Adaptive strategies of sponges to deoxygenated oceans

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Abstract

Ocean deoxygenation is one of the major consequences of climate change. In coastal waters, this process can be exacerbated by eutrophication, which is contributing to an alarming increase in the so-called "dead zones" globally. Despite its severity, the effect of reduced dissolved oxygen has only been studied for a very limited number of organisms, compared to other climate change impacts such as ocean acidification and warming. Here we experimentally assessed the response of sponges to moderate and severe simulated hypoxic events. We ran three laboratory experiments on four species from two different temperate oceans (NE Atlantic and SW Pacific). Sponges were exposed to a total of five hypoxic treatments, with increasing severity (3.3, 1.6, 0.5, 0.4 and 0.13 mg O_2 L^{-1}, over 7–12-days). We found that sponges are generally very tolerant of hypoxia. All the sponges survived in the experimental conditions, except *Polymastia crocea,* which showed significant mortality at the lowest oxygen concentration (0.13 mg O_2 L^{-1}, lethal median time: 286 h). In all species except *Suberites carnosus*, hypoxic conditions do not significantly affect respiration rate down to 0.4 mg O_2 L^{-1}, showing that sponges can uptake oxygen at very low concentrations in the surrounding environment. Importantly, sponges displayed species-specific phenotypic modifications in response to the hypoxic treatments, including physiological, morphological, and behavioural changes. This phenotypic plasticity likely represents an adaptive strategy to live in reduced or low oxygen water. Our results also show that a single sponge species (i.e., *Suberites australiensis*) can display different strategies at different oxygen concentrations. Compared to other sessile organisms, sponges

generally showed higher tolerance to hypoxia, suggesting that sponges could be favoured and survive in future deoxygenated oceans.

KEYWORDS

climate change, Porifera, evolution, marine benthic hypoxia, hypoxic events, oxygen depletion, eutrophication, sessile organisms, dead zones, phenotypic plasticity

1 INTRODUCTION

Anthropogenic emissions of carbon dioxide and other greenhouse gasses have increased exponentially since the industrial revolution, causing significant changes in the Earth's climate (Raupach & Canadell, 2010; IPCC, 2021). Climate change has three main effects on the marine environment: warming, acidification, and oxygen decline (Bijma et al., 2013). While most ecological and physiological research has targeted the first two stressors, deoxygenation remains comparatively neglected (Limburg et al., 2017). Despite the scant attention, recent research shows that oxygen loss is a major anthropogenic stressor for marine biota that may exceed the severity of the combined effects of ocean warming and acidification (Sampaio et al., 2021).

Oxygen is essential to all aerobic life, and ocean deoxygenation has the potential to affect all biogeochemical and biological processes within the oceans (Semenza, 2007; Levin & Breitburg, 2015). In the open sea, warming is considered the main cause of O_2 reduction: an increase in sea temperature leads to decreased O_2 solubility, increased water stratification, and alterations to oceanic circulation, which reduces O_2 supply to the ocean interior (Doney, 2010; Keeling et al., 2010). Higher temperatures also enhance microbial respiration, which can further deplete oxygen in marine ecosystems (Altieri & Diaz, 2019; Robinson, 2019). Oxygen levels in the global oceans have already declined by 2% during the last 50 years, with more significant O_2 declines in the North Pacific and tropical oxygen minimum zones (OMZ) (Levin & Breitburg, 2015). This is likely to get worse in the future, with models predicting a global ocean reduction in O_2 of up to 7% by the end of the century (Keeling et al., 2010).

In coastal waters, climate-driven deoxygenation can be intensified by eutrophication (Nixon, 1995; Altieri & Gedan, 2015). The input of anthropogenic nutrients, such as fertilizers and human/livestock wastes, can increase algal growth resulting in an accumulation of organic material on the seafloor. This excess of organic matter is then degraded by bacteria, causing O_2 depletion that can lead to hypoxic conditions (Smith et al., 2006). In shallow and well-mixed waters, eutrophication-driven hypoxia is generally caused by nocturnal heterotrophic respiration, resulting in daily oscillations in

oxygen concentration. In contrast, long-term hypoxic events are more likely to occur in enclosed seas or basins (Levin et al., 2009). Hypoxia has widespread and severe impacts across taxonomic and functional groups. The intensity and duration of oxygen depletion are the main factors influencing the severity of hypoxic events on benthic organisms (Levin et al., 2009; Altieri & Diaz, 2019). Mild hypoxia can alter behavioural patterns, decrease feeding rates and cause changes in physiological processes (Vaquer-Sunyer & Duarte, 2008). Severe hypoxic events can cause mass mortalities, leading to the formation of the so-called "dead zones", areas largely devoid of macrofauna (Diaz & Rosenberg, 2008). Dead zones have been reported in small water bodies such as harbours, fjord and inlets, and large basins, such as the Baltic Sea, spreading over 60,000 km^2 (Altieri & Diaz, 2019). As climate and land use continue to change, coastal hypoxia is expected to worsen, with the increased occurrence, frequency, intensity, and duration of hypoxic events (Diaz & Rosenberg, 2011).

Despite the extent of the problem and the dramatic effects caused by ocean deoxygenation, the response of many groups of organisms to hypoxia is still poorly studied. This lack of knowledge limits our ability to model the effects of declining oxygen availability on marine ecosystems (Seibel, 2011). To date, research on tolerance to reduced levels of dissolved oxygen has primarily focused on fish, crustaceans and molluscs (Vaquer-Sunyer & Duarte, 2008), while very little is known about other groups, especially sessile organisms. Sessile organisms are particularly vulnerable to hypoxic events because they cannot move or migrate to well-oxygenated water. Furthermore, sessile organisms include many important habitat-forming species, so any change in their abundance could have major consequences for the ecosystems they support (Vergés et al., 2019; Woodhead et al., 2019; Piazzi et al., 2021). Therefore, it is critical to understand how these organisms respond to hypoxia to predict possible future changes and effectively manage marine ecosystems.

Sponges are the dominant sessile organisms in many marine ecosystems and are found in high abundance in tropical, temperate, and polar ecosystems (Ayling, 1983, Bell et al., 2020). They perform many important ecological functions, including contributing to nutrient cycling, bioerosion, enhancing ecosystem complexity and providing habitats for a wide range of associated organisms (Wulff, 2006; Bell, 2008; Maldonado et al., 2012). Despite being important components of marine ecosystems, sponge tolerance to hypoxia has been poorly investigated to date. Mills et al. (2014) showed that *Halichondria panicea* can feed and respire with oxygen levels down to 4% of air saturation. However, the authors did not provide information on the duration of the treatments and replication; furthermore, in the same study, information on the temperature and salinity of the water was unavailable, so it is not possible to derive the actual oxygen concentrations to which sponges were exposed. Two other relevant experiments have investigated the short-term response of sponges to hypoxia. Mills et al. (2018) exposed *Tethya wilhelma* to a step decreasing oxygen

concentration (30–40 h with O_2 lower than 10% a.s., 0.7 mg L^{-1}). They found that sponges continued to perform periodic full-body contractions down to 0.27 mg O_2 L^{-1}, but ceased below that concentration. Leys & Kahn (2018) exposed *Geodia barretti* to 6.5 h of hypoxia (7% air saturation, 0.6 mg O_2 L^{-1}), and found that sponge respiration rate remained unchanged. However, filtration rates dropped almost immediately after the oxygen level was reduced. Despite these earlier studies, we still have very little insight into how sponges may cope with hypoxic events caused by ocean and coastal deoxygenation.

Here we provide the first comprehensive assessment of sponge response to hypoxia. Specifically, we experimentally investigated the physiological, behavioural, and morphological responses of four temperate sponge species to moderate and severe hypoxic conditions. We ran the first experiment to expose sponges to moderate hypoxic conditions for seven days, including a wide range of dissolved oxygen concentrations (0.5, 1.6 and 3.3 mg O_2 L^{-1}). Subsequently, we investigated sponge response to severe hypoxia (0.13 and 0.4 mg O_2 L^{-1}) for 12 days with two additional experiments. Finally, we discuss sponge tolerance to low dissolved oxygen compared to other sessile organisms in the context of future climatic conditions.

2 MATERIALS AND METHODS

2.1 Study area and species

Experiment 1 (moderate hypoxia) was performed in Ireland (Renouf Laboratory, Lough Hyne) on two abundant North-East Atlantic sponge species: *Cliona celata* Grant, 1826 and *Suberites carnosus* (Johnston, 1842). Experiments 2 and 3 (severe hypoxia) were performed in New Zealand (WUCEL, Wellington) on two abundant temperate Australasian species: *Polymastia crocea* Kelly-Borges and Bergquist, 1997 and *Suberites australiensis* Bergquist, 1968.

2.2 Experiment 1: moderate hypoxia

In the first experiment, we investigated the response of *Cliona celata* and *Suberites carnosus* to a wide range of oxygen concentrations, using an air-tight system with a continuous flow of seawater. Sponges were exposed to ~95% (7.71 ± 0.19 mg O_2 L^{-1}), ~40% (3.34 ± 0.17 mg O_2 L^{-1}), ~20% (1.56 ± 0.19 mg O_2 L^{-1}) and ~6% (0.48 ± 0.09 mg O_2 L^{-1}) air saturation (a.s.) for seven days (a summary of the seawater parameters is provided in Table S1).

