Adaptive strategies of sponges to deoxygenated oceans

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Global Change Biology

26. Apr. 2024

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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 O2 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 croceus, which showed significant mortality at the lowest oxygen concentration (0.13 mg O2 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 O2 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. |
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Adaptive strategies of sponges to deoxygenated oceans

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2   **Abstract**

3   Ocean deoxygenation is one of the major consequences of climate change. In coastal waters, this process

4   can be exacerbated by eutrophication, which is contributing to an alarming increase in the so-called "dead

5   zones" globally. Despite its severity, the effect of reduced dissolved oxygen has only been studied for a very

6   limited number of organisms, compared to other climate change impacts such as ocean acidification and

7   warming. Here we experimentally assessed the response of sponges to moderate and severe simulated

8   hypoxic events. We ran three laboratory experiments on four species from two different temperate oceans

9   (NE Atlantic and SW Pacific). Sponges were exposed to a total of five hypoxic treatments, with increasing

10   severity (3.3, 1.6, 0.5, 0.4 and 0.13 mg O_2 L^{-1}, over ~~a~~ 7–12-day~~s period~~). We found that sponges are

11   generally very tolerant of hypoxia. All the sponges survived in the experimental conditions, except

12   *Polymastia croceus,* which showed significant mortality at the lowest oxygen concentration (0.13 mg O_2 L^{-1},

13   lethal median time: 286 h). In all species except *Suberites carnosus*, hypoxic conditions do not significantly

14   affect respiration rate down to 0.4 mg O_2 L^{-1}, showing that sponges can uptake oxygen at very low

15   concentrations in the surrounding environment. Importantly, sponges displayed species-specific phenotypic

16   modifications in response to the hypoxic treatments, including physiological, morphological, and

17   behavioural changes. This phenotypic plasticity likely represents an adaptive strategy to live in reduced or

18   low oxygen water. Our results also show that a single sponge species (i.e., *Suberites australiensis*) can

19   display different strategies at different oxygen concentrations. Compared to other sessile organisms,

20   sponges generally showed higher tolerance to hypoxia, suggesting that sponges could be favoured and

21   survive in future deoxygenated oceans.

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~~little~~ 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

51   widespread and severe impacts across taxonomic and functional groups. The intensity and duration of

52   oxygen depletion are the main factors influencing the severity of hypoxic events on benthic organisms

53   (Levin et al., 2009; Altieri & Diaz, 2019). Mild hypoxia can alter behavioural patterns, decrease feeding rates

54   and cause changes in physiological processes (Vaquer-Sunyer & Duarte, 2008). Severe hypoxic events can

55   cause mass mortalities, leading to the formation of the so-called "dead zones", areas largely devoid of

56   macrofauna (Diaz & Rosenberg, 2008). Dead zones have been reported in small water bodies such as

57   harbours, fjord and inlets, and large basins, such as the Baltic Sea, spreading over 60,000 km^2 (Altieri & Diaz,

58   2019). As climate and land use continue to change, coastal hypoxia is expected to worsen, with the

59   increased occurrence, frequency, intensity, and duration of hypoxic events (Diaz & Rosenberg, 2011).

60   Despite the extent of the problem and the dramatic effects caused by ocean deoxygenation, the response

61   of many groups of organisms to hypoxia is still poorly studied. This lack of knowledge limits our ability to

62   model the effects of declining oxygen availability on marine ecosystems (Seibel, 2011). To date, research on

63   tolerance to reduced levels of dissolved oxygen has primarily focused on fish, crustaceans and molluscs

64   (Vaquer-Sunyer & Duarte, 2008), while very little is known about other groups, especially sessile organisms.

65   Sessile organisms are particularly vulnerable to hypoxic events because they cannot move or migrate to

66   well-oxygenated water. Furthermore, sessile organisms include many important habitat-forming species, so

67   any change in their abundance could have major consequences for the ecosystems they support (Vergés et

68   al., 2019; Woodhead et al., 2019; Piazzi et al., 2021). Therefore, it is critical to understand how these

69   organisms respond to hypoxia to predict possible future changes and effectively manage marine

70   ecosystems.

71   Sponges are the dominant sessile organisms in many marine ecosystems and are found in high abundance

72   in tropical, temperate, and polar ecosystems (Ayling, 1983, Bell et al., 2020). They perform many important

73   ecological functions, including contributing to nutrient cycling, bioerosion, enhancing ecosystem complexity

74   and providing habitats for a wide range of associated organisms (Wulff, 2006; Bell, 2008; Maldonado et al.,

75   2012). Despite being important components of marine ecosystems, sponge tolerance to hypoxia has been

76   poorly investigated to date. Mills et al. (2014) showed that *Halichondria panicea* can feed and respire with

77   oxygen levels down to 4% of air saturation. However, the authors did not provide information on the

78   duration of the treatments and replication; furthermore, in the same study, information on the

79   temperature and salinity of the water was unavailable, so it is not possible to derive the actual oxygen

80   concentrations to which sponges were exposed. Two other relevant experiments have investigated the

81   short-term response of sponges to hypoxia. Mills et al. (2018) exposed *Tethya wilhelma* to a step

82   decreasing oxygen concentration (30–40 h with O_2 lower than 10% a.s., 0.7 mg L^{-1}). They found that

83   sponges continued to perform periodic full-body contractions down to 0.27 mg O_2 L^{-1}, but ceased below

84   that concentration. Leys & Kahn (2018) exposed *Geodia barretti* to 6.5 h of hypoxia (7% air saturation, 0.6

85   mg O_2 L^{-1}), and found that sponge respiration rate remained largely unchanged. However, filtration rates

86   dropped almost immediately after the oxygen level was reduced. Despite these earlier studies, we still have

87   very little insight into how sponges may cope with hypoxic events caused by ocean and coastal

88   deoxygenation.

89   Here we provide the first comprehensive assessment of sponge response to hypoxia. Specifically, we

90   experimentally investigated the physiological, behavioural, and morphological responses of four temperate

91   sponge species to moderate and severe hypoxic conditions. We ran the first experiment to expose sponges

92   to moderate hypoxic conditions for seven days, including a wide range of dissolved oxygen concentrations

93   (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

94   mg O_2 L^{-1}) for 12 days with two additional experiments. Finally, we discuss sponge tolerance to low

95   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 (Wellington University Coastal Ecology Laboratory, Wellington) on two abundant temperate Australasian species: *Polymastia croceus* Kelly-Borges and Bergquist, 1997 and *Suberites australiensis* Bergquist, 1968.

2.1 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.

121 *Cliona celata* was collected from the Kedges (51°27'41.4 "N 9°20'44.2 "W), whereas *Suberites* ~~S.~~ *carnosus*

122 was collected from the rocky cliffs of Lough Hyne (51°30'00.4"N 9°18'03.9"W). For both species, sampling

123 was carried out at 10–18 m in June 2019 and sponges collected were at least 2 m apart. Sponges were then

124 left to recover for two months from harvesting stress in a 1 m^3 underwater cage placed at 8 m of depth.

125 Sponges were then transferred to the experimental system and randomly distributed across the

126 experimental chambers. The experimental design consisted of 32 experimental chambers (two replicate

127 chambers for each species for each replicate module, and two replicate modules for each treatment). A

128 diagram of the experimental design is reported in Figure S2. Three sponges were placed in each chamber (6

129 sponges in total for each replicate module and 12 for each treatment). Sponges belonging to different

130 species were not mixed but kept in separate chambers. Sponges were left to acclimate with oxygen

131 saturated air for five days before oxygen was lowered by introducing hypoxic water into the chambers.

132 Oxygen concentration was lowered in 24h and was then maintained for seven days until the end of the

133 experiment (a graph showing the oxygen concentration in the different treatments over time is provided in

134 Figure S3). In natural ecosystems, hypoxic conditions can develop in short times ranging from hours to a

135 few days (Breitburg, 1990; Nezlin et al., 2009), so we consider these acclimation times appropriate and

136 ecologically relevant. Temperature and oxygen concentration inside the experimental chamber were

137 measured twice a day using a Fibox 4 oxygen meter with a dipping probe (Presence GmbH, Germany). A

138 two-spot calibration was performed on the oxygen probe every three days, using sodium dithionite for 0 %

139 oxygen and air-saturated water for 100% oxygen.

140 **2.2 Experiments 2 and 3: severe hypoxia**

141 We also investigated the response of sponges (*Polymastia* ~~P.~~ *croceus* and *Suberites* ~~S.~~ *australiensis*) to

142 severe hypoxia through two separate experiments using an air-tight system. In experiment 2, sponges were

143 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 ~~e~~Experiment 3,

144 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

145 oxygen concentration in the controls was due to the small difference in temperature between the two

146   experiments (13.3 ± 0.5 °C in experiment 2 compared to 14.3 ± 0.6 °C in experiment 3). A summary of the

147   seawater parameters is provided in Table S1.

148   Sponges were kept in independent cylindrical air-sealed polypropylene chambers (10 L), randomly

149   distributed inside a water bath. Every two days, ~70% of the water was replaced using 10-μm-filtered

150   seawater, preconditioned to the desired oxygen concentration in independent conditioning tanks. Oxygen

151   concentration was then maintained by bubbling air or air-N$_2$ blends through glass-ceramic diffusers (see

152   section 2.1.2 for more details on gas blends). Custom made de-bubbler devices were used to eliminate

153   bubbles coming from the ceramic diffusers that could affect sponges (Figure S4). The sponges were fed

154   twice a day with *Nannochloropsis* microalgae (1–2 μm cell diameter; Nanno 3600™ Reed Mariculture, US.).

155   Water circulation within each experimental chamber was provided by the de-bubbler device and an

156   additional water pump located on the side of the chamber, which provided a constant circular water flow.

157   The chambers were placed in a water bath to control water temperature.

158   *Polymastia croceus* was collected from Barrett Reef (Wellington South Coast, 41°20'31.1"S 174°50'09.7"E)

159   by cutting fragments (~8 cm^3) from separate sponges (at least 5 m apart). Whole specimens of *Suberites S.*

160   *australiensis* were collected from Mahanga Bay (Wellington Harbour, 41°17'32.2"S 174°50'06.5"E),

161   attached to a fragment of their respective substrate. Sponges were then left to recover for three weeks

162   after sampling and cutting stress in water tables with 10-μm-filtered flow-through seawater.

163   Sponges were then transferred to the experimental system, consisting of 12 experimental chambers (3

164   independent replicate chambers for each species and treatment combination). Five sponges were placed in

165   each chamber (15 sponges in total for each treatment). Sponges were left to acclimate with oxygen

166   saturated air for five days. Oxygen was then lowered by bubbling a specific Air-N$_2$ mixture. In experiment 2,

167   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

168   the experiment. While in eExperiment 3, oxygen was firstly gradually lowered to an intermediate

169   concentration (10% a.s., 1 mg O$_2$ L^{-1}) for two days, then decreased lowered to 1.5% a.s. (0.13 mg O$_2$ L^{-1}) in

170   ~72 hours (which included a preacclimation at 10% a.s., 1 mg O$_2$ L^{-1}) and maintained for 12 days until the

171   end of the experiment (Fig. S3). This further acclimation in hypoxic conditions was made because of the

172 very low O_2 concentration of the treatment. In experiment 3, due to the very low concentration of O_2 (0.13

173 ± 0.02 mg L^{-1}), O_2 increased to ~0.3 mg L^{-1} for about 20 minutes during daily examinations. Temperature

174 and oxygen concentration inside the experimental chamber were measured twice a day using Fibox 4

175 oxygen meter with a dipping probe (Presence GmbH, Germany). A two-spot calibration was performed on

176 the oxygen probe every three days, using sodium dithionite for 0% oxygen and air-saturated water for

177 100% oxygen.

178 **2.3 Response variables**

179 **2.3.1 Survival and health monitoring**

180 Sponge health was monitored daily during the experiment. Sponges showing ≥ 25% of external necrosis

181 were considered dead and immediately removed from their treatment tanks during the daily checks, so as

182 not to impact other sponges in the treatments. At the end of the experiments, all sponges were sectioned

183 to assess the presence of any internal necrosis.

184 **2.3.2 Respiration Rate**

185 For all the experiments, respiration rate was measured on the same specimens at $T0$ (before the beginning

186 of the experiment), $T1/2$ (after two days from the beginning of the final treatment in experiment 1, and

187 after five days in experiments 2 and 3) and $T\text{-}end$ (end of the experiment). In experiment 1 (moderate

188 hypoxia), we measured respiration rates of three sponges in each replicate module (n = 6 for each

189 treatment). In experiments 2 and 3 (severe hypoxia), respiration rates were measured on three sponges in

190 each experimental chamber (n = 9 for each treatment). To measure respiration rate, sponges were placed

191 in sealed cylindrical glass respiration chambers (150 ml for _Cliona C. celata_; 80 ml for _Suberites S. carnosus_;

192 250 ml for _Polymastia P. croceus_ and _Suberites S. australiensis_) with PreSens oxygen sensor spots (SP-PSt3-

193 NAU) attached to their inner surface. Experimental chambers contained either oxygen saturated water

194 (pre-experimental measurements and controls) or water at a slightly higher oxygen concentration than the

195 experimental treatment (+20–50%, depending on the treatment) collected from the respective header

196 tanks. Respiration rates were not performed on sponges from eExperiment 3 (1.5% a.s.). The incubations

197 were performed in controlled temperature (water bath) and dark conditions. The water inside the

198   respirometry chambers was gently stirred using a magnetic stir bar. After 20 min of acclimation, oxygen

199   concentration inside the chambers was measured every 10 min for 1 hour, using a Fibox 4 oxygen meter

200   with a polymer optical fibre (POF). Respiration measurements were ended prematurely if the oxygen level

201   fell below 70% of the treatment concentration to avoid any detrimental effect on the sponges. Blank

202   incubations, containing only seawater were performed every respiration run and used to correct for any

203   microbial community respiration in the seawater. A two-point calibration was performed on the oxygen

204   sensor spots before each measurement session.

205   Respiration measurements were standardized to sponge ash-free dry weight (*AFDW*) from buoyant weight

206   (*BW*) measurements (Fig. S5). For *Suberites* ~~S.~~ *australiensis*, it was not possible to estimate *AFDW* from *BW*

207   due to the abundant external material accumulated by the sponge inside the tissue that influences the *BW*.

208   For this species, we measured the *AFDW* of all the specimens used in the respirations at the *T-end*, and we

209   assumed that sponges had the same weight at *T*0 and *T*1/2.

210   **2.3.3 Changes in weight, size, and morphology**

211   Changes in weight and size over time relative to the initial values were estimated by calculating the

212   buoyant weight variation (*BWV*) and contracted area variation (*CAV*). For all of the experiments, buoyant

213   weights (*BW*) of all experimental sponges (except *Suberites* ~~S.~~ *australiensis*) were taken at *T*0 and *T-end* and

214   used to calculate relative buoyant weight variation as $BWV = [(BW_{T\text{-}end} - BW_{T0}) / BW_{T0}] \cdot 100$. Buoyant weight

215   was measured with a digital scale (A&D FX-200i) following the methods of Osinga et al. (1999). For

216   experiments 2 and 3 (severe hypoxia), photographs of contracted sponges were taken at *T*0 and *T-end* to

217   measure sponge contracted area (*CA*) and calculate contracted area variation as $CAV = [(CA_{T\text{-}end} - CA_{T0}) /$

218    $CA_{T0}] \cdot 100$ (following Osinga et al., 1999). Contraction was achieved by disturbing sponges with a blunt

219   plastic rod (being careful not to damage the sponge) and waiting for one hour for the sponge to react to the

220   stimulus. All the photographs were analysed using ImageJ (US National Institutes of Health, Bethesda, Md,

221   USA).

222 During experiments 2 and 3, treatment conditions induced the development of peculiar morphological

223 structures in some specimens of both _Polymastia P. croceus_ and _Suberites S. australiensis_. Sponges were

224 photographed and monitored daily to calculate the percentage of specimens developing these structures

225 and the median time of occurrence.

2.3.4 Sponge contractile behaviour

227 During experiments 2 (5% a.s.) and 3 (1.5% a.s.), sponge contractile behaviour was monitored daily from $T0$

228 to $T\text{-}end$, on all experimental sponges through photographic analysis. For _Suberites S. australiensis_, the

229 contractile behaviour was estimated using an "expansion ratio" ($EXPR$) calculated as $EXPR = A_{Ti} / CA_{T0}$,

230 where A_{Ti} is the area occupied at T_i and CA_{T0} is the contracted area at $T0$. Area was preferred over volume

231 because of the low invasiveness of the measurements. In _Polymastia P. croceus,_ contraction/expansion

232 mainly occur at the papillae level, so the contractile behaviour was estimated from the ratio of expanded

233 papillae (REP) calculated as $REP = P_E/P_{tot}$, where P_E is the number of visible expanded papillae and P_{tot} is

234 total number of visible papillae. Expanded papillae were defined as papillae whose length was at least two

235 and a half times the width.

2.3.5 Pumping rate

237 Pumping rate was only calculated for _Suberites S. australiensis_ from experiments 2 and 3 (severe hypoxia).

238 Having only one osculum of relatively large size, this species was particularly suitable for investigating

239 changes in pumping rate. To minimize sponge disturbance during the experiment, pumping rate (PR) was

240 derived from the measurement of the sponge osculum cross-sectional area (OSA). In sponges, pumping

241 rate (PR) is correlated with OSA (e.g. Goldstein et al., 2019; Morganti et al., 2021). In the case of _S._

242 _australiensis_, this relationship was calculated on 20 sponges (following Yahel et al., 2005) and was found as

243 $PR = 6.55 \cdot OSA^{1.43}$ (Fig. S6). Photographs of the oscula with scale were taken daily from $T0$ to $T\text{-}end$, on

244 three sponges in each experimental chamber (the same specimens each time point, n = 9 per treatment).

245 Since _S. australiensis_ has only one osculum, pumping rate was then standardized per sponge volume.

2.3.6 Histology

Histological sections of *Suberites* ~~S.~~ *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.

2.4 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* ~~S.~~ *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* ~~P.~~ *croceus* in ~~e~~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. croceus* from experiment 2 and expansion ratio in *S. australiensis*

272   from eExperiment 3 were investigated using repeated measure univariate PERMANOVA (Anderson, 2001;

273   2014), because did not meet the normality assumption for mixed-effects models. Pairwise tests were then

274   calculated using permutation *t*-tests (*R* package *RVAideMemoire*; Hervé, 2021). PERMANOVA and

275   permutation *t*-tests were also used to supplement mixed-effects models when there were concerns about

276   the normality of the residuals (pumping rate in *S. australiensis*). Change over time relative to the initial

277   value of buoyant weight and contracted area, and differences in percentage occupied by the aquiferous

278   system were investigated using Kruskal–Wallis H tests, Welch's t-tests or Wilcoxon Signed-Rank Tests,

279   depending on the variable. Respiration rates from experiment 1 were log (x + 1) transformed, and pumping

280   rates were square square-root transformed to meet normality assumptions. The goodness of fit, normality

281   and homoscedasticity of the errors were checked for all models by inspecting plots of the normalized

282   residuals and the quantile-quantile plots. All the multiple comparisons were corrected using Benjamini-

283   Hochberg Procedure, but uncorrected p-values are reported in the text. All the statistical analyses made for

284   each variable are reported and summarized in Table S2**.**

285   Time to event analysis for sponge survival and development of peculiar morphological structures (modified

286   papillae and protruding oscular membranes) was performed using Kaplan-Meier Method, and *p*-values

287   were calculated using the Log Rank Test implemented in the survival *R* package (Therneau, 2021). Median

288   lethal time (LT_{50}) and median time to the development of modified morphological structures were

289   calculated using a logistic model.

290   **3 RESULTS**

291   **3.1 Sponge responses to moderate hypoxia**

292   All the sponges of experiment 1 survived the seven days of treatment, except one specimen of _Suberites S._

293   _carnosus_ in the lower DO treatment (6% a.s.)_,_ which presented internal necrosis on the final day of the

294   experiment.

295   Mean buoyant weight variation between _T_0 and _T-end_ ranged between -1% and -1.6% for _ClionaC. celata_

296   and +2.1% and -0.5% in _Suberites S. carnosus_. There were no differences in in buoyant weight variation

297   among treatments for both species, but for _C. celata_ there was a significant slight decrease in weight in the

298   40% a.s. (-1.6%, _p_ = 0.008) and 20% a.s. (-1.4%, _p_ = 0.008) treatments (Tab. S3; Fig. S7).

299   For _Cliona C. celata_, there was no significant effect of time or treatment on the respiration rate (Tab. S4).

300   However, pairwise comparisons ~~found~~ revealed a significant decrease (_p_ = 0.028) in the 20% a.s. treatment

301   between day 0 and 7, and a significant increase (_p_ = 0.029) in the 6% a.s. treatment between day 2 and day

302   7, but both became non-significant after the correction for multiple comparisons (Tab. S4). However, the

303   data suggest a coherent temporal pattern in the respiration rate in both 20% a.s. and 6% a.s. treatments. _C._

304   _celata_ respiration rate decreased after two days from the start of the experiment and then increased until

305   the end of the experiment. In contrast, in both the 100% a.s. and 40% a.s. treatments, respiration rate

306   remained stable for the whole duration of the experiment (Fig. 1a).

307   For _Suberites S. carnosus_, there was a significant ~~effect of time (_p_ = 0.002), and the~~ interaction of time and

308   treatment (_p_ = 0.007) on the respiration rate (Tab. S5). Pairwise comparisons revealed~~found~~ a significant

309   decrease in respiration rate between day 0 and 7 (_p_ < 0.0001), and day 2 and 7 (_p_ < 0.0001) (Tab. S5). The

310   respiration rate also slightly decreased towards the end of the experiment in the 20% a.s. treatment (but

311   not significantly), while in both the 100% a.s. and 40% a.s. treatments, respiration rate remained stable for

312   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 S. australiensis* more tolerant than *Polymastia P. croceus*. No mortality was observed for *S. australiensis* in both experiments 2 (5% a.s.) and 3 (1.5% a.s.). In contrast, for *P. croceus*, 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. croceus* in the 5% a.s. treatment.

3.2.2 Change in weight and size

For *Polymastia P. croceus*, buoyant weight variation between *T*0 and *T-end* differed among treatments in eExperiment 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 *T*0 and *T-end* differed significantly between treatments and controls for both *Polymastia P. croceus* (W = 12, p < 0.0001) and *Suberites S. australiensis* (W = 13, p < 0.0001), but only in experiment 2 (5% a.s.). Both *P. croceus* 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 P. croceus*, the ratio of expanded papillae was significantly affected by time and the interaction between time and treatment in both experiments 2 (p = 0.0001) and 3 (p < 0.0001 and p = 0.03) experiments (Tab. S8–9). During experiment 2 (5% a.s.), the treatment induced a progressive expansion of papillae from day 2. The ratio of expanded

338   papillae in sponges from the hypoxic treatment ~~become~~ became significantly higher than control sponges

339   from day 6 to the end of the experiment (p = 0.0002–0.005) (Tab. S8; Fig. 2a). A similar trend was found in

340   ~~e~~Experiment 3 (1.5% a.s.), but the ratio of expanded papillae of the treatment sponges was more variable

341   and became significantly different only at day 9 (p = 0.003) (Tab. S9; Fig. 2b). In this experiment, we also

342   found a correlation between the ratio of expanded papillae and mortality. Sponges that survived the

343   treatment had a significantly higher maximum ratio of expanded papillae compared to sponges that died,

344   both when the maximum ratio was calculated at the end of the experiment (Welch t-test: t = 5.3, p =

345   0.0005) and at day ten, before sponges started to die (Welch t-test: t = 4.6, p = 0.0007).

346   In *Suberites S. australiensis*, there was a significant interactive effect ~~effect~~ of treatment and ~~(p = 0.001),~~

347   time ~~(p < 0.0001) and their interaction~~ (p < 0.0001) on the expansion ratio in experiment 2 (5% a.s.), but

348   only an effect of time (p = 0.01) in ~~e~~Experiment 3 (1.5% a.s.) (Tab. S10–11). For experiment 2 (5% a.s.),

349   pairwise comparisons found significant expansion in sponges (+60%, p < 0.0001) between day 0 and 1.

350   Sponges then remained expanded for the whole duration of the experiment, and the expansion ratio was

351   significantly higher in the treatments compared to the controls from the first to the last day of the

352   experiment (p < 0.0003) (Tab. S10; Fig. 2c, d).

353   **3.2.4 Morphological modifications**

354   During experiments 2 (5% a.s.) and 3 (1.5% a.s.), some *Polymastia P. croceus* and *Suberites S. australiensis*

355   sponges exposed to hypoxic treatments underwent morphological modifications (Fig. 3; S12). In some *P.*

356   *croceus*, the conical papillae showed a progressive elongation, flattening, and, in some cases, spiralization

357   (Fig. S12a–f). This process occurred in both the 5% a.s. and 1.5% a.s. treatments, but morphological

358   changes were more pronounced in lower DO treatment (Fig. S12d-e). Exposed to the 1.5% a.s. treatment,

359   some sponges developed papillae so slender that they could not sustain their weight (Fig. S12d-e). The

360   development of these modified papillae was also associated with an apparent increase in the porosity of

361   the sponge external surface (Fig. S12e). In the 5% a.s. treatment, 73% of the sponges developed modified

362   papillae, starting from day 6. In the 1.5% a.s. treatment, 60% of sponges developed modified papillae,

363   starting from day 2, from the beginning of the final treatment (day 4 considering hypoxic acclimation

364 period) (Fig. S13). The median time of development of these morphological structures (considering only the

365 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%

366 a.s. treatment (Fig. S14). Although not significant (χ^2 = 3.62, p = 0.057), a relationship between modified

367 papillae and survival was found. Among the *P. croceus* that survived the 1.5% a.s. treatment, six had

368 developed modified papillae, while one had not. While among the sponges that died following the 1.5% a.s.

369 treatment, three had developed modified papillae, while five had not.

370 In the the 5% a.s. treatment, 53% of *Suberites ~~S.~~ australiensis* developed a semi-transparent protruding

371 membrane surrounding the oscula. This membrane progressively reduced the oscular-cross sectional area

372 (Fig. 3; S12g–i). The median time it took for these protruding oscular membranes to ~~became~~ become

373 noticeable (considering only the sponges that developed them) was 5.1 ± 0.2 days (Fig. S14). By the end of

374 the experiment, 53% of sponges had developed these structures (Fig. S13).

