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

| | also show that a single sponge species (i.e., Suberites australiensis) can display different strategies at different oxygen concentrations. Compared to other sessile organisms, sponges generally showed higher tolerance to hypoxia, suggesting that sponges could be favoured and survive in future deoxygenated oceans. |
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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

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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.

96   **2 MATERIALS AND METHODS**

97   **2.1 Study area and species**

98   Experiment 1 (moderate hypoxia) was performed in Ireland (Renouf Laboratory, Lough Hyne) on two

99   abundant North-East Atlantic sponge species: *Cliona celata* Grant, 1826 and *Suberites carnosus* (Johnston,

100   1842). Experiments 2 and 3 (severe hypoxia) were performed in New Zealand (Wellington University

101   Coastal Ecology Laboratory, Wellington) on two abundant temperate Australasian species: *Polymastia*

102   *croceus* Kelly-Borges and Bergquist, 1997 and *Suberites australiensis* Bergquist, 1968.

103   **2.1 Experiment 1: moderate hypoxia**

104   In the first experiment, we investigated the response of *Cliona. celata* and *Suberites. carnosus* to a wide

105   range of oxygen concentrations, using an air-tight system with a continuous flow of seawater. Sponges

106   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})

107   and ~6% (0.48 ± 0.09 mg O_2 L^{-1}) air saturation (a.s.) for seven days (a summary of the seawater parameters

108   is provided in Table S1).

109   The experimental set-up (see scheme in Figure S1) consisted of two independent replicate modules for

110   each treatment, randomly distributed in the experimental set-up. To condition water, we used two header

111   tanks for each experimental module: one providing water and one reservoir. Header tanks were filled with

112   10-μm-filtered seawater. The oxygen level was then lowered and maintained to the desired dissolved

113   oxygen concentration by bubbling specific mixtures of N_2 (BOC, food-grade) and air, through glass-ceramic

114   diffusers. Hypoxic gas blends were prepared by decanting food-grade N_2 and air in 15 L scuba cylinders

115   using an oxygen decanting assembly (Undersea Ltd, 5215) with a DPM-300 digital gauge (0.25% accuracy).

116   Oxygen concentration was then checked with a Nuvair Pro O_2 Analyser and adjusted, if necessary.

117   Conditioned water was delivered to two replicate experimental chambers (2.3 L) for each system at a rate

118   of 25 L per day, ensuring 100% water replacement every 2 h and 15 min. Water circulation within each

119   experimental chamber was provided by the gravity-driven water flow (~3 cm/s). Temperature was kept

120   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 m³ 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 eExperiment 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     $\pm$ 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 $\geq$ 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-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 ~~e~~Experiment 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.

226 **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.

236 **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.

246   **2.3.6 Histology**

247   Histological sections of _Suberites_ ~~S.~~ _australiensis_ from the severe hypoxia experiments were analyzed to

248   calculate the percentage of the sponge body occupied by the aquiferous system (system of connected

249   water channels inside the sponge). At _T-end_, two contracted sponges for each experimental chamber (n = 6

250   per treatment) were fixed and processed following the methods of Strano et al. (2021). Three replicate

251   sections for each sponge were then photographed under a dissecting microscope (Olympus SZ61) and

252   photographed using a Canon EOS 70D digital camera. To calculate the area occupied by the aquiferous

253   system, pictures were analyzed using ImageJ.

254   **2.4 Data analysis**

255   All the statistical data analyses were performed in R version 3.1.3 (R Core Team, 2013), except

256   PERMANOVA models, which were performed using PRIMER v7 with PERMANOVA+ add-on (Anderson et al.,

257   2008; Clarke & Gorley, 2015). Experiments 2 and 3 were analyzed separately. To investigate respiration

258   rate, pumping rate and expansion ratio in _Suberites_ ~~S.~~ _australiensis_ from experiment 2, we used linear

259   mixed-effects models with normally distributed errors and random intercepts (lmer, _lme4_ package; Bates et

260   al., 2015). For pumping rate, we added a constant variance function structure (varIdent) to the linear

261   mixed-effects models to allow different variances for each treatment at each time point (lme, _R_ package

262   _nlme_; Pinheiro et al., 2021). The constant variance function structure was necessary because the variance

263   of the response variable differed across treatments and experimental days. To investigate the effect of time

264   and treatment on the expansion ratio of _Polymastia_ ~~P.~~ _croceus_ in ~~e~~Experiment 3, we used a generalized

265   linear mixed model with beta regression and logit link (_glmmTMB_, Brooks et al. 2017). In all the mixed

266   models, treatment and time were considered fixed effects, while experimental chamber and sponge

267   specimen were considered random effects. The experimental chamber effect was included to address

268   pseudo-replication. For these models, fixed- and random-effect terms were tested using the function anova

269   and ranova (_R_ package _lmerTest_, Kuznetsova et al., 2017), respectively; while post hoc pairwise

270   comparisons were computed on estimated marginal means using emmeans (_R_ package _emmeans_; Lenth,

271   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 *Cliona*~~C.~~ *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).

313 **3.2 Sponge responses to severe hypoxia**

314 **3.2.1 Survival**

315 Sponge survival differed among species, with *Suberites S. australiensis* more tolerant than *Polymastia P.*

316 *croceus*. No mortality was observed for *S. australiensis* in both experiments 2 (5% a.s.) and 3 (1.5% a.s.). In

317 contrast, for *P. croceus*, significant mortality (p = 0.001) was observed in sponges exposed to the 1.5% a.s.

318 treatment, starting from day 10 (day 12 when including the hypoxic acclimation), and with a median lethal

319 time of 11.9 ± 0.3 days (Fig S8–9). Eight out of 15 sponges had died by the end of the experiment. No

320 mortality was observed for *P. croceus* in the 5% a.s. treatment.

321 **3.2.2 Change in weight and size**

322 For *Polymastia P. croceus*, buoyant weight variation between T0 and T-end differed among treatments in

323 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.

324 treatment experienced a significant decrease in buoyant weight (-7.1%, t = -5.17, p = 0.002), while the

325 controls did not experience any significant change (Tab. S6; Fig. S10).

326 The relative variation in area of contracted sponges (after stimulating contraction) between T0 and T-end

327 differed significantly between treatments and controls for both *Polymastia P. croceus* (W = 12, p < 0.0001)

328 and *Suberites S. australiensis* (W = 13, p < 0.0001), but only in experiment 2 (5% a.s.). Both *P. croceus* and *S.*

329 *australiensis*, from the 5% a.s. treatment, experienced an increase in contracted area (+18.9%, W = 120, p =

330 0.0001 and +18.4%, W = 105, p = 0.008, respectively). While *S. australiensis* from the control treatment

331 (experiment 2) experienced a decrease in contracted area (-15.3%, W = 3, p = 0.0003) (Tab. S7; Fig. S11).