The experimental set-up (see scheme in Figure S1) consisted of two independent replicate modules for each treatment, randomly distributed in the experimental set-up. To condition water, we used two header tanks for each experimental module: one providing water and one reservoir. Header tanks were filled with 10-µm-filtered seawater. The oxygen level was then lowered and maintained to the desired dissolved oxygen concentration by bubbling specific mixtures of N_2 (BOC, food-grade) and air, through glass-ceramic diffusers. Hypoxic gas blends were prepared by decanting food-grade N_2 and air in 15 L scuba cylinders using an oxygen decanting assembly (Undersea Ltd, 5215) with a DPM-300 digital gauge (0.25% accuracy). Oxygen concentration was then checked with a Nuvair Pro O_2 Analyser and adjusted, if necessary.

Conditioned water was delivered to two replicate experimental chambers (2.3 L) for each system at a rate of 25 L per day, ensuring 100% water replacement every 2 h and 15 min. Water circulation within each experimental chamber was provided by the gravity-driven water flow (~3 cm/s). Temperature was kept constant using a water bath controlled by an aquarium chiller.

Cliona celata was collected from the Kedges (51°27'41.4 "N 9°20'44.2 "W), whereas *Suberites carnosus* was collected from the rocky cliffs of Lough Hyne (51°30'00.4"N 9°18'03.9"W). For both species, sampling was carried out at 10–18 m in June 2019 and sponges collected were at least 2 m apart. Sponges were then left to recover for two months from harvesting stress in a 1 m^3 underwater cage placed at 8 m of depth.

Sponges were then transferred to the experimental system and randomly distributed across the experimental chambers. The experimental design consisted of 32 experimental chambers (two replicate chambers for each species for each replicate module, and two replicate modules for each treatment). A diagram of the experimental design is reported in Figure S2. Three sponges were placed in each chamber (6 sponges in total for each replicate module and 12 for each treatment). Sponges belonging to different species were not mixed but kept in separate chambers. Sponges were left to acclimate with oxygen saturated air for five days before oxygen was lowered by introducing hypoxic water into the chambers. Oxygen concentration was lowered in 24h and was then maintained for seven days until the end of the experiment (a graph showing the oxygen concentration in the different treatments over time is provided in Figure S3). In natural ecosystems, hypoxic conditions can develop in short times ranging from hours to a few days (Breitburg, 1990; Nezlin et al., 2009), so we consider these acclimation times appropriate and ecologically relevant. Temperature and oxygen concentration inside the experimental chamber were measured twice a day using a Fibox 4 oxygen meter with a dipping probe (Presence GmbH, Germany). A two-spot calibration was performed on the oxygen probe every three days, using sodium dithionite for 0 % oxygen and air-saturated water for 100% oxygen.

2.3 Experiments 2 and 3: severe hypoxia

We also investigated the response of sponges (*Polymastia crocea* and *Suberites australiensis*) to severe hypoxia through two separate experiments using an air-tight system. In experiment 2, sponges were exposed to ~5% (0.4 ± 0.04 mg O2 L^{-1}), and ~100% a.s. (8.34 ± 0.13 mg O_2 L^{-1}), while in experiment 3, sponges were exposed ~1.5% (0.13 ± 0.02 mg O_2 L^{-1}), and ~100% a.s. (8.15 ± 0.16 mg O_2 L^{-1}). The different oxygen concentration in the controls was due to the small difference in temperature between the two experiments (13.3 ± 0.5 °C in experiment 2 compared to 14.3 ± 0.6 °C in experiment 3). A summary of the seawater parameters is provided in Table S1.

Sponges were kept in independent cylindrical air-sealed polypropylene chambers (10 L), randomly distributed inside a water bath. Every two days, ~70% of the water was replaced using 10-µm-filtered seawater, preconditioned to the desired oxygen concentration in independent conditioning tanks. Oxygen concentration was then maintained by bubbling air or air-N_2 blends through glass-ceramic diffusers (see section 2.1.2 for more details on gas blends). Custom made de-bubbler devices were used to eliminate bubbles coming from the ceramic diffusers that could affect sponges (Figure S4). The sponges were fed twice a day with *Nannochloropsis* microalgae (1–2 µm cell diameter; Nanno 3600™ Reed Mariculture, US.). Water circulation within each experimental chamber was provided by the de-bubbler device and an additional water pump located on the side

of the chamber, which provided a constant circular water flow. The chambers were placed in a water bath to control water temperature.

Polymastia crocea was collected from Barrett Reef (Wellington South Coast, 41°20'31.1"S 174°50'09.7"E) by cutting fragments (~8 cm^3) from separate sponges (at least 5 m apart). Whole specimens of *Suberites australiensis* were collected from Mahanga Bay (Wellington Harbour, 41°17'32.2"S 174°50'06.5"E), attached to a fragment of their respective substrate. Sponges were then left to recover for three weeks after sampling and cutting stress in water tables with 10-µm-filtered flow-through seawater.

Sponges were then transferred to the experimental system, consisting of 12 experimental chambers (3 independent replicate chambers for each species and treatment combination). Five sponges were placed in each chamber (15 sponges in total for each treatment). Sponges were left to acclimate with oxygen saturated air for five days. Oxygen was then lowered by bubbling a specific Air-N_2 mixture. In experiment 2, oxygen was lowered in ~24 hours and then maintained at 5% a.s. (0.4 mg O_2 L^{-1}) for 12 days until the end of the experiment. While in experiment 3, oxygen was lowered to 1.5% a.s. (0.13 mg O_2 L^{-1}) in ~72 hours (which included a preacclimation at 10% a.s., 1 mg O_2 L^{-1}) and maintained for 12 days until the end of the experiment (Fig. S3). This further acclimation in hypoxic conditions was made because of the very low O_2 concentration of the treatment. In experiment 3, due to the very low concentration of O_2 (0.13 ± 0.02 mg L^{-1}), oxygen increased to ~0.3 mg L^{-1} for about 20 minutes during daily examinations. Temperature and oxygen concentration inside the experimental chamber were measured twice a day using Fibox 4 oxygen meter with a dipping probe (Presence GmbH, Germany). A two-spot calibration was performed on the oxygen probe every three days, using sodium dithionite for 0% oxygen and air-saturated water for 100% oxygen.

2.4 Response variables

2.4.1 Survival and health monitoring

Sponge health was monitored daily during the experiment. Sponges showing ≥ 25% of external necrosis were considered dead and removed from their treatment tanks during the daily checks, so as not to impact other sponges in the treatments. At the end of the experiments, all sponges were sectioned to assess the presence of any internal necrosis.

2.4.2 Respiration Rate

For all the experiments, respiration rate was measured on the same specimens at *T*0 (before the beginning of the experiment), *T*1/2 (after two days from the beginning of the final treatment in experiment 1, and after five days in experiments 2 and 3) and *T-end* (end of the experiment). In experiment 1 (moderate hypoxia), we measured respiration rates of three sponges in each replicate module (n = 6 for each treatment). In experiments 2 and 3 (severe hypoxia), respiration rates were measured on three sponges in each experimental chamber (n = 9 for each treatment). To measure respiration rate, sponges were placed in sealed cylindrical glass respiration chambers (150 ml for *Cliona celata*; 80 ml for *Suberites carnosus*; 250 ml for *Polymastia crocea* and *Suberites australiensis*) with PreSens oxygen sensor spots (SP-PSt3-NAU) attached to their inner surface. Experimental chambers contained either oxygen saturated water (pre-experimental measurements and controls) or water at a slightly higher oxygen concentration than the experimental treatment (+20–50%, depending on the treatment) collected from the respective header tanks. Respiration rates were not performed on sponges from experiment 3 (1.5% a.s.). The incubations were performed in controlled temperature (water bath) and dark conditions. The water inside the respirometry chambers was gently stirred using a magnetic stir bar. After 20 min of acclimation, oxygen concentration inside the chambers was measured every 10 min for 1 hour, using a Fibox 4 oxygen meter with a polymer optical fibre (POF). Respiration measurements were ended prematurely if the oxygen level fell below 70% of the treatment concentration to avoid any detrimental effect on the sponges. Blank incubations, containing only seawater were performed every respiration run and used to correct for any microbial community respiration in the seawater. A two-point calibration was performed on the oxygen sensor spots before each measurement session.

Respiration measurements were standardized to sponge ash-free dry weight (*AFDW*) from buoyant weight (*BW*) measurements (Fig. S5). For *Suberites australiensis*, it was not possible to estimate *AFDW* from *BW* due to the abundant external material accumulated by the sponge inside the tissue that influences the *BW*. For this species, we measured the *AFDW* of all the specimens used in the respirations at the *T-end*, and we assumed that sponges had the same weight at *T*0 and *T*1/2.

2.4.3 Changes in weight, size, and morphology

Changes in weight and size over time relative to the initial values were estimated by calculating the buoyant weight variation (*BWV*) and contracted area variation (*CAV*). For all of the experiments, buoyant weights (*BW*) of all experimental sponges (except *Suberites australiensis*) were taken at *T*0 and *T-end* and used to calculate relative buoyant weight variation as $BWV = [(BW_{T\text{-}end} - BW_{T0}) / BW_{T0}]$

· 100. Buoyant weight was measured with a digital scale (A&D FX-200i) following the methods of Osinga et al. (1999). For experiments 2 and 3 (severe hypoxia), photographs of contracted sponges were taken at *T*0 and *T-end* to measure sponge contracted area (*CA*) and calculate contracted area variation as $CAV = [(CA_{T\text{-}end} - CA_{T0}) / CA_{T0}] \cdot 100$ (following Osinga et al., 1999). Contraction was achieved by disturbing sponges with a blunt plastic rod (being careful not to hurt the sponge) and waiting for one hour for the sponge to react to the stimulus. All the photographs were analysed using ImageJ (US National Institutes of Health, Bethesda, Md, USA).

