumala and Canfield 2018). This similarity reinforces the idea that respiration is limited by the diffusive flux across the outer sponge surface during periods of osculum closure (Hoffmann et al. 2008; Kumala and Canfield 2018).

We observed that oxygenation of the explant interior tended to occur in canal-like structures of 100–300 μm diameter pervading the concentric anoxic area (Figs. 5, 6, 7). Contractile behaviour of the osculum is associated with contraction–expansion of the internal water canal system in these single-osculum *H. panicea* explants (cf. Kumala et al. 2017; Goldstein et al. 2020), as well as in other demosponge species, such as *Ephydatia muelleri* and *Tethya wilhelma* (Elliott and Leys 2007; Ellwanger et al. 2007; Nickel et al. 2011). During reoxygenation, we observed the deep penetration of oxygenated water into the sponge explant through pumping. We also note that rates of deoxygenation were faster than rates of reoxygenation of the sponge explant, which is explained by the fact that the O_2 decrease in the explant interior during full contraction is driven by respiration, while the oxygen increase during expansion is affected by both, ongoing respiration and advective water transport through the aquiferous system.

Regulation of internal O₂ dynamics

Our findings visually demonstrate that internal oxygen dynamics in *H. panicea* demosponge explants are driven by their contractile behaviour, which includes considerable fluctuations in oxygen levels in major parts of the sponge explant body. As noted above, for most of the observation time, our single-osculum *H. panicea* explants revealed internal oxygen levels approaching that of the ambient seawater (Figs. 2, 4). Therefore, during periods of maximal pumping activity, the advective flux through the internal water canal system saturates the explant interior with oxygen, thus providing ample oxygen to respiring sponge cells and the internal microbiome.

In *H. panicea* sponge explants, osculum closure is linked to a considerable compression of aquiferous space including the in-/excurrent canals and water-pumping choanocyte chambers (Goldstein et al. 2020), eliminating internal water flow (Kumala et al. 2017; Goldstein et al. 2019). These periods of pumping cessation result in a gradual depletion of oxygen reserves in the sponge interior by respiration (Hazelhoff 1938; Kumala and Canfield 2018). At steady state, molecular diffusion of oxygen across the outer-explant body remains the only mechanism for oxygen supply to the explant tissue (Fig. 5; Hoffmann et al. 2005a, b, 2008; Schläppy et al. 2007, 2010a; Kumala and Canfield 2018). The diffusive oxygen uptake (*DOU*) of $1.4 \pm 0.4 \mu\text{mol O}_2 \text{ cm}^2 \text{ d}^{-1}$ by contracted *H. panicea* sponge explants in the present experiments is in the lower range of those determined from oxygen profile measurements using oxygen-sensitive microsensors across the exopinacoderm in other (non-pumping) demosponges, such as *Aplysina aerophoba* (Hoffmann et al. 2005a, 2008; Schläppy et al. 2007). In *A. aerophoba* and *Dysidea avara*, for instance, rates of oxygen uptake of $4.5\text{--}4.7 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ d}^{-1}$ and $4.2 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ d}^{-1}$, respectively, were measured during pumping cessation. The lower *DOU* in our *H. panicea* explants may be explained by possible differences in the distribution of structural elements, porosity and sponge tissues which can strongly vary among sponge species. In fact, oxygen consumption/uptake rates in sponges generally appear to be species-specific and may additionally be influenced by the temperature conditions used in laboratory experiments (Osinga et al. 1999).

It is not clear to what extent our observations on single-osculum explants can be applied to sponges in general, particularly to those with multiple exhalant openings, i.e. oscula. However, if we view water canal systems within sponges as individual aquiferous modules (Frey 1970, 1979; Ereskovskii 2003), with advective water output through an osculum opening, then it would stand to reason that oscula closure (or contraction of various parts of the aquiferous system, e.g. inhalant pores) would encourage deoxygenation of the sponge interior. If this is true, internal sponge anoxia

might be common, but not necessarily synchronous through the whole sponge body. If individual water canal systems are not coupled and oscula closing is non-synchronous, as observed in multi-oscula *H. panicea* sponges (Riisgård et al. 2016), then some parts of the sponge body may experience anoxia while other parts are fully oxygenated. We observed localized oxygen depletion that expanded throughout the sponge explant (Fig. 5), indicating that single-osculum sponges may regulate oxygen even in specific parts of the sponge interior which could result in heterogenous oxygenation of sponge tissue. However, if water can be actively advected through the pinacoderm of a more porous sponge (Gatti et al. 2002; Schläppy et al. 2010a), then internal anoxia may not be achieved. For instance, primmorphs of the demosponge *Suberites domuncula*, in which internal structures have not yet developed, solely depend on advective water flow to oxygenate their interior (Gatti et al. 2002).