332 **3.2.3 Sponge contractile behaviour**

333 Low DO treatments generally induced sponge expansion, but the response differed between species, and it

334 was generally more marked in the 5% a.s. treatment. In *Polymastia P. croceus*, the ratio of expanded

335 papillae was significantly affected by time and the interaction between time and treatment in both

336 experiments 2 (p = 0.0001) and 3 (p < 0.0001 and p = 0.03) experiments (Tab. S8–9). During experiment 2

337 (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).

395   **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$_2$S) 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 (Burnett & 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 they~~They 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 M~~modern sponges might have

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

595 more likely that s~~S~~ponges 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).

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619 **AUTHOR CONTRIBUTIONS**

620 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.

621 conducted the experiments. V.M. and L.W. analyzed the data. V.M. and J.J.B wrote the original draft. All the

622 authors participated in interpreting the results and contributed to the revision of the manuscript.

623 **DATA AVAILABILITY STATEMENT**

624 All data and the R code used in this paper are available on Figshare repository:

625 https://doi.org/10.6084/m9.figshare.15169662.

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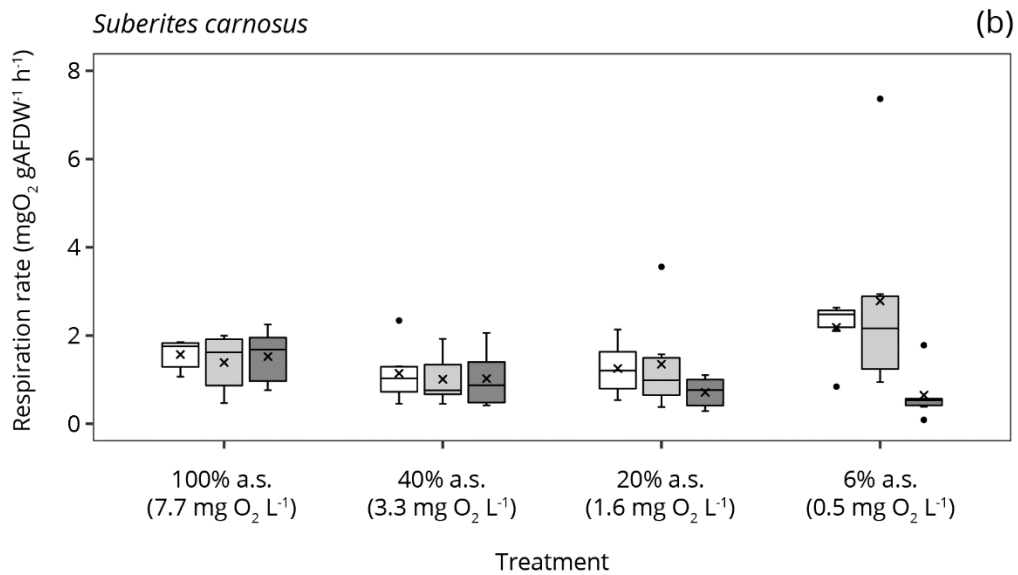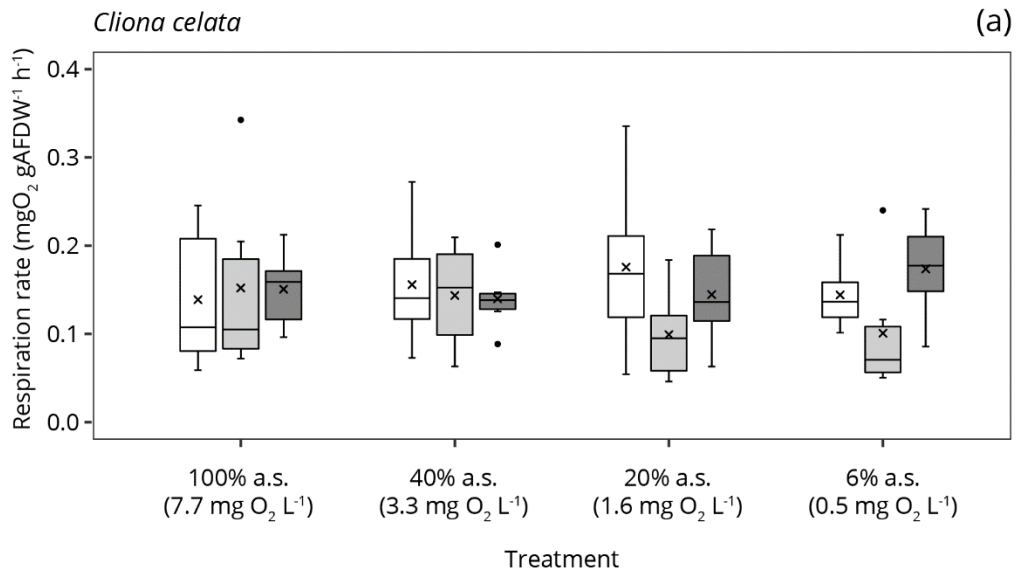
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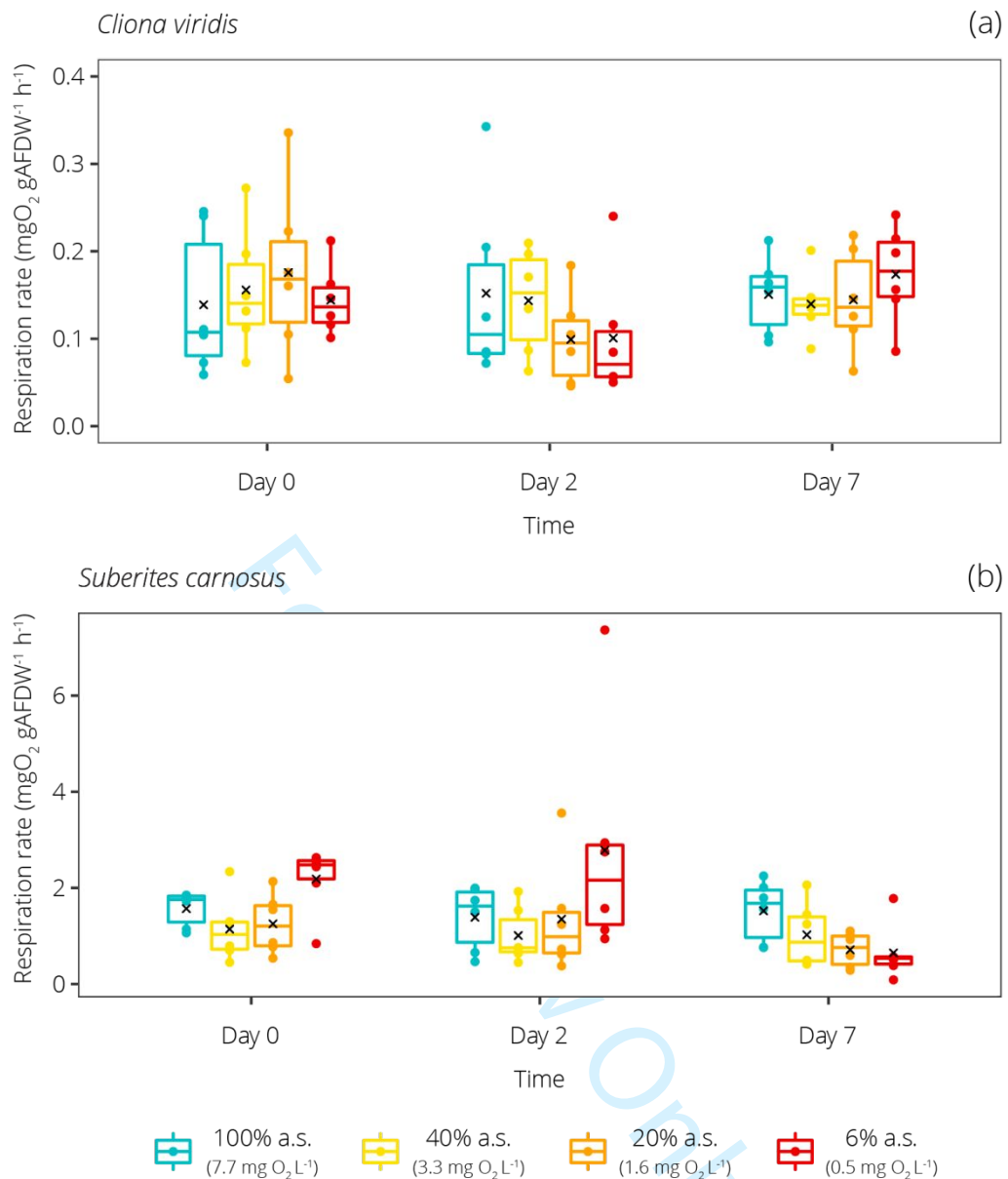944   46–58. https://doi.org/10.4319/lom.2005.3.46