During experiments 2 and 3, treatment conditions induced the development of peculiar morphological structures in some specimens of both *Polymastia crocea* and *Suberites australiensis*. Sponges were photographed and monitored daily to calculate the percentage of specimens developing these structures and the median time of occurrence.

2.4.4 Sponge contractile behaviour

During experiments 2 (5% a.s.) and 3 (1.5% a.s.), sponge contractile behaviour was monitored daily from *T*0 to *T-end*, on all experimental sponges through photographic analysis. For *Suberites australiensis*, the contractile behaviour was estimated using an "expansion ratio" (*EXPR*) calculated as $EXPR = A_{Ti} / CA_{T0}$, where A_{Ti} is the area occupied at T_i and CA_{T0} is the contracted area at *T*0. Area was preferred over volume because of the low invasiveness of the measurements. In *Polymastia crocea,* contraction/expansion mainly occur at the papillae level, so the contractile behaviour was estimated from the ratio of expanded papillae (*REP*) calculated as $REP = P_E/P_{tot}$, where P_E is the number of visible expanded papillae and P_{tot} is total number of visible papillae. Expanded papillae were defined as papillae whose length was at least two and a half times the width.

2.4.5 Pumping rate

Pumping rate was only calculated for *Suberites australiensis* from experiments 2 and 3 (severe hypoxia). Having only one osculum of relatively large size, this species was particularly suitable for investigating changes in pumping rate. To minimize sponge disturbance during the experiment, pumping rate (PR) was derived from the measurement of the sponge osculum cross-sectional area (OSA). In sponges, pumping rate (PR) is correlated with OSA (e.g. Goldstein et al., 2019; Morganti et al., 2021). In the case of *S. australiensis*, this relationship was calculated on 20 sponges (following Yahel et al., 2005) and was found as $PR = 6.55 \cdot OSA^{1.43}$ (Fig. S6). Photographs of the oscula with scale were taken daily from *T*0 to *T-end*, on three sponges in each experimental chamber (the same specimens each time point, n = 9 per treatment). Since *S. australiensis* has only one osculum, pumping rate was then standardized per sponge volume.

2.4.6 Histology

Histological sections of *Suberites australiensis* from the severe hypoxia experiments were analyzed to calculate the percentage of the sponge body occupied by the aquiferous system (system of connected water channels inside the sponge). At *T-end*, two contracted sponges for each experimental chamber (n = 6 per treatment) were fixed and processed following the methods of Strano et al. (2021). Three replicate sections for each sponge were then photographed under a dissecting microscope (Olympus SZ61) and photographed using a Canon EOS 70D digital camera. To calculate the area occupied by the aquiferous system, pictures were analyzed using ImageJ.

2.5 Data analysis

All the statistical data analyses were performed in R version 3.1.3 (R Core Team, 2013), except PERMANOVA models, which were performed using PRIMER v7 with PERMANOVA+ add-on (Anderson et al., 2008; Clarke & Gorley, 2015). Experiments 2 and 3 were analyzed separately. To investigate respiration rate, pumping rate and expansion ratio in *Suberites australiensis* from experiment 2, we used linear mixed-effects models with normally distributed errors and random intercepts (lmer, *lme4* package; Bates et al., 2015). For pumping rate, we added a constant variance function structure (varIdent) to the linear mixed-effects models to allow different variances for each treatment at each time point (lme, *R* package *nlme*; Pinheiro et al., 2021). The constant variance function structure was necessary because the variance of the response variable differed across treatments and experimental days. To investigate the effect of time and treatment on the expansion ratio of *Polymastia crocea* in experiment 3, we used a generalized linear mixed model with beta regression and logit link (*glmmTMB*, Brooks et al. 2017). In all the mixed models, treatment and time were considered fixed effects, while experimental chamber and sponge specimen were considered random effects. The experimental chamber effect was included to address pseudo-replication. For these models, fixed- and random-effect terms were tested using the function anova and ranova (*R* package *lmerTest*, Kuznetsova et al., 2017), respectively; while post hoc pairwise comparisons were computed on estimated marginal means using emmeans (*R* package *emmeans*; Lenth, 2021). The ratio of expanded papillae in *P. crocea* from experiment 2 and expansion ratio in *S. australiensis* from experiment 3 were investigated using repeated measure univariate PERMANOVA (Anderson, 2001; 2014), because did not meet the normality assumption for mixed-effects models. Pairwise tests were then calculated using permutation *t*-tests (*R* package *RVAideMemoire*; Hervé, 2021). PERMANOVA and permutation *t*-tests were also used to supplement mixed-effects models when there were concerns about the normality of the residuals (pumping rate in *S. australiensis*). Change over time relative to the initial value of buoyant weight and contracted area, and differences in

percentage occupied by the aquiferous system were investigated using Kruskal–Wallis H tests, Welch's t-tests or Wilcoxon Signed-Rank Tests, depending on the variable. Respiration rates from experiment 1 were log (x + 1) transformed, and pumping rates were square-root transformed to meet normality assumptions. The goodness of fit, normality and homoscedasticity of the errors were checked for all models by inspecting plots of the normalized residuals and the quantile-quantile plots. All the multiple comparisons were corrected using Benjamini-Hochberg Procedure, but uncorrected p-values are reported in the text. All the statistical analyses made for each variable are reported and summarized in Table S2.

Time to event analysis for sponge survival and development of peculiar morphological structures (modified papillae and protruding oscular membranes) was performed using Kaplan-Meier Method, and *p*-values were calculated using the Log Rank Test implemented in the *survival R* package (Therneau, 2021). Median lethal time (LT_{50}) and median time to the development of modified morphological structures were calculated using a logistic model.

3 RESULTS

3.1 Sponge responses to moderate hypoxia

All the sponges of experiment 1 survived the seven days of treatment, except one specimen of *Suberites carnosus* in the lower DO treatment (6% a.s.), which presented internal necrosis on the final day of the experiment.

Mean buoyant weight variation between *T*0 and *T-end* ranged between -1% and -1.6% for *Cliona celata* and +2.1% and -0.5% in *Suberites carnosus*. There were no differences in in buoyant weight variation among treatments for both species, but for *C. celata* there was a significant slight decrease in weight in the 40% a.s. (-1.6%, $p = 0.008$) and 20% a.s. (-1.4%, $p = 0.008$) treatments (Tab. S3; Fig. S7).

For *Cliona celata*, there was no significant effect of time or treatment on the respiration rate (Tab. S4). However, pairwise comparisons revealed a significant decrease ($p = 0.028$) in the 20% a.s. treatment between day 0 and 7, and a significant increase ($p = 0.029$) in the 6% a.s. treatment between day 2 and day 7, but both became non-significant after the correction for multiple comparisons (Tab. S4). However, the data suggest a coherent temporal pattern in the respiration rate in both 20% a.s. and 6% a.s. treatments. *C. celata* respiration rate decreased after two days from the start of the experiment and then increased at the end of the experiment. In contrast, in both the 100% a.s. and 40% a.s. treatments, respiration rate remained stable for the whole duration of the experiment (Fig. 1a).

For *Suberites carnosus*, there was a significant interaction of time and treatment ($p = 0.007$) on the respiration rate (Tab. S5). Pairwise comparisons revealed a significant decrease in respiration rate between day 0 and 7 ($p < 0.0001$), and day 2 and 7 ($p < 0.0001$) (Tab. S5). The respiration rate also slightly decreased towards the end of the experiment in the 20% a.s. treatment (but not significantly), while in both the 100% a.s. and 40% a.s. treatments, respiration rate remained stable for the duration of the experiment (Fig. 1b).

3.2 Sponge responses to severe hypoxia

3.2.1 Survival

Sponge survival differed among species, with *Suberites australiensis* more tolerant than *Polymastia crocea*. No mortality was observed for *S. australiensis* in both experiments 2 (5% a.s.) and 3 (1.5% a.s.). In contrast, for *P. crocea*, significant mortality ($p = 0.001$) was observed in sponges exposed to

the 1.5% a.s. treatment, starting from day 10 (day 12 when including the hypoxic acclimation), and with a median lethal time of 11.9 ± 0.3 days (Fig S8–9). Eight out of 15 sponges had died by the end of the experiment. No mortality was observed for *P. crocea* in the 5% a.s. treatment.

3.2.2 Change in weight and size

For *Polymastia crocea*, buoyant weight variation between *T0* and *T-end* differed among treatments in experiment 3 (1.5% a.s.) ($t = 2.82$, $p = 0.012$), but not in experiment 2 (5% a.s.). Sponges from the 1.5% a.s. treatment experienced a significant decrease in buoyant weight (-7.1%, $t = -5.17$, $p = 0.002$), while the controls did not experience any significant change (Tab. S6; Fig. S10).