375 **3.2.5 Histology**

376 Histological analyses indicated that hypoxia influences the percentage of the sponge body occupied by the

377 aquiferous system in *Suberites ~~S.~~ australiensis*. At the end of experiment 2 (5% a.s.), treatment sponges had

378 a significantly higher percentage of aquiferous system (t = -9.82, p < 0.0001), compared to the controls

379 (35.9 ± 7.1% vs 6.4 ± 1.8 %). No significant differences were found for ~~e~~Experiment 3 (1.5% a.s.) (Fig. 3;

380 S12j–m; S15).

381 **3.2.6 Pumping rate**

382 Oxygen concentration significantly affected the pumping rate of *Suberites ~~S.~~ australiensis* in both

383 experiments 2 (5% a.s.) and 3 (1.5% a.s.) (Tab. S12–15). In experiment 2 (5% a.s.), there was significant

384 ~~effect~~ interaction of treatment and~~(p = 0.02),~~ time (p = 0.0001~~) and the interaction between time and~~

385 ~~treatment (p = 0.0001)~~ (linear mixed-effects model~~;,~~ Tab. S12). Pumping rate significantly increased from

386 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)

387 and from day 3 to 4 (p = 0.005) (Tab. S12). Sponges from the 5% a.s. treatment had a significantly higher

388 pumping rate than the control at day 1 (p = 0.007) and 2 (p = 0.002) (Tab. S12; Fig. 2e). Similar results were

389　given by PERMANOVA (Tab. S13). For eExperiment 3 (1.5% a.s.), both the linear mixed-effects model and

390　PERMANOVA revealedfound significant effect of time ($p < 0.0001$ and $p = 0.0006$, respectively) and the

391　interaction between time and treatment ($p = 0.049$ and $p = 0.002$, respectively) on the pumping rate of *S.*

392　*australiensis* (Tab. S14–15)*.* However, differences were less marked compared to experiment 2 and

393　pairwise comparisons only found revealed a slight decrease of pumping rate of treatment sponges between

394　day 0 and 14 ($p = 0.0002$) (Tab. S14; Fig. 2f).

3.2.7 Respiration rate

396　In experiment 2 (5% a.s.), linear mixed-effects models only revealedfound a significant effect of time on the

397　respiration rate but not treatment or interaction between treatment and time, for both *Polymastia P.*

398　*croceus* and *Suberites S. australiensiscarnosus* (Tab. S16–17; Fig. 4). In *P. croceus*, pairwise comparisons

399　revealedfound a slightly higher respiration rate of the controls at day 12 compared to day 0 ($p = 0.008$) and

400　day 5 ($p = 0.004$), but no differences between controls and treatments at any time. In *S. australiensis,*

401　pairwise comparisons revealedfound a slightly lower respiration rate at day 12 compared to day 0 ($p =$

402　0.008) and day 5 ($p = 0.005$) in control sponges; while in treatment sponges, respiration rate was slightly

403　lower at day 5 ($p = 0.032$) and 12 ($p = 0.016$) compared to day 0, but also in this case, there was no

404　significant difference between treatments and controls at any time point.

405   **4 DISCUSSION**

406   Hypoxia has become an increasingly common problem in the marine environment and will likely become

407   worse in the future (Diaz & Rosenberg, 2011). Nevertheless, the direct effects of hypoxia on marine

408   organisms are still very poorly studied (Vaquer-Sunyer & Duarte, 2008). We describe the first multi-species

409   experiment from two oceans to test sponge tolerance, behaviour, and physiological responses to oxygen

410   concentrations as low as 1.5% a.s. (0.13 mg O_2 L^{-1}) for up to 12 days. We found that ~~irrespective of species~~

411   ~~or location, sponges are generally very tolerant to low DO~~sponges are generally very tolerant to low DO

412   irrespective of species or location. Only~~, and only~~ _Polymastia_ ~~P.~~ _croceus_ showed mortality in the lower DO

413   treatment (0.13 mg O_2 L^{-1}, LT$_{50}$ = 286 h). Furthermore, our results suggest that sponges can display species-

414   specific acclimation, including physiological, morphological and behavioural changes, in response to severe

415   hypoxia that might help them survive periods of very low oxygen. Our study also suggests that the same

416   species can show different adaptive strategies for different degrees of hypoxia.

417   **4.1 Sponge response to hypoxia**

418   Our results suggest that sub-lethal oxygen thresholds for most sponges are in the range of 6–20% a.s.

419   (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

420   evidence are consistent with Mills et al. (2014) for _Halichondria panicea_, which showed a sub-lethal

421   response starting from 17% air saturation. However, our results contrast with Mills et al. (2018) studying

422   _Tethya wilhelma_, which did not show any response down to 4% a.s. (0.27 mg O_2 L^{-1}). The very high

423   tolerance of _T. wilhelma_ could be explained by the extremely low metabolism of _Tethya_ species generally

424   (Leys & Kahn, 2018), and by their very small size (0.5–1 cm) (Sarà et al., 2001). Of the two species we

425   exposed to the lowest DO concentration (1.5% a.s., 0.13 mg O_2 L^{-1}), only _Polymastia_ ~~P.~~ _croceus_ showed

426   mortality, while all the _Suberites_ ~~S.~~ _australiensis_ survived the 12 days of treatment conditions. This

427   differential response could be due to the different habitats where these species are usually found.

428   _Polymastia croceus_ lives on rocky reefs, while _S. australiensis_ lives on sediments in bays and semi-enclosed

429   basins, where hypoxic events are more likely to occur (Diaz & Rosenberg, 2008; de Cook, 2010).

430   ~~Therefore, it is possible that _S. australiensis_ have evolved adaptations to survive with very little oxygen.~~

431   Some sponges can live in anoxic conditions for several months, such as the sponges of the family

432   Raspailidae found in the deeper cliffs of Lough Hyne (Bell & Barnes, 2000; McAllen et al., 2009). Schuster et

433   al. (2021) suggested that this tolerance could be conferred by specific bacterial symbionts, which are able

434   to carry out anaerobic metabolism. In addition, these sponges living in anoxia are all thin crusts, with a very

435   high surface-to-volume ratio, which could favour the exchange of gases and the release of metabolic waste

436   (Levin et al., 1991). Other examples of sponges living in very low oxygen conditions are the ones found at

437   the edges of Oxygen Minimum Zones (OMZ) (Mosch et al., 2012). These sponges can live with a consistent

438   oxygen concentration as low as 0.13mg O_2 L^{-1} (Wishner et al., 1995, Murty et al., 2009). Sponges are not the

439   only organisms able to live in OMZs. Many representatives of other phyla live in these extremely hypoxic

440   conditions, where they benefit from the rich supply of organic matter. However, since OMZs have existed

441   over geological timescales, organisms have had the time to evolve specific adaptations to cope with

442   permanent hypoxia (Levin, 2003). Therefore, these organisms cannot be used to generalize tolerance to

443   periodic hypoxic events experienced by organisms usually living in fully oxygenated waters.

444   The degree of hypoxia tolerance in sponges could also be influenced by the abundance and diversity of

445   sponge-associated microbial symbionts-. Based on -bacterial biomass, sponges are generally divided into

446   "low microbial abundance" (LMA) or "high microbial abundance" (HMA) species (Hentschel et al., 2003).

447   Bacterial densities in HMA sponges are generally two to four orders of magnitude higher than in LMA

448   sponges and can constitute up to 35% of the total sponge biomass (Vacelet, 1975; Hentschel et al., 2006).

449   Sponges with HMA tend to have a lower choanocyte chamber density, and a slower pumping rate

450   compared LMA sponges (Lavy et al., 2016), which means HMA species might have a lower ability to

451   ventilate in low oxygen conditions. Furthermore, HMA species generally have a higher metabolic cost than

452   LMA species, and therefore a higher oxygen requirement (Leys & Kahn, 2018). Although these differences

453   suggest that LMA sponges might be better adapted to hypoxic conditions, HMA species have a higher

454   diversity of microbial symbionts that could help them cope with low oxygen conditions (Hoffmann et al.,

455   2005, Lavy et al., 2016). All the sponges for which responses to hypoxia has been investigated so far are

456   LMA species (or are likely to be, based on known congenerics, see Kamke et al., 2010; Mills et al., 2014;

457   Moitinho-Silva et al., 2017). Therefore, future research is needed to investigate the response of HMA

458   sponges to hypoxia and shed light on possible differences between LMA and HMA sponges and the

459   mechanisms involved.

460   Some organism's abilities to tolerate hypoxia result from their physiological ability to lower metabolism and

461   oxygen demand (McAllen et al., 1999; Altieri, 2019). Instead, other species switch from aerobic to

462   anaerobic metabolism or a combination of the two (Altieri & Diaz, 2019). Our results suggest that all our

463   species (except *Suberites. carnosus*) have respiration rates at 5-6% a.s. that are comparable to sponges in

464   normoxic conditions. This is consistent with what was found in *Geodia barretti* and *H. panicea*, suggesting

465   that sponges have a common ability to uptake oxygen at very low concentrations in the surrounding

466   environment (Leys & Kahn, 2018). In *Cliona C. celata*, hypoxic water initially resulted in a decrease in the

467   respiration rate, which then increased back to pre-treatment levels after seven days of exposure. This

468   suggests that the sponges gradually adjusted to hypoxic conditions. In *S. carnosus,* instead, the respiration

469   rate remained stable after two days of exposure to low dissolved oxygen, but it more than halved after

470   seven days. This response may allow *S. carnosus* to cope with long periods of hypoxia, in which sponges

471   decrease their metabolism, as has been reported for other organisms (Hagerman, 1998; Mentel et al.,

472   2014). Although our study shows that sponges can perform aerobic metabolism when exposed to

473   extremely low oxygen concentrations, the presence of anaerobic metabolism cannot be excluded and

474   needs further investigation.

475   Sponge species exposed to the lowest DO concentrations (0.4 and 0.13 mg O_2 L^{-1}) also showed other

476   phenotypic modifications that could represent adaptive strategies to cope with hypoxia. In *Suberites S.*

477   *australiensis*, hypoxic water (0.4 mg O_2 L^{-1}) induced expansion of the sponge body and the aquiferous

478   system that lasted for the duration of the experiment. This expansion was likely semi-permanent as it

479   persisted after inducing the contraction and corresponded to a reorganization of the sponge aquiferous

480   system at the histological level. These behavioural and morphological changes are likely beneficial for the

481   sponge, as higher internal water flow corresponds to an increase in oxygen that can be taken up. The body

482   expansion was accompanied by a marked increase in the pumping rate that then dropped after two days.

483   The pumping rate increase could be a strategy to increase ventilation and oxygen availability, similarly to

484   other animals when exposed to hypoxic waters (Hagerman, 1998). However, the successive decrease in

485   pumping rate (after two days) and the gradual production of a membrane to close the oscula remains

486   unclear but could represent a trade-off between increasing ventilation and keeping the energetic coast of

487   pumping reasonable. In *S. australiensis*, body expansion is correlated with an increase in osculum area, and

488   osculum area is the main determinant of pumping rate in this and many other species (Morganti et al.,

489   2021; Goldstein et al., 2019). Perhaps the increase in pumping rate only represents a physiological

490   consequence of the body expansion and is then quickly brought back to normal, decreasing the osculum

491   size by producing an oscular membrane. These physiological and morphological changes of *S. australiensis*

492   described above was not present on sponges exposed to more severe hypoxia (0.13 mg O_2 L^{-1}). This could

493   mean that the same sponge species may display different adaptive strategies to cope with decreased

494   oxygen depending on the oxygen concentration. At 0.4 mg L^{-1}, oxygen might still be sufficient to support

495   regular metabolism, but sponges may need to increase the amount of water flowing through their bodies

496   to absorb the oxygen needed. However, 0.13 mg L^{-1} might be too low a DO concentration, and sponges

497   might decrease their metabolism to cope with lack of oxygen, similarly to other metazoans (Hagerman

498   1998, Mentel et al., 2014).

499   *Polymastia croceus* also showed a behavioural change in response to hypoxic conditions: hypoxic water at

500   0.4 mg L^{-1} induced the progressive expansion of sponge papillae (where inhalant and exhalant channels are

501   found), that was significantly greater than in the control sponges. It is unlikely that the papillae expansion

502   represents an increase in sponge filtering activity because the respiration rate was very similar in the

503   treatments and the controls. Therefore, sponges might expand their papillae to increase the volume

504   occupied by the aquiferous systems, as in the case of *Suberites* ~~S.~~ *australiensis*, but also to access more

505   oxygenated water further from the bottom. A similar response occurred in sponges exposed to 0.13 mg L^{-1}

506   but with much more variability across specimens, and the statistical test did not detect any change.

507   Interestingly, sponges that survived after the 12-day treatment had a significantly higher ratio of expanded

508   papillae than sponges that died, suggesting that expansion might help cope with severe hypoxic conditions.

509 Along with behavioural changes, *Polymastia* underwent morphological modifications that could help to

510 tolerate low DO. Papillae become thinner and flattened, and some even spiralized. These modifications of

511 the papillae could increase the surface-to-volume ratio and help oxygen diffusion (Levin et al., 1991). The

512 elongation of papillae, which accompanies the thinning, could be an evolutionary relic of a process that

513 moved the inhalant pores of the papillae as far as possible from the surface. However, in the lowest DO

514 treatment, papillae often lost their vertical orientation and laid horizontally on the sponge surface. We

515 hypothesized that the new orientation of papillae was a consequence of their thinning process: probably

516 papillae become became so thin that they could not support their weight anymore. Interestingly, sponges

517 that developed modified papillae showed less mortality than sponges that did not, although the evidence is

518 not strong enough to claim this with confidence (p = 0.057). Therefore, these structures may not only

519 represent a stress response, but could provide an advantage to the sponge. Further research is needed on

520 this topic needed to elucidate the function of these structures.

521 Despite the remarkable tolerance of sponges to hypoxia observed in laboratory conditions, field

522 observations suggest that severe hypoxic/anoxic events can catastrophically affect sponge populations.

523 Mass mortalities of sponges following hypoxic/anoxic events have been reported both in temperate and

524 tropical ecosystems (Stachowitsch, 1984, Altieri et al., 2017; Chu et al., 2018; Johnson et al., 2018; Kealoha

525 et al., 2020). For example, in a hypoxic/anoxic event in the Gulf of Trieste, all the sponges living in several

526 hundred km^2 died within 2-3 days (Stachowitsch, 1984). Some anemones survived up to a week, but

527 virtually all organisms were dead within two weeks from the onset. Altieri et al. (2017) also reported

528 widespread mortality of sponges and corals following a hypoxic event (~0.5 mg O$_2$ L^{-1}) that occurred in

529 Bocas del Toro, Panama. Since this study focused on corals, it is unclear what proportion of the sponges

530 were affected and if some species were more tolerant than others. These reports highlight that hypoxic

531 events, in their most severe form, leave no survivors.

532 Furthermore, it is possible that in natural conditions, other factors combine with low dissolved oxygen. For

533 example, a recent meta-analysis showed that in marine organisms, increased temperature reduces survival

534 times under hypoxia by 74% on average and increased median lethal concentration by 16% on average

535   (Vaquer-Sunyer & Duarte, 2011). Another meta-analysis showed that hydrogen sulphide (H_2S) also reduces

536   survival time of marine organisms under hypoxia by an average of 30% (Vaquer-Sunyer & Duarte, 2010).

537   Acidification was shown to have additive or synergistic negative effects combined with hypoxia (Gobler &

538   Baumann, 2016; Steckbauer et al., 2020). Since all these factors usually co-occur during hypoxic events, *in*

539   *situ* sponge thresholds to hypoxia could be lower than determined through single stressor laboratory

540   experiments (Diaz & Rosenberg, 1995; Steckbauer et al., 2020). Future experiments that evaluate the

541   combined effect of these factors will be crucial to understand the full response of sponges to hypoxia in

542   natural ecosystems.

543   Diel oxygen variation is another factor that could influence anorganism' tolerance to hypoxia in natural

544   conditions. In the photic zone of marine ecosystems, dissolved oxygen generally increases during the day

545   because of photosynthesis and decreases at night because of aerobic respiration (Kroeker et al. 2019). The

546   amplitude of these diel fluctuations can sometimes lead to hypoxia or complete anoxia at night and,

547   supersaturation in peak sunny hours, or both (Diaz & Breitburg, 2009). These extreme oxygen dynamics

548   have been reported from a wide variety of macro- and micro--habitats from both tropical and temperate

549   ecosystems, such as intertidal reef platforms, tide pools, semi-enclosed basins, tropical lagoons and the

550   boundary layer around macroalgal canopies (Morris and Taylor, 1983, Frieder et al. 2012, Cornwall et al.,

551   2013, Gruber et al., 2017, Trowbridge et al. 2017, Hughes et al., 2020). Diurnal fluctuations in oxygen can

552   produce different responses from static exposure in a laboratory experiments, which may either

553   overestimate or underestimate the emergent effects of hypoxia in natural environments (Bumett & Stickle,

554   2001). Therefore, future experiments will need to account for current and future temporal variability in

555   oxygen concentration to accurately forecast the emergent ecological effects of deoxygenation (Kroeker et

556   al., 2019).

557   **4.2 Hypoxia Tolerance of sponges compared to other sessile organisms**

558   Marine organisms have very variable tolerance to low dissolved oxygen, with lethal thresholds ranging from

559   8.6 mg O_2 L^{-1} for the first larval zoea stage of the crustacean *Cancer irroratus*, to resistance to complete

560   anoxia as in the case of the sea anemone *Metridium senile* and the oyster *Crassostrea virginica* (Wahl 1984;

561   Vaquer-Sunyer and Duarte, 2008). Sessile organisms are generally more tolerant than mobile ones, which is

562   likely due to them not being able to escape hypoxic conditions (Altieri & Diaz, 2019). Therefore, sessile

563   organisms that experience these conditions must have evolved other adaptive strategies to cope with

564   reduced oxygen (Diaz & Rosenberg, 1995).

565   Here we provide new evidence to support the hypothesis that sponges are one of the groups of sessile

566   organisms that are more tolerant to hypoxia and could be favoured in future deoxygenated oceans. Other

567   phyla, such as cnidarians and bivalves, include very tolerant species that can cope with prolonged periods

568   of anoxia (Fig. 13). This is not surprising since tolerance to severe hypoxia/anoxia is a widespread feature in

569   the animal world, and many organisms independently evolved this feature to cope with local conditions

570   (Hochachka & Lutz, 2001; Nilsson & Renshaw, 2004; Vaquer-Sunyer & Duarte, 2008). This ability is not

571   restricted to invertebrates and includes higher animals such as fish and reptiles (Milton & Prentice, 2007;

572   Vornanen et al., 2009).

573   What makes sponges unique as a phylum is their widespread tolerance to hypoxia, with all the species

574   investigated so far being. All the species investigated so far have been shown to cope with very low levels

575   of dissolved oxygen. In contrast, other phyla have a much wider range of tolerances, with some species

576   resistant to anoxia and others very sensitive to decreased oxygen (Fig. 5). For example, in sessile cnidarians,

577   lethal hypoxia thresholds range between 0 and 4 mg $O_2 L^{-1}$, while sublethal ones are between 0.71 and 4.56

578   mg $O_2 L^{-1}$ (Mangum, 1980; Dodds et al., 2007). In sessile bivalves, lethal thresholds range between 0 and 2

579   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.,

580   1991; Woo et al., 2013). Sponges, instead, show much less variation with known lethal thresholds that are

581   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.,

582   2014; 2018) (Fig. 5). It is worth noting that lethal thresholds are highly dependent on the time of exposure.

583   In the studies we considered, these ranged from a few days to weeks. However, there were no noticeable

584   differences evidencein the experimental duration and the median lethal time for the different organisms.

585   Therefore, we believe that differences in time of exposure do not represent a bias in our comparison. In

586   contrast, sublethal responses (e.g. changes in respiration rate, behaviour, and feeding activity) usually have

587   rapid time-to-onset, so they will likely be independent of exposure time.

588   The high tolerance of sponges to hypoxia compared to other organisms can be explained by the

589   evolutionary history of this group. Sponges are one of the most ancient groups of metazoans. There is some

590   evidence that theyThey likely evolved before the Marinoan glaciation (657-645 million years

591   ago)Neoproterozoic oxygenation event (635–630 Mya), when oxygen was perhaps less than 10% of present

592   atmospheric concentration -(Kump, 2008;Love et al., 2009; Maloof et al., 2010; Brocks et al., 2017; Whelan

593   et al., 2017; Cole et al., 2020; Turner, 2021).). Therefore, it is possible that Mmodern sponges might have

594   retained an ancestral condition concerning oxygen requirements (Mills et al., 2014; 2018). Therefore, it is

595   more likely that sSponges unable to survive severe hypoxia today (e.g., *P. croceus*) have lost certain key

596   ancestral adaptations to hypoxia, rather than hypoxia-tolerant lineages (e.g., *S. australiensis*) having

597   evolved relatively new capacities for hypoxia tolerance (Müller et al., 2012). Likewise, other animals which

598   might have evolved in similar conditions, such as ctenophores, also show great resistance to hypoxia

599   (Thuesen et al., 2005). Therefore, we speculate that sponges' long evolutionary history could give these

600   organisms an adaptive advantage in future deoxygenated oceans, since they may have experienced similar

601   conditions in past geological eras.

602

603

604 **CONCLUSIONS**

605 Overall, sponges show high tolerance to low dissolved oxygen compared to all the other phyla of sessile

606 marine organisms that have been studied. Species-specific phenotypic plasticity appears to help these

607 organisms to overcome hypoxic events, and future research will need to elucidate the mechanisms behind

608 these changes. This exceptional adaptive capacity of sponges could derive from their ancient evolutionary

609 origin and could confer sponges a competitive advantage in future deoxygenated oceans over other

610 organisms (Mills et al., 2014; Schuster et al., 2021).

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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633   **REFERENCES**

634   Altieri, A. H., & Diaz, R. J. (2019). Dead zones: oxygen depletion in coastal ecosystems. In: C. Sheppard

635   (Ed.), *World Seas: An Environmental Evaluation* (pp. 453–473). Academic Press.

636   https://doi.org/10.1016/b978-0-12-805052-1.00021-8

637   Altieri, A. H., & Gedan, K. B. (2015). Climate change and dead zones. *Global Change Biology*, 21(4),

638   1395–1406. https://doi.org/10.1111/gcb.12754

639   Altieri, A. H., Harrison, S. B., Seemann, J., Collin, R., Diaz, R. J., & Knowlton, N. (2017). Tropical dead

640   zones and mass mortalities on coral reefs. *Proceedings of the National Academy of Sciences, 114*(14),

641   3660–3665. https://doi.org/10.1073/pnas.1621517114

642   Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral

643   Ecology, 26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

644   Anderson, M. J. (2014). Permutational multivariate analysis of variance (PERMANOVA). *Wiley statsref:

645   statistics reference online*, 1–15. https://doi.org/10.1002/9781118445112.stat07841

646   Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and

647   statistical methods*. PRIMER-E, Plymouth, UK.

648   Ayling, A. L. (1983). Factors affecting the spatial distributions of thinly encrusting sponges from

649   temperate waters. *Oecologia, 60*(3), 412–418. https://doi.org/10.1007/bf00376861

650   Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4.

651   *Journal of Statistical Software, 67*(1), 1–48. https://doi:10.18637/jss.v067.i01

652   Bell, J. J. (2008). The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science, 79*(3),

653   341–353. https://doi.org/10.1016/j.ecss.2008.05.002

654   Bell, J. J., & Barnes, D. K. (2000). The distribution and prevalence of sponges in relation to

655   environmental gradients within a temperate sea lough: vertical cliff surfaces. *Diversity and*

656   *Distributions, 6*(6), 283–303. https://doi.org/10.1046/j.1472-4642.2000.00091.x

657   Bell, J. J., McGrath, E., Kandler, N. M., Marlow, J., Beepat, S. S., Bachtiar, R., Shaffer, M. R., Mortimer, C.,

658   Micaroni, V., Mobilia, V., Rovellini, A., Harris, B., Farnham, E., Strano, F., & Carballo, J. L. (2020).

659   Interocean patterns in shallow water sponge assemblage structure and function. *Biological*

660   *Reviews, 95*(6), 1720–1758. https://doi.org/10.1111/brv.12637

661   Bijma, J., Pörtner, H. O., Yesson, C., & Rogers, A. D. (2013). Climate change and the oceans–What does

662   the future hold?. *Marine Pollution Bulletin, 74*(2), 495–505.

663   https://doi.org/10.1016/j.marpolbul.2013.07.022

664   Breitburg, D. L. (1990). Near-shore hypoxia in the Chesapeake Bay: patterns and relationships among

665   physical factors. *Estuarine, Coastal and Shelf Science, 30*(6), 593–609. https://doi.org/10.1016/0272-

666   7714(90)90095-9

667   Brocks, J. J., Jarrett, A. J., Sirantoine, E., Hallmann, C., Hoshino, Y., & Liyanage, T. (2017). The rise of

668   algae in Cryogenian oceans and the emergence of animals. *Nature, 548*(7669), 578-581.

669   https://doi.org/doi:10.1038/nature23457

670   Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J.,

671   Machler, M. & Bolker, B.M. (2017). glmmTMB balances speed and flexibility among packages for zero-

672   inflated generalized linear mixed modeling. *The R Journal, 9*(2), 378–400. https://doi.org/10.32614/rj-

673   2017-066

674   Bumett, L. E., & Stickle, W. B. (2001). Physiological responses to hypoxia. In: N. N. Rabalais, R. E. Turner

675   (Eds). *Coastal and Estuarine Studies, Volume 58. Coastal hypoxia: consequences for living resources and*

676   *ecosystems*, (pp. 101–114). AGU Publications. https://doi.org/10.1029/ce058p0101

677   Chu, J. W., Curkan, C., & Tunnicliffe, V. (2018). Drivers of temporal beta diversity of a benthic

678   community in a seasonally hypoxic fjord. *Royal Society Open Science, 5*(4), 172284.

679   https://doi.org/10.1098/rsos.172284

680   Clarke, K. R., & Gorley, R. N. (2015). *Getting started with PRIMER v7*. PRIMER-E, Plymouth, UK.

681   Cole, D. B., Mills, D. B., Erwin, D. H., Sperling, E. A., Porter, S. M., Reinhard, C. T., & Planavsky, N. J.

682   (2020). On the co-evolution of surface oxygen levels and animals. *Geobiology, 18*(3), 260–281.