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946

947 **FIGURES**

Cliona celata   (a)

Suberites carnosus   (b)

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Figure 1. Respiration rates in (a) *Cliona. celata* and (b) *Suberites. carnosus* from experiment 1 (moderate

hypoxia) measured at *T*0, *T*1/2 and *T-end*. Note: x-axis and y-axis scales differ between species. 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 dotsPoints represent outliersdata points.
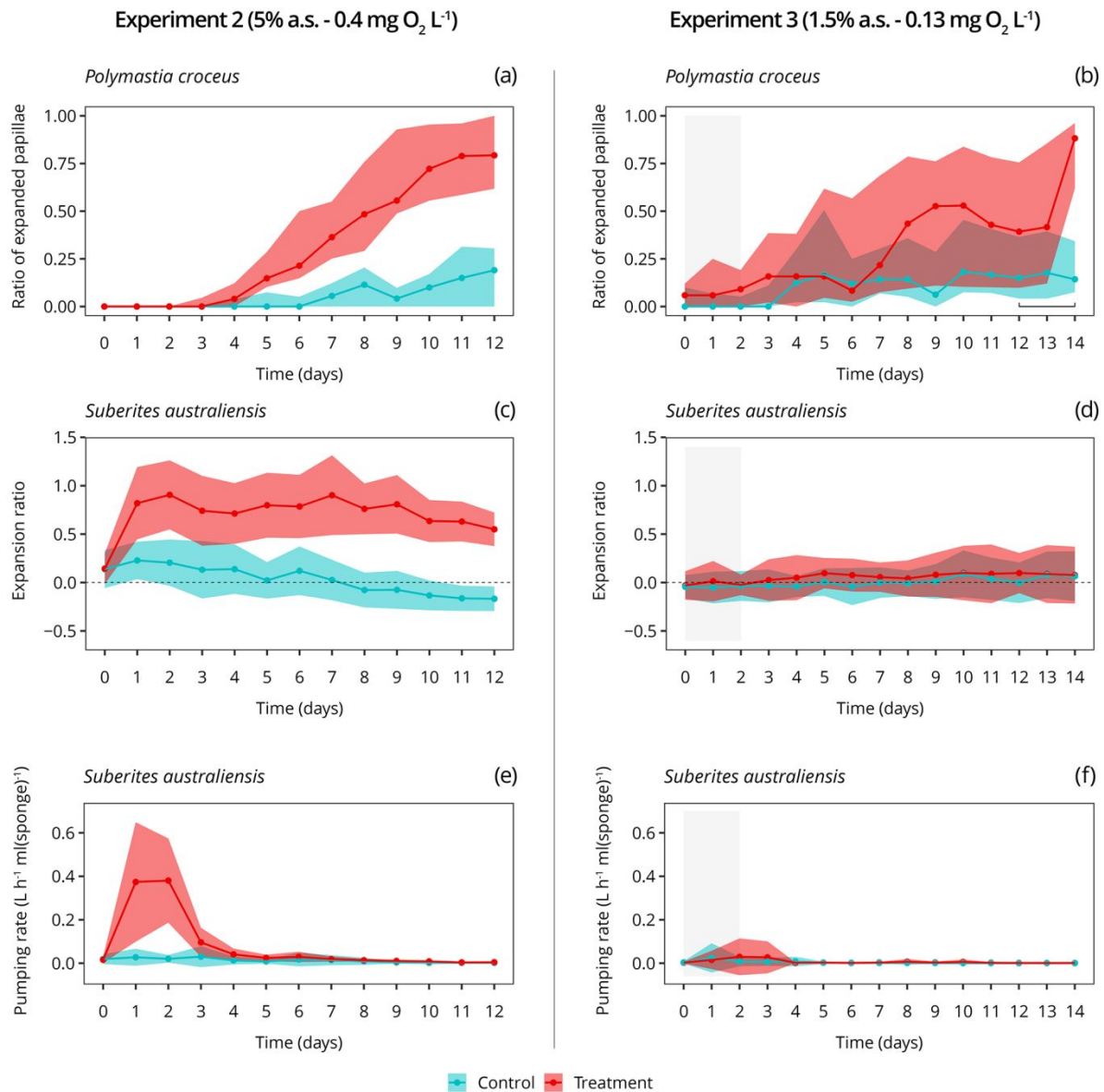
Figure 2. Contractile behaviour and pumping rate during experiments 2 and 3 (severe hypoxic conditions).