The relative variation in area of contracted sponges (after stimulating contraction) between *T0* and *T-end* differed significantly between treatments and controls for both *Polymastia crocea* ($W = 12$, $p < 0.0001$) and *Suberites australiensis* ($W = 13$, $p < 0.0001$), but only in experiment 2 (5% a.s.). Both *P. crocea* and *S. australiensis*, from the 5% a.s. treatment, experienced an increase in contracted area (+18.9%, $W = 120$, $p = 0.0001$ and +18.4%, $W = 105$, $p = 0.008$, respectively). While *S. australiensis* from the control treatment (experiment 2) experienced a decrease in contracted area (-15.3%, $W = 3$, $p = 0.0003$) (Tab. S7; Fig. S11).

3.2.3 Sponge contractile behaviour

Low DO treatments generally induced sponge expansion, but the response differed between species, and it was generally more marked in the 5% a.s. treatment. In *Polymastia crocea*, the ratio of expanded papillae was significantly affected by the interaction between time and treatment in both experiments 2 ($p = 0.0001$) and 3 ($p < 0.0001$ and $p = 0.03$) (Tab. S8–9). During experiment 2 (5% a.s.), the treatment induced a progressive expansion of papillae from day 2. The ratio of expanded papillae in sponges from the hypoxic treatment became significantly higher than control sponges from day 6 to the end of the experiment ($p = 0.0002$–0.005) (Tab. S8; Fig. 2a). A similar trend was found in experiment 3 (1.5% a.s.), but the ratio of expanded papillae of the treatment sponges was more variable and became significantly different only at day 9 ($p = 0.003$) (Tab. S9; Fig. 2b). In this experiment, we also found a correlation between the ratio of expanded papillae and mortality. Sponges that survived the treatment had a significantly higher maximum ratio of expanded papillae compared to sponges that died, both when the maximum ratio was calculated at the end of the experiment (Welch *t*-test: $t = 5.3$, $p = 0.0005$) and at day ten, before sponges started to die (Welch *t*-test: $t = 4.6$, $p = 0.0007$).

In *Suberites australiensis*, there was a significant interactive effect of treatment and time ($p < 0.0001$) on the expansion ratio in experiment 2 (5% a.s.), but only an effect of time ($p = 0.01$) in experiment 3 (1.5% a.s.) (Tab. S10–11). For experiment 2 (5% a.s.), pairwise comparisons found significant expansion in sponges (+60%, $p < 0.0001$) between day 0 and 1. Sponges then remained expanded for the whole duration of the experiment, and the expansion ratio was significantly higher in the treatments compared to the controls from the first to the last day of the experiment ($p < 0.0003$) (Tab. S10; Fig. 2c, d).

3.2.4 Morphological modifications

During experiments 2 (5% a.s.) and 3 (1.5% a.s.), some *Polymastia crocea* and *Suberites australiensis* sponges exposed to hypoxic treatments underwent morphological modifications (Fig. 3; S12). In some *P. crocea*, the conical papillae showed a progressive elongation, flattening, and, in some cases, spiralization (Fig. S12a–f). This process occurred in both the 5% a.s. and 1.5% a.s. treatments, but morphological changes were more pronounced in lower DO treatment (Fig. S12d-e). Exposed to the 1.5% a.s. treatment, some sponges developed papillae so slender that they could not sustain their weight (Fig. S12d-e). The development of these modified papillae was also associated with an apparent increase in the porosity of the sponge external surface (Fig. S12e). In the 5% a.s. treatment, 73% of *P. crocea* developed modified papillae, starting from day 6. In the 1.5% a.s. treatment, 60% of sponges developed modified papillae, starting from day 2, from the beginning of the final treatment (day 4 considering hypoxic acclimation period) (Fig. S13). The median time of development of these morphological structures (considering only the sponges that developed them) was 7.2 ± 0.2 days in the 5% a.s. treatment and 4.6 ± 0.4 days in the 1.5% a.s. treatment (Fig. S14). Although not significant ($\chi^2 = 3.62$, $p = 0.057$), a relationship between modified papillae and survival was found: among the *P. crocea* that survived the 1.5% a.s. treatment, six had developed modified papillae, while one had not; while among the sponges that died following the 1.5% a.s. treatment, three had developed modified papillae, while five had not.

In the the 5% a.s. treatment, 53% of *Suberites australiensis* developed a semi-transparent protruding membrane surrounding the oscula. This membrane progressively reduced the oscular-cross sectional area (Fig. 3; S12g–i). The median time it took for these protruding oscular membranes to become noticeable (considering only the sponges that developed them) was 5.1 ± 0.2 days (Fig. S14). By the end of the experiment, 53% of *S. australiensis* had developed these structures (Fig. S13).

3.2.5 Histology

Histological analyses indicated that hypoxia influences the percentage of the sponge body occupied by the aquiferous system in *Suberites australiensis*. At the end of experiment 2 (5% a.s.), treatment sponges had a significantly higher percentage of aquiferous system (t = -9.82, p < 0.0001), compared to the controls (35.9 ± 7.1% vs 6.4 ± 1.8 %). No significant differences were found for experiment 3 (1.5% a.s.) (Fig. 3; S12j–m; S15).

3.2.6 Pumping rate

Oxygen concentration significantly affected the pumping rate of *Suberites australiensis* in both experiments 2 (5% a.s.) and 3 (1.5% a.s.) (Tab. S12–15). In experiment 2 (5% a.s.), there was significant interaction of treatment and time (p = 0.0001, linear mixed-effects model; Tab. S12). Pumping rate significantly increased from day 0 to 1 (p < 0.0001), remained stable from day 1 to 2, and then decreased from day 2 to 3 (p < 0.0001) and from day 3 to 4 (p = 0.005) (Tab. S12). Sponges from the 5% a.s. treatment had a significantly higher pumping rate than the control at day 1 (p = 0.007) and 2 (p = 0.002) (Tab. S12; Fig. 2e). Similar results were given by PERMANOVA (Tab. S13). For experiment 3 (1.5% a.s.), both the linear mixed-effects model and PERMANOVA revealed a significant interaction between time and treatment (p = 0.049 and p = 0.002, respectively) on the pumping rate of *S. australiensis* (Tab. S14–15). However, differences were less marked than experiment 2 and pairwise comparisons only revealed a slight decrease in the pumping rate of treatment sponges between day 0 and 14 (p = 0.0002) (Tab. S14; Fig. 2f).

3.2.7 Respiration rate

In experiment 2 (5% a.s.), linear mixed-effects models only revealed a significant effect of time on the respiration rate, for both *Polymastia crocea* and *Suberites australiensis* (Tab. S16–17; Fig. 4). In *P. crocea*, pairwise comparisons revealed a slightly higher respiration rate of the controls at day 12 compared to day 0 (p = 0.008) and day 5 (p = 0.004), but no differences between controls and treatments at any time. In *S. australiensis,* pairwise comparisons revealed a slightly lower respiration rate at day 12 compared to day 0 (p = 0.008) and day 5 (p = 0.005) in control sponges; while in treatment sponges, respiration rate was slightly lower at day 5 (p = 0.032) and 12 (p = 0.016) compared to day 0, but also in this case, there was no significant difference between treatments and controls at any time point.

4 DISCUSSION

Hypoxia has become an increasingly common problem in the marine environment and will likely become worse in the future (Diaz & Rosenberg, 2011). Nevertheless, the direct effects of hypoxia on marine organisms are still very poorly studied (Vaquer-Sunyer & Duarte, 2008). We describe the first multi-species experiment from two oceans to test sponge tolerance, behaviour, and physiological responses to oxygen concentrations as low as 1.5% a.s. (0.13 mg O_2 L^{-1}) for up to 12 days. We found that sponges are generally very tolerant to low DO irrespective of species or location. Only *Polymastia crocea* showed mortality in the lower DO treatment (0.13 mg O_2 L^{-1}, LT_{50} = 286 h). Furthermore, our results suggest that sponges can display species-specific acclimation, including physiological, morphological and behavioural changes, in response to severe hypoxia that might help them survive periods of very low oxygen. Our study also suggests that the same species can show different adaptive strategies for different degrees of hypoxia.

4.1 Sponge response to hypoxia

Our results suggest that sub-lethal oxygen thresholds for most sponges are in the range of 6–20% a.s. (0.48–1.56 mg O_2 L^{-1}), while lethal thresholds are lower than 5% a.s. (0.4 mg O_2 L^{-1}). These pieces of evidence are consistent with Mills et al. (2014) for *Halichondria panicea*, which showed a sub-lethal response starting from 17% air saturation. However, our results contrast with Mills et al. (2018) studying *Tethya wilhelma*, which did not show any response down to 4% a.s. (0.27 mg O_2 L^{-1}). The very high tolerance of *T. wilhelma* could be explained by the low metabolism of *Tethya* species generally (Leys & Kahn, 2018), and by their very small size (0.5–1 cm) (Sarà et al., 2001). Of the two species we exposed to the lowest DO concentration (1.5% a.s., 0.13 mg O_2 L^{-1}), only *Polymastia crocea* showed mortality, while all the *Suberites australiensis* survived the 12 days of treatment conditions. This differential response could be due to the different habitats where these species are usually found. *Polymastia crocea* lives on rocky reefs, while *S. australiensis* lives on sediments in bays and semi-enclosed basins, where hypoxic events are more likely to occur (Diaz & Rosenberg, 2008; de Cook, 2010).