683   https://doi.org/10.1111/gbi.12382

684   Cook, S. D. C. (2010). *New Zealand coastal marine invertebrates*. Canterbury University Press.

685   Cornwall, C. E., Hepburn, C. D., Pilditch, C. A., & Hurd, C. L. (2013). Concentration boundary layers

686   around complex assemblages of macroalgae: Implications for the effects of ocean acidification on

687   understory coralline algae. *Limnology and Oceanography, 58*(1), 121–130.

688   https://doi.org/10.4319/lo.2013.58.1.0121

689   De Zwaan, A., Cortesi, P., Van den Thillart, G., Roos, J., & Storey, K. B. (1991). Differential sensitivities to

690   hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Marine Biology, 111*(3), 343–

691   351. https://doi.org/10.1007/bf01319405

692   Desai, D. V., & Prakash, S. (2009). Physiological responses to hypoxia and anoxia in *Balanus amphitrite*

693   (Cirripedia: Thoracica). *Marine Ecology Progress Series, 390*, 157–166.

694   https://doi.org/10.3354/meps08155

695   Diaz, R. J., & Breitburg, D. L. (2009). The hypoxic environment. In: J. G. Richards, A. P. Farrell, C. J.

696   Brauner (Eds) *Fish physiology, Volume 27* (pp. 1–23). Academic Press. https://doi.org/10.1016/b978-0-

697   12-809633-8.03213-1

698   Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the

699   behavioural responses of benthic macrofauna. *Oceanography and marine biology. An annual review,*

700   *33*, 245–03.

701   Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine

702   ecosystems. *Science, 321*(5891), 926–929. https://doi.org/10.1126/science.1156401

703   Diaz, R. J., & Rosenberg, R. (2011). Introduction to environmental and economic consequences of

704   hypoxia. *International Journal of Water Resources Development, 27*(1), 71–82.

705   https://doi.org/10.1080/07900627.2010.531379

706   Dodds, L. A., Roberts, J. M., Taylor, A. C., & Marubini, F. (2007). Metabolic tolerance of the cold-water

707   coral *Lophelia pertusa* (Scleractinia) to temperature and dissolved oxygen change. *Journal of*

708   *Experimental Marine Biology and Ecology, 349*(2), 205–214.

709   https://doi.org/10.1016/j.jembe.2007.05.013

710   Doney, S. C. (2010). The growing human footprint on coastal and open-ocean biogeochemistry. *Science,*

711   *328*(5985), 1512–1516. https://doi.org/10.1126/science.1185198

712   Ellington, W. R. (1982). Metabolic responses of the sea anemone *Bunodosoma cavernata* (Bosc) to

713   declining oxygen tensions and anoxia. *Physiological Zoology, 55*(3), 240–249.

714   https://doi.org/10.1086/physzool.55.3.30157888

715   Frieder, C. A., Nam, S. H., Martz, T. R., & Levin, L. A. (2012). High temporal and spatial variability of

716   dissolved oxygen and pH in a nearshore California kelp forest. *Biogeosciences, 9*(10), 3917–3930.

717   https://doi.org/10.5194/bg-9-3917-2012

718   Gobler, C. J., & Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics

719   and effects on marine life. *Biology Letters, 12*(5), 20150976. https://doi.org/10.1098/rsbl.2015.0976

720   Goldstein, J., Riisgård, H. U., & Larsen, P. S. (2019). Exhalant jet speed of single-osculum explants of the

721   demosponge *Halichondria panicea* and basic properties of the sponge-pump. *Journal of Experimental*

722   *Marine Biology and Ecology, 511*, 82–90. https://doi.org/10.1016/j.jembe.2018.11.009

723   Gruber, R. K., Lowe, R. J., & Falter, J. L. (2017). Metabolism of a tide-dominated reef platform subject to

724   extreme diel temperature and oxygen variations. *Limnology and Oceanography, 62*(4), 1701–1717.

725   https://doi.org/10.1002/lno.10527

726   Haas, A. F., Smith, J. E., Thompson, M., & Deheyn, D. D. (2014). Effects of reduced dissolved oxygen

727   concentrations on physiology and fluorescence of hermatypic corals and benthic algae. *PeerJ, 2*, e235.

728   https://doi.org/10.7717/peerj.235

729   Hagerman, L. (1998). Physiological flexibility; a necessity for life in anoxic and sulphidic habitats.

730   *Hydrobiologia, 375*, 241–254. https://doi.org/10.1007/978-94-017-2864-5_20

731   Hervé, M. (2021). *RVAideMemoire: testing and plotting procedures for biostatistics*. R package version

732   0.9-79. https://cran.r-project.org/web/packages/RVAideMemoire/index.html

733   Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., & Horn, M. (2003). Microbial

734   diversity of marine sponges. In: W. E. G. Müller (Ed) *Progress in Molecular and Subcellular Biology.*

735   *Sponges (Porifera)* (pp 59–88). Springer. https://doi.org/10.1007/978-3-642-55519-0_3

736   Hentschel, U., Usher, K. M., & Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS*

737   *microbiology ecology, 55*(2), 167–177. https://doi.org/10.1111/j.1574-6941.2005.00046.x

738   Hochachka, P. W., & Lutz, P. L. (2001). Mechanism, origin, and evolution of anoxia tolerance in animals.

739   *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 130*(4), 435–459.

740   https://doi.org/10.1016/s1096-4959(01)00408-0

741   Hoffmann, F., Larsen, O., Thiel, V., Rapp, H. T., Pape, T., Michaelis, W., & Reitner, J. (2005). An

742   anaerobic world in sponges. *Geomicrobiology Journal, 22*(1–2), 1–10.

743   https://doi.org/10.1080/01490450590922505

744   Hughes, D. J., Alderdice, R., Cooney, C., Kühl, M., Pernice, M., Voolstra, C. R., & Suggett, D. J. (2020).

745   Coral reef survival under accelerating ocean deoxygenation. *Nature Climate Change, 10*(4), 296–307.

746   https://doi.org/10.1038/s41558-020-0737-9

747 IPCC (2021). Summary for Policymakers. In: V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C.

748 Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R.

749 Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu & B. Zhou (Eds.). *Climate Change 2021: The*

750 *Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the*

751 *Intergovernmental Panel on Climate Change*. Cambridge University Press. In Press.

752 Johnson, M. D., Rodriguez, L. M., & Altieri, A. H. (2018). Shallow-water hypoxia and mass mortality on a

753 Caribbean coral reef. *Bulletin of Marine Science, 94*(1), 143–144.

754 https://doi.org/10.5343/bms.2017.1163

755 Johnson, P. D., & McMahon, R. F. (1998). Effects of temperature and chronic hypoxia on survivorship of

756 the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). *Canadian Journal of*

757 *Fisheries and Aquatic Sciences, 55*(7), 1564–1572. https://doi.org/10.1139/f98-030

758 Kamke, J., Taylor, M. W., & Schmitt, S. (2010). Activity profiles for marine sponge-associated bacteria

759 obtained by 16S rRNA vs 16S rRNA gene comparisons. *The ISME Journal, 4*(4), 498–508.

760 https://doi.org/10.1038/ismej.2009.143

761 Kealoha, A. K., Doyle, S. M., Shamberger, K. E., Sylvan, J. B., Hetland, R. D., & Di Marco, S. F. (2020).

762 Localized hypoxia may have caused coral reef mortality at the Flower Garden Banks. *Coral Reefs, 39*(1),

763 119–132. https://doi.org/10.1007/s00338-019-01883-9

764 Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. *Annual*

765 *Review of Marine Science, 2*, 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

766 Kroeker, K. J., Bell, L. E., Donham, E. M., Hoshijima, U., Lummis, S., Toy, J. A., & Willis-Norton, E. (2020).

767 Ecological change in dynamic environments: Accounting for temporal environmental variability in

768 studies of ocean change biology. *Global change biology, 26*(1), 54–67.

769 https://doi.org/10.1111/gcb.14868

770 Kump, L. R. (2008). The rise of atmospheric oxygen. *Nature, 451*(7176), 277–278.

771 https://doi.org/10.1038/nature06587

772   Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed

773   effects models. *Journal of Statistical Software, 82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

774   Lavy, A., Keren, R., Yahel, G., & Ilan, M. (2016). Intermittent hypoxia and prolonged suboxia measured

775   in situ in a marine sponge. *Frontiers in Marine Science, 3*, 263.

776   https://doi.org/10.3389/fmars.2016.00263

777   Lenth, R. (2021). *emmeans: estimated marginal means, aka least-squares means*. R package version

778   1.6.0. https://CRAN.R-project.org/package=emmeans

779   Levin, L. A. (2003). Oxygen minimum zone benthos: adaptation and community response to hypoxia.

780   *Oceanography and Marine Biology: An Annual Review. 41*, 1–45.

781   https://doi.org/10.1201/9780203180570-3

782   Levin, L. A., & Breitburg, D. L. (2015). Linking coasts and seas to address ocean deoxygenation. *Nature*

783   *Climate Change, 5*(5), 401–403. https://doi.org/10.1038/nclimate2595

784   Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., Neira, C., Rabalais, N.

785   N. & Zhang, J. (2009). Effects of natural and human-induced hypoxia on coastal benthos.

786   *Biogeosciences, 6*(10), 2063–2098. https://doi.org/10.5194/bg-6-2063-2009

787   Levin, L. A., Huggett, C. L., & Wishner, K. F. (1991). Control of deep-sea benthic community structure by

788   oxygen and organic-matter gradients in the eastern Pacific Ocean. *Journal of Marine Research, 49*(4),

789   763–800. https://doi.org/10.1357/002224091784995756

790   Leys, S. P., & Kahn, A. S. (2018). Oxygen and the energetic requirements of the first multicellular

791   animals. *Integrative and Comparative Biology, 58*(4), 666–676. https://doi.org/10.1093/icb/icy051

792   Limburg, K. E., Breitburg, D., & Levin, L. A. (2017). Ocean deoxygenation-a climate-related problem.

793   *Frontiers in Ecology and the Environment, 15*(9), 479–479. https://doi.org/10.1002/fee.1728

794   Love, G.D., Grosjean, E., Stalvies, C., Fike, D.A., Grotzinger, J.P., Bradley, A.S., Kelly, A.E., Bhatia, M.,

795   Meredith, W., Snape, C.E. and Bowring, S.A., Condon, D. J., & Summons, R. E. (2009). Fossil steroids

796   record the appearance of Demospongiae during the Cryogenian period. *Nature, 457*(7230), 718–721.

797   https://doi.org/10.1038/nature07673

798   Lunden, J. J., McNicholl, C. G., Sears, C. R., Morrison, C. L., & Cordes, E. E. (2014). Acute survivorship of

799   the deep-sea coral *Lophelia pertusa* from the Gulf of Mexico under acidification, warming, and

800   deoxygenation. *Frontiers in Marine Science, 1*, 78. https://doi.org/10.3389/fmars.2014.00078

801   Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: biology, budgets,

802   and ecological implications. *Advances in Marine Biology, 62*, 113–182. https://doi.org/10.1016/b978-0-

803   12-394283-8.00003-5

804   Maloof, A. C., Rose, C. V., Beach, R., Samuels, B. M., Calmet, C. C., Erwin, D. H., Poirier, G. R., Yao, N., &

805   Simons, F.J. (2010). Possible animal-body fossils in pre-Marinoan limestones from South Australia.

806   *Nature Geoscience, 3*(9), 653–659. https://doi.org/10.1038/ngeo934

807   Mangum, D. C. (1980). Sea anemone neuromuscular responses in anaerobic conditions. *Science,*

808   *208*(4448), 1177–1178. https://doi.org/10.1126/science.6103580

809   McAllen, R., Davenport, J., Bredendieck, K., & Dunne, D. (2009). Seasonal structuring of a benthic

810   community exposed to regular hypoxic events. *Journal of Experimental Marine Biology and Ecology,*

811   *368*(1), 67–74. https://doi.org/10.1016/j.jembe.2008.10.019

812   McAllen, R., Taylor, A. C., & Davenport, J. (1999). The effects of temperature and oxygen partial

813   pressure on the rate of oxygen consumption of the high-shore rock pool copepod *Tigriopus brevicornis*.

814   *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 123*(2), 195–202.

815   https://doi.org/10.1016/s1095-6433(99)00050-1

816   Mentel, M., Röttger, M., Leys, S., Tielens, A. G., & Martin, W. F. (2014). Of early animals, anaerobic

817   mitochondria, and a modern sponge. *Bioessays, 36*(10), 924–932.

818   https://doi.org/10.1002/bies.201400060

819 Mills, D. B., Francis, W. R., Vargas, S., Larsen, M., Elemans, C. P., Canfield, D. E., & Wörheide, G. (2018).

820 The last common ancestor of animals lacked the HIF pathway and respired in low-oxygen

821 environments. *Elife, 7*, e31176. https://doi.org/10.7554/eLife.31176.001

822 Mills, D. B., Ward, L. M., Jones, C., Sweeten, B., Forth, M., Treusch, A. H., & Canfield, D. E. (2014).

823 Oxygen requirements of the earliest animals. *Proceedings of the National Academy of Sciences,*

824 *111*(11), 4168–4172. https://doi.org/10.1073/pnas.1400547111

825 Milton, S. L., & Prentice, H. M. (2007). Beyond anoxia: the physiology of metabolic downregulation and

826 recovery in the anoxia-tolerant turtle. *Comparative Biochemistry and Physiology Part A: Molecular and*

827 *Integrative Physiology, 147*(2), 277–290. https://doi.org/10.1016/j.cbpa.2006.08.041

828 Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C., Wu, Y. C., McCormack, G. P., López-Legentil,

829 S., Marchant, R., Webster, N., Thomas, T., & Hentschel, U. (2017). Predicting the HMA-LMA status in

830 marine sponges by machine learning. *Frontiers in microbiology, 8*, 752.

831 https://doi.org/10.3389/fmicb.2017.00752

832 Morganti, T. M., Ribes, M., Moskovich, R., Weisz, J. B., Yahel, G., & Coma, R. (2021). In situ Pumping

833 Rate of 20 Marine Demosponges Is a Function of Osculum Area. *Frontiers in Marine Science, 8*, 583188.

834 https://doi.org/10.3389/fmars.2021.583188

835 Morris, S., & Taylor, A. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within

836 intertidal rock pools. *Estuarine, Coastal and Shelf Science, 17*, 339–355. https://doi.org/10.1016/0272-

837 7714(83)90026-4

838 Mosch, T., Sommer, S., Dengler, M., Noffke, A., Bohlen, L., Pfannkuche, O., Liebetrau, V. & Wallmann, K.

839 (2012). Factors influencing the distribution of epibenthic megafauna across the Peruvian oxygen

840 minimum zone. *Deep Sea Research Part I: Oceanographic Research Papers, 68*, 123–135.

841 https://doi.org/10.1016/j.dsr.2012.04.014

842 Müller, M., Mentel, M., van Hellemond, J. J., Henze, K., Woehle, C., Gould, S. B., Yu, R.Y., van der

843 Giezen, M., Tielens, A.G., Martin, W. F. (2012). Biochemistry and evolution of anaerobic energy

844 metabolism in eukaryotes. *Microbiology and Molecular Biology Reviews, 76*(2), 444–495.

845 https://doi.org/10.1128/MMBR.05024-11

846 Murty, S. J., Bett, B. J., & Gooday, A. J. (2009). Megafaunal responses to strong oxygen gradients on the

847 Pakistan margin of the Arabian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography, 56*(6-

848 7), 472–487. https://doi.org/10.1016/j.dsr2.2008.05.029

849 Nagasoe, S., Tokunaga, T., Yurimoto, T., & Matsuyama, Y. (2020). Survival and behavior patterns

850 associated with hypoxia at different life stages of the pen shell *Atrina* cf. *japonica*. *Aquatic Toxicology,*

851 *227*, 105610. https://doi.org/10.1016/j.aquatox.2020.105610

852 Nezlin, N. P., Kamer, K., Hyde, J., & Stein, E. D. (2009). Dissolved oxygen dynamics in a eutrophic

853 estuary, Upper Newport Bay, California. *Estuarine, Coastal and Shelf Science*, 82(1), 139–151.

854 https://doi.org/10.1016/j.ecss.2009.01.004

855 Nilsson, G. E., & Renshaw, G. M. (2004). Hypoxic survival strategies in two fishes: extreme anoxia

856 tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark.

857 *Journal of Experimental Biology, 207*(18), 3131–3139. https://doi.org/10.1242/jeb.00979

858 Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns.

859 *Ophelia, 41*(1), 199–219. https://doi.org/10.1080/00785236.1995.10422044

860 Osinga, R., Tramper, J., & Wijffels, R. H. (1999). Cultivation of marine sponges. *Marine Biotechnology,*

861 *1*(6), 509–532. https://doi.org/10.1007/PL00011807

862 Piazzi, L., Atzori, F., Cadoni, N., Cinti, M. F., Frau, F., Pansini, A., Pinna, F., Stipcich, P. & Ceccherelli, G.

863 (2021). Animal Forest Mortality: Following the Consequences of a Gorgonian Coral Loss on a

864 Mediterranean Coralligenous Assemblage. *Diversity, 13*(3), 133. https://doi.org/10.3390/d13030133

865 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team (2021). *nlme: Linear and Nonlinear Mixed*

866 *Effects Models*. R package version 3.1-152. https://CRAN.R-project.org/package=nlme.

867 R Core Team (2013). *R: A language and environment for statistical computing.*

868   Rao, D. P., & Ganapati, P. N. (1968). Respiration as a function of oxygen concentration in intertidal

869   barnacles. *Marine Biology, 1*(4), 309–310. https://doi.org/10.1007/BF00360781

870   Raupach, M. R., & Canadell, J. G. (2010). Carbon and the Anthropocene. *Current Opinion in*

871   *Environmental Sustainability, 2*(4), 210–218. https://doi.org/10.1016/j.cosust.2010.04.003

872   Robinson, C. (2019). Microbial respiration, the engine of ocean deoxygenation. *Frontiers in Marine*

873   *Science, 5*, 533. https://doi.org/10.3389/fmars.2018.00533

874   Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H. O., Duarte, C. M., Levin, L. A. & Rosa, R.

875   (2021). Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nature*

876   *Ecology and Evolution, 5*(3), 311–321. https://doi.org/10.1038/s41559-020-01370-3

877   Sarà, M., Sarà, A., Nickel, M., & Brümmer, F. (2001). Three New Species of *Tethya* (Porifera:

878   Demospongiae) From German Aquaria. *Stuttgarter Beiträge zur Naturkunde 631*, 1–15.

879   Sassaman, C., & Mangum, C. P. (1972). Adaptations to environmental oxygen levels in infaunal and

880   epifaunal sea anemones. *The Biological Bulletin, 143*(3), 657–678. https://doi.org/10.2307/1540189

881   Schuster, A., Strehlow, B. W., Eckford-Soper, L., McAllen, R., & Canfield, D. E. (2021). Effects of Seasonal

882   Anoxia on the Microbial Community Structure in Demosponges in a Marine Lake in Lough Hyne, Ireland.

883   *Msphere, 6(*1), e00991-20. https://doi.org/10.1101/2020.09.09.290791

884   Seibel, B. A. (2011). Critical oxygen levels and metabolic suppression in oceanic oxygen minimum

885   zones. *Journal of Experimental Biology, 214*(2), 326–336. https://doi.org/10.1242/jeb.049171

886   Semenza, G. L. (2007). Life with oxygen. *Science, 318*(5847), 62–64.

887   https://doi.org/10.1126/science.1147949

888   Smith, V. H., Joye, S. B., & Howarth, R. W. (2006). Eutrophication of freshwater and marine ecosystems.

889   *Limnology and Oceanography, 51*, 351–355. https://doi.org/10.1007/978-90-481-3385-7_1

890   Stachowitsch, M. (1984). Mass mortality in the Gulf of Trieste: the course of community destruction.

891   *Marine Ecology, 5*(3), 243–264. https://doi.org/10.1111/j.1439-0485.1984.tb00124.x

892   Steckbauer, A., Klein, S. G., & Duarte, C. M. (2020). Additive impacts of deoxygenation and acidification

893   threaten marine biota. *Global Change Biology, 26*(10), 5602–5612. https://doi.org/10.1111/gcb.15252

894   Stickle, W. B., Kapper, M. A., Liu, L. L., Gnaiger, E., & Wang, S. Y. (1989). Metabolic adaptations of

895   several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. The

896   *Biological Bulletin, 177*(2), 303–312. https://doi.org/10.2307/1541945

897   Strano, F., Micaroni, V., Davy, S. K., Maldonado, M., & Bell, J. J. (2021). Reproduction and early life

898   stages of the poecilosclerid sponge *Crella incrustans*. *Invertebrate Biology*, e12335.

899   https://doi.org/10.1111/ivb.12335

900   Therneau, T. (2021). *A Package for Survival Analysis in R*. R package version 3.2-10. https://CRAN.R-

901   project.org/package=survival.

902   Thuesen, E. V., Rutherford Jr, L. D., & Brommer, P. L. (2005). The role of aerobic metabolism and

903   intragel oxygen in hypoxia tolerance of three ctenophores: *Pleurobrachia bachei*, *Bolinopsis*

904   *infundibulum* and *Mnemiopsis leidyi*. *Journal of the Marine Biological Association of the United*

905   *Kingdom, 85*(3), 627. https://doi.org/10.1017/s0025315405011550

906   Trowbridge, C. D., Davenport, J., Cottrell, D. M., Harman, L., Plowman, C. Q., Little, C., & McAllen, R.

907   (2017). Extreme oxygen dynamics in shallow water of a fully marine Irish sea lough. *Regional Studies in*

908   *Marine Science, 11*, 9–16. https://doi.org/10.1016/j.rsma.2017.01.008

909   Turner, E. C. (2021). Possible poriferan body fossils in early Neoproterozoic microbial reefs. *Nature*,

910   *596*, 87–91. https://doi.org/10.1038/s41586-021-03773-z

911   Vacelet, J. (1975). Étude en microscopie électronique de l'association entre bactéries et spongiaires du

912   genre Verongia (Dictyoceratida).

913   Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings*

914   *of the National Academy of Sciences, 105*(40), 15452–15457.

915   https://doi.org/10.1073/pnas.0803833105

916 Vaquer-Sunyer, R., & Duarte, C. M. (2010). Sulfide exposure accelerates hypoxia-driven mortality.

917 *Limnology and Oceanography, 55*(3), 1075–1082. https://doi.org/10.4319/lo.2010.55.3.1075

918 Vaquer-Sunyer, R., & Duarte, C. M. (2011). Temperature effects on oxygen thresholds for hypoxia in

919 marine benthic organisms. *Global Change Biology, 17*(5), 1788–1797. https://doi.org/10.1111/j.1365-

920 2486.2010.02343.x

921 Vergés, A., McCosker, E., Mayer-Pinto, M., Coleman, M. A., Wernberg, T., Ainsworth, T., & Steinberg, P.

922 D. (2019). Tropicalisation of temperate reefs: implications for ecosystem functions and management

923 actions. *Functional Ecology, 33*(6), 1000–1013. https://doi.org/10.1111/1365-2435.13310

924 Vornanen, M., Stecyk, J. A., & Nilsson, G. E. (2009). The anoxia-tolerant crucian carp (*Carassius*

925 *carassius* L.). In: J. Richards, A. Farrell, & C. Brauner (Eds.), *Fish Physiology: Hypoxia, Volume 27* (pp.

926 397–441). Academic Press. https://doi.org/10.1016/s1546-5098(08)00009-5

927 Whelan, N. V., Kocot, K. M., Moroz, T. P., Mukherjee, K., Williams, P., Paulay, G., Moroz, L. L. &

928 Halanych, K. M. (2017). Ctenophore relationships and their placement as the sister group to all other

929 animals. *Nature Ecology & Evolution, 1*(11), 1737–1746. https://doi.org/10.1038/s41559-017-0331-3

930 Wishner, K. F., Ashjian, C. J., Gelfman, C., Gowing, M. M., Kann, L., Levin, L. A., Mullineaux, L. S. &

931 Saltzman, J. (1995). Pelagic and benthic ecology of the lower interface of the Eastern Tropical Pacific

932 oxygen minimum zone. *Deep Sea Research Part I: Oceanographic Research Papers, 42*(1), 93–115.

933 https://doi.org/10.1016/0967-0637(94)00021-j

934 Woo, S., Denis, V., Won, H., Shin, K., Lee, G., Lee, T. K., & Yum, S. (2013). Expressions of oxidative stress-

935 related genes and antioxidant enzyme activities in *Mytilus galloprovincialis* (Bivalvia, Mollusca) exposed

936 to hypoxia. *Zoological Studies, 52*(1), 1–8. https://doi.org/10.1186/1810-522x-52-15

937 Woodhead, A. J., Hicks, C. C., Norström, A. V., Williams, G. J., & Graham, N. A. (2019). Coral reef

938 ecosystem services in the Anthropocene. *Functional Ecology, 33*(6), 1023–1034.