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*	

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	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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978

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.~~

Cnidarians

Bunodosoma cavernatum
Bunodosoma cavernatum
Acropora yongei
Cerianthiopsis americana
Metridium senile
Desmophyllum pertusum
Desmophyllum pertusum

Cirripeds

Amphibalanus amphitrite
Amphibalanus amphitrite
Megabalanus tintinnabulum

Bivalves

Dreissena polymorpha
Atrina japonica
Crassostrea virginica
Mytilus galloprovincialis
Mytilus galloprovincialis
Mytilus galloprovincialis

Algae and seagrasses

Bryopsis pennata
Zoostera marina

Sponges

Halichondria panicea
Polymastia croceus
Cliona celata
Suberites carnosus
Tethya wilhelma

○ Sublethal threshold
● Lethal threshold

Dissolved Oxygen (mg L^{-1})

984

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 $T0$ and $T1/2$.

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 $T0$ 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 $T0$ 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.

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 $T\text{-}end$, 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.

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 $T\text{-}end$, 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.

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.

287   **3 RESULTS**

288   **3.1 Sponge responses to moderate hypoxia**

289   All the sponges of experiment 1 survived the seven days of treatment, except one specimen of *Suberites*

290   *carnosus* in the lower DO treatment (6% a.s.)*,* which presented internal necrosis on the final day of the

291   experiment.

292   Mean buoyant weight variation between *T*0 and *T-end* ranged between -1% and -1.6% for *Cliona celata* and

293   +2.1% and -0.5% in *Suberites carnosus*. There were no differences in in buoyant weight variation among

294   treatments for both species, but for *C. celata* there was a significant slight decrease in weight in the 40%

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

296   For *Cliona celata*, there was no significant effect of time or treatment on the respiration rate (Tab. S4).

297   However, pairwise comparisons revealed a significant decrease (p = 0.028) in the 20% a.s. treatment

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

299   7, but both became non-significant after the correction for multiple comparisons (Tab. S4). However, the

300   data suggest a coherent temporal pattern in the respiration rate in both 20% a.s. and 6% a.s. treatments. *C.*

301   *celata* respiration rate decreased after two days from the start of the experiment and then increased until

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

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

304   For *Suberites carnosus*, there was a significant interaction of time and treatment (p = 0.007) on the

305   respiration rate (Tab. S5). Pairwise comparisons revealed a significant decrease in respiration rate between

306   day 0 and 7 (p < 0.0001), and day 2 and 7 (p < 0.0001) (Tab. S5). The respiration rate also slightly decreased

307   towards the end of the experiment in the 20% a.s. treatment (but not significantly), while in both the 100%

308   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$_2$S) 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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931 **FIGURES**
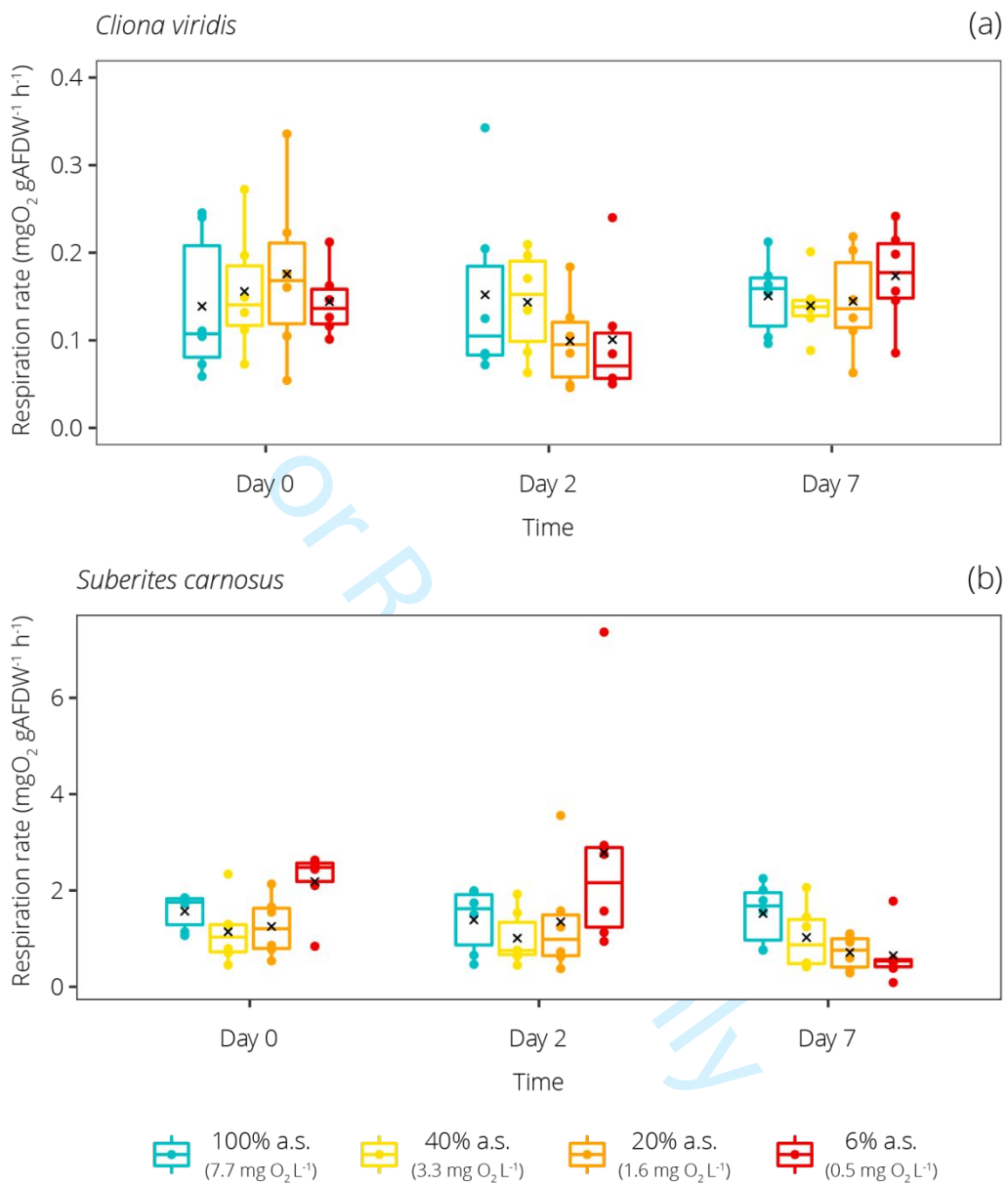
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934 Figure 1. Respiration rates in (a) *Cliona celata* and (b) *Suberites carnosus* from experiment 1 (moderate