Implications of contractile behaviour and subsequent oxygen dynamics

The observed contractile behaviour and subsequent changes in O₂ level in the sponge interior may strongly affect sponge metabolism and the activity of the highly diverse microbial consortia in sponges. Irregular, as well as regular, contraction–expansion of compartments of the water canal system (ostia, water canals and oscula) in response to disturbances (e.g. agitation, Elliot and Leys 2007), or as part of their natural behaviour (Reiswig 1971), may influence filter-feeding (Kumala et al. 2017) and the internal redox state. To our knowledge, sponges lack the ability for anaerobic metabolism but are host to functional microbes that are capable of mediating nutrient cycling during periods of internal anoxia (reviewed in Zhang et al. 2019). Aerobic and anaerobic processes have been detected in both high microbial abundance (HMA) and low microbial abundance (LMA) sponges (Schläppy et al. 2010b). For instance, nitrification, denitrification and anaerobic ammonia oxidation (‘anammox’) may occur in the HMA sponge *Geodia baretii* (Hoffmann et al. 2009), highlighting the benefit of coupled aerobic and anaerobic niches for nutrient cycling within the sponge holobiont. Spatial and temporal oxygen depletion in the sponge body, as observed in our explants and other sponge species (Hoffmann et al. 2005a, 2008; Schläppy et al. 2010a), may maintain and regulate the activity of the sponge microbiome, removing toxic metabolic waste (e.g. ammonium) produced by the sponge during active filter-feeding and digestion (cf. Kumala and Canfield 2018; Hudspith et al. 2021). Contractile behaviour, and subsequent pumping cessation, may hence be considered as a nutrient conserving mechanism that limits nutrient loss to mediate mutualistic relationships between sponges and their microbiomes (Hudspith et al. 2021).

The shifting redox state in the sponge interior, in response to changing pumping dynamics, may favour the abundance and distinct distribution of functional microbial groups according to their metabolic oxygen requirements. Distinct bacterial phylotypes involved in nutrient cycling may occur in varying numbers in different sections of the sponge body (Thiel et al. 2007; Yang and Li 2012; but see Sipkema and Blanch 2010), which can also be hotspots for metabolic activity. In the intertidal demosponge *Cinachyrella cavernosa*, for instance, active nitrifiers were found to be abundantly located in the sponge cortex where 2 times higher nitrification rates were detected compared to intra- or extracellular sections of the sponge specimen (Subina et al. 2018). We hypothesize that both anaerobic as well as facultative anaerobic microbes are likely to occur in areas that regularly experience anoxia during osculum contraction, such as the concentric anoxic area, as observed in our explants. Conversely, obligate and facultative aerobic microorganisms may be closely associated with (outer) regions of the sponge (e.g. cortex/exopinacoderm), that are sufficiently supplied with oxygen during periods of pumping cessation. The active ‘control’ of internal shifting between oxygen levels via contractile behaviour may also enable sponges to maintain or ‘farm’ distinct microbes (‘bacterial farming’), as suggested for *H. panicea* (Knobloch et al. 2019) and other demosponge species that exhibit varying internal oxygen levels, such as *Theonella swinhoei* (Lavy et al. 2016). *H. panicea* sponges from Icelandic waters, for instance, are hosts for a dominant and temporally-stable alphaproteobacterium (‘*Candidatus* Halichondribacter symbioticus’; Knobloch et al. 2019), whose draft gene repertoire points towards the dynamic exchange of metabolites between this symbiont and the sponge–host environment, including ammonia assimilation or the production of a putative bacteriocin (Knobloch et al. 2020).

Perspective

Our study demonstrates that modular luminescence life-time imaging is well suited to investigate 2-dimensional and temporal oxygen dynamics in sponges. Shifting oxygen levels in the sponge mesohyl, as observed in the present study, reflect a combination of effects from changes in the sponge host pumping activity and internal respiration by both sponge cells and microbial cells. We found that contraction of the sponge osculum leads to concentric anoxia in the innermost part of the sponge explant and an oxygen gradient with increasing oxygen levels towards the explant circumference. Our findings support a previous hypothesis that the subsequent diffusive flux of oxygen across the pinacoderm of *H. panicea* explants facilitates oxygenation of major parts of sponge tissue during non-pumping periods

(Kumala and Canfield 2018). We hypothesize that contraction of select compartments of the aquiferous system may enable sponges to regulate oxygen in specific parts of the sponge interior, which may result in heterogeneous oxygenation of sponge tissue. Spatial and temporal shifting between internal oxygen levels may facilitate distinct microbes to colonize niches in the sponge interior according to their metabolic oxygen requirements, enabling intertwined metabolic networking between sponges and their internal microbiomes. New 3-dimensional O₂ measuring approaches, such as O₂-sensitive nanoparticles (Koren et al. 2016; Brodersen et al. 2020), combined with the laser-induced fluorescence techniques (Murniati et al. 2016, 2017), could facilitate the 3-D mapping of O₂ around sponges and even in the interior of a transparent sponge. This would enable a holistic assessment of O₂ consumption and subsequent fluxes across external as well as internal sponge–seawater interfaces, complementing the temporal and spatial oxygen dynamics revealed in the present study.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflict of interests to declare that are relevant to the content of this article.

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

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