939 https://doi.org/10.1111/1365-2435.13331

940   Wulff, J. L. (2006). Ecological interactions of marine sponges. *Canadian Journal of Zoology, 84*(2), 146–

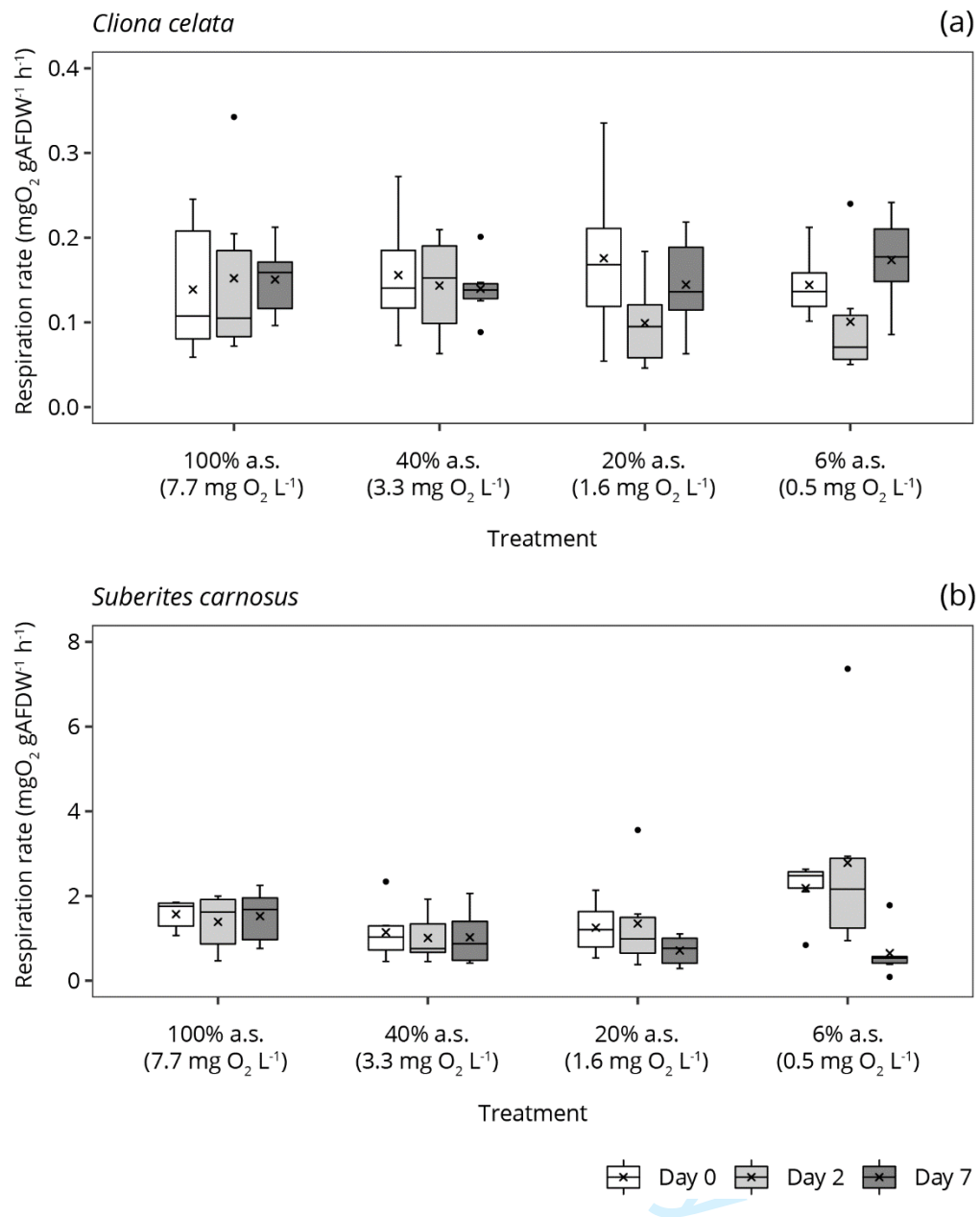941   166. https://doi.org/10.1139/z06-019

942   Yahel, G., Marie, D., & Genin, A. (2005). InEx—a direct in situ method to measure filtration rates,

943   nutrition, and metabolism of active suspension feeders. *Limnology and Oceanography: Methods, 3*(2),

944   46–58. https://doi.org/10.4319/lom.2005.3.46

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946

947   **FIGURES**

Cliona celata   (a)

Suberites carnosus   (b)
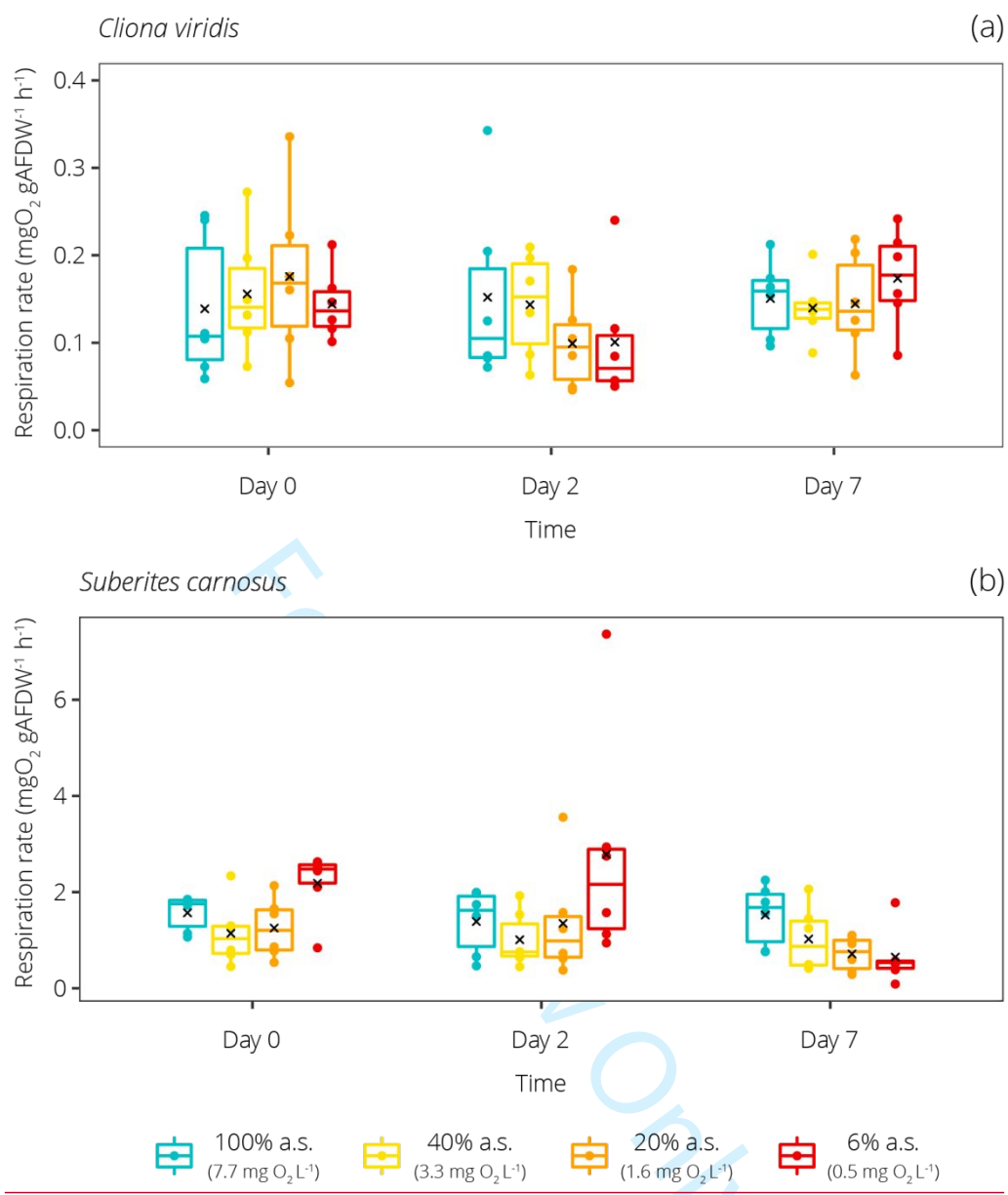

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Figure 1. Respiration rates in (a) *Cliona̶. celata* and (b) *Suberites̶. carnosus* from experiment 1 (moderate hypoxia) measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. ~~The oxygen concentration of the different treatments is expressed as % air saturation (% a.s.).~~ 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. ~~Lower and upper whiskers represent the smallest and largest values, respectively. Single dots~~Points represent ~~outliers~~data points.
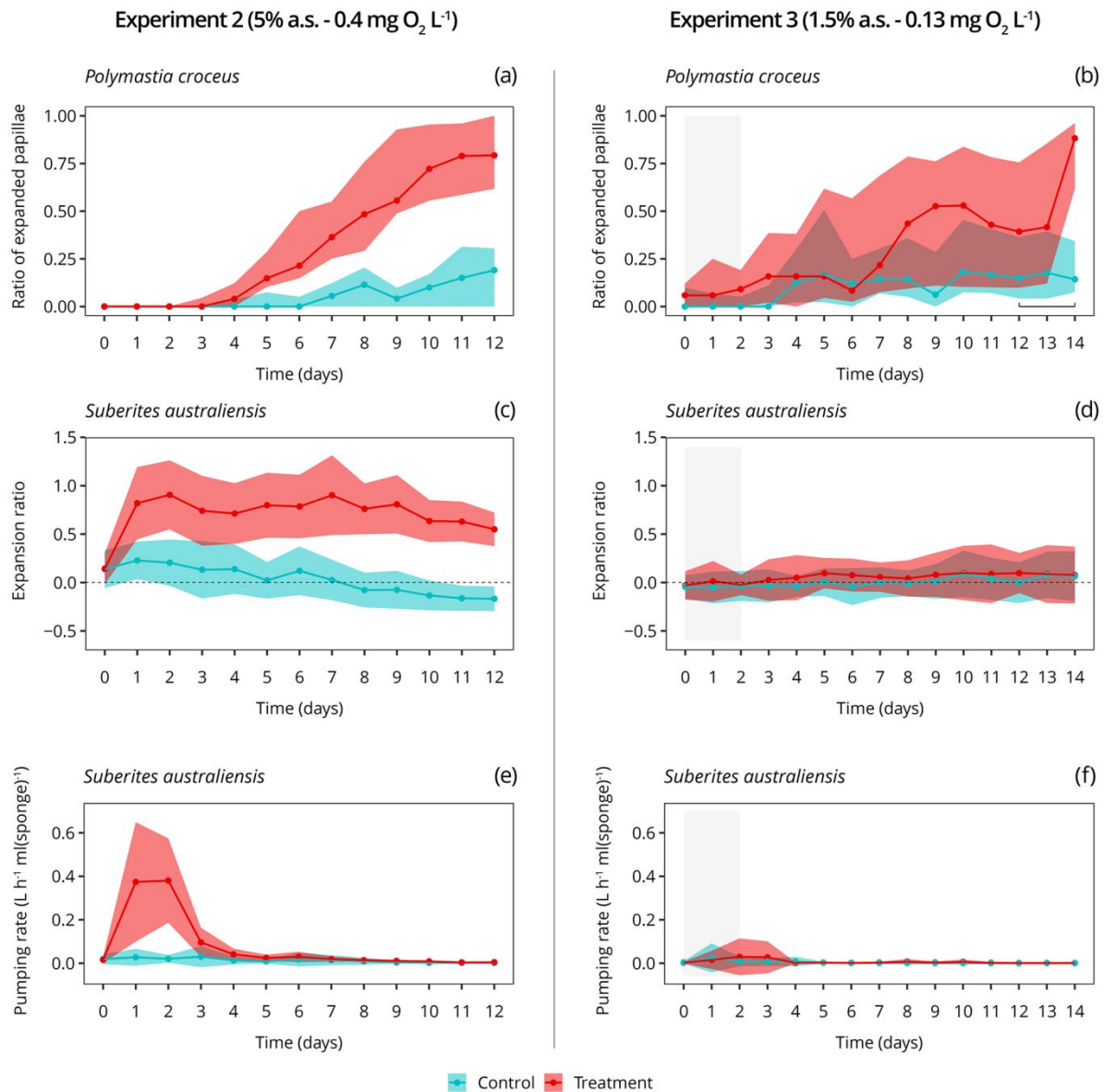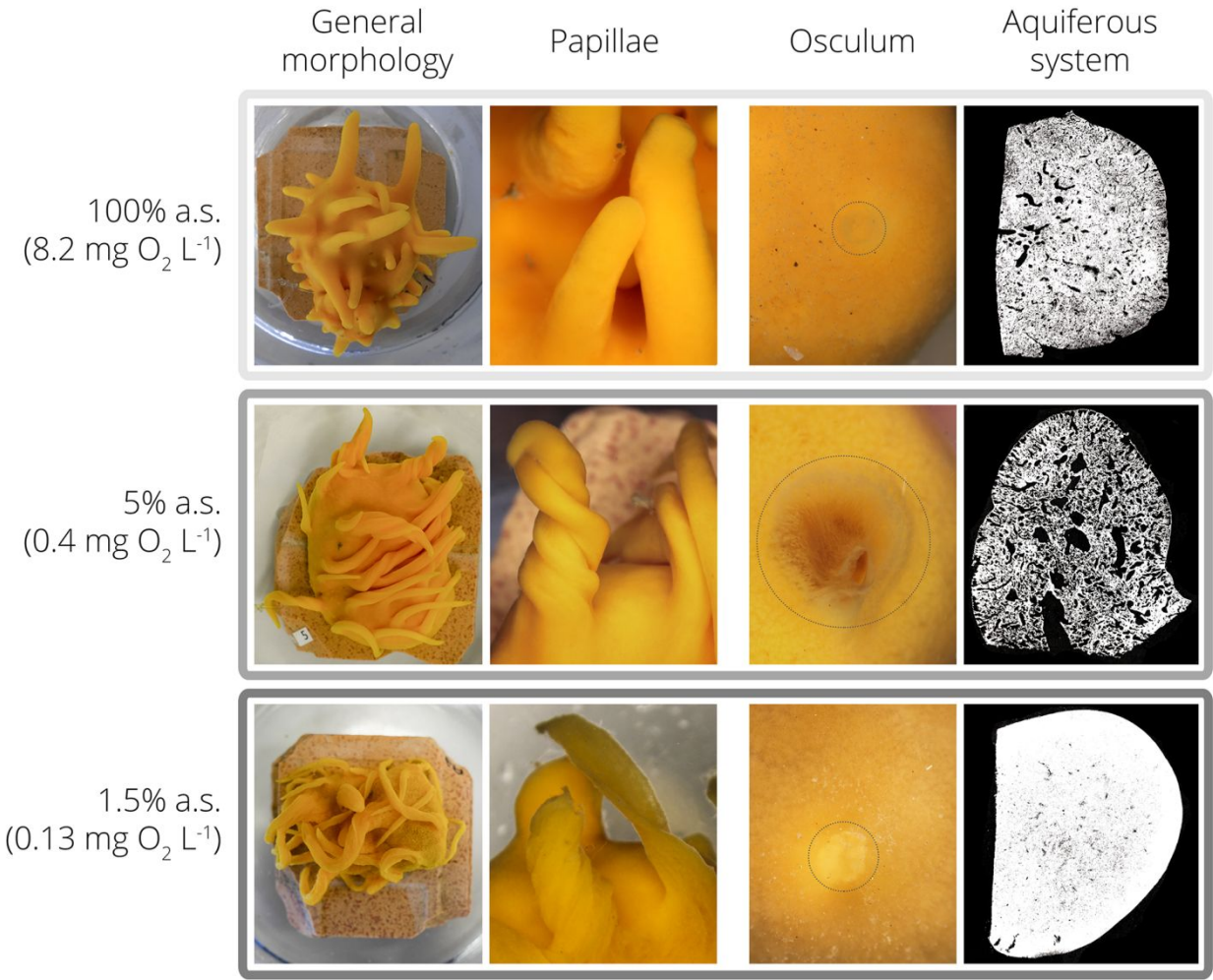
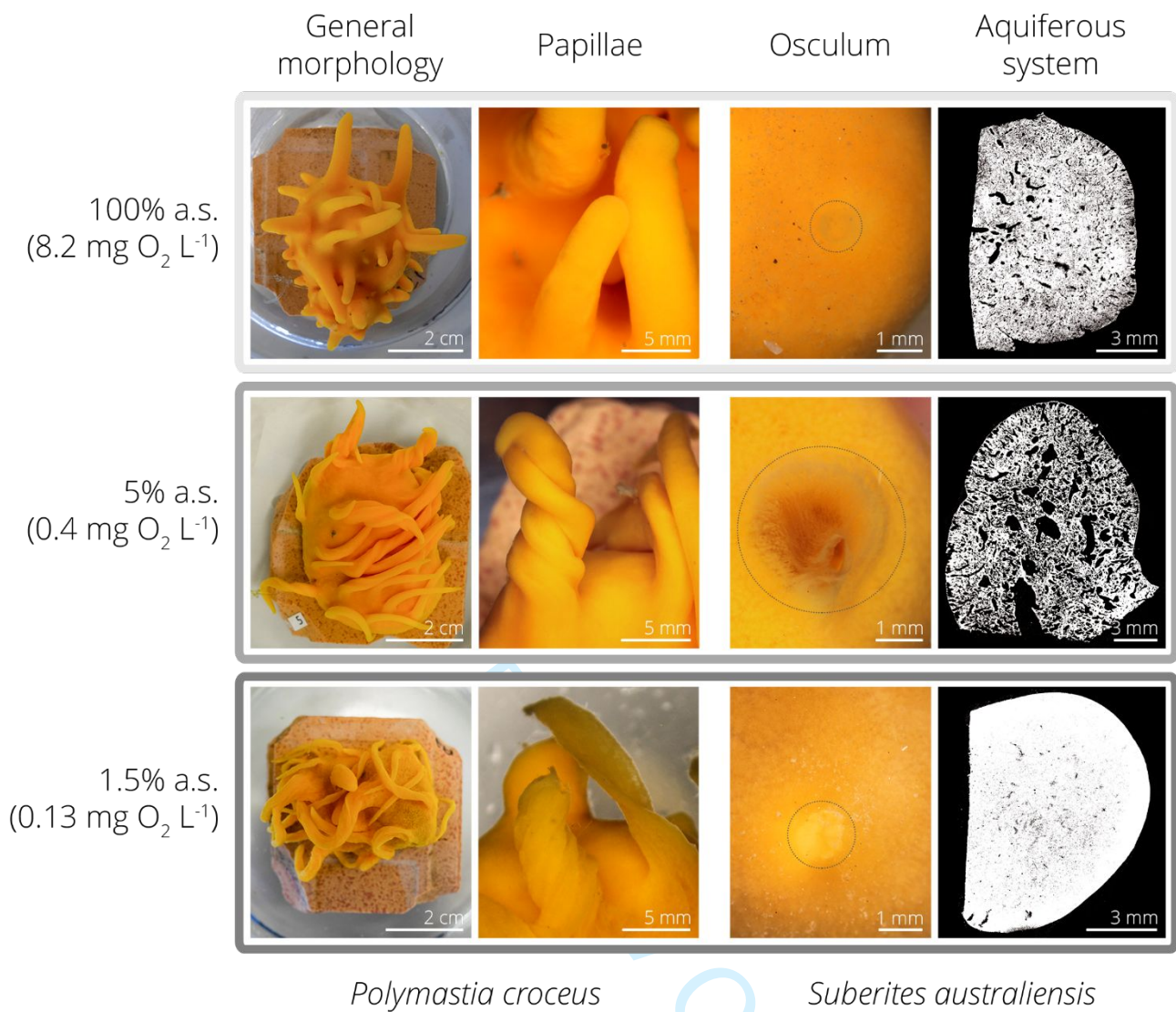Figure 2. Contractile behaviour and pumping rate during experiments 2 and 3 (severe hypoxic conditions). Changes in the ratio of expanded papillae over time in _Polymastia P. croceus_ in each treatment in experiments 2 (a) and 3 (b). Changes in the expansion ratio over time in _Suberites S. australiensis_ 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.

	General morphology	Papillae	Osculum	Aquiferous system
100% a.s. (8.2 mg O_2 L^{-1})				
5% a.s. (0.4 mg O_2 L^{-1})				
1.5% a.s. (0.13 mg O_2 L^{-1})				

Polymastia croceus   *Suberites australiensis*

968

	General morphology	Papillae	Osculum	Aquiferous system
100% a.s. (8.2 mg O$_2$ L^{-1})				
5% a.s. (0.4 mg O$_2$ L^{-1})				
1.5% a.s. (0.13 mg O$_2$ L^{-1})				
	Polymastia croceus		*Suberites australiensis*	

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 P. croceus*; 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 S. australiensis*. An extended version of this figure is found in the supplemental material (Fig. S12).
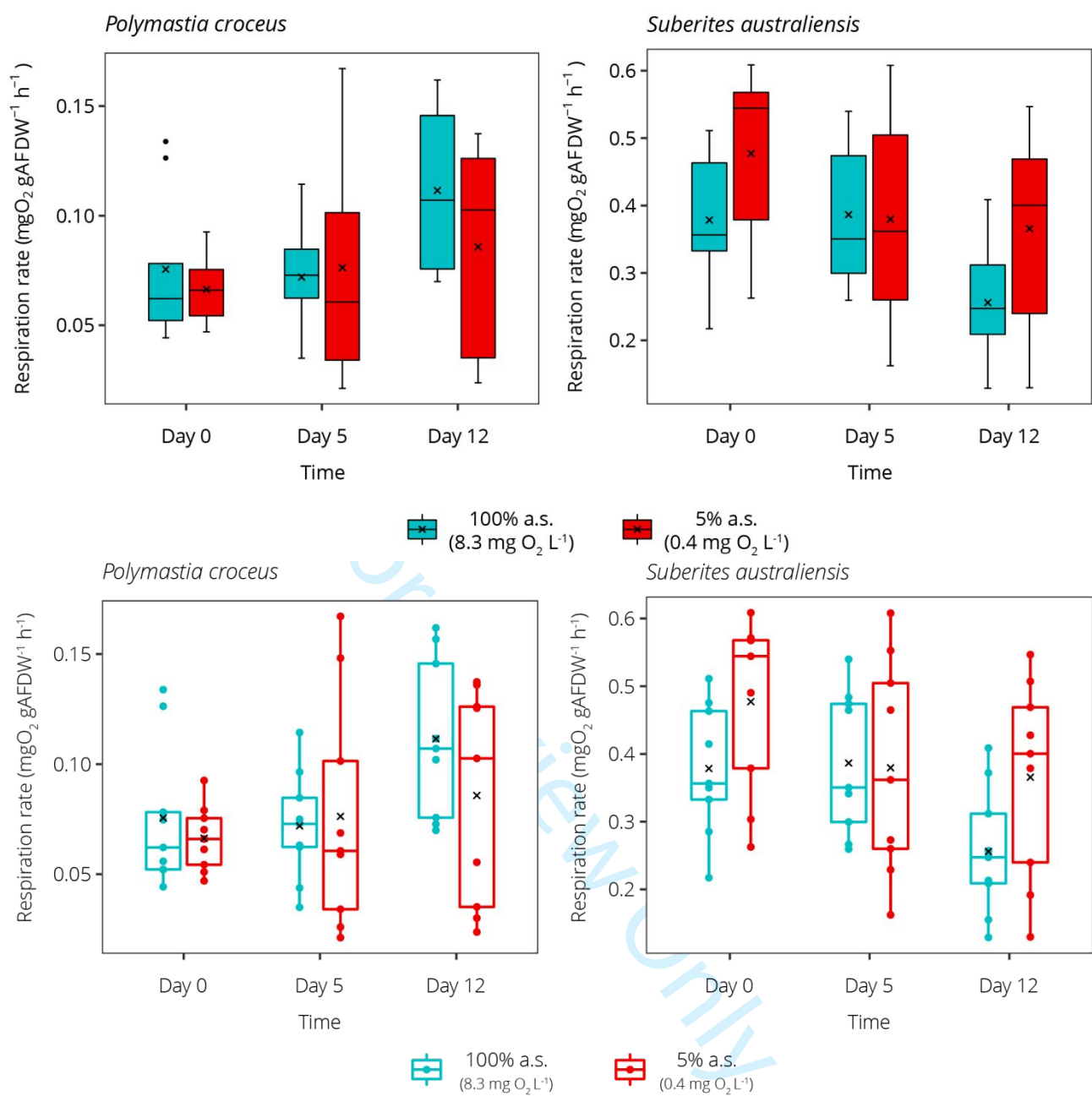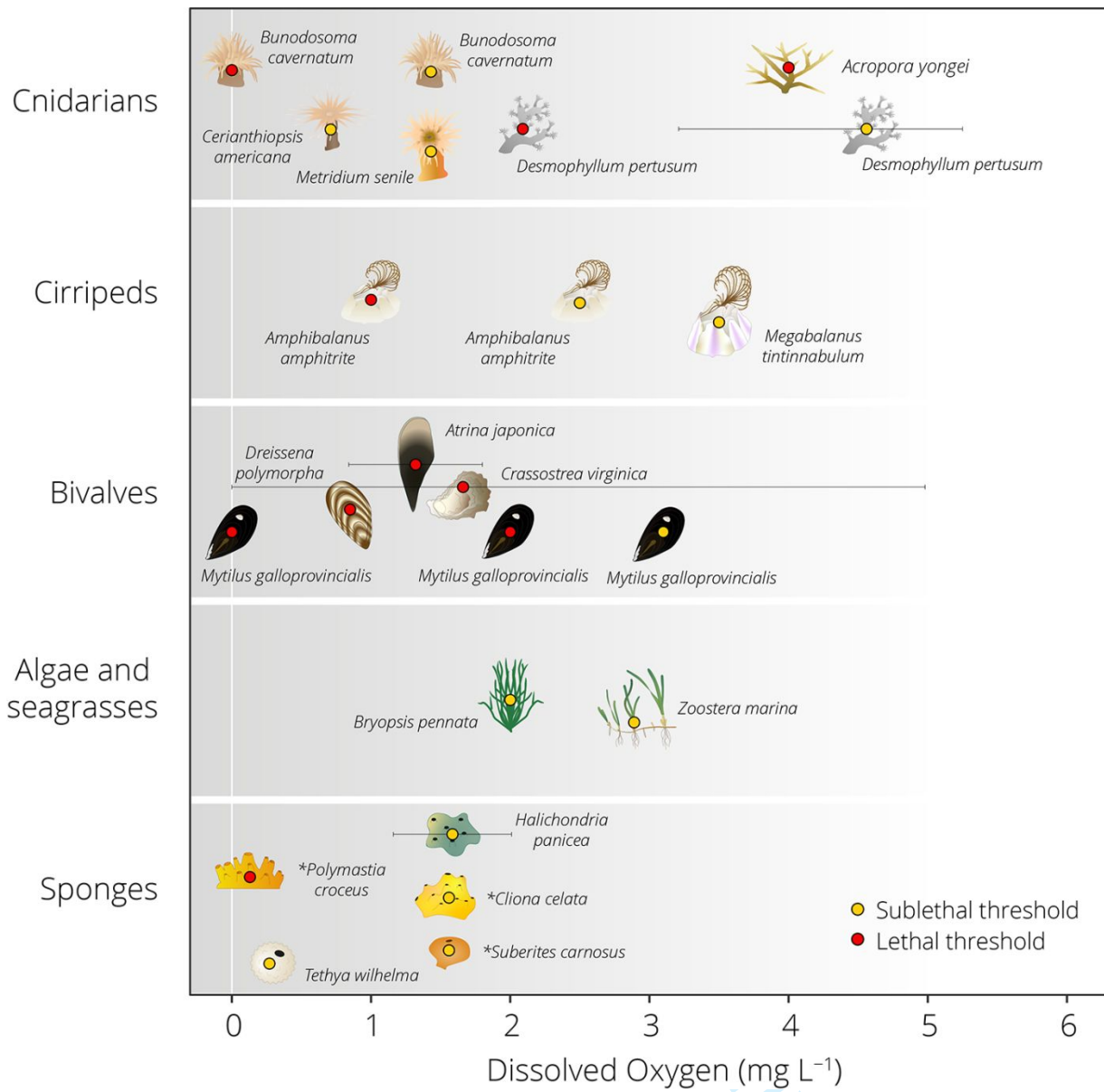
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979   Figure 4. Respiration rates in *Polymastia* ~~P.~~ *croceus* and *Suberites* ~~S.~~ *australiensis* from experiment 2 (5%