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.
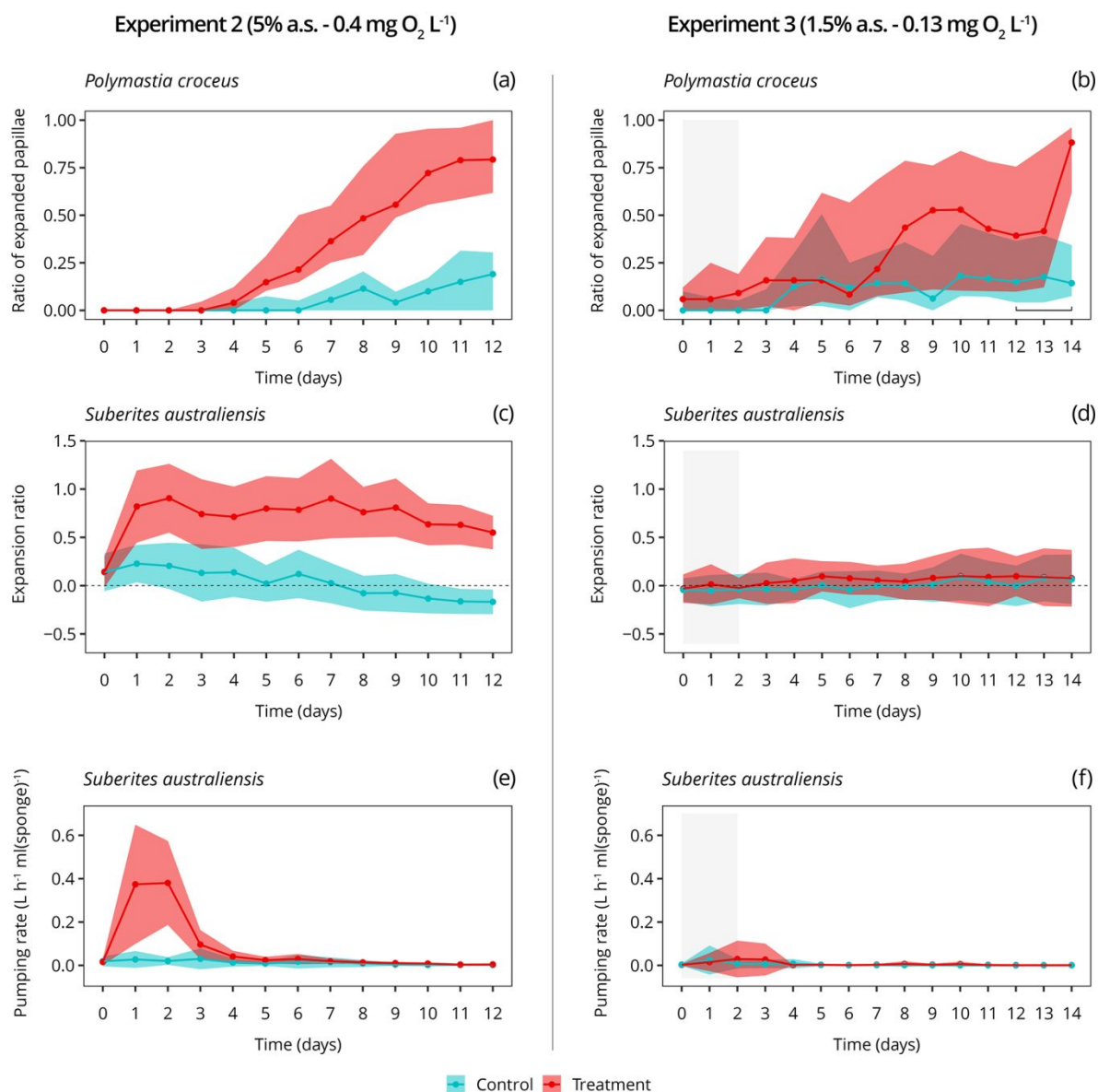
Figure 2. Contractile behaviour and pumping rate during experiments 2 and 3 (severe hypoxic conditions). Changes in the ratio of expanded papillae over time in *Polymastia croceus* in each treatment in experiments 2 (a) and 3 (b). Changes in the expansion ratio over time in *Suberites australiensis* 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.

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).

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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