Some sponges can live in anoxic conditions for several months, such as the sponges of the family Raspailidae found in the deeper cliffs of Lough Hyne (Bell & Barnes, 2000; McAllen et al., 2009; Micaroni et al., 2021). Schuster et al. (2021) suggested that this tolerance could be conferred by specific bacterial symbionts, which are able to carry out anaerobic metabolism. In addition, these sponges living in anoxia are all thin crusts, with a very high surface-to-volume ratio, which could favour the exchange of gases and the release of metabolic waste (Levin et al., 1991). Other examples

of sponges living in very low oxygen conditions are the ones found at the edges of Oxygen Minimum Zones (OMZ) (Mosch et al., 2012). These sponges can live with a consistent oxygen concentration as low as 0.13mg O_2 L^{-1} (Wishner et al., 1995, Murty et al., 2009). Sponges are not the only organisms able to live in OMZs. Many representatives of other phyla live in these extremely hypoxic conditions, where they benefit from the rich supply of organic matter. However, since OMZs have existed over geological timescales, organisms have had the time to evolve specific adaptations to cope with permanent hypoxia (Levin, 2003). Therefore, these organisms cannot be used to generalize tolerance to periodic hypoxic events experienced by organisms usually living in fully oxygenated waters.

The degree of hypoxia tolerance in sponges could also be influenced by the abundance and diversity of sponge-associated microbial symbionts. Based on bacterial biomass, sponges are generally divided into "low microbial abundance" (LMA) or "high microbial abundance" (HMA) species (Hentschel et al., 2003). Bacterial densities in HMA sponges are generally two to four orders of magnitude higher than in LMA sponges and can constitute up to 35% of the total sponge biomass (Vacelet, 1975; Hentschel et al., 2006). Sponges with HMA tend to have a lower choanocyte chamber density, and a slower pumping rate compared LMA sponges (Lavy et al., 2016), which means HMA species might have a lower ability to ventilate in low oxygen conditions. Furthermore, HMA species generally have a higher metabolic cost than LMA species, and therefore a higher oxygen requirement (Leys & Kahn, 2018). Although these differences suggest that LMA sponges might be better adapted to hypoxic conditions, HMA species have a higher diversity of microbial symbionts that could help them cope with low oxygen conditions (Hoffmann et al., 2005, Lavy et al., 2016). All the sponges for which responses to hypoxia has been investigated so far are LMA species (or are likely to be, based on known congenerics, see Kamke et al., 2010; Mills et al., 2014; Moitinho-Silva et al., 2017). Therefore, future research is needed to investigate the response of HMA sponges to hypoxia and shed light on possible differences between LMA and HMA sponges and the mechanisms involved.

Some organism's abilities to tolerate hypoxia result from their physiological ability to lower metabolism and oxygen demand (McAllen et al., 1999; Altieri, 2019). Instead, other species switch from aerobic to anaerobic metabolism or a combination of the two (Altieri & Diaz, 2019). Our results suggest that all our species (except *Suberites carnosus*) have respiration rates at 5–6% a.s. that are comparable to sponges in normoxic conditions. This is consistent with what was found in *Geodia barretti* and *H. panicea*, suggesting that sponges have a common ability to uptake oxygen at very low concentrations in the surrounding environment (Leys & Kahn, 2018). In *Cliona celata*, hypoxic water initially resulted in a decrease in the respiration rate, which then increased back to pre-treatment

levels after seven days of exposure. This suggests that the sponges gradually adjusted to hypoxic conditions. In *S. carnosus,* instead, the respiration rate remained stable after two days of exposure to low dissolved oxygen, but it more than halved after seven days. This response may allow *S. carnosus* to cope with long periods of hypoxia, in which sponges decrease their metabolism, as has been reported for other organisms (Hagerman, 1998; Mentel et al., 2014). Although our study shows that sponges can perform aerobic metabolism when exposed to extremely low oxygen concentrations, the presence of anaerobic metabolism cannot be excluded and needs further investigation.

Sponge species exposed to the lowest DO concentrations (0.4 and 0.13 mg O_2 L^{-1}) also showed other phenotypic modifications that could represent adaptive strategies to cope with hypoxia. In *Suberites australiensis*, hypoxic water (0.4 mg O_2 L^{-1}) induced expansion of the sponge body and the aquiferous system that lasted for the duration of the experiment. This expansion was likely semi-permanent as it persisted after inducing the contraction and corresponded to a reorganization of the sponge aquiferous system at the histological level. These behavioural and morphological changes are likely beneficial for the sponge, as higher internal water flow corresponds to an increase in oxygen that can be taken up. The body expansion was accompanied by a marked increase in the pumping rate that then dropped after two days. The pumping rate increase could be a strategy to increase ventilation and oxygen availability, similarly to other animals when exposed to hypoxic waters (Hagerman, 1998). However, the successive decrease in pumping rate (after two days) and the gradual production of a membrane to close the oscula remains unclear but could represent a trade-off between increasing ventilation and keeping the energetic coast of pumping reasonable. In *S. australiensis*, body expansion is correlated with an increase in osculum area, and osculum area is the main determinant of pumping rate in this and many other species (Morganti et al., 2021; Goldstein et al., 2019). Perhaps the increase in pumping rate only represents a physiological consequence of the body expansion and is then quickly brought back to normal, decreasing the osculum size by producing an oscular membrane. These physiological and morphological changes of *S. australiensis* described above was not present on sponges exposed to more severe hypoxia (0.13 mg O_2 L^{-1}). This could mean that the same sponge species may display different adaptive strategies to cope with decreased oxygen depending on the oxygen concentration. At 0.4 mg L^{-1}, oxygen might still be sufficient to support regular metabolism, but sponges may need to increase the amount of water flowing through their bodies to absorb the oxygen needed. However, 0.13 mg L^{-1} might be too low a DO concentration, and sponges might decrease their metabolism to cope with lack of oxygen, similarly to other metazoans (Hagerman 1998, Mentel et al., 2014).

Polymastia crocea also showed a behavioural change in response to hypoxic conditions: hypoxic water at 0.4 mg L^{-1} induced the progressive expansion of sponge papillae (where inhalant and exhalant channels are found), that was significantly greater than in the control sponges. It is unlikely that the papillae expansion represents an increase in sponge filtering activity because the respiration rate was very similar in the treatments and the controls. Therefore, sponges might expand their papillae to increase the volume occupied by the aquiferous systems, as in the case of *Suberites australiensis*, but also to access more oxygenated water further from the bottom. A similar response occurred in sponges exposed to 0.13 mg L^{-1} but with much more variability across specimens, and the statistical test did not detect any change. Interestingly, sponges that survived after the 12-day treatment had a significantly higher ratio of expanded papillae than sponges that died, suggesting that expansion might help cope with severe hypoxic conditions.

Along with behavioural changes, *Polymastia* underwent morphological modifications that could help to tolerate low DO. Papillae become thinner and flattened, and some even spiralized. These modifications of the papillae could increase the surface-to-volume ratio and help oxygen diffusion (Levin et al., 1991). The elongation of papillae, which accompanies the thinning, could be an evolutionary relic of a process that moved the inhalant pores of the papillae as far as possible from the surface. However, in the lowest DO treatment, papillae often lost their vertical orientation and laid horizontally on the sponge surface. We hypothesized that the new orientation of papillae was a consequence of their thinning process: probably papillae became so thin that they could not support their weight anymore. Interestingly, sponges that developed modified papillae showed less mortality than sponges that did not, although the evidence is not strong enough to claim this with confidence ($p = 0.057$). Therefore, these structures may not only represent a stress response, but could provide an advantage to the sponge. Further research is needed on this topic needed to elucidate the function of these structures.

Despite the remarkable tolerance of sponges to hypoxia observed in laboratory conditions, field observations suggest that severe hypoxic/anoxic events can catastrophically affect sponge populations. Mass mortalities of sponges following hypoxic/anoxic events have been reported both in temperate and tropical ecosystems (Stachowitsch, 1984, Altieri et al., 2017; Chu et al., 2018; Johnson et al., 2018; Kealoha et al., 2020). For example, in a hypoxic/anoxic event in the Gulf of Trieste, all the sponges living in several hundred km^2 died within 2-3 days (Stachowitsch, 1984). Some anemones survived up to a week, but virtually all organisms were dead within two weeks from the onset. Altieri et al. (2017) also reported widespread mortality of sponges and corals following a hypoxic event (~0.5 mg O_2 L^{-1}) that occurred in Bocas del Toro, Panama. Since this study focused on corals, it is unclear what proportion of the sponges were affected and if some species were more

tolerant than others. These reports highlight that hypoxic events, in their most severe form, leave no survivors.

Furthermore, it is possible that in natural conditions, other factors combine with low dissolved oxygen. For example, a recent meta-analysis showed that in marine organisms, increased temperature reduces survival times under hypoxia by 74% on average and increased median lethal concentration by 16% on average (Vaquer-Sunyer & Duarte, 2011). Another meta-analysis showed that hydrogen sulphide (H_2S) also reduces survival time of marine organisms under hypoxia by an average of 30% (Vaquer-Sunyer & Duarte, 2010). Acidification was shown to have additive or synergistic negative effects combined with hypoxia (Gobler & Baumann, 2016; Steckbauer et al., 2020). Since all these factors usually co-occur during hypoxic events, *in situ* sponge thresholds to hypoxia could be lower than determined through single stressor laboratory experiments (Diaz & Rosenberg, 1995; Steckbauer et al., 2020). Future experiments that evaluate the combined effect of these factors will be crucial to understand the full response of sponges to hypoxia in natural ecosystems.