980   a.s.) measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. Horizontal bars

981   inside the boxplots represent medians; the symbol × represents means. Lower and upper hinges of the

982   boxplots correspond to the first and third quartiles, respectively. ~~Lower and upper whiskers represent the~~

983   ~~smallest and largest values, respectively.~~ Points represent data points.~~Single dots represent outliers.~~
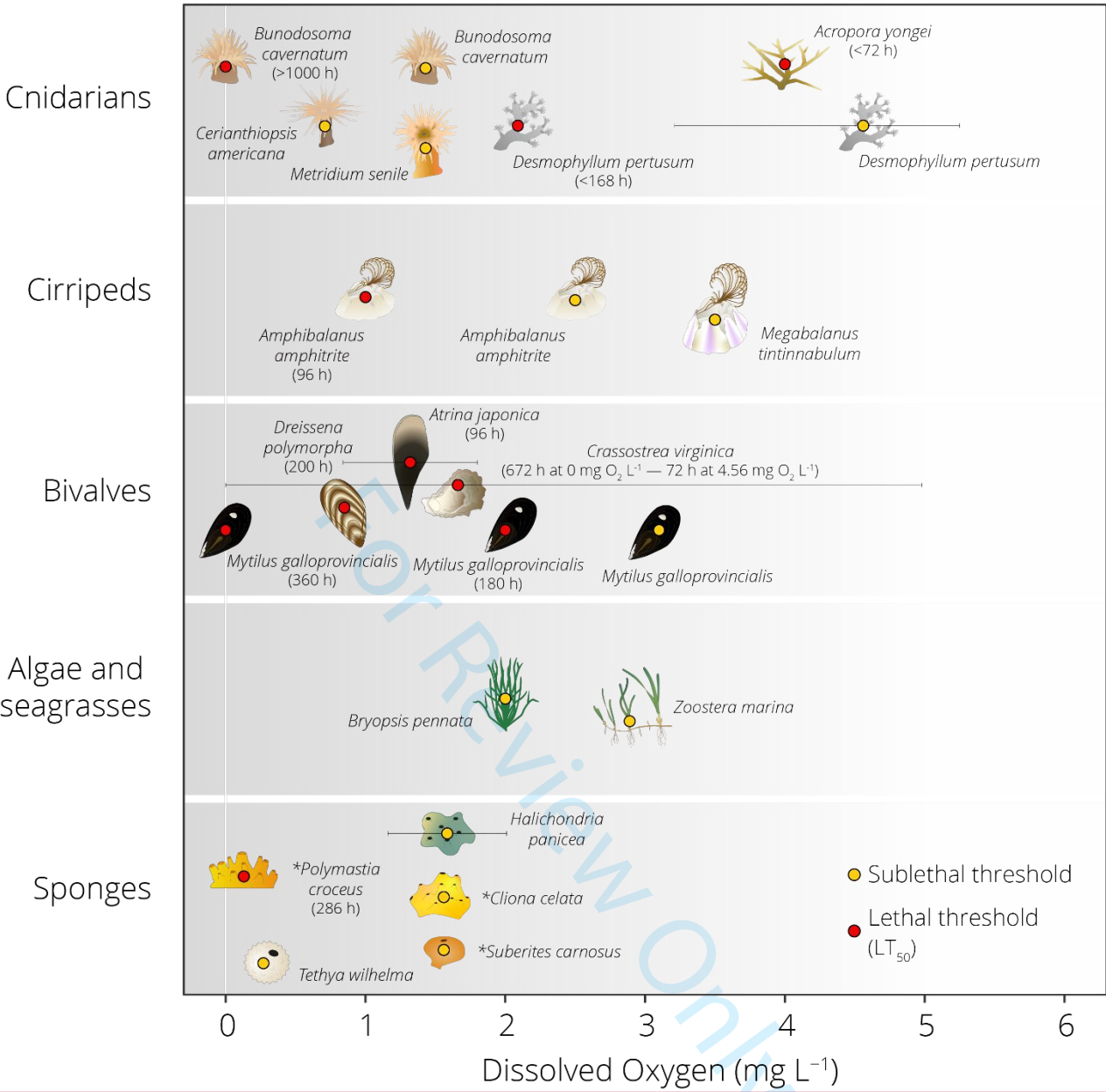
Figure 5. The tolerance of marine sessile organisms to hypoxia. Red dots indicate lethal thresholds, while yellow dots indicate sub-lethal thresholds. Organisms whose values were found in the present study are labelled with an asterisk. For studies that report multiple values for the same species according 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

995 the experiment. For *H. panicea,* Mills et al. (2014) only report oxygen measurement as per cent air

996 saturation without reporting temperature and salinity, so the actual oxygen concentration is unknown. We,

997 therefore, estimated the oxygen content using the range of temperatures and salinity found where these

998 sponges were sampled (Salinity 8.9–29.5; Temperature: 5–25 °C; Thomassen & Riisgård, 1995) and we

999 provide the mean and the range of possible values. List of references associated with each species: *A.*

1000 *yongei* (Haas et al., 2014), *A. amphitrite* (Rao & Ganapati, 1968; Desai & Prakash, 2009), *A. japonica*

1001 (Nagasoe et al., 2020), *B. pennata* (Haas et al., 2014), *B. cavernatum* (Mangum, 1980; Ellington, 1982), *C.*

1002 *americana* (Vaquer-Sunyer & Duarte, 2008), *C. virginica* (Stickle et al., 1989), *D. pertusum* (Dodds et al.,

1003 2007; Lunden et al., 2014), *D. polymorpha* (Johnson & McMahon, 1998), *H. panicea* (Mills et al., 2014), *M.*

1004 *tintinnabulum* (Rao and Ganapati 1968), *M. senile* (Sassaman & Mangum 1972), *M. galloprovincialis* (De

1005 Zwaan et al., 1991; Woo et al., 2013), *T. wilhelma* (Mills et al., 2018), *Z. marina* (Hughes et al., 2020). Figure

1006 inspired by Hughes et al. (2020).

1   **Adaptive strategies of sponges to deoxygenated oceans**

2   **Abstract**

3   Ocean deoxygenation is one of the major consequences of climate change. In coastal waters, this process

4   can be exacerbated by eutrophication, which is contributing to an alarming increase in the so-called "dead

5   zones" globally. Despite its severity, the effect of reduced dissolved oxygen has only been studied for a very

6   limited number of organisms, compared to other climate change impacts such as ocean acidification and

7   warming. Here we experimentally assessed the response of sponges to moderate and severe simulated

8   hypoxic events. We ran three laboratory experiments on four species from two different temperate oceans

9   (NE Atlantic and SW Pacific). Sponges were exposed to a total of five hypoxic treatments, with increasing

10   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

11   tolerant of hypoxia. All the sponges survived in the experimental conditions, except *Polymastia croceus,*

12   which showed significant mortality at the lowest oxygen concentration (0.13 mg O_2 L^{-1}, lethal median time:

13   286 h). In all species except *Suberites carnosus*, hypoxic conditions do not significantly affect respiration

14   rate down to 0.4 mg O_2 L^{-1}, showing that sponges can uptake oxygen at very low concentrations in the

15   surrounding environment. Importantly, sponges displayed species-specific phenotypic modifications in

16   response to the hypoxic treatments, including physiological, morphological, and behavioural changes. This

17   phenotypic plasticity likely represents an adaptive strategy to live in reduced or low oxygen water. Our

18   results also show that a single sponge species (i.e., *Suberites australiensis*) can display different strategies at

19   different oxygen concentrations. Compared to other sessile organisms, sponges generally showed higher

20   tolerance to hypoxia, suggesting that sponges could be favoured and survive in future deoxygenated

21   oceans.

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

51   oxygen depletion are the main factors influencing the severity of hypoxic events on benthic organisms

52   (Levin et al., 2009; Altieri & Diaz, 2019). Mild hypoxia can alter behavioural patterns, decrease feeding rates

53   and cause changes in physiological processes (Vaquer-Sunyer & Duarte, 2008). Severe hypoxic events can

54   cause mass mortalities, leading to the formation of the so-called "dead zones", areas largely devoid of

55   macrofauna (Diaz & Rosenberg, 2008). Dead zones have been reported in small water bodies such as

56   harbours, fjord and inlets, and large basins, such as the Baltic Sea, spreading over 60,000 km^2 (Altieri & Diaz,

57   2019). As climate and land use continue to change, coastal hypoxia is expected to worsen, with the

58   increased occurrence, frequency, intensity, and duration of hypoxic events (Diaz & Rosenberg, 2011).

59   Despite the extent of the problem and the dramatic effects caused by ocean deoxygenation, the response

60   of many groups of organisms to hypoxia is still poorly studied. This lack of knowledge limits our ability to

61   model the effects of declining oxygen availability on marine ecosystems (Seibel, 2011). To date, research on

62   tolerance to reduced levels of dissolved oxygen has primarily focused on fish, crustaceans and molluscs

63   (Vaquer-Sunyer & Duarte, 2008), while very little is known about other groups, especially sessile organisms.

64   Sessile organisms are particularly vulnerable to hypoxic events because they cannot move or migrate to

65   well-oxygenated water. Furthermore, sessile organisms include many important habitat-forming species, so

66   any change in their abundance could have major consequences for the ecosystems they support (Vergés et

67   al., 2019; Woodhead et al., 2019; Piazzi et al., 2021). Therefore, it is critical to understand how these

68   organisms respond to hypoxia to predict possible future changes and effectively manage marine

69   ecosystems.

70   Sponges are the dominant sessile organisms in many marine ecosystems and are found in high abundance

71   in tropical, temperate, and polar ecosystems (Ayling, 1983, Bell et al., 2020). They perform many important

72   ecological functions, including contributing to nutrient cycling, bioerosion, enhancing ecosystem complexity

73   and providing habitats for a wide range of associated organisms (Wulff, 2006; Bell, 2008; Maldonado et al.,

74   2012). Despite being important components of marine ecosystems, sponge tolerance to hypoxia has been

75   poorly investigated to date. Mills et al. (2014) showed that *Halichondria panicea* can feed and respire with

76   oxygen levels down to 4% of air saturation. However, the authors did not provide information on the

77   duration of the treatments and replication; furthermore, in the same study, information on the

78   temperature and salinity of the water was unavailable, so it is not possible to derive the actual oxygen

79   concentrations to which sponges were exposed. Two other relevant experiments have investigated the

80   short-term response of sponges to hypoxia. Mills et al. (2018) exposed *Tethya wilhelma* to a step

81   decreasing oxygen concentration (30–40 h with O_2 lower than 10% a.s., 0.7 mg L^{-1}). They found that

82   sponges continued to perform periodic full-body contractions down to 0.27 mg O_2 L^{-1}, but ceased below

83   that concentration. Leys & Kahn (2018) exposed *Geodia barretti* to 6.5 h of hypoxia (7% air saturation, 0.6

84   mg O_2 L^{-1}), and found that sponge respiration rate remained largely unchanged. However, filtration rates

85   dropped almost immediately after the oxygen level was reduced. Despite these earlier studies, we still have

86   very little insight into how sponges may cope with hypoxic events caused by ocean and coastal

87   deoxygenation.

88   Here we provide the first comprehensive assessment of sponge response to hypoxia. Specifically, we

89   experimentally investigated the physiological, behavioural, and morphological responses of four temperate

90   sponge species to moderate and severe hypoxic conditions. We ran the first experiment to expose sponges

91   to moderate hypoxic conditions for seven days, including a wide range of dissolved oxygen concentrations

92   (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

93   mg O_2 L^{-1}) for 12 days with two additional experiments. Finally, we discuss sponge tolerance to low

94   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 (Wellington University Coastal Ecology Laboratory, Wellington) on two abundant temperate Australasian species: *Polymastia croceus* Kelly-Borges and Bergquist, 1997 and *Suberites australiensis* Bergquist, 1968.

2.1 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.

120 *Cliona celata* was collected from the Kedges (51°27'41.4 "N 9°20'44.2 "W), whereas *Suberites carnosus* was

121 collected from the rocky cliffs of Lough Hyne (51°30'00.4"N 9°18'03.9"W). For both species, sampling was

122 carried out at 10–18 m in June 2019 and sponges collected were at least 2 m apart. Sponges were then left

123 to recover for two months from harvesting stress in a 1 m³ underwater cage placed at 8 m of depth.

124 Sponges were then transferred to the experimental system and randomly distributed across the

125 experimental chambers. The experimental design consisted of 32 experimental chambers (two replicate

126 chambers for each species for each replicate module, and two replicate modules for each treatment). A

127 diagram of the experimental design is reported in Figure S2. Three sponges were placed in each chamber (6

128 sponges in total for each replicate module and 12 for each treatment). Sponges belonging to different

129 species were not mixed but kept in separate chambers. Sponges were left to acclimate with oxygen

130 saturated air for five days before oxygen was lowered by introducing hypoxic water into the chambers.

131 Oxygen concentration was lowered in 24h and was then maintained for seven days until the end of the

132 experiment (a graph showing the oxygen concentration in the different treatments over time is provided in

133 Figure S3). In natural ecosystems, hypoxic conditions can develop in short times ranging from hours to a

134 few days (Breitburg, 1990; Nezlin et al., 2009), so we consider these acclimation times appropriate and

135 ecologically relevant. Temperature and oxygen concentration inside the experimental chamber were

136 measured twice a day using a Fibox 4 oxygen meter with a dipping probe (Presence GmbH, Germany). A

137 two-spot calibration was performed on the oxygen probe every three days, using sodium dithionite for 0 %

138 oxygen and air-saturated water for 100% oxygen.

139 **2.2 Experiments 2 and 3: severe hypoxia**

140 We also investigated the response of sponges (*Polymastia croceus* and *Suberites australiensis*) to severe

141 hypoxia through two separate experiments using an air-tight system. In experiment 2, sponges were

142 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,

143 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

144 oxygen concentration in the controls was due to the small difference in temperature between the two

145   experiments (13.3 ± 0.5 °C in experiment 2 compared to 14.3 ± 0.6 °C in experiment 3). A summary of the

146   seawater parameters is provided in Table S1.

147   Sponges were kept in independent cylindrical air-sealed polypropylene chambers (10 L), randomly

148   distributed inside a water bath. Every two days, ~70% of the water was replaced using 10-μm-filtered

149   seawater, preconditioned to the desired oxygen concentration in independent conditioning tanks. Oxygen

150   concentration was then maintained by bubbling air or air-N_2 blends through glass-ceramic diffusers (see

151   section 2.1.2 for more details on gas blends). Custom made de-bubbler devices were used to eliminate

152   bubbles coming from the ceramic diffusers that could affect sponges (Figure S4). The sponges were fed

153   twice a day with *Nannochloropsis* microalgae (1–2 μm cell diameter; Nanno 3600™ Reed Mariculture, US.).

154   Water circulation within each experimental chamber was provided by the de-bubbler device and an

155   additional water pump located on the side of the chamber, which provided a constant circular water flow.

156   The chambers were placed in a water bath to control water temperature.

157   *Polymastia croceus* was collected from Barrett Reef (Wellington South Coast, 41°20'31.1"S 174°50'09.7"E)

158   by cutting fragments (~8 cm^3) from separate sponges (at least 5 m apart). Whole specimens of *Suberites*

159   *australiensis* were collected from Mahanga Bay (Wellington Harbour, 41°17'32.2"S 174°50'06.5"E),

160   attached to a fragment of their respective substrate. Sponges were then left to recover for three weeks

161   after sampling and cutting stress in water tables with 10-μm-filtered flow-through seawater.

162   Sponges were then transferred to the experimental system, consisting of 12 experimental chambers (3

163   independent replicate chambers for each species and treatment combination). Five sponges were placed in

164   each chamber (15 sponges in total for each treatment). Sponges were left to acclimate with oxygen

165   saturated air for five days. Oxygen was then lowered by bubbling a specific Air-N_2 mixture. In experiment 2,

166   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

167   the experiment. While in experiment 3, oxygen was lowered to 1.5% a.s. (0.13 mg O_2 L^{-1}) in ~72 hours

168   (which included a preacclimation at 10% a.s., 1 mg O_2 L^{-1}) and maintained for 12 days until the end of the

169   experiment (Fig. S3). This further acclimation in hypoxic conditions was made because of the very low O_2

170   concentration of the treatment. In experiment 3, due to the very low concentration of O_2 (0.13 ± 0.02 mg L$^-$

171   ^{1}), O$_2$ increased to ~0.3 mg L^{-1} for about 20 minutes during daily examinations. Temperature and oxygen

172   concentration inside the experimental chamber were measured twice a day using Fibox 4 oxygen meter

173   with a dipping probe (Presence GmbH, Germany). A two-spot calibration was performed on the oxygen

174   probe every three days, using sodium dithionite for 0% oxygen and air-saturated water for 100% oxygen.

175   **2.3 Response variables**

176   **2.3.1 Survival and health monitoring**

177   Sponge health was monitored daily during the experiment. Sponges showing ≥ 25% of external necrosis

178   were considered dead and removed from their treatment tanks during the daily checks, so as not to impact

179   other sponges in the treatments. At the end of the experiments, all sponges were sectioned to assess the

180   presence of any internal necrosis.

181   **2.3.2 Respiration Rate**

182   For all the experiments, respiration rate was measured on the same specimens at *T*0 (before the beginning

183   of the experiment), *T*1/2 (after two days from the beginning of the final treatment in experiment 1, and

184   after five days in experiments 2 and 3) and *T-end* (end of the experiment). In experiment 1 (moderate

185   hypoxia), we measured respiration rates of three sponges in each replicate module (n = 6 for each

186   treatment). In experiments 2 and 3 (severe hypoxia), respiration rates were measured on three sponges in

187   each experimental chamber (n = 9 for each treatment). To measure respiration rate, sponges were placed

188   in sealed cylindrical glass respiration chambers (150 ml for *Cliona celata*; 80 ml for *Suberites carnosus*; 250

189   ml for *Polymastia croceus* and *Suberites australiensis*) with PreSens oxygen sensor spots (SP-PSt3-NAU)

190   attached to their inner surface. Experimental chambers contained either oxygen saturated water (pre-

191   experimental measurements and controls) or water at a slightly higher oxygen concentration than the

192   experimental treatment (+20–50%, depending on the treatment) collected from the respective header

193   tanks. Respiration rates were not performed on sponges from experiment 3 (1.5% a.s.). The incubations

194   were performed in controlled temperature (water bath) and dark conditions. The water inside the

195   respirometry chambers was gently stirred using a magnetic stir bar. After 20 min of acclimation, oxygen

196   concentration inside the chambers was measured every 10 min for 1 hour, using a Fibox 4 oxygen meter

197 with a polymer optical fibre (POF). Respiration measurements were ended prematurely if the oxygen level

198 fell below 70% of the treatment concentration to avoid any detrimental effect on the sponges. Blank

199 incubations, containing only seawater were performed every respiration run and used to correct for any

200 microbial community respiration in the seawater. A two-point calibration was performed on the oxygen

201 sensor spots before each measurement session.

202 Respiration measurements were standardized to sponge ash-free dry weight (*AFDW*) from buoyant weight

203 (*BW*) measurements (Fig. S5). For *Suberites australiensis*, it was not possible to estimate *AFDW* from *BW*

204 due to the abundant external material accumulated by the sponge inside the tissue that influences the *BW*.

205 For this species, we measured the *AFDW* of all the specimens used in the respirations at the *T-end*, and we

206 assumed that sponges had the same weight at *T*0 and *T*1/2.

207 **2.3.3 Changes in weight, size, and morphology**

208 Changes in weight and size over time relative to the initial values were estimated by calculating the

209 buoyant weight variation (*BWV*) and contracted area variation (*CAV*). For all of the experiments, buoyant

210 weights (*BW*) of all experimental sponges (except *Suberites australiensis*) were taken at *T*0 and *T-end* and

211 used to calculate relative buoyant weight variation as $BWV = [(BW_{T\text{-}end} - BW_{T0}) / BW_{T0}] \cdot 100$. Buoyant weight

212 was measured with a digital scale (A&D FX-200i) following the methods of Osinga et al. (1999). For

213 experiments 2 and 3 (severe hypoxia), photographs of contracted sponges were taken at *T*0 and *T-end* to

214 measure sponge contracted area (*CA*) and calculate contracted area variation as $CAV = [(CA_{T\text{-}end} - CA_{T0}) /$

215 $CA_{T0}] \cdot 100$ (following Osinga et al., 1999). Contraction was achieved by disturbing sponges with a blunt

216 plastic rod (being careful not to damage the sponge) and waiting for one hour for the sponge to react to the

217 stimulus. All the photographs were analysed using ImageJ (US National Institutes of Health, Bethesda, Md,

218 USA).

219 During experiments 2 and 3, treatment conditions induced the development of peculiar morphological

220 structures in some specimens of both *Polymastia croceus* and *Suberites australiensis*. Sponges were

221   photographed and monitored daily to calculate the percentage of specimens developing these structures

222   and the median time of occurrence.

223   **2.3.4 Sponge contractile behaviour**

224   During experiments 2 (5% a.s.) and 3 (1.5% a.s.), sponge contractile behaviour was monitored daily from $T0$

225   to T-end, on all experimental sponges through photographic analysis. For *Suberites australiensis*, the

226   contractile behaviour was estimated using an "expansion ratio" (*EXPR*) calculated as $EXPR = A_{Ti} / CA_{T0}$,

227   where A_{Ti} is the area occupied at T_i and CA_{T0} is the contracted area at $T0$. Area was preferred over volume

228   because of the low invasiveness of the measurements. In *Polymastia croceus,* contraction/expansion mainly

229   occur at the papillae level, so the contractile behaviour was estimated from the ratio of expanded papillae

230   (*REP*) calculated as $REP = P_E/P_{tot}$, where P_E is the number of visible expanded papillae and P_{tot} is total

231   number of visible papillae. Expanded papillae were defined as papillae whose length was at least two and a

232   half times the width.

233   **2.3.5 Pumping rate**

234   Pumping rate was only calculated for *Suberites australiensis* from experiments 2 and 3 (severe hypoxia).

235   Having only one osculum of relatively large size, this species was particularly suitable for investigating

236   changes in pumping rate. To minimize sponge disturbance during the experiment, pumping rate (PR) was

237   derived from the measurement of the sponge osculum cross-sectional area (OSA). In sponges, pumping

238   rate (PR) is correlated with OSA (e.g. Goldstein et al., 2019; Morganti et al., 2021). In the case of *S.*

239   *australiensis*, this relationship was calculated on 20 sponges (following Yahel et al., 2005) and was found as

240    $PR = 6.55 \cdot OSA^{1.43}$ (Fig. S6). Photographs of the oscula with scale were taken daily from $T0$ to T-end, on

241   three sponges in each experimental chamber (the same specimens each time point, n = 9 per treatment).

242   Since *S. australiensis* has only one osculum, pumping rate was then standardized per sponge volume.

243   **2.3.6 Histology**

244   Histological sections of *Suberites australiensis* from the severe hypoxia experiments were analyzed to

245   calculate the percentage of the sponge body occupied by the aquiferous system (system of connected

246   water channels inside the sponge). At *T-end*, two contracted sponges for each experimental chamber (n = 6

247   per treatment) were fixed and processed following the methods of Strano et al. (2021). Three replicate

248   sections for each sponge were then photographed under a dissecting microscope (Olympus SZ61) and

249   photographed using a Canon EOS 70D digital camera. To calculate the area occupied by the aquiferous

250   system, pictures were analyzed using ImageJ.

251   **2.4 Data analysis**

252   All the statistical data analyses were performed in R version 3.1.3 (R Core Team, 2013), except

253   PERMANOVA models, which were performed using PRIMER v7 with PERMANOVA+ add-on (Anderson et al.,

254   2008; Clarke & Gorley, 2015). Experiments 2 and 3 were analyzed separately. To investigate respiration

255   rate, pumping rate and expansion ratio in *Suberites australiensis* from experiment 2, we used linear mixed-

256   effects models with normally distributed errors and random intercepts (lmer, *lme4* package; Bates et al.,

257   2015). For pumping rate, we added a constant variance function structure (varIdent) to the linear mixed-

258   effects models to allow different variances for each treatment at each time point (lme, *R* package *nlme*;

259   Pinheiro et al., 2021). The constant variance function structure was necessary because the variance of the

260   response variable differed across treatments and experimental days. To investigate the effect of time and

261   treatment on the expansion ratio of *Polymastia croceus* in experiment 3, we used a generalized linear

262   mixed model with beta regression and logit link (*glmmTMB*, Brooks et al. 2017). In all the mixed models,

263   treatment and time were considered fixed effects, while experimental chamber and sponge specimen were

264   considered random effects. The experimental chamber effect was included to address pseudo-replication.

265   For these models, fixed- and random-effect terms were tested using the function anova and ranova (*R*

266   package *lmerTest*, Kuznetsova et al., 2017), respectively; while post hoc pairwise comparisons were

267   computed on estimated marginal means using emmeans (*R* package *emmeans*; Lenth, 2021). The ratio of

268   expanded papillae in *P. croceus* from experiment 2 and expansion ratio in *S. australiensis* from experiment

269   3 were investigated using repeated measure univariate PERMANOVA (Anderson, 2001; 2014), because did

270   not meet the normality assumption for mixed-effects models. Pairwise tests were then calculated using

271   permutation *t*-tests (*R* package *RVAideMemoire*; Hervé, 2021). PERMANOVA and permutation *t*-tests were

272   also used to supplement mixed-effects models when there were concerns about the normality of the

273   residuals (pumping rate in *S. australiensis*). Change over time relative to the initial value of buoyant weight

274   and contracted area, and differences in percentage occupied by the aquiferous system were investigated

275   using Kruskal–Wallis H tests, Welch's t-tests or Wilcoxon Signed-Rank Tests, depending on the variable.

276   Respiration rates from experiment 1 were log (x + 1) transformed, and pumping rates were square-root

277   transformed to meet normality assumptions. The goodness of fit, normality and homoscedasticity of the

278   errors were checked for all models by inspecting plots of the normalized residuals and the quantile-quantile

279   plots. All the multiple comparisons were corrected using Benjamini-Hochberg Procedure, but uncorrected

280   p-values are reported in the text. All the statistical analyses made for each variable are reported and

281   summarized in Table S2**.**

282   Time to event analysis for sponge survival and development of peculiar morphological structures (modified

283   papillae and protruding oscular membranes) was performed using Kaplan-Meier Method, and *p*-values

284   were calculated using the Log Rank Test implemented in the survival *R* package (Therneau, 2021). Median

285   lethal time (LT_{50}) and median time to the development of modified morphological structures were

286   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 until 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).