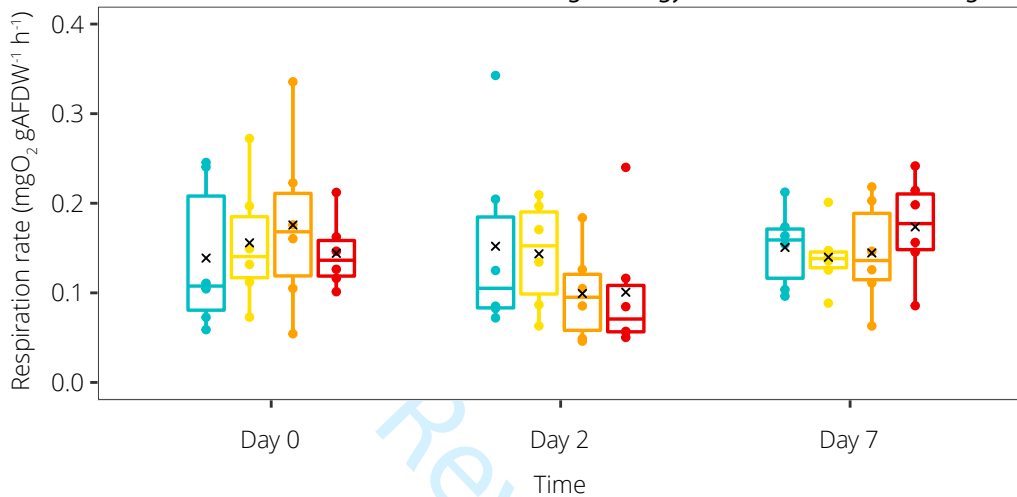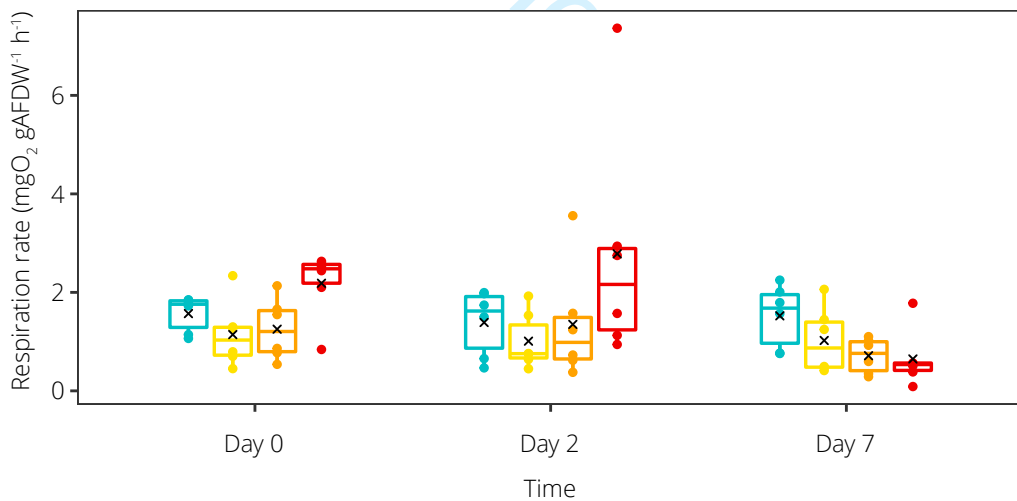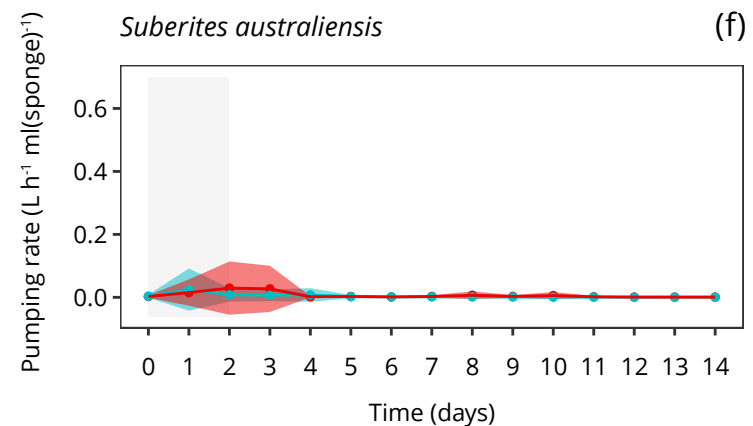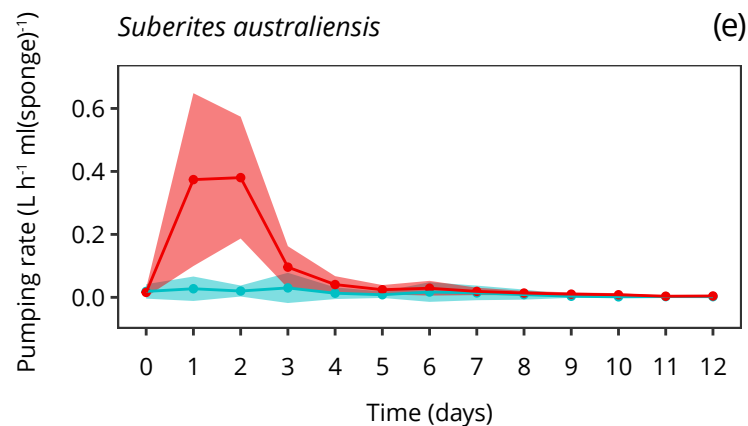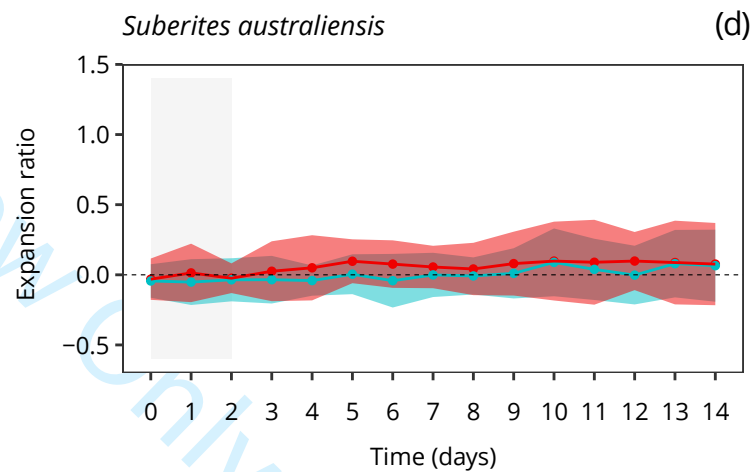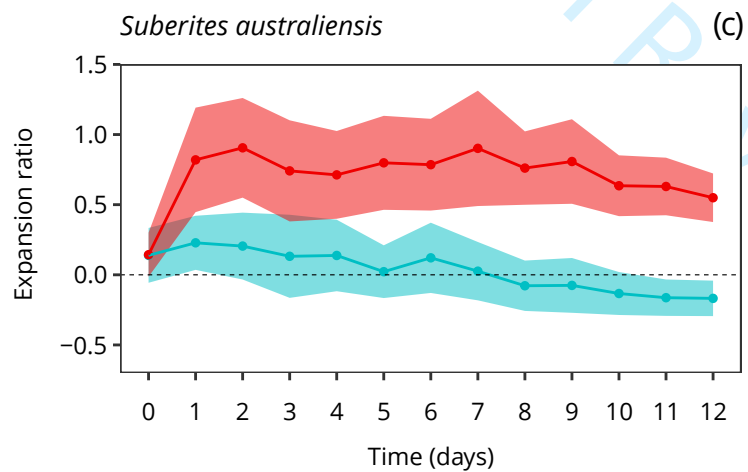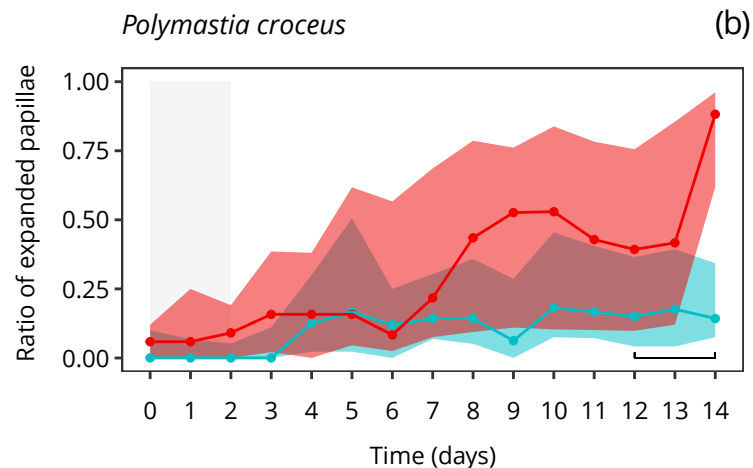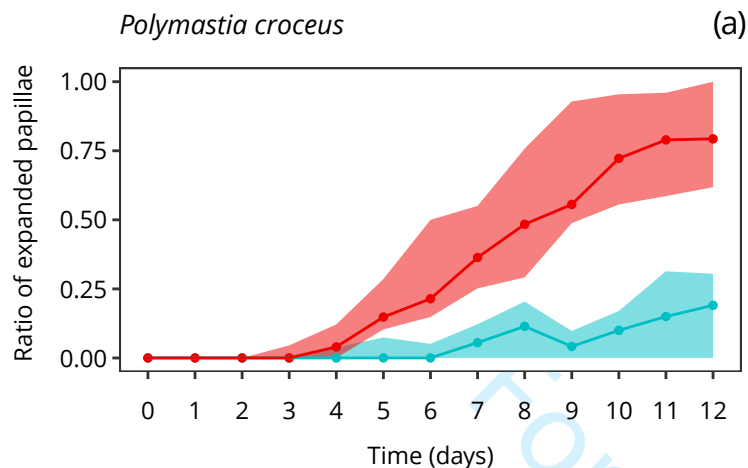961   boxplots correspond to the first and third quartiles, respectively. Points represent data points.