Diel oxygen variation is another factor that could influence organism tolerance to hypoxia in natural conditions. In the photic zone of marine ecosystems, dissolved oxygen generally increases during the day because of photosynthesis and decreases at night because of aerobic respiration (Kroeker et al. 2019). The amplitude of these diel fluctuations can sometimes lead to hypoxia or complete anoxia at night and supersaturation in peak sunny hours, or both (Diaz & Breitburg, 2009). These extreme oxygen dynamics have been reported from a wide variety of macro- and micro-habitats from both tropical and temperate ecosystems, such as intertidal reef platforms, tide pools, semi-enclosed basins, tropical lagoons and the boundary layer around macroalgal canopies (Morris and Taylor, 1983, Frieder et al. 2012, Cornwall et al., 2013, Gruber et al., 2017, Trowbridge et al. 2017, Hughes et al., 2020). Diurnal fluctuations in oxygen can produce different responses from static exposure in laboratory experiments, which may either overestimate or underestimate the emergent effects of hypoxia in natural environments (Bumett & Stickle, 2001). Therefore, future experiments will need to account for current and future temporal variability in oxygen concentration to accurately forecast the emergent ecological effects of deoxygenation (Kroeker et al., 2019).

4.2 Hypoxia Tolerance of sponges compared to other sessile organisms

Marine organisms have very variable tolerance to low dissolved oxygen, with lethal thresholds ranging from 8.6 mg O_2 L^{-1} for the first larval zoea stage of the crustacean *Cancer irroratus*, to resistance to complete anoxia as in the case of the sea anemone *Metridium senile* and the oyster

Crassostrea virginica (Wahl 1984; Vaquer-Sunyer and Duarte, 2008). Sessile organisms are generally more tolerant than mobile ones, which is likely due to them not being able to escape hypoxic conditions (Altieri & Diaz, 2019). Therefore, sessile organisms that experience these conditions must have evolved other adaptive strategies to cope with reduced oxygen (Diaz & Rosenberg, 1995).

Here we provide new evidence to support the hypothesis that sponges are one of the groups of sessile organisms that are more tolerant to hypoxia and could be favoured in future deoxygenated oceans. Other phyla, such as cnidarians and bivalves, include very tolerant species that can cope with prolonged periods of anoxia (Fig. 5). This is not surprising since tolerance to severe hypoxia/anoxia is a widespread feature in the animal world, and many organisms independently evolved this feature to cope with local conditions (Hochachka & Lutz, 2001; Nilsson & Renshaw, 2004; Vaquer-Sunyer & Duarte, 2008). This ability is not restricted to invertebrates and includes higher animals such as fish and reptiles (Milton & Prentice, 2007; Vornanen et al., 2009).

What makes sponges unique as a phylum is their widespread tolerance to hypoxia. All the species investigated so far have been shown to cope with very low levels of dissolved oxygen. In contrast, other phyla have a much wider range of tolerances, with some species resistant to anoxia and others very sensitive to decreased oxygen (Fig. 5). For example, in sessile cnidarians, lethal hypoxia thresholds range between 0 and 4 mg O_2 L^{-1}, while sublethal ones are between 0.71 and 4.56 mg O_2 L^{-1} (Mangum, 1980; Dodds et al., 2007). In sessile bivalves, lethal thresholds range between 0 and 2 mg O_2 L^{-1}, with the sub-lethal threshold being 3.1 mg O_2 L^{-1} for *Mytilus galloprovincialis* (de Zwaan et al., 1991; Woo et al., 2013). Sponges, instead, show much less variation with known lethal thresholds that are lower than 0.5 mg O_2 L^{-1}, and sublethal thresholds that range between 0.27 and 1.56 mg O_2 L^{-1} (Mills et al., 2014; 2018) (Fig. 5). It is worth noting that lethal thresholds are highly dependent on the time of exposure. In the studies we considered, these ranged from a few days to weeks. However, there were no noticeable differences in the experimental duration and the median lethal time for the different organisms. Therefore, we believe that differences in time of exposure do not represent a bias in our comparison. In contrast, sublethal responses (e.g. changes in respiration rate, behaviour, and feeding activity) usually have rapid time-to-onset, so they will likely be independent of exposure time.

The high tolerance of sponges to hypoxia compared to other organisms can be explained by the evolutionary history of this group. Sponges are one of the most ancient groups of metazoans. They likely evolved before the Marinoan glaciation (657-645 million years ago), when oxygen was perhaps less than 10% of present atmospheric concentration (Love et al., 2009; Maloof et al., 2010; Brocks et al., 2017; Whelan et al., 2017; Cole et al., 2020; Turner, 2021). Modern sponges might have retained

an ancestral condition concerning oxygen requirements (Mills et al., 2014; 2018). Therefore, it is more likely that sponges unable to survive severe hypoxia today (e.g., *P. crocea*) have lost certain key ancestral adaptations to hypoxia, rather than hypoxia-tolerant lineages (e.g., *S. australiensis*) having evolved relatively new capacities for hypoxia tolerance (Müller et al., 2012). Likewise, other animals which might have evolved in similar conditions, such as ctenophores, also show great resistance to hypoxia (Thuesen et al., 2005). Therefore, we speculate that sponges' long evolutionary history could give these organisms an adaptive advantage in future deoxygenated oceans, since they may have experienced similar conditions in past geological eras.

CONCLUSIONS

Overall, sponges show high tolerance to low dissolved oxygen compared to all the other phyla of sessile marine organisms that have been studied. Species-specific phenotypic plasticity appears to help these organisms to overcome hypoxic events, and future research will need to elucidate the mechanisms behind these changes. This exceptional adaptive capacity of sponges could derive from their ancient evolutionary origin and could confer sponges a competitive advantage in future deoxygenated oceans over other organisms (Mills et al., 2014; Schuster et al., 2021).

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AUTHOR CONTRIBUTIONS

V.M., J.J.B and R.M. designed the study. V.M., F.S. and L.H. realized the experimental set-up. V.M. and F.S. conducted the experiments. V.M. and L.W. analyzed the data. V.M. and J.J.B wrote the original draft. All the authors participated in interpreting the results and contributed to the revision of the manuscript.

DATA AVAILABILITY STATEMENT

All data and the R code used in this paper are available on Figshare repository: https://doi.org/10.6084/m9.figshare.15169662.

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FIGURES

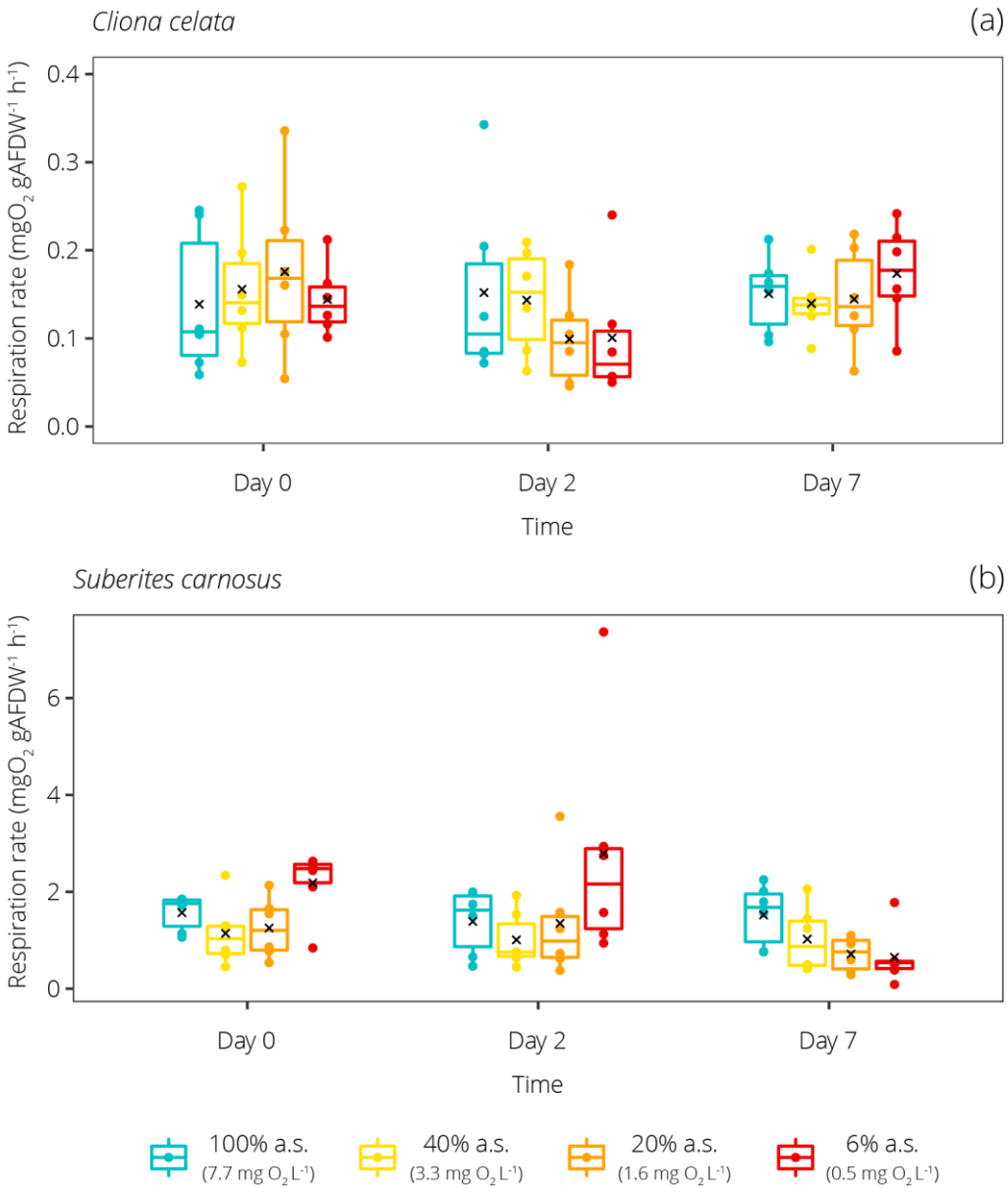

Figure 1. Respiration rates in (a) *Cliona celata* and (b) *Suberites carnosus* from experiment 1 (moderate hypoxia) measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. Horizontal bars inside the boxplots represent medians; the symbol × represents means. Lower and upper hinges of the boxplots correspond to the first and third quartiles, respectively. Points represent data points.