309 **3.2 Sponge responses to severe hypoxia**

310 **3.2.1 Survival**

311 Sponge survival differed among species, with *Suberites australiensis* more tolerant than *Polymastia croceus*.

312 No mortality was observed for *S. australiensis* in both experiments 2 (5% a.s.) and 3 (1.5% a.s.). In contrast,

313 for *P. croceus*, significant mortality ($p = 0.001$) was observed in sponges exposed to the 1.5% a.s. treatment,

314 starting from day 10 (day 12 when including the hypoxic acclimation), and with a median lethal time of 11.9

315 ± 0.3 days (Fig S8–9). Eight out of 15 sponges had died by the end of the experiment. No mortality was

316 observed for *P. croceus* in the 5% a.s. treatment.

317 **3.2.2 Change in weight and size**

318 For *Polymastia croceus*, buoyant weight variation between *T*0 and *T-end* differed among treatments in

319 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.

320 treatment experienced a significant decrease in buoyant weight (-7.1%, $t = -5.17$, $p = 0.002$), while the

321 controls did not experience any significant change (Tab. S6; Fig. S10).

322 The relative variation in area of contracted sponges (after stimulating contraction) between *T*0 and *T-end*

323 differed significantly between treatments and controls for both *Polymastia croceus* ($W = 12$, $p < 0.0001$)

324 and *Suberites australiensis* ($W = 13$, $p < 0.0001$), but only in experiment 2 (5% a.s.). Both *P. croceus* and *S.*

325 *australiensis*, from the 5% a.s. treatment, experienced an increase in contracted area (+18.9%, $W = 120$, $p =$

326 0.0001 and +18.4%, $W = 105$, $p = 0.008$, respectively). While *S. australiensis* from the control treatment

327 (experiment 2) experienced a decrease in contracted area (-15.3%, $W = 3$, $p = 0.0003$) (Tab. S7; Fig. S11).

328 **3.2.3 Sponge contractile behaviour**

329 Low DO treatments generally induced sponge expansion, but the response differed between species, and it

330 was generally more marked in the 5% a.s. treatment. In *Polymastia croceus*, the ratio of expanded papillae

331 was significantly affected by the interaction between time and treatment in both experiments 2 ($p =$

332 0.0001) and 3 ($p < 0.0001$ and $p = 0.03$) (Tab. S8–9). During experiment 2 (5% a.s.), the treatment induced a

333 progressive expansion of papillae from day 2. The ratio of expanded papillae in sponges from the hypoxic

334   treatment became significantly higher than control sponges from day 6 to the end of the experiment (p =

335   0.0002–0.005) (Tab. S8; Fig. 2a). A similar trend was found in experiment 3 (1.5% a.s.), but the ratio of

336   expanded papillae of the treatment sponges was more variable and became significantly different only at

337   day 9 (p = 0.003) (Tab. S9; Fig. 2b). In this experiment, we also found a correlation between the ratio of

338   expanded papillae and mortality. Sponges that survived the treatment had a significantly higher maximum

339   ratio of expanded papillae compared to sponges that died, both when the maximum ratio was calculated at

340   the end of the experiment (Welch t-test: t = 5.3, p = 0.0005) and at day ten, before sponges started to die

341   (Welch t-test: t = 4.6, p = 0.0007).

342   In *Suberites australiensis*, there was a significant interactive effect of treatment and time (p < 0.0001) on

343   the expansion ratio in experiment 2 (5% a.s.), but only an effect of time (p = 0.01) in experiment 3 (1.5%

344   a.s.) (Tab. S10–11). For experiment 2 (5% a.s.), pairwise comparisons found significant expansion in sponges

345   (+60%, p < 0.0001) between day 0 and 1. Sponges then remained expanded for the whole duration of the

346   experiment, and the expansion ratio was significantly higher in the treatments compared to the controls

347   from the first to the last day of the experiment (p < 0.0003) (Tab. S10; Fig. 2c, d).

348   **3.2.4 Morphological modifications**

349   During experiments 2 (5% a.s.) and 3 (1.5% a.s.), some *Polymastia croceus* and *Suberites australiensis*

350   sponges exposed to hypoxic treatments underwent morphological modifications (Fig. 3; S12). In some *P.*

351   *croceus*, the conical papillae showed a progressive elongation, flattening, and, in some cases, spiralization

352   (Fig. S12a–f). This process occurred in both the 5% a.s. and 1.5% a.s. treatments, but morphological

353   changes were more pronounced in lower DO treatment (Fig. S12d-e). Exposed to the 1.5% a.s. treatment,

354   some sponges developed papillae so slender that they could not sustain their weight (Fig. S12d-e). The

355   development of these modified papillae was also associated with an apparent increase in the porosity of

356   the sponge external surface (Fig. S12e). In the 5% a.s. treatment, 73% of the sponges developed modified

357   papillae, starting from day 6. In the 1.5% a.s. treatment, 60% of sponges developed modified papillae,

358   starting from day 2, from the beginning of the final treatment (day 4 considering hypoxic acclimation

359   period) (Fig. S13). The median time of development of these morphological structures (considering only the

360 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%

361 a.s. treatment (Fig. S14). Although not significant (χ^2 = 3.62, p = 0.057), a relationship between modified

362 papillae and survival was found. Among the *P. croceus* that survived the 1.5% a.s. treatment, six had

363 developed modified papillae, while one had not. While among the sponges that died following the 1.5% a.s.

364 treatment, three had developed modified papillae, while five had not.

365 In the the 5% a.s. treatment, 53% of *Suberites australiensis* developed a semi-transparent protruding

366 membrane surrounding the oscula. This membrane progressively reduced the oscular-cross sectional area

367 (Fig. 3; S12g–i). The median time it took for these protruding oscular membranes to become noticeable

368 (considering only the sponges that developed them) was 5.1 ± 0.2 days (Fig. S14). By the end of the

369 experiment, 53% of sponges had developed these structures (Fig. S13).

370 **3.2.5 Histology**

371 Histological analyses indicated that hypoxia influences the percentage of the sponge body occupied by the

372 aquiferous system in *Suberites australiensis*. At the end of experiment 2 (5% a.s.), treatment sponges had a

373 significantly higher percentage of aquiferous system (t = -9.82, p < 0.0001), compared to the controls (35.9

374 ± 7.1% vs 6.4 ± 1.8 %). No significant differences were found for experiment 3 (1.5% a.s.) (Fig. 3; S12j–m;

375 S15).

376 **3.2.6 Pumping rate**

377 Oxygen concentration significantly affected the pumping rate of *Suberites australiensis* in both experiments

378 2 (5% a.s.) and 3 (1.5% a.s.) (Tab. S12–15). In experiment 2 (5% a.s.), there was significant interaction of

379 treatment and time (p = 0.0001, linear mixed-effects model; Tab. S12). Pumping rate significantly increased

380 from day 0 to 1 (p < 0.0001), remained stable from day 1 to 2, and then decreased from day 2 to 3 (p <

381 0.0001) and from day 3 to 4 (p = 0.005) (Tab. S12). Sponges from the 5% a.s. treatment had a significantly

382 higher pumping rate than the control at day 1 (p = 0.007) and 2 (p = 0.002) (Tab. S12; Fig. 2e). Similar

383 results were given by PERMANOVA (Tab. S13). For experiment 3 (1.5% a.s.), both the linear mixed-effects

384 model and PERMANOVA revealed significant interaction between time and treatment (p = 0.049 and p =

385   0.002, respectively) on the pumping rate of *S. australiensis* (Tab. S14–15)*.* However, differences were less

386   marked compared to experiment 2 and pairwise comparisons only revealed a slight decrease of pumping

387   rate of treatment sponges between day 0 and 14 (p = 0.0002) (Tab. S14; Fig. 2f).

388   **3.2.7 Respiration rate**

389   In experiment 2 (5% a.s.), linear mixed-effects models only revealed a significant effect of time on the

390   respiration rate, for both *Polymastia croceus* and *Suberites australiensis* (Tab. S16–17; Fig. 4). In *P. croceus*,

391   pairwise comparisons revealed a slightly higher respiration rate of the controls at day 12 compared to day 0

392   (p = 0.008) and day 5 (p = 0.004), but no differences between controls and treatments at any time. In *S.*

393   *australiensis,* pairwise comparisons revealed a slightly lower respiration rate at day 12 compared to day 0

394   (p = 0.008) and day 5 (p = 0.005) in control sponges; while in treatment sponges, respiration rate was

395   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

396   significant difference between treatments and controls at any time point.

397   **4 DISCUSSION**

398   Hypoxia has become an increasingly common problem in the marine environment and will likely become

399   worse in the future (Diaz & Rosenberg, 2011). Nevertheless, the direct effects of hypoxia on marine

400   organisms are still very poorly studied (Vaquer-Sunyer & Duarte, 2008). We describe the first multi-species

401   experiment from two oceans to test sponge tolerance, behaviour, and physiological responses to oxygen

402   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

403   very tolerant to low DO irrespective of species or location. Only *Polymastia croceus* showed mortality in the

404   lower DO treatment (0.13 mg O_2 L^{-1}, LT_{50} = 286 h). Furthermore, our results suggest that sponges can

405   display species-specific acclimation, including physiological, morphological and behavioural changes, in

406   response to severe hypoxia that might help them survive periods of very low oxygen. Our study also

407   suggests that the same species can show different adaptive strategies for different degrees of hypoxia.

408   **4.1 Sponge response to hypoxia**

409   Our results suggest that sub-lethal oxygen thresholds for most sponges are in the range of 6–20% a.s.

410   (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

411   evidence are consistent with Mills et al. (2014) for *Halichondria panicea*, which showed a sub-lethal

412   response starting from 17% air saturation. However, our results contrast with Mills et al. (2018) studying

413   *Tethya wilhelma*, which did not show any response down to 4% a.s. (0.27 mg O_2 L^{-1}). The very high

414   tolerance of *T. wilhelma* could be explained by the extremely low metabolism of *Tethya* species generally

415   (Leys & Kahn, 2018), and by their very small size (0.5–1 cm) (Sarà et al., 2001). Of the two species we

416   exposed to the lowest DO concentration (1.5% a.s., 0.13 mg O_2 L^{-1}), only *Polymastia croceus* showed

417   mortality, while all the *Suberites australiensis* survived the 12 days of treatment conditions. This differential

418   response could be due to the different habitats where these species are usually found. *Polymastia croceus*

419   lives on rocky reefs, while *S. australiensis* lives on sediments in bays and semi-enclosed basins, where

420   hypoxic events are more likely to occur (Diaz & Rosenberg, 2008; de Cook, 2010).

421   Some sponges can live in anoxic conditions for several months, such as the sponges of the family

422   Raspailidae found in the deeper cliffs of Lough Hyne (Bell & Barnes, 2000; McAllen et al., 2009). Schuster et

423   al. (2021) suggested that this tolerance could be conferred by specific bacterial symbionts, which are able

424   to carry out anaerobic metabolism. In addition, these sponges living in anoxia are all thin crusts, with a very

425   high surface-to-volume ratio, which could favour the exchange of gases and the release of metabolic waste

426   (Levin et al., 1991). Other examples of sponges living in very low oxygen conditions are the ones found at

427   the edges of Oxygen Minimum Zones (OMZ) (Mosch et al., 2012). These sponges can live with a consistent

428   oxygen concentration as low as 0.13mg O_2 L^{-1} (Wishner et al., 1995, Murty et al., 2009). Sponges are not the

429   only organisms able to live in OMZs. Many representatives of other phyla live in these extremely hypoxic

430   conditions, where they benefit from the rich supply of organic matter. However, since OMZs have existed

431   over geological timescales, organisms have had the time to evolve specific adaptations to cope with

432   permanent hypoxia (Levin, 2003). Therefore, these organisms cannot be used to generalize tolerance to

433   periodic hypoxic events experienced by organisms usually living in fully oxygenated waters.

434   The degree of hypoxia tolerance in sponges could also be influenced by the abundance and diversity of

435   sponge-associated microbial symbionts. Based on bacterial biomass, sponges are generally divided into

436   "low microbial abundance" (LMA) or "high microbial abundance" (HMA) species (Hentschel et al., 2003).

437   Bacterial densities in HMA sponges are generally two to four orders of magnitude higher than in LMA

438   sponges and can constitute up to 35% of the total sponge biomass (Vacelet, 1975; Hentschel et al., 2006).

439   Sponges with HMA tend to have a lower choanocyte chamber density, and a slower pumping rate

440   compared LMA sponges (Lavy et al., 2016), which means HMA species might have a lower ability to

441   ventilate in low oxygen conditions. Furthermore, HMA species generally have a higher metabolic cost than

442   LMA species, and therefore a higher oxygen requirement (Leys & Kahn, 2018). Although these differences

443   suggest that LMA sponges might be better adapted to hypoxic conditions, HMA species have a higher

444   diversity of microbial symbionts that could help them cope with low oxygen conditions (Hoffmann et al.,

445   2005, Lavy et al., 2016). All the sponges for which responses to hypoxia has been investigated so far are

446   LMA species (or are likely to be, based on known congenerics, see Kamke et al., 2010; Mills et al., 2014;

447   Moitinho-Silva et al., 2017). Therefore, future research is needed to investigate the response of HMA

448   sponges to hypoxia and shed light on possible differences between LMA and HMA sponges and the

449   mechanisms involved.

450   Some organism's abilities to tolerate hypoxia result from their physiological ability to lower metabolism and

451   oxygen demand (McAllen et al., 1999; Altieri, 2019). Instead, other species switch from aerobic to

452   anaerobic metabolism or a combination of the two (Altieri & Diaz, 2019). Our results suggest that all our

453   species (except *Suberites carnosus*) have respiration rates at 5-6% a.s. that are comparable to sponges in

454   normoxic conditions. This is consistent with what was found in *Geodia barretti* and *H. panicea*, suggesting

455   that sponges have a common ability to uptake oxygen at very low concentrations in the surrounding

456   environment (Leys & Kahn, 2018). In *Cliona celata*, hypoxic water initially resulted in a decrease in the

457   respiration rate, which then increased back to pre-treatment levels after seven days of exposure. This

458   suggests that the sponges gradually adjusted to hypoxic conditions. In *S. carnosus,* instead, the respiration

459   rate remained stable after two days of exposure to low dissolved oxygen, but it more than halved after

460   seven days. This response may allow *S. carnosus* to cope with long periods of hypoxia, in which sponges

461   decrease their metabolism, as has been reported for other organisms (Hagerman, 1998; Mentel et al.,

462   2014). Although our study shows that sponges can perform aerobic metabolism when exposed to

463   extremely low oxygen concentrations, the presence of anaerobic metabolism cannot be excluded and

464   needs further investigation.

465   Sponge species exposed to the lowest DO concentrations (0.4 and 0.13 mg O_2 L^{-1}) also showed other

466   phenotypic modifications that could represent adaptive strategies to cope with hypoxia. In *Suberites*

467   *australiensis*, hypoxic water (0.4 mg O_2 L^{-1}) induced expansion of the sponge body and the aquiferous

468   system that lasted for the duration of the experiment. This expansion was likely semi-permanent as it

469   persisted after inducing the contraction and corresponded to a reorganization of the sponge aquiferous

470   system at the histological level. These behavioural and morphological changes are likely beneficial for the

471   sponge, as higher internal water flow corresponds to an increase in oxygen that can be taken up. The body

472   expansion was accompanied by a marked increase in the pumping rate that then dropped after two days.

473   The pumping rate increase could be a strategy to increase ventilation and oxygen availability, similarly to

474   other animals when exposed to hypoxic waters (Hagerman, 1998). However, the successive decrease in

475   pumping rate (after two days) and the gradual production of a membrane to close the oscula remains

476   unclear but could represent a trade-off between increasing ventilation and keeping the energetic coast of

477   pumping reasonable. In *S. australiensis*, body expansion is correlated with an increase in osculum area, and

478   osculum area is the main determinant of pumping rate in this and many other species (Morganti et al.,

479   2021; Goldstein et al., 2019). Perhaps the increase in pumping rate only represents a physiological

480   consequence of the body expansion and is then quickly brought back to normal, decreasing the osculum

481   size by producing an oscular membrane. These physiological and morphological changes of *S. australiensis*

482   described above was not present on sponges exposed to more severe hypoxia (0.13 mg O_2 L^{-1}). This could

483   mean that the same sponge species may display different adaptive strategies to cope with decreased

484   oxygen depending on the oxygen concentration. At 0.4 mg L^{-1}, oxygen might still be sufficient to support

485   regular metabolism, but sponges may need to increase the amount of water flowing through their bodies

486   to absorb the oxygen needed. However, 0.13 mg L^{-1} might be too low a DO concentration, and sponges

487   might decrease their metabolism to cope with lack of oxygen, similarly to other metazoans (Hagerman

488   1998, Mentel et al., 2014).

489   *Polymastia croceus* also showed a behavioural change in response to hypoxic conditions: hypoxic water at

490   0.4 mg L^{-1} induced the progressive expansion of sponge papillae (where inhalant and exhalant channels are

491   found), that was significantly greater than in the control sponges. It is unlikely that the papillae expansion

492   represents an increase in sponge filtering activity because the respiration rate was very similar in the

493   treatments and the controls. Therefore, sponges might expand their papillae to increase the volume

494   occupied by the aquiferous systems, as in the case of *Suberites australiensis*, but also to access more

495   oxygenated water further from the bottom. A similar response occurred in sponges exposed to 0.13 mg L^{-1}

496   but with much more variability across specimens, and the statistical test did not detect any change.

497   Interestingly, sponges that survived after the 12-day treatment had a significantly higher ratio of expanded

498   papillae than sponges that died, suggesting that expansion might help cope with severe hypoxic conditions.

499   Along with behavioural changes, *Polymastia* underwent morphological modifications that could help to

500   tolerate low DO. Papillae become thinner and flattened, and some even spiralized. These modifications of

501   the papillae could increase the surface-to-volume ratio and help oxygen diffusion (Levin et al., 1991). The

502   elongation of papillae, which accompanies the thinning, could be an evolutionary relic of a process that

503   moved the inhalant pores of the papillae as far as possible from the surface. However, in the lowest DO

504   treatment, papillae often lost their vertical orientation and laid horizontally on the sponge surface. We

505   hypothesized that the new orientation of papillae was a consequence of their thinning process: probably

506   papillae became so thin that they could not support their weight anymore. Interestingly, sponges that

507   developed modified papillae showed less mortality than sponges that did not, although the evidence is not

508   strong enough to claim this with confidence (p = 0.057). Therefore, these structures may not only represent

509   a stress response, but could provide an advantage to the sponge. Further research is needed on this topic

510   needed to elucidate the function of these structures.

511   Despite the remarkable tolerance of sponges to hypoxia observed in laboratory conditions, field

512   observations suggest that severe hypoxic/anoxic events can catastrophically affect sponge populations.

513   Mass mortalities of sponges following hypoxic/anoxic events have been reported both in temperate and

514   tropical ecosystems (Stachowitsch, 1984, Altieri et al., 2017; Chu et al., 2018; Johnson et al., 2018; Kealoha

515   et al., 2020). For example, in a hypoxic/anoxic event in the Gulf of Trieste, all the sponges living in several

516   hundred km^2 died within 2-3 days (Stachowitsch, 1984). Some anemones survived up to a week, but

517   virtually all organisms were dead within two weeks from the onset. Altieri et al. (2017) also reported

518   widespread mortality of sponges and corals following a hypoxic event (~0.5 mg O_2 L^{-1}) that occurred in

519   Bocas del Toro, Panama. Since this study focused on corals, it is unclear what proportion of the sponges

520   were affected and if some species were more tolerant than others. These reports highlight that hypoxic

521   events, in their most severe form, leave no survivors.

522   Furthermore, it is possible that in natural conditions, other factors combine with low dissolved oxygen. For

523   example, a recent meta-analysis showed that in marine organisms, increased temperature reduces survival

524   times under hypoxia by 74% on average and increased median lethal concentration by 16% on average

525 (Vaquer-Sunyer & Duarte, 2011). Another meta-analysis showed that hydrogen sulphide (H_2S) also reduces

526 survival time of marine organisms under hypoxia by an average of 30% (Vaquer-Sunyer & Duarte, 2010).

527 Acidification was shown to have additive or synergistic negative effects combined with hypoxia (Gobler &

528 Baumann, 2016; Steckbauer et al., 2020). Since all these factors usually co-occur during hypoxic events, *in*

529 *situ* sponge thresholds to hypoxia could be lower than determined through single stressor laboratory

530 experiments (Diaz & Rosenberg, 1995; Steckbauer et al., 2020). Future experiments that evaluate the

531 combined effect of these factors will be crucial to understand the full response of sponges to hypoxia in

532 natural ecosystems.

533 Diel oxygen variation is another factor that could influence organism tolerance to hypoxia in natural

534 conditions. In the photic zone of marine ecosystems, dissolved oxygen generally increases during the day

535 because of photosynthesis and decreases at night because of aerobic respiration (Kroeker et al. 2019). The

536 amplitude of these diel fluctuations can sometimes lead to hypoxia or complete anoxia at night and

537 supersaturation in peak sunny hours, or both (Diaz & Breitburg, 2009). These extreme oxygen dynamics

538 have been reported from a wide variety of macro- and micro-habitats from both tropical and temperate

539 ecosystems, such as intertidal reef platforms, tide pools, semi-enclosed basins, tropical lagoons and the

540 boundary layer around macroalgal canopies (Morris and Taylor, 1983, Frieder et al. 2012, Cornwall et al.,

541 2013, Gruber et al., 2017, Trowbridge et al. 2017, Hughes et al., 2020). Diurnal fluctuations in oxygen can

542 produce different responses from static exposure in laboratory experiments, which may either

543 overestimate or underestimate the emergent effects of hypoxia in natural environments (Bumett & Stickle,

544 2001). Therefore, future experiments will need to account for current and future temporal variability in

545 oxygen concentration to accurately forecast the emergent ecological effects of deoxygenation (Kroeker et

546 al., 2019).

547 **4.2 Hypoxia Tolerance of sponges compared to other sessile organisms**

548 Marine organisms have very variable tolerance to low dissolved oxygen, with lethal thresholds ranging from

549 8.6 mg O_2 L^{-1} for the first larval zoea stage of the crustacean *Cancer irroratus*, to resistance to complete

550 anoxia as in the case of the sea anemone *Metridium senile* and the oyster *Crassostrea virginica* (Wahl 1984;

551   Vaquer-Sunyer and Duarte, 2008). Sessile organisms are generally more tolerant than mobile ones, which is

552   likely due to them not being able to escape hypoxic conditions (Altieri & Diaz, 2019). Therefore, sessile

553   organisms that experience these conditions must have evolved other adaptive strategies to cope with

554   reduced oxygen (Diaz & Rosenberg, 1995).

555   Here we provide new evidence to support the hypothesis that sponges are one of the groups of sessile

556   organisms that are more tolerant to hypoxia and could be favoured in future deoxygenated oceans. Other

557   phyla, such as cnidarians and bivalves, include very tolerant species that can cope with prolonged periods

558   of anoxia (Fig. 13). This is not surprising since tolerance to severe hypoxia/anoxia is a widespread feature in

559   the animal world, and many organisms independently evolved this feature to cope with local conditions

560   (Hochachka & Lutz, 2001; Nilsson & Renshaw, 2004; Vaquer-Sunyer & Duarte, 2008). This ability is not

561   restricted to invertebrates and includes higher animals such as fish and reptiles (Milton & Prentice, 2007;

562   Vornanen et al., 2009).

563   What makes sponges unique as a phylum is their widespread tolerance to hypoxia. All the species

564   investigated so far have been shown to cope with very low levels of dissolved oxygen. In contrast, other

565   phyla have a much wider range of tolerances, with some species resistant to anoxia and others very

566   sensitive to decreased oxygen (Fig. 5). For example, in sessile cnidarians, lethal hypoxia thresholds range

567   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;

568   Dodds et al., 2007). In sessile bivalves, lethal thresholds range between 0 and 2 mg $O_2 L^{-1}$, with the sub-

569   lethal threshold being 3.1 mg $O_2 L^{-1}$ for *Mytilus galloprovincialis* (de Zwaan et al., 1991; Woo et al., 2013).

570   Sponges, instead, show much less variation with known lethal thresholds that are lower than 0.5 mg $O_2 L^{-1}$,

571   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

572   worth noting that lethal thresholds are highly dependent on the time of exposure. In the studies we

573   considered, these ranged from a few days to weeks. However, there were no noticeable differences in the

574   experimental duration and the median lethal time for the different organisms. Therefore, we believe that

575   differences in time of exposure do not represent a bias in our comparison. In contrast, sublethal responses

576   (e.g. changes in respiration rate, behaviour, and feeding activity) usually have rapid time-to-onset, so they

577 will likely be independent of exposure time.The high tolerance of sponges to hypoxia compared to other

578 organisms can be explained by the evolutionary history of this group. Sponges are one of the most ancient

579 groups of metazoans. They likely evolved before the Marinoan glaciation (657-645 million years ago), when

580 oxygen was perhaps less than 10% of present atmospheric concentration (Love et al., 2009; Maloof et al.,

581 2010; Brocks et al., 2017; Whelan et al., 2017; Cole et al., 2020; Turner, 2021). Modern sponges might have

582 retained an ancestral condition concerning oxygen requirements (Mills et al., 2014; 2018). Therefore, it is

583 more likely that sponges unable to survive severe hypoxia today (e.g., *P. croceus*) have lost certain key

584 ancestral adaptations to hypoxia, rather than hypoxia-tolerant lineages (e.g., *S. australiensis*) having

585 evolved relatively new capacities for hypoxia tolerance (Müller et al., 2012). Likewise, other animals which

586 might have evolved in similar conditions, such as ctenophores, also show great resistance to hypoxia

587 (Thuesen et al., 2005). Therefore, we speculate that sponges' long evolutionary history could give these

588 organisms an adaptive advantage in future deoxygenated oceans, since they may have experienced similar

589 conditions in past geological eras.