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).

Cliona viridis (a)

Suberites carnosus (b)

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})**

(a) *Polymastia croceus* — Ratio of expanded papillae vs Time (days)

(b) *Polymastia croceus* — Ratio of expanded papillae vs Time (days)

(c) *Suberites australiensis* — Expansion ratio vs Time (days)

(d) *Suberites australiensis* — Expansion ratio vs Time (days)

(e) *Suberites australiensis* — Pumping rate (L h^{-1} ml(sponge)$^{-1}$) vs Time (days)

(f) *Suberites australiensis* — Pumping rate (L h^{-1} ml(sponge)$^{-1}$) vs Time (days)
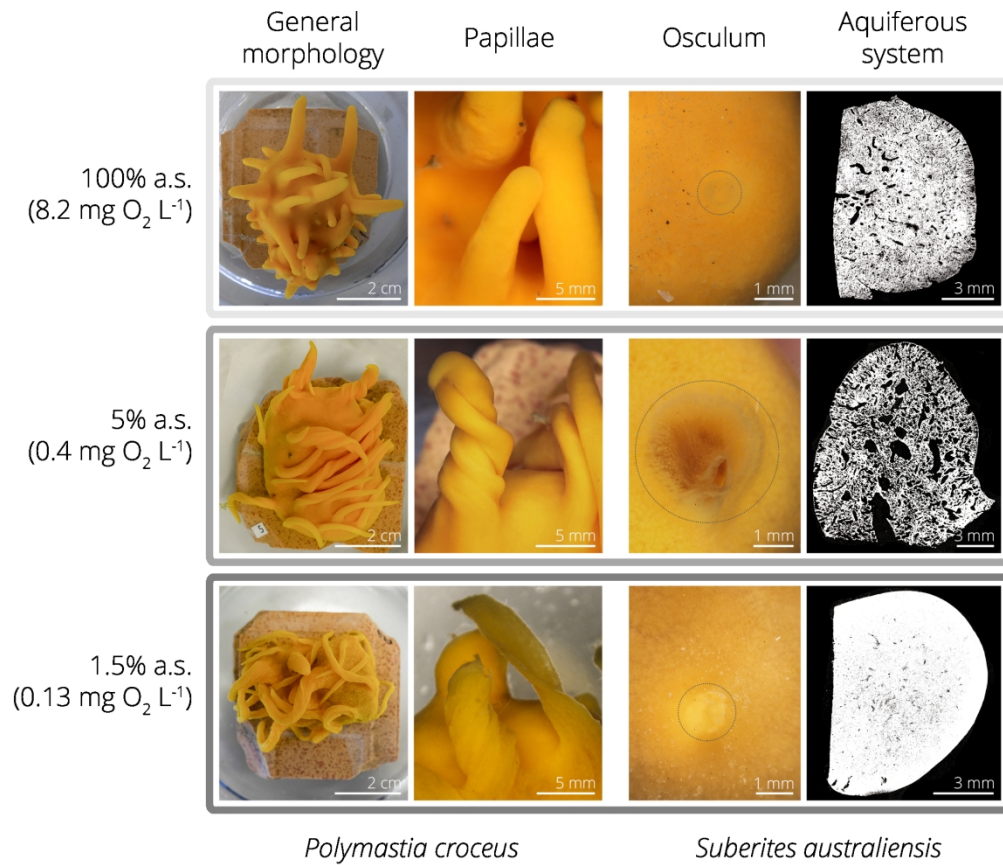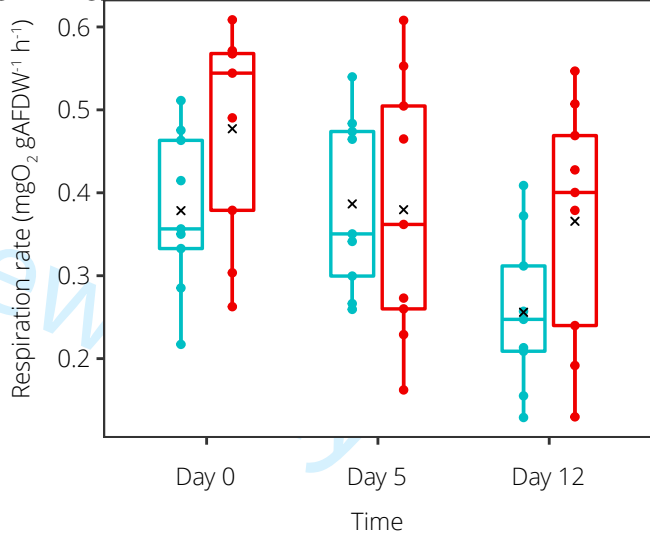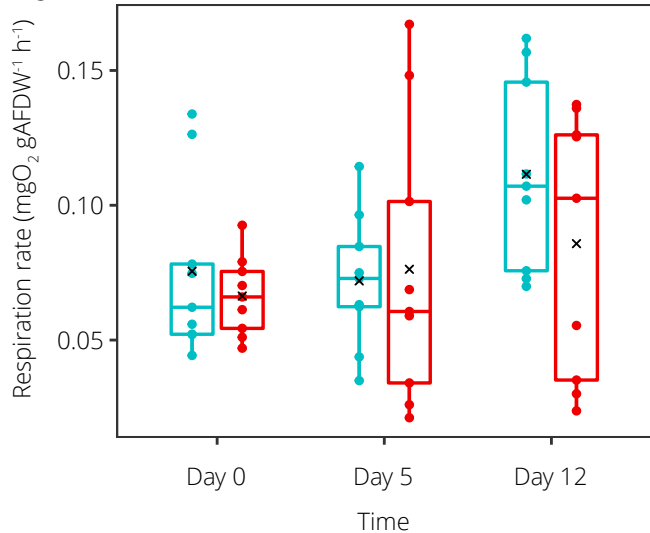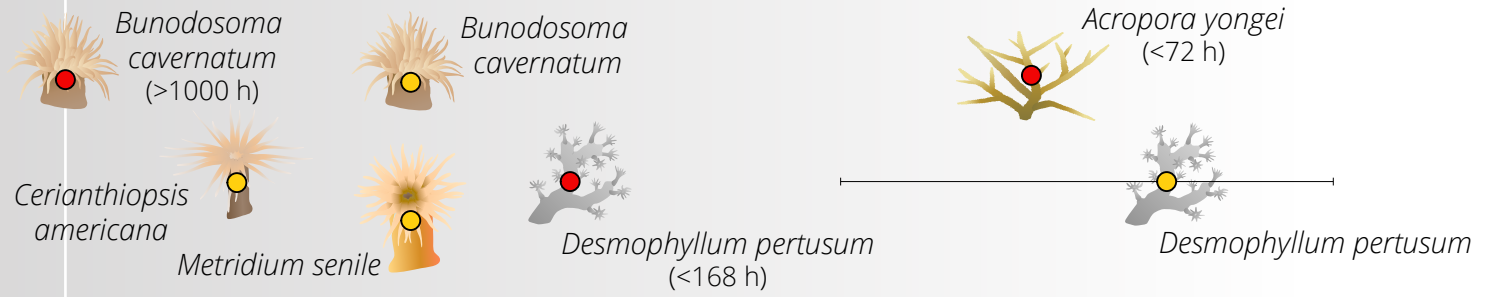
Control   Treatment