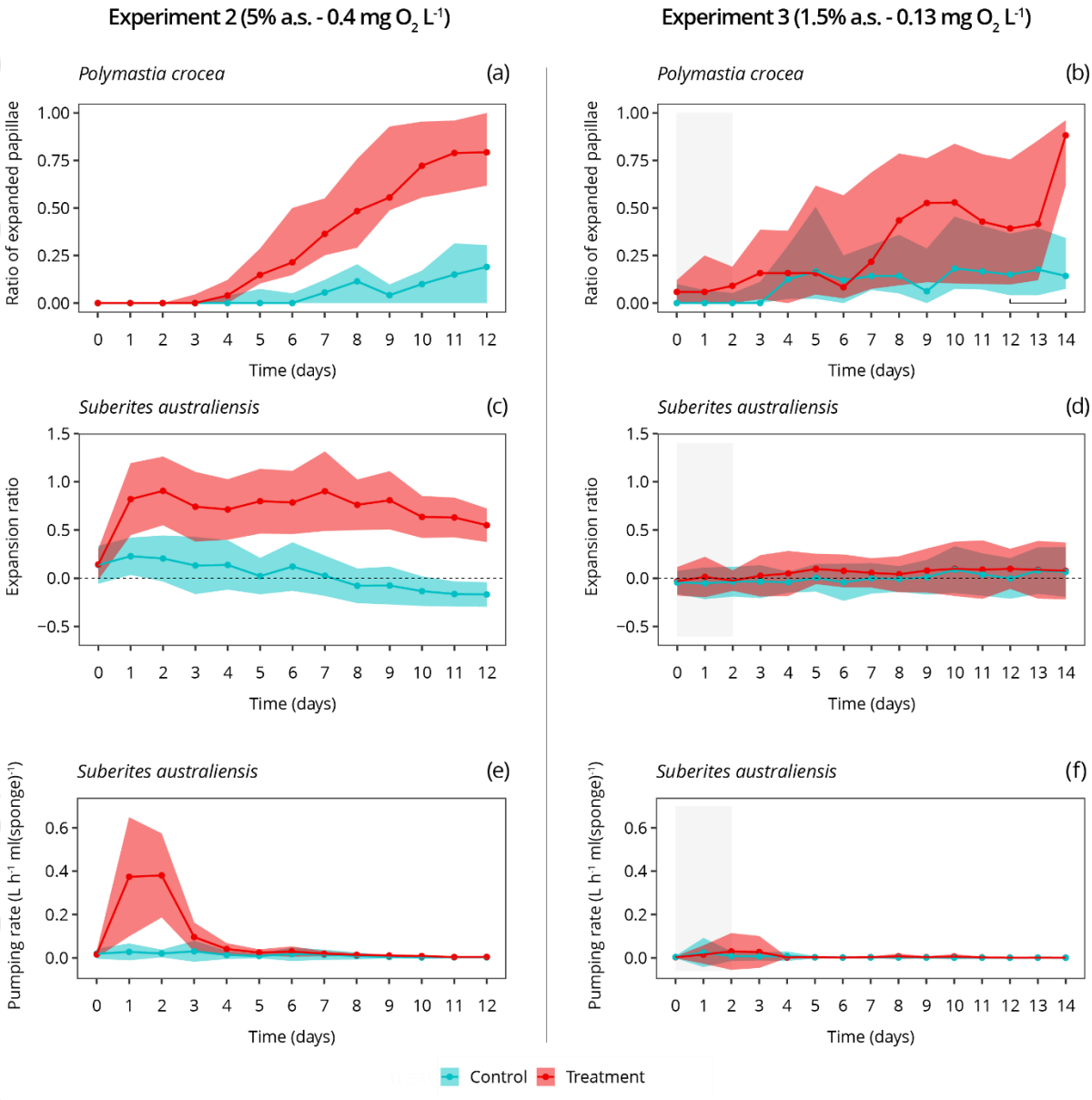

Figure 2. Contractile behaviour and pumping rate during experiments 2 and 3 (severe hypoxic conditions). Changes in the ratio of expanded papillae over time in *Polymastia crocea* in each treatment in experiments 2 (a) and 3 (b). Changes in the expansion ratio over time in *Suberites australiensis* in each treatment in experiments 2 (c) and 3 (d). Changes in the pumping rate over time (estimated from the osculum cross-sectional area) in *S. australiensis* in each treatment in experiments 2 (e) and 3 (f). In (a) and (b), points represent the median, while lower and upper edges of the ribbons represent the 75th and 25th percentile, respectively. In (c), (d), (e) and (f), points represent the means while lower and upper edges of the ribbon represent the standard deviation. Days of hypoxic acclimation (10% a.s.) are highlighted in grey. In (b), a black line is used to highlight days when sponges experienced mortality.

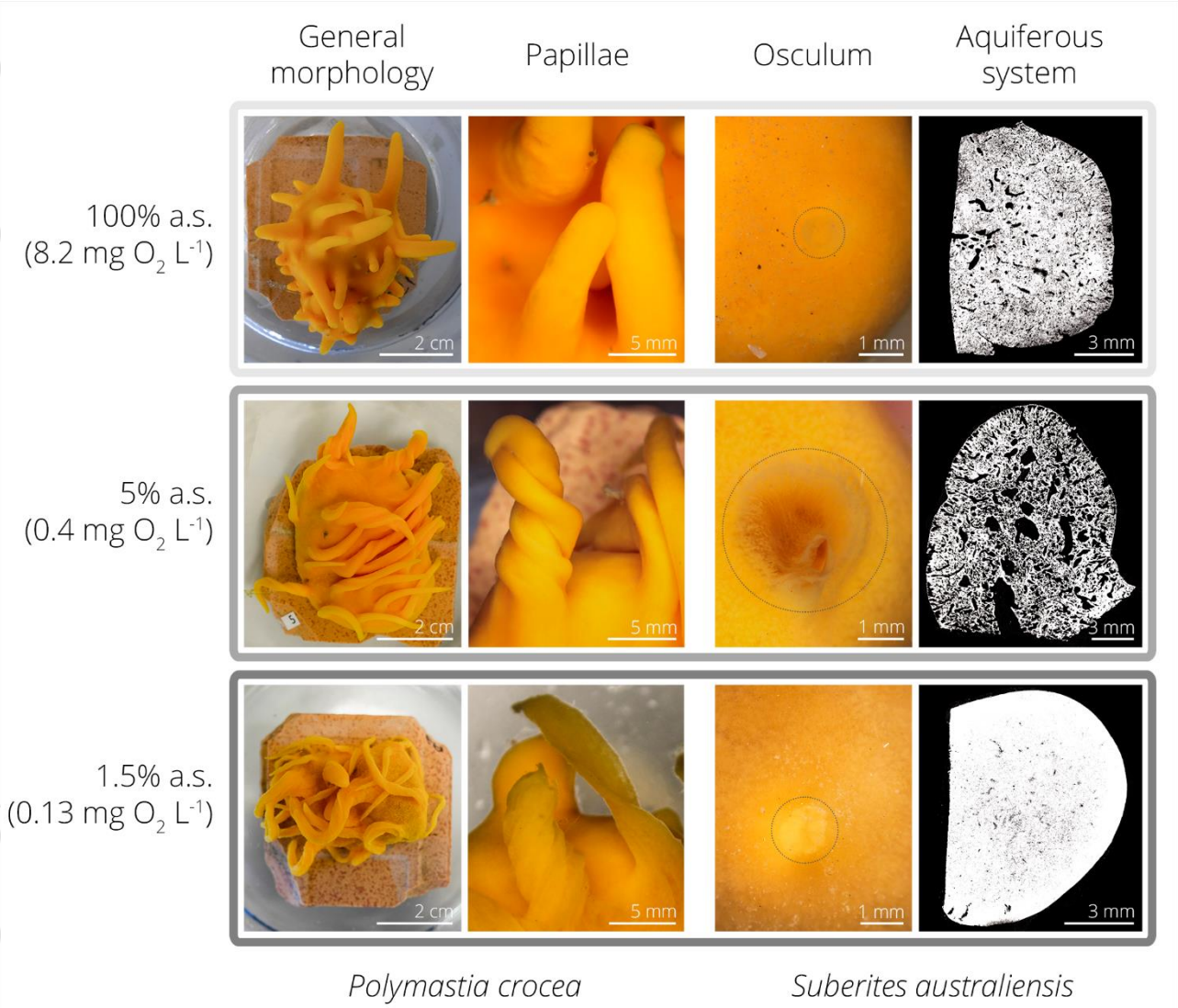

Figure 3. Examples of the morphological modifications reported in sponges exposed to low dissolved oxygen in the severe hypoxia treatments compared to the controls. From left to right: general external morphology, and details of papillae in *Polymastia crocea*; details of the osculum (evidenced with a dotted line), and transverse histological section (sponge tissue is in white and empty spaces representing the aquiferous system are in black) in *Suberites australiensis*. An extended version of this figure is found in the supplemental material (Fig. S12).