590

591 **CONCLUSIONS**

592 Overall, sponges show high tolerance to low dissolved oxygen compared to all the other phyla of sessile

593 marine organisms that have been studied. Species-specific phenotypic plasticity appears to help these

594 organisms to overcome hypoxic events, and future research will need to elucidate the mechanisms behind

595 these changes. This exceptional adaptive capacity of sponges could derive from their ancient evolutionary

596 origin and could confer sponges a competitive advantage in future deoxygenated oceans over other

597 organisms (Mills et al., 2014; Schuster et al., 2021).

606 **AUTHOR CONTRIBUTIONS**

607 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.

608 conducted the experiments. V.M. and L.W. analyzed the data. V.M. and J.J.B wrote the original draft. All the

609 authors participated in interpreting the results and contributed to the revision of the manuscript.

610 **DATA AVAILABILITY STATEMENT**

611 All data and the R code used in this paper are available on Figshare repository:

612 https://doi.org/10.6084/m9.figshare.15169662.

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REFERENCES

Altieri, A. H., & Diaz, R. J. (2019). Dead zones: oxygen depletion in coastal ecosystems. In: C. Sheppard (Ed.), *World Seas: An Environmental Evaluation* (pp. 453–473). Academic Press. https://doi.org/10.1016/b978-0-12-805052-1.00021-8

Altieri, A. H., & Gedan, K. B. (2015). Climate change and dead zones. *Global Change Biology*, 21(4), 1395–1406. https://doi.org/10.1111/gcb.12754

Altieri, A. H., Harrison, S. B., Seemann, J., Collin, R., Diaz, R. J., & Knowlton, N. (2017). Tropical dead zones and mass mortalities on coral reefs. *Proceedings of the National Academy of Sciences, 114*(14), 3660–3665. https://doi.org/10.1073/pnas.1621517114

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anderson, M. J. (2014). Permutational multivariate analysis of variance (PERMANOVA). *Wiley statsref: statistics reference online*, 1–15. https://doi.org/10.1002/9781118445112.stat07841

Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. PRIMER-E, Plymouth, UK.

Ayling, A. L. (1983). Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. *Oecologia, 60*(3), 412–418. https://doi.org/10.1007/bf00376861

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi:10.18637/jss.v067.i01

Bell, J. J. (2008). The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science, 79*(3), 341–353. https://doi.org/10.1016/j.ecss.2008.05.002

641   Bell, J. J., & Barnes, D. K. (2000). The distribution and prevalence of sponges in relation to

642   environmental gradients within a temperate sea lough: vertical cliff surfaces. *Diversity and*

643   *Distributions, 6*(6), 283–303. https://doi.org/10.1046/j.1472-4642.2000.00091.x

644   Bell, J. J., McGrath, E., Kandler, N. M., Marlow, J., Beepat, S. S., Bachtiar, R., Shaffer, M. R., Mortimer, C.,

645   Micaroni, V., Mobilia, V., Rovellini, A., Harris, B., Farnham, E., Strano, F., & Carballo, J. L. (2020).

646   Interocean patterns in shallow water sponge assemblage structure and function*. Biological*

647   *Reviews, 95*(6), 1720–1758. https://doi.org/10.1111/brv.12637

648   Bijma, J., Pörtner, H. O., Yesson, C., & Rogers, A. D. (2013). Climate change and the oceans–What does

649   the future hold?. *Marine Pollution Bulletin, 74*(2), 495–505.

650   https://doi.org/10.1016/j.marpolbul.2013.07.022

651   Breitburg, D. L. (1990). Near-shore hypoxia in the Chesapeake Bay: patterns and relationships among

652   physical factors. *Estuarine, Coastal and Shelf Science, 30*(6), 593–609. https://doi.org/10.1016/0272-

653   7714(90)90095-9

654   Brocks, J. J., Jarrett, A. J., Sirantoine, E., Hallmann, C., Hoshino, Y., & Liyanage, T. (2017). The rise of

655   algae in Cryogenian oceans and the emergence of animals. *Nature, 548*(7669), 578-581.

656   https://doi.org/doi:10.1038/nature23457

657   Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J.,

658   Machler, M. & Bolker, B.M. (2017). glmmTMB balances speed and flexibility among packages for zero-

659   inflated generalized linear mixed modeling*. The R Journal, 9*(2), 378–400. https://doi.org/10.32614/rj-

660   2017-066

661   Bumett, L. E., & Stickle, W. B. (2001). Physiological responses to hypoxia. In: N. N. Rabalais, R. E. Turner

662   (Eds). *Coastal and Estuarine Studies, Volume 58. Coastal hypoxia: consequences for living resources and*

663   *ecosystems*, (pp. 101–114). AGU Publications. https://doi.org/10.1029/ce058p0101

664 Chu, J. W., Curkan, C., & Tunnicliffe, V. (2018). Drivers of temporal beta diversity of a benthic

665 community in a seasonally hypoxic fjord. *Royal Society Open Science, 5*(4), 172284.

666 https://doi.org/10.1098/rsos.172284

667 Clarke, K. R., & Gorley, R. N. (2015). *Getting started with PRIMER v7*. PRIMER-E, Plymouth, UK.

668 Cole, D. B., Mills, D. B., Erwin, D. H., Sperling, E. A., Porter, S. M., Reinhard, C. T., & Planavsky, N. J.

669 (2020). On the co-evolution of surface oxygen levels and animals. *Geobiology, 18*(3), 260–281.

670 https://doi.org/10.1111/gbi.12382

671 Cook, S. D. C. (2010). *New Zealand coastal marine invertebrates*. Canterbury University Press.

672 Cornwall, C. E., Hepburn, C. D., Pilditch, C. A., & Hurd, C. L. (2013). Concentration boundary layers

673 around complex assemblages of macroalgae: Implications for the effects of ocean acidification on

674 understory coralline algae. *Limnology and Oceanography, 58*(1), 121–130.

675 https://doi.org/10.4319/lo.2013.58.1.0121

676 De Zwaan, A., Cortesi, P., Van den Thillart, G., Roos, J., & Storey, K. B. (1991). Differential sensitivities to

677 hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Marine Biology, 111*(3), 343–

678 351. https://doi.org/10.1007/bf01319405

679 Desai, D. V., & Prakash, S. (2009). Physiological responses to hypoxia and anoxia in *Balanus amphitrite*

680 (Cirripedia: Thoracica). *Marine Ecology Progress Series, 390*, 157–166.

681 https://doi.org/10.3354/meps08155

682 Diaz, R. J., & Breitburg, D. L. (2009). The hypoxic environment. In: J. G. Richards, A. P. Farrell, C. J.

683 Brauner (Eds) *Fish physiology, Volume 27* (pp. 1–23). Academic Press. https://doi.org/10.1016/b978-0-

684 12-809633-8.03213-1

685 Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the

686 behavioural responses of benthic macrofauna. *Oceanography and marine biology. An annual review,*

687 *33*, 245–03.

688   Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine

689   ecosystems. *Science, 321*(5891), 926–929. https://doi.org/10.1126/science.1156401

690   Diaz, R. J., & Rosenberg, R. (2011). Introduction to environmental and economic consequences of

691   hypoxia. *International Journal of Water Resources Development, 27*(1), 71–82.

692   https://doi.org/10.1080/07900627.2010.531379

693   Dodds, L. A., Roberts, J. M., Taylor, A. C., & Marubini, F. (2007). Metabolic tolerance of the cold-water

694   coral *Lophelia pertusa* (Scleractinia) to temperature and dissolved oxygen change. *Journal of*

695   *Experimental Marine Biology and Ecology, 349*(2), 205–214.

696   https://doi.org/10.1016/j.jembe.2007.05.013

697   Doney, S. C. (2010). The growing human footprint on coastal and open-ocean biogeochemistry. *Science,*

698   *328*(5985), 1512–1516. https://doi.org/10.1126/science.1185198

699   Ellington, W. R. (1982). Metabolic responses of the sea anemone *Bunodosoma cavernata* (Bosc) to

700   declining oxygen tensions and anoxia. *Physiological Zoology, 55*(3), 240–249.

701   https://doi.org/10.1086/physzool.55.3.30157888

702   Frieder, C. A., Nam, S. H., Martz, T. R., & Levin, L. A. (2012). High temporal and spatial variability of

703   dissolved oxygen and pH in a nearshore California kelp forest. *Biogeosciences, 9*(10), 3917–3930.

704   https://doi.org/10.5194/bg-9-3917-2012

705   Gobler, C. J., & Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics

706   and effects on marine life. *Biology Letters, 12*(5), 20150976. https://doi.org/10.1098/rsbl.2015.0976

707   Goldstein, J., Riisgård, H. U., & Larsen, P. S. (2019). Exhalant jet speed of single-osculum explants of the

708   demosponge *Halichondria panicea* and basic properties of the sponge-pump. *Journal of Experimental*

709   *Marine Biology and Ecology, 511*, 82–90. https://doi.org/10.1016/j.jembe.2018.11.009

710 Gruber, R. K., Lowe, R. J., & Falter, J. L. (2017). Metabolism of a tide-dominated reef platform subject to

711 extreme diel temperature and oxygen variations. *Limnology and Oceanography, 62*(4), 1701–1717.

712 https://doi.org/10.1002/lno.10527

713 Haas, A. F., Smith, J. E., Thompson, M., & Deheyn, D. D. (2014). Effects of reduced dissolved oxygen

714 concentrations on physiology and fluorescence of hermatypic corals and benthic algae. *PeerJ, 2*, e235.

715 https://doi.org/10.7717/peerj.235

716 Hagerman, L. (1998). Physiological flexibility; a necessity for life in anoxic and sulphidic habitats.

717 *Hydrobiologia, 375*, 241–254. https://doi.org/10.1007/978-94-017-2864-5_20

718 Hervé, M. (2021). *RVAideMemoire: testing and plotting procedures for biostatistics*. R package version

719 0.9-79. https://cran.r-project.org/web/packages/RVAideMemoire/index.html

720 Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., & Horn, M. (2003). Microbial

721 diversity of marine sponges. In: W. E. G. Müller (Ed) *Progress in Molecular and Subcellular Biology.*

722 *Sponges (Porifera)* (pp 59–88). Springer. https://doi.org/10.1007/978-3-642-55519-0_3

723 Hentschel, U., Usher, K. M., & Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS*

724 *microbiology ecology, 55*(2), 167–177. https://doi.org/10.1111/j.1574-6941.2005.00046.x

725 Hochachka, P. W., & Lutz, P. L. (2001). Mechanism, origin, and evolution of anoxia tolerance in animals.

726 *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 130*(4), 435–459.

727 https://doi.org/10.1016/s1096-4959(01)00408-0

728 Hoffmann, F., Larsen, O., Thiel, V., Rapp, H. T., Pape, T., Michaelis, W., & Reitner, J. (2005). An

729 anaerobic world in sponges. *Geomicrobiology Journal, 22*(1–2), 1–10.

730 https://doi.org/10.1080/01490450590922505

731 Hughes, D. J., Alderdice, R., Cooney, C., Kühl, M., Pernice, M., Voolstra, C. R., & Suggett, D. J. (2020).

732 Coral reef survival under accelerating ocean deoxygenation. *Nature Climate Change, 10*(4), 296–307.

733 https://doi.org/10.1038/s41558-020-0737-9

734   IPCC (2021). Summary for Policymakers. In: V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C.

735   Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R.

736   Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu & B. Zhou (Eds.). *Climate Change 2021: The*

737   *Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the*

738   *Intergovernmental Panel on Climate Change*. Cambridge University Press. In Press.

739   Johnson, M. D., Rodriguez, L. M., & Altieri, A. H. (2018). Shallow-water hypoxia and mass mortality on a

740   Caribbean coral reef. *Bulletin of Marine Science, 94*(1), 143–144.

741   https://doi.org/10.5343/bms.2017.1163

742   Johnson, P. D., & McMahon, R. F. (1998). Effects of temperature and chronic hypoxia on survivorship of

743   the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). *Canadian Journal of*

744   *Fisheries and Aquatic Sciences, 55*(7), 1564–1572. https://doi.org/10.1139/f98-030

745   Kamke, J., Taylor, M. W., & Schmitt, S. (2010). Activity profiles for marine sponge-associated bacteria

746   obtained by 16S rRNA vs 16S rRNA gene comparisons. *The ISME Journal, 4*(4), 498–508.

747   https://doi.org/10.1038/ismej.2009.143

748   Kealoha, A. K., Doyle, S. M., Shamberger, K. E., Sylvan, J. B., Hetland, R. D., & Di Marco, S. F. (2020).

749   Localized hypoxia may have caused coral reef mortality at the Flower Garden Banks. *Coral Reefs, 39*(1),

750   119–132. https://doi.org/10.1007/s00338-019-01883-9

751   Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. *Annual*

752   *Review of Marine Science, 2*, 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

753   Kroeker, K. J., Bell, L. E., Donham, E. M., Hoshijima, U., Lummis, S., Toy, J. A., & Willis-Norton, E. (2020).

754   Ecological change in dynamic environments: Accounting for temporal environmental variability in

755   studies of ocean change biology. *Global change biology, 26*(1), 54–67.

756   https://doi.org/10.1111/gcb.14868

757   Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed

758   effects models. *Journal of Statistical Software, 82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

759   Lavy, A., Keren, R., Yahel, G., & Ilan, M. (2016). Intermittent hypoxia and prolonged suboxia measured

760   in situ in a marine sponge. *Frontiers in Marine Science, 3*, 263.

761   https://doi.org/10.3389/fmars.2016.00263

762   Lenth, R. (2021). *emmeans: estimated marginal means, aka least-squares means*. R package version

763   1.6.0. https://CRAN.R-project.org/package=emmeans

764   Levin, L. A. (2003). Oxygen minimum zone benthos: adaptation and community response to hypoxia.

765   *Oceanography and Marine Biology: An Annual Review. 41*, 1–45.

766   https://doi.org/10.1201/9780203180570-3

767   Levin, L. A., & Breitburg, D. L. (2015). Linking coasts and seas to address ocean deoxygenation. *Nature*

768   *Climate Change, 5*(5), 401–403. https://doi.org/10.1038/nclimate2595

769   Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., Neira, C., Rabalais, N.

770   N. & Zhang, J. (2009). Effects of natural and human-induced hypoxia on coastal benthos.

771   *Biogeosciences, 6*(10), 2063–2098. https://doi.org/10.5194/bg-6-2063-2009

772   Levin, L. A., Huggett, C. L., & Wishner, K. F. (1991). Control of deep-sea benthic community structure by

773   oxygen and organic-matter gradients in the eastern Pacific Ocean. *Journal of Marine Research, 49*(4),

774   763–800. https://doi.org/10.1357/002224091784995756

775   Leys, S. P., & Kahn, A. S. (2018). Oxygen and the energetic requirements of the first multicellular

776   animals. *Integrative and Comparative Biology, 58*(4), 666–676. https://doi.org/10.1093/icb/icy051

777   Limburg, K. E., Breitburg, D., & Levin, L. A. (2017). Ocean deoxygenation-a climate-related problem.

778   *Frontiers in Ecology and the Environment, 15*(9), 479–479. https://doi.org/10.1002/fee.1728

779   Love, G.D., Grosjean, E., Stalvies, C., Fike, D.A., Grotzinger, J.P., Bradley, A.S., Kelly, A.E., Bhatia, M.,

780   Meredith, W., Snape, C.E. and Bowring, S.A., Condon, D. J., & Summons, R. E. (2009). Fossil steroids

781   record the appearance of Demospongiae during the Cryogenian period. *Nature, 457*(7230), 718–721.

782   https://doi.org/10.1038/nature07673

783   Lunden, J. J., McNicholl, C. G., Sears, C. R., Morrison, C. L., & Cordes, E. E. (2014). Acute survivorship of

784   the deep-sea coral *Lophelia pertusa* from the Gulf of Mexico under acidification, warming, and

785   deoxygenation. *Frontiers in Marine Science, 1*, 78. https://doi.org/10.3389/fmars.2014.00078

786   Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: biology, budgets,

787   and ecological implications. *Advances in Marine Biology, 62*, 113–182. https://doi.org/10.1016/b978-0-

788   12-394283-8.00003-5

789   Maloof, A. C., Rose, C. V., Beach, R., Samuels, B. M., Calmet, C. C., Erwin, D. H., Poirier, G. R., Yao, N., &

790   Simons, F.J. (2010). Possible animal-body fossils in pre-Marinoan limestones from South Australia.

791   *Nature Geoscience, 3*(9), 653–659. https://doi.org/10.1038/ngeo934

792   Mangum, D. C. (1980). Sea anemone neuromuscular responses in anaerobic conditions. *Science,*

793   *208*(4448), 1177–1178. https://doi.org/10.1126/science.6103580

794   McAllen, R., Davenport, J., Bredendieck, K., & Dunne, D. (2009). Seasonal structuring of a benthic

795   community exposed to regular hypoxic events. *Journal of Experimental Marine Biology and Ecology,*

796   *368*(1), 67–74. https://doi.org/10.1016/j.jembe.2008.10.019

797   McAllen, R., Taylor, A. C., & Davenport, J. (1999). The effects of temperature and oxygen partial

798   pressure on the rate of oxygen consumption of the high-shore rock pool copepod *Tigriopus brevicornis*.

799   *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 123*(2), 195–202.

800   https://doi.org/10.1016/s1095-6433(99)00050-1

801   Mentel, M., Röttger, M., Leys, S., Tielens, A. G., & Martin, W. F. (2014). Of early animals, anaerobic

802   mitochondria, and a modern sponge. *Bioessays, 36*(10), 924–932.

803   https://doi.org/10.1002/bies.201400060

804   Mills, D. B., Francis, W. R., Vargas, S., Larsen, M., Elemans, C. P., Canfield, D. E., & Wörheide, G. (2018).

805   The last common ancestor of animals lacked the HIF pathway and respired in low-oxygen

806   environments. *Elife, 7*, e31176. https://doi.org/10.7554/eLife.31176.001

807   Mills, D. B., Ward, L. M., Jones, C., Sweeten, B., Forth, M., Treusch, A. H., & Canfield, D. E. (2014).

808   Oxygen requirements of the earliest animals. *Proceedings of the National Academy of Sciences,*

809   *111*(11), 4168–4172. https://doi.org/10.1073/pnas.1400547111

810   Milton, S. L., & Prentice, H. M. (2007). Beyond anoxia: the physiology of metabolic downregulation and

811   recovery in the anoxia-tolerant turtle. *Comparative Biochemistry and Physiology Part A: Molecular and*

812   *Integrative Physiology, 147*(2), 277–290. https://doi.org/10.1016/j.cbpa.2006.08.041

813   Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C., Wu, Y. C., McCormack, G. P., López-Legentil,

814   S., Marchant, R., Webster, N., Thomas, T., & Hentschel, U. (2017). Predicting the HMA-LMA status in

815   marine sponges by machine learning. *Frontiers in microbiology, 8*, 752.

816   https://doi.org/10.3389/fmicb.2017.00752

817   Morganti, T. M., Ribes, M., Moskovich, R., Weisz, J. B., Yahel, G., & Coma, R. (2021). In situ Pumping

818   Rate of 20 Marine Demosponges Is a Function of Osculum Area. *Frontiers in Marine Science, 8*, 583188.

819   https://doi.org/10.3389/fmars.2021.583188

820   Morris, S., & Taylor, A. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within

821   intertidal rock pools. *Estuarine, Coastal and Shelf Science, 17*, 339–355. https://doi.org/10.1016/0272-

822   7714(83)90026-4

823   Mosch, T., Sommer, S., Dengler, M., Noffke, A., Bohlen, L., Pfannkuche, O., Liebetrau, V. & Wallmann, K.

824   (2012). Factors influencing the distribution of epibenthic megafauna across the Peruvian oxygen

825   minimum zone. *Deep Sea Research Part I: Oceanographic Research Papers, 68*, 123–135.

826   https://doi.org/10.1016/j.dsr.2012.04.014

827   Müller, M., Mentel, M., van Hellemond, J. J., Henze, K., Woehle, C., Gould, S. B., Yu, R.Y., van der

828   Giezen, M., Tielens, A.G., Martin, W. F. (2012). Biochemistry and evolution of anaerobic energy

829   metabolism in eukaryotes. *Microbiology and Molecular Biology Reviews, 76*(2), 444–495.

830   https://doi.org/10.1128/MMBR.05024-11

831		Murty, S. J., Bett, B. J., & Gooday, A. J. (2009). Megafaunal responses to strong oxygen gradients on the

832		Pakistan margin of the Arabian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography, 56*(6-

833		7), 472–487. https://doi.org/10.1016/j.dsr2.2008.05.029

834		Nagasoe, S., Tokunaga, T., Yurimoto, T., & Matsuyama, Y. (2020). Survival and behavior patterns

835		associated with hypoxia at different life stages of the pen shell *Atrina* cf. *japonica*. *Aquatic Toxicology,*

836		*227*, 105610. https://doi.org/10.1016/j.aquatox.2020.105610

837		Nezlin, N. P., Kamer, K., Hyde, J., & Stein, E. D. (2009). Dissolved oxygen dynamics in a eutrophic

838		estuary, Upper Newport Bay, California. *Estuarine, Coastal and Shelf Science*, 82(1), 139–151.

839		https://doi.org/10.1016/j.ecss.2009.01.004

840		Nilsson, G. E., & Renshaw, G. M. (2004). Hypoxic survival strategies in two fishes: extreme anoxia

841		tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark.

842		*Journal of Experimental Biology, 207*(18), 3131–3139. https://doi.org/10.1242/jeb.00979

843		Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns.

844		*Ophelia, 41*(1), 199–219. https://doi.org/10.1080/00785236.1995.10422044

845		Osinga, R., Tramper, J., & Wijffels, R. H. (1999). Cultivation of marine sponges. *Marine Biotechnology,*

846		*1*(6), 509–532. https://doi.org/10.1007/PL00011807

847		Piazzi, L., Atzori, F., Cadoni, N., Cinti, M. F., Frau, F., Pansini, A., Pinna, F., Stipcich, P. & Ceccherelli, G.

848		(2021). Animal Forest Mortality: Following the Consequences of a Gorgonian Coral Loss on a

849		Mediterranean Coralligenous Assemblage. *Diversity, 13*(3), 133. https://doi.org/10.3390/d13030133

850		Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team (2021). *nlme: Linear and Nonlinear Mixed*

851		*Effects Models*. R package version 3.1-152. https://CRAN.R-project.org/package=nlme.

852		R Core Team (2013). *R: A language and environment for statistical computing.*

853		Rao, D. P., & Ganapati, P. N. (1968). Respiration as a function of oxygen concentration in intertidal

854		barnacles. *Marine Biology, 1*(4), 309–310. https://doi.org/10.1007/BF00360781

855   Raupach, M. R., & Canadell, J. G. (2010). Carbon and the Anthropocene. *Current Opinion in*

856   *Environmental Sustainability, 2*(4), 210–218. https://doi.org/10.1016/j.cosust.2010.04.003

857   Robinson, C. (2019). Microbial respiration, the engine of ocean deoxygenation. *Frontiers in Marine*

858   *Science, 5*, 533. https://doi.org/10.3389/fmars.2018.00533

859   Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H. O., Duarte, C. M., Levin, L. A. & Rosa, R.

860   (2021). Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nature*

861   *Ecology and Evolution, 5*(3), 311–321. https://doi.org/10.1038/s41559-020-01370-3

862   Sarà, M., Sarà, A., Nickel, M., & Brümmer, F. (2001). Three New Species of *Tethya* (Porifera:

863   Demospongiae) From German Aquaria. *Stuttgarter Beiträge zur Naturkunde 631*, 1–15.

864   Sassaman, C., & Mangum, C. P. (1972). Adaptations to environmental oxygen levels in infaunal and

865   epifaunal sea anemones. *The Biological Bulletin, 143*(3), 657–678. https://doi.org/10.2307/1540189

866   Schuster, A., Strehlow, B. W., Eckford-Soper, L., McAllen, R., & Canfield, D. E. (2021). Effects of Seasonal

867   Anoxia on the Microbial Community Structure in Demosponges in a Marine Lake in Lough Hyne, Ireland.

868   *Msphere, 6(*1), e00991-20. https://doi.org/10.1101/2020.09.09.290791

869   Seibel, B. A. (2011). Critical oxygen levels and metabolic suppression in oceanic oxygen minimum

870   zones. *Journal of Experimental Biology, 214*(2), 326–336. https://doi.org/10.1242/jeb.049171

871   Semenza, G. L. (2007). Life with oxygen. *Science, 318*(5847), 62–64.

872   https://doi.org/10.1126/science.1147949

873   Smith, V. H., Joye, S. B., & Howarth, R. W. (2006). Eutrophication of freshwater and marine ecosystems.

874   *Limnology and Oceanography, 51*, 351–355. https://doi.org/10.1007/978-90-481-3385-7_1

875   Stachowitsch, M. (1984). Mass mortality in the Gulf of Trieste: the course of community destruction.

876   *Marine Ecology, 5*(3), 243–264. https://doi.org/10.1111/j.1439-0485.1984.tb00124.x

877   Steckbauer, A., Klein, S. G., & Duarte, C. M. (2020). Additive impacts of deoxygenation and acidification

878   threaten marine biota. *Global Change Biology, 26*(10), 5602–5612. https://doi.org/10.1111/gcb.15252

879   Stickle, W. B., Kapper, M. A., Liu, L. L., Gnaiger, E., & Wang, S. Y. (1989). Metabolic adaptations of

880   several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. The

881   *Biological Bulletin, 177*(2), 303–312. https://doi.org/10.2307/1541945

882   Strano, F., Micaroni, V., Davy, S. K., Maldonado, M., & Bell, J. J. (2021). Reproduction and early life

883   stages of the poecilosclerid sponge *Crella incrustans*. *Invertebrate Biology*, e12335.

884   https://doi.org/10.1111/ivb.12335

885   Therneau, T. (2021). *A Package for Survival Analysis in R*. R package version 3.2-10. https://CRAN.R-

886   project.org/package=survival.

887   Thuesen, E. V., Rutherford Jr, L. D., & Brommer, P. L. (2005). The role of aerobic metabolism and

888   intragel oxygen in hypoxia tolerance of three ctenophores: *Pleurobrachia bachei*, *Bolinopsis*

889   *infundibulum* and *Mnemiopsis leidyi*. *Journal of the Marine Biological Association of the United*

890   *Kingdom, 85*(3), 627. https://doi.org/10.1017/s0025315405011550

891   Trowbridge, C. D., Davenport, J., Cottrell, D. M., Harman, L., Plowman, C. Q., Little, C., & McAllen, R.

892   (2017). Extreme oxygen dynamics in shallow water of a fully marine Irish sea lough. *Regional Studies in*

893   *Marine Science, 11*, 9–16. https://doi.org/10.1016/j.rsma.2017.01.008

894   Turner, E. C. (2021). Possible poriferan body fossils in early Neoproterozoic microbial reefs. *Nature*,

895   *596*, 87–91. https://doi.org/10.1038/s41586-021-03773-z

896   Vacelet, J. (1975). Étude en microscopie électronique de l'association entre bactéries et spongiaires du

897   genre Verongia (Dictyoceratida).