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).
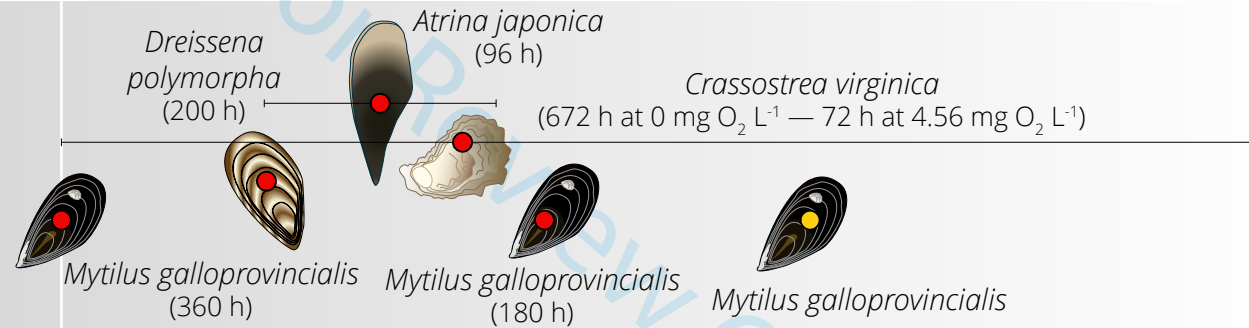
Cnidarians

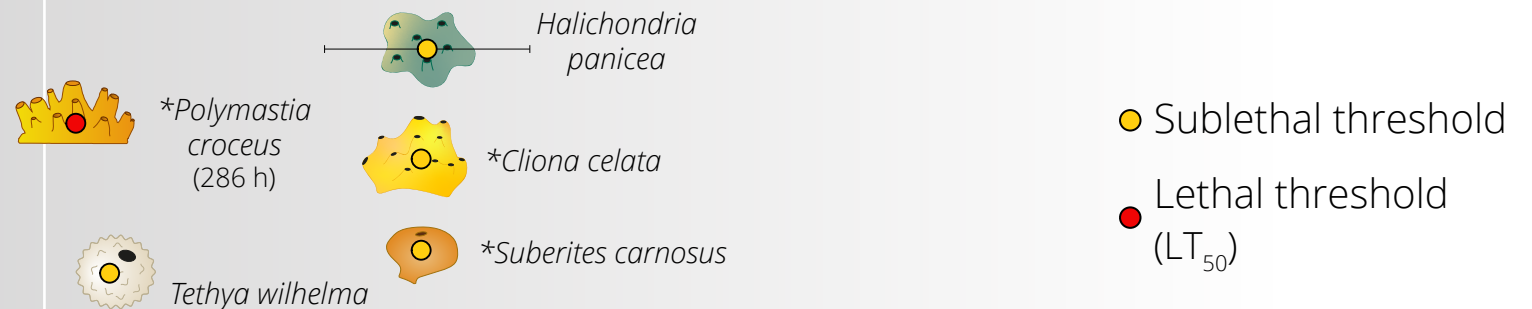*Bunodosoma cavernatum* (>1000 h)

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

○ Sublethal threshold

● Lethal threshold (LT$_{50}$)

Tethya wilhelma

Suberites carnosus

Dissolved Oxygen (mg L^{-1})