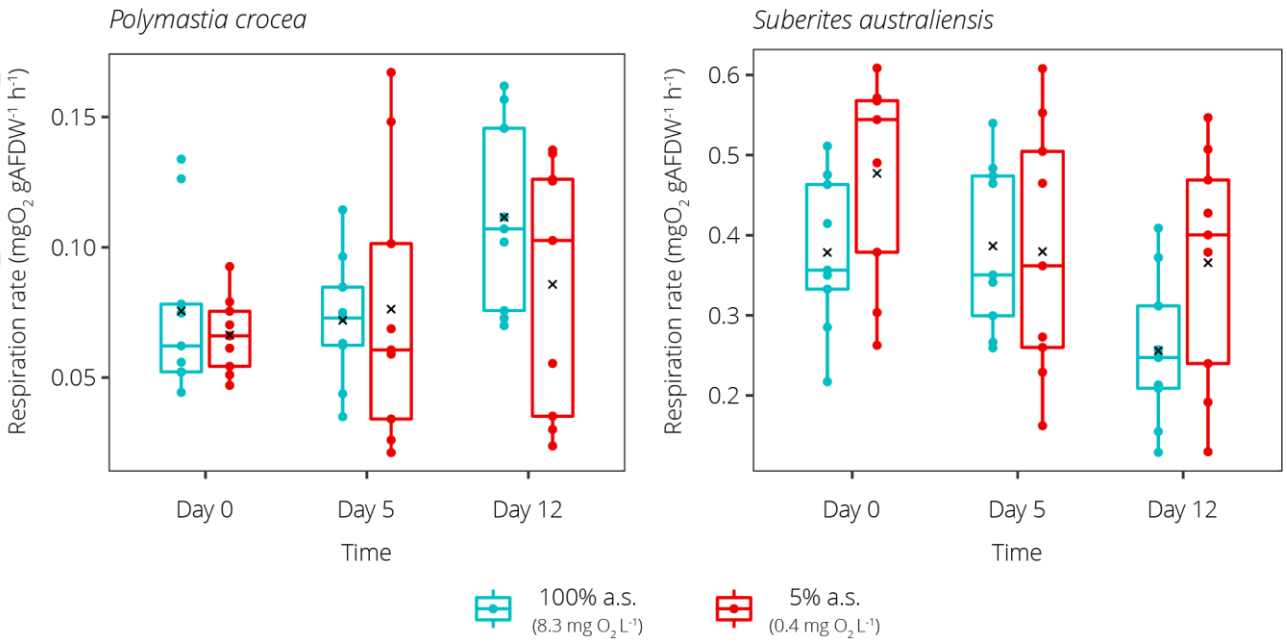

Figure 4. Respiration rates in *Polymastia crocea* and *Suberites australiensis* from experiment 2 (5% a.s.) measured at *T0*, *T1/2* and *T-end*. Note: x-axis and y-axis scales differ between species. Horizontal bars inside the boxplots represent medians; the symbol × represents means. Lower and upper hinges of the boxplots correspond to the first and third quartiles, respectively. Points represent data points.

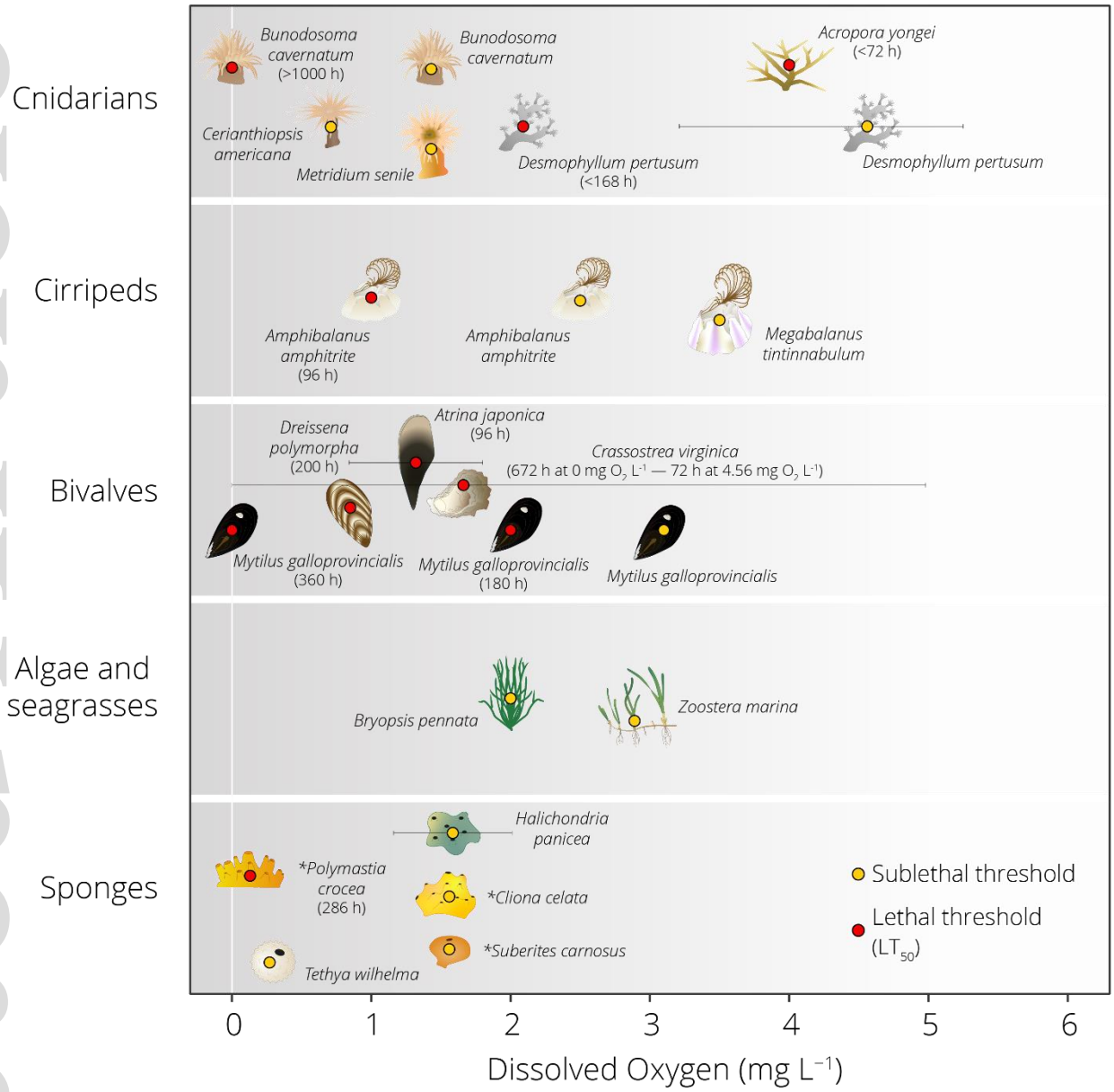

Figure 5. The tolerance of marine sessile organisms to hypoxia. Red dots indicate lethal thresholds, while yellow dots indicate sub-lethal thresholds. Organisms for which threshold values were found in the present study are labelled with an asterisk. For studies that report multiple values for the same species according to other abiotic conditions (i.e., temperature and salinity), we report a range where the dots represent the mean value, and the edges of the whiskers represent minimum and maximum values. For lethal thresholds, we report in bracket the median Lethal time (LT_{50}, hours) at that specific oxygen concentration (or at the extremes of the range). The symbol < was used when LT_{50} was not reported, but more than 50% of the organisms died after a certain amount of time; while > was used when LT_{50} was not reached by the end of the experiment. For *H. panicea,* Mills et al. (2014) only report oxygen measurement as per cent air saturation without reporting temperature

and salinity, so the actual oxygen concentration is unknown. We , therefore, estimated the oxygen content using the range of temperatures and salinity found where these sponges were sampled (Salinity 8.9–29.5; Temperature: 5–25 °C; Thomassen & Riisgård, 1995) and we provide the mean and the range of possible values. List of references associated with each species: *A. yongei* (Haas et al., 2014), *A. amphitrite* (Rao & Ganapati, 1968; Desai & Prakash, 2009), *A. japonica* (Nagasoe et al., 2020), *B. pennata* (Haas et al., 2014), *B. cavernatum* (Mangum, 1980; Ellington, 1982), *C. americana* (Vaquer-Sunyer & Duarte, 2008), *C. virginica* (Stickle et al., 1989), *D. pertusum* (Dodds et al., 2007; Lunden et al., 2014), *D. polymorpha* (Johnson & McMahon, 1998), *H. panicea* (Mills et al., 2014), *M. tintinnabulum* (Rao and Ganapati 1968), *M. senile* (Sassaman & Mangum 1972), *M. galloprovincialis* (De Zwaan et al., 1991; Woo et al., 2013), *T. wilhelma* (Mills et al., 2018), *Z. marina* (Hughes et al., 2020). Figure inspired by Hughes et al. (2020).