898   Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings*

899   *of the National Academy of Sciences, 105*(40), 15452–15457.

900   https://doi.org/10.1073/pnas.0803833105

901   Vaquer-Sunyer, R., & Duarte, C. M. (2010). Sulfide exposure accelerates hypoxia-driven mortality.

902   *Limnology and Oceanography, 55*(3), 1075–1082. https://doi.org/10.4319/lo.2010.55.3.1075

903   Vaquer-Sunyer, R., & Duarte, C. M. (2011). Temperature effects on oxygen thresholds for hypoxia in

904   marine benthic organisms. *Global Change Biology, 17*(5), 1788–1797. https://doi.org/10.1111/j.1365-

905   2486.2010.02343.x

906   Vergés, A., McCosker, E., Mayer-Pinto, M., Coleman, M. A., Wernberg, T., Ainsworth, T., & Steinberg, P.

907   D. (2019). Tropicalisation of temperate reefs: implications for ecosystem functions and management

908   actions. *Functional Ecology, 33*(6), 1000–1013. https://doi.org/10.1111/1365-2435.13310

909   Vornanen, M., Stecyk, J. A., & Nilsson, G. E. (2009). The anoxia-tolerant crucian carp (*Carassius*

910   *carassius* L.). In: J. Richards, A. Farrell, & C. Brauner (Eds.), *Fish Physiology: Hypoxia, Volume 27* (pp.

911   397–441). Academic Press. https://doi.org/10.1016/s1546-5098(08)00009-5

912   Whelan, N. V., Kocot, K. M., Moroz, T. P., Mukherjee, K., Williams, P., Paulay, G., Moroz, L. L. &

913   Halanych, K. M. (2017). Ctenophore relationships and their placement as the sister group to all other

914   animals. *Nature Ecology & Evolution, 1*(11), 1737–1746. https://doi.org/10.1038/s41559-017-0331-3

915   Wishner, K. F., Ashjian, C. J., Gelfman, C., Gowing, M. M., Kann, L., Levin, L. A., Mullineaux, L. S. &

916   Saltzman, J. (1995). Pelagic and benthic ecology of the lower interface of the Eastern Tropical Pacific

917   oxygen minimum zone. *Deep Sea Research Part I: Oceanographic Research Papers, 42*(1), 93–115.

918   https://doi.org/10.1016/0967-0637(94)00021-j

919   Woo, S., Denis, V., Won, H., Shin, K., Lee, G., Lee, T. K., & Yum, S. (2013). Expressions of oxidative stress-

920   related genes and antioxidant enzyme activities in *Mytilus galloprovincialis* (Bivalvia, Mollusca) exposed

921   to hypoxia. *Zoological Studies, 52*(1), 1–8. https://doi.org/10.1186/1810-522x-52-15

922   Woodhead, A. J., Hicks, C. C., Norström, A. V., Williams, G. J., & Graham, N. A. (2019). Coral reef

923   ecosystem services in the Anthropocene. *Functional Ecology, 33*(6), 1023–1034.

924   https://doi.org/10.1111/1365-2435.13331

925   Wulff, J. L. (2006). Ecological interactions of marine sponges. *Canadian Journal of Zoology, 84*(2), 146–

926   166. https://doi.org/10.1139/z06-019

927   Yahel, G., Marie, D., & Genin, A. (2005). InEx—a direct in situ method to measure filtration rates,

928   nutrition, and metabolism of active suspension feeders. *Limnology and Oceanography: Methods, 3*(2),

929   46–58. https://doi.org/10.4319/lom.2005.3.46

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931   **FIGURES**
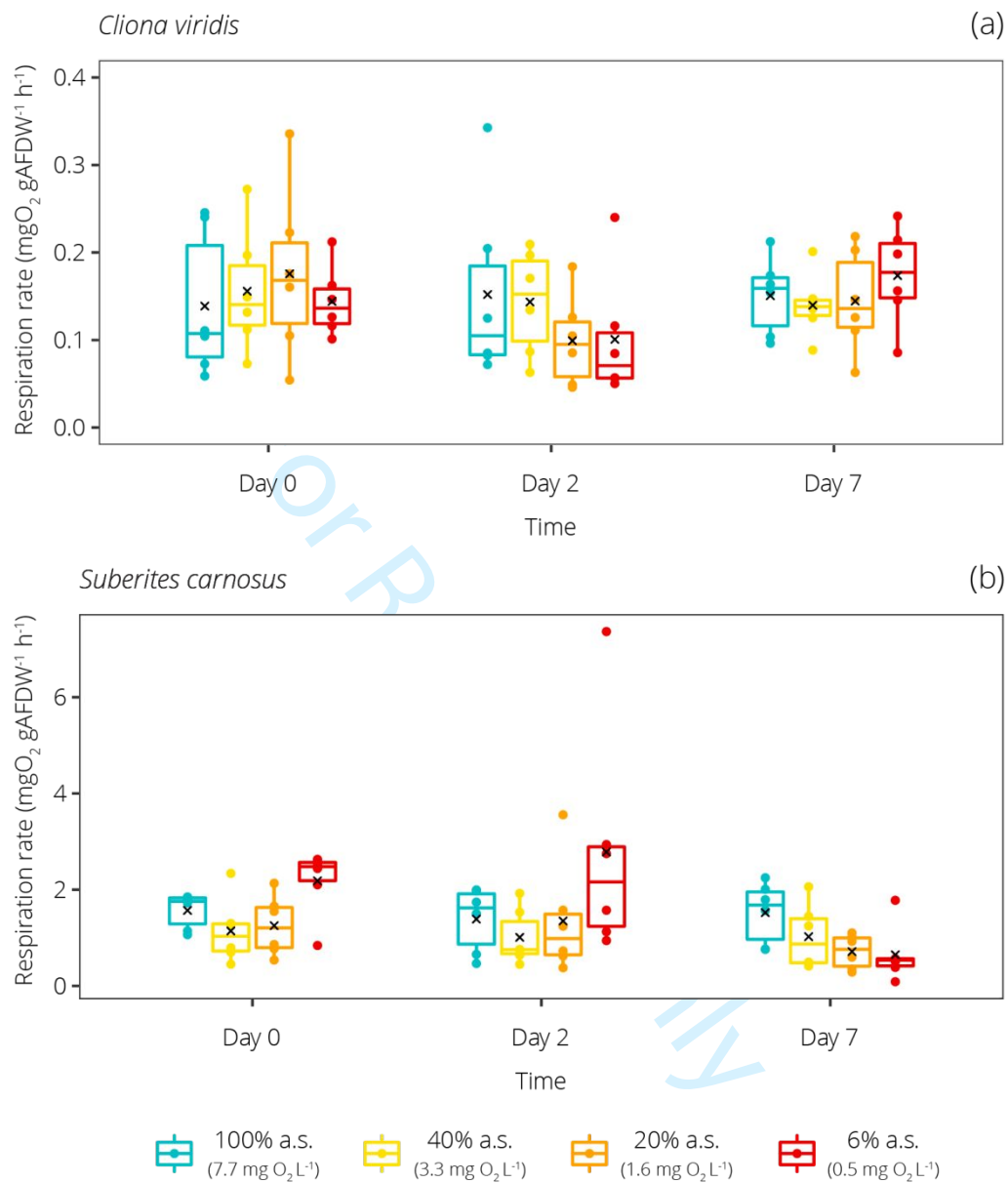
934   Figure 1. Respiration rates in (a) *Cliona celata* and (b) *Suberites carnosus* from experiment 1 (moderate

935   hypoxia) measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. Horizontal

936   bars inside the boxplots represent medians; the symbol × represents means. Lower and upper hinges of the

937   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 croceus* in each treatment in experiments
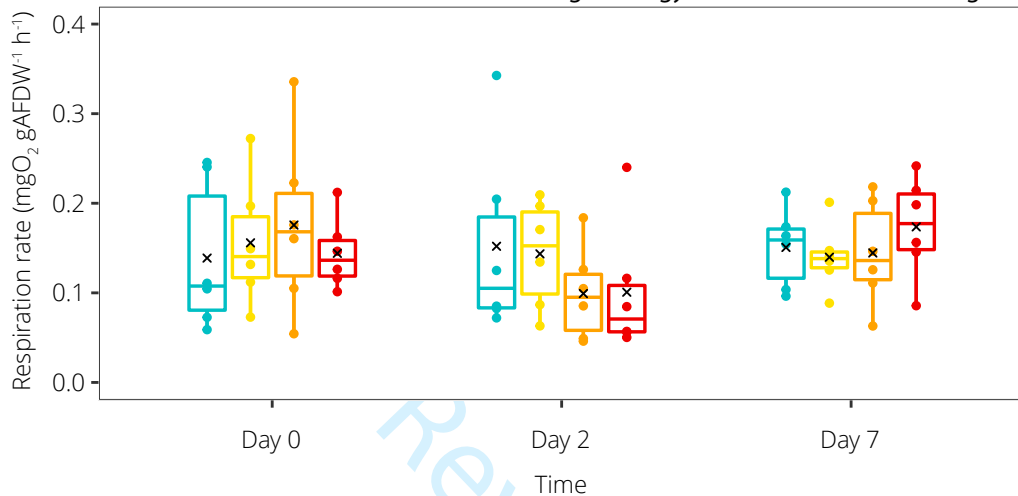
2 (a) and 3 (b). Changes in the expansion ratio over time in *Suberites australiensis* in each treatment in

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.

	General morphology	Papillae	Osculum	Aquiferous system
100% a.s. (8.2 mg O₂ L⁻¹)				
5% a.s. (0.4 mg O₂ L⁻¹)				
1.5% a.s. (0.13 mg O₂ L⁻¹)				
	Polymastia croceus		*Suberites australiensis*	

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 croceus*; 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).

956

957

958   Figure 4. Respiration rates in *Polymastia croceus* and *Suberites australiensis* from experiment 2 (5% a.s.)

959   measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. Horizontal bars

960   inside the boxplots represent medians; the symbol × represents means. Lower and upper hinges of the

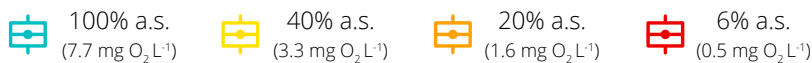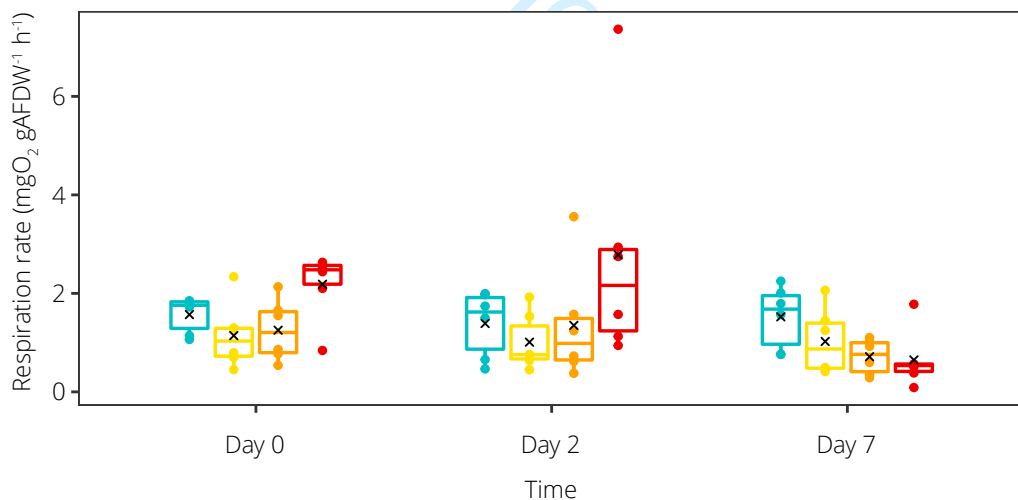961   boxplots correspond to the first and third quartiles, respectively. Points represent data points.

Figure 5. The tolerance of marine sessile organisms to hypoxia. Red dots indicate lethal thresholds, while yellow dots indicate sub-lethal thresholds. Organisms whose 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

972   the experiment. For *H. panicea,* Mills et al. (2014) only report oxygen measurement as per cent air

973   saturation without reporting temperature and salinity, so the actual oxygen concentration is unknown. We,

974   therefore, estimated the oxygen content using the range of temperatures and salinity found where these

975   sponges were sampled (Salinity 8.9–29.5; Temperature: 5–25 °C; Thomassen & Riisgård, 1995) and we

976   provide the mean and the range of possible values. List of references associated with each species: *A.*

977   *yongei* (Haas et al., 2014), *A. amphitrite* (Rao & Ganapati, 1968; Desai & Prakash, 2009), *A. japonica*

978   (Nagasoe et al., 2020), *B. pennata* (Haas et al., 2014), *B. cavernatum* (Mangum, 1980; Ellington, 1982), *C.*

979   *americana* (Vaquer-Sunyer & Duarte, 2008), *C. virginica* (Stickle et al., 1989), *D. pertusum* (Dodds et al.,

980   2007; Lunden et al., 2014), *D. polymorpha* (Johnson & McMahon, 1998), *H. panicea* (Mills et al., 2014), *M.*

981   *tintinnabulum* (Rao and Ganapati 1968), *M. senile* (Sassaman & Mangum 1972), *M. galloprovincialis* (De

982   Zwaan et al., 1991; Woo et al., 2013), *T. wilhelma* (Mills et al., 2018), *Z. marina* (Hughes et al., 2020). Figure
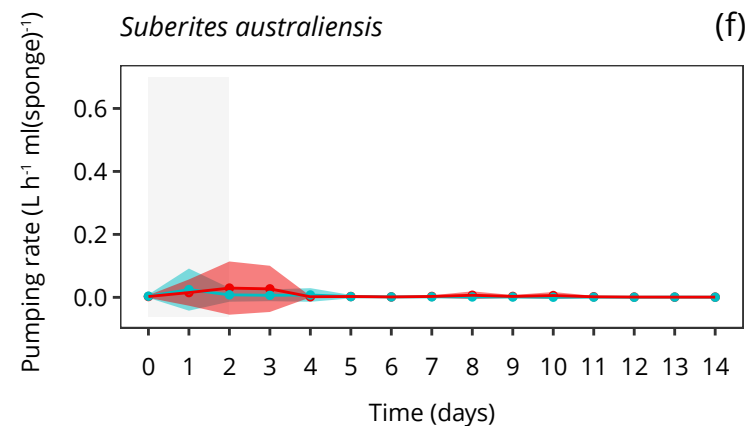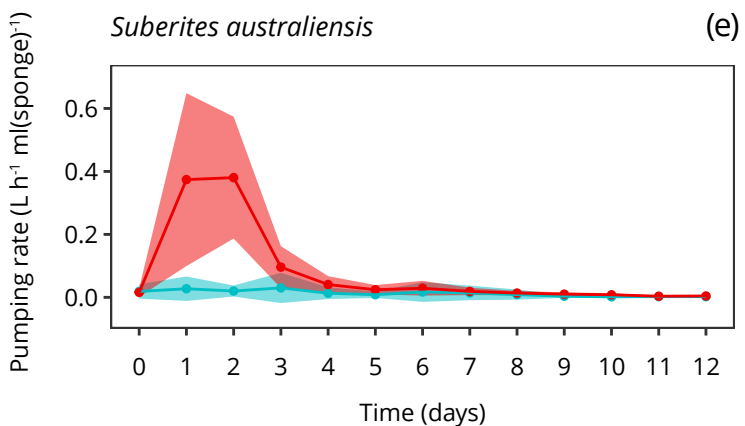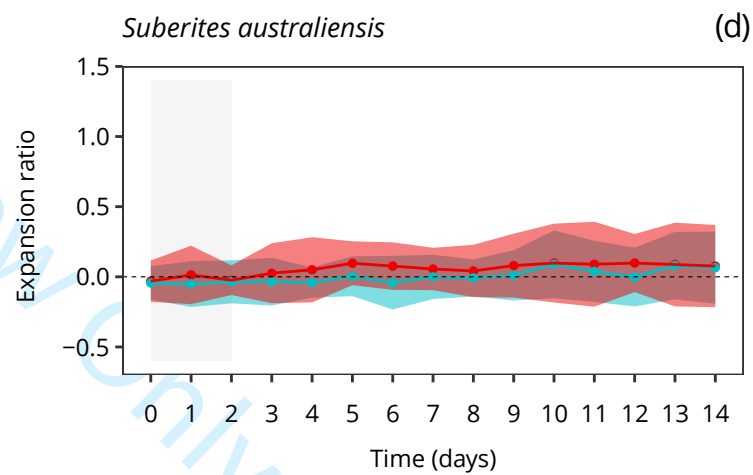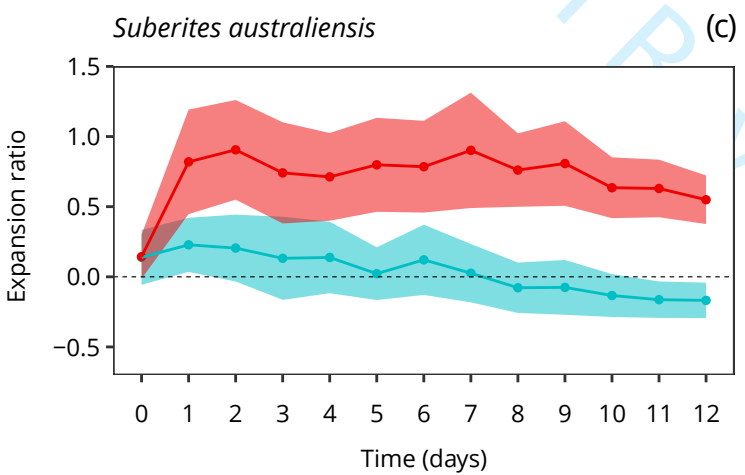
983   inspired by Hughes et al. (2020).

(a) *Cliona viridis*

(b) *Suberites carnosus*

Legend:
- 100% a.s. (7.7 mg O$_2$ L^{-1})
- 40% a.s. (3.3 mg O$_2$ L^{-1})
- 20% a.s. (1.6 mg O$_2$ L^{-1})
- 6% a.s. (0.5 mg O$_2$ L^{-1})

Experiment 2 (5% a.s. - 0.4 mg O$_2$ L^{-1})

Experiment 3 (1.5% a.s. - 0.13 mg O$_2$ L^{-1})

Polymastia croceus (a)

Polymastia croceus (b)

Suberites australiensis (c)

Suberites australiensis (d)

Suberites australiensis (e)
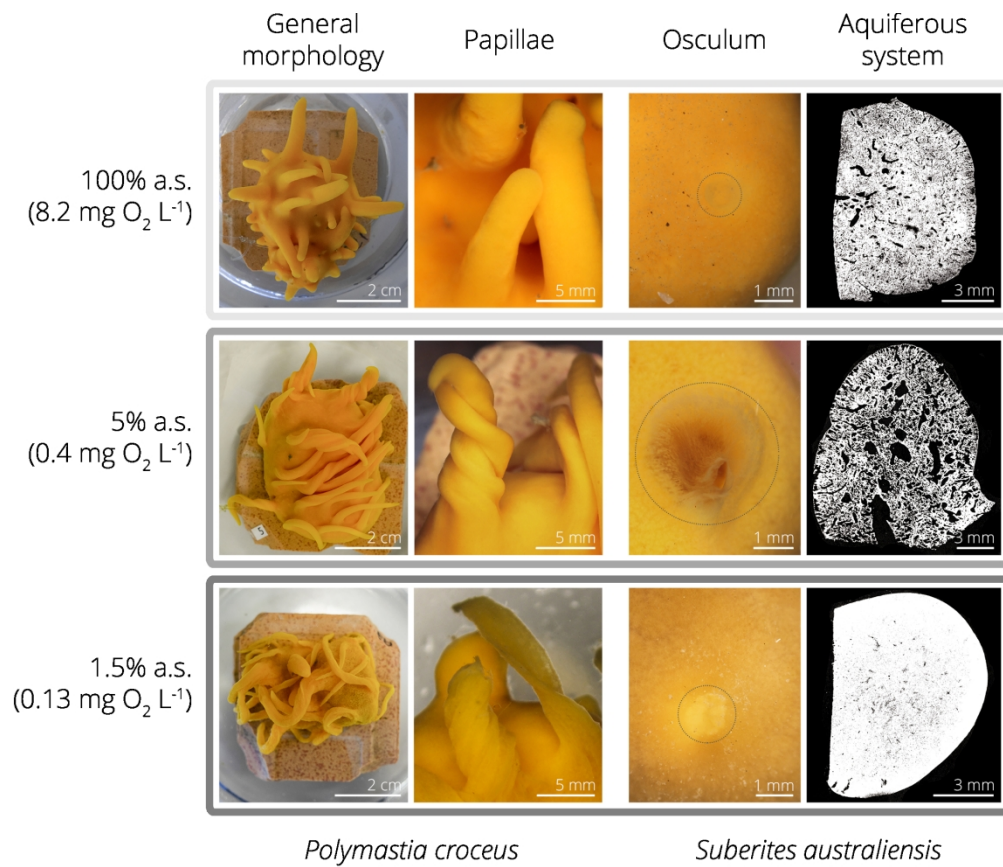
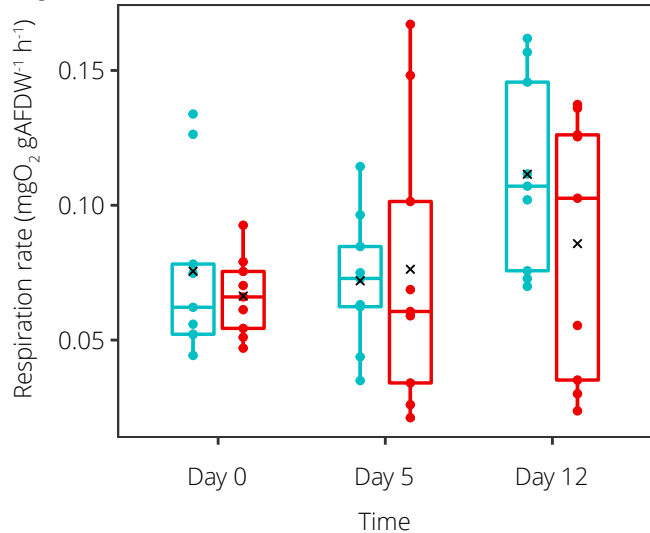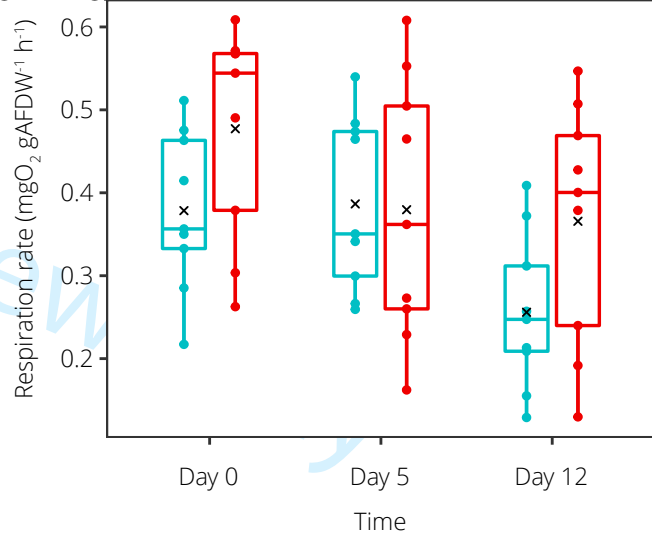*Suberites australiensis* (f)

Control — Treatment

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 croceus; 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).
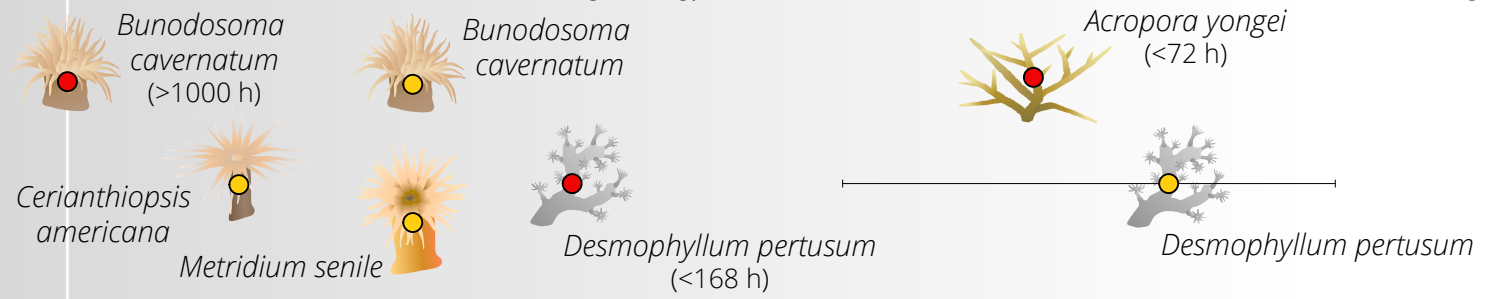
Polymastia croceus

Suberites australiensis

Cnidarians

Bunodosoma cavernatum (>1000 h)

Bunodosoma cavernatum

Acropora yongei (<72 h)

Cerianthiopsis americana

Metridium senile

Desmophyllum pertusum (<168 h)

Desmophyllum pertusum

Cirripeds

Amphibalanus amphitrite (96 h)

Amphibalanus amphitrite

Megabalanus tintinnabulum

Bivalves

Dreissena polymorpha (200 h)

Atrina japonica (96 h)

Crassostrea virginica (672 h at 0 mg O_2 L^{-1} — 72 h at 4.56 mg O_2 L^{-1})

Mytilus galloprovincialis (360 h)

Mytilus galloprovincialis (180 h)

Mytilus galloprovincialis

Algae and seagrasses

Bryopsis pennata

Zoostera marina

Sponges

Halichondria panicea

Polymastia croceus (286 h)

Cliona celata

Tethya wilhelma

Suberites carnosus

○ Sublethal threshold

● Lethal threshold (LT_{50})

Dissolved Oxygen (mg L^{-1})

0   1   2   3   4   5   6