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REPORT

Coral larvae avoid substratum exploration and settlement in low-oxygen environments

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Abstract Oxygen is a critical resource that mediates a multitude of essential processes and interactions at multiple scales on coral reefs. In adult corals, it can directly or indirectly impact physiological processes, such as photosynthesis, respiration, and calcification. Moreover, many corals bleach as a consequence of being exposed to low oxygen. The sessile adult phase of corals makes habitat selection crucial for post-settlement survival and thus their pelagic larvae use a diverse array of cues to determine a suitable spot for settlement. However, the effects of oxygen on the early life stages of corals are still poorly known. This study investigated the importance of oxygen as a potential settlement cue and its effect on swimming and settlement behavior of coral larvae of two *Acropora* species. Two experiments were performed, one investigating coral larval swimming behavior under different oxygen conditions and the other studying coral larval settlement along an oxygen gradient. Bottom exploration, expressed as the percent of *A. cytherea* and *A. pulchra* larvae in the bottom section of experimental cylinders, was reduced by 96% and 100%, respectively, in hypoxic water compared to normoxic water. When offered the choice to settle on an otherwise preferred settlement substrate (*Titanoderma prototypum*) along an oxygen gradient, larvae of both coral species settled almost exclusively on *T. prototypum* fragments placed in well-oxygenated environments, with settlement rates increasing nonlinearly with oxygen concentrations. These results suggest that low-oxygen areas can negatively influence the settlement success of coral larvae and that oxygen concentration may be used as a cue for coral larval swimming and settlement behavior.

Keywords Oxygen · Hypoxia · Settlement · Coral larvae · Larvae behavior · Acropora

Introduction

Oxygen is a fundamental driver of the functioning and health of coral reef ecosystems, and the dynamics of coral reefs cannot be understood without comprehending the role of oxygen (Altieri and Gedan 2015; Nelson and Altieri 2019). It has recently been called the universal currency of coral reefs, as it is produced by photosynthetic organisms such as corals and algae, consumed by nearly all reef species, exchanged between mutualists, and suppressed by competitors and disease agents (Nelson and Altieri 2019). However, research on the drivers behind the degradation and decline of tropical coral reefs has so far mainly focused on elevated ocean temperatures, ocean acidification, eutrophication and overfishing (Pandolfi et al. 2003; Bruno et al. 2007; Hoegh-Guldberg et al. 2007; Mumby and Steneck 2008; Jackson et al. 2015; McCauley et al. 2015). It is only recently that low dissolved oxygen levels and hypoxia have gained attention (Mumby and Steneck 2008; Vaquer-Sunyer and Duarte 2008; Diaz and Breitburg 2009; Wangpraseurt et al. 2012; Côte and Knowlton 2013; Jorissen et al. 2016; Altieri et al. 2017; Nelson and Altieri 2019).

An analysis of global databases by Altieri et al. (2017) found that more than 10% of all coral reefs are at risk of

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hypoxic-related mortality events and that in all likeliness these events on tropical coral reefs are under-reported. Hypoxia-related mortality on coral reefs typically occurs because of a combination of factors that increase oxygen demand and/or prevent re-oxygenation (Nelson and Altieri 2019). These factors include coral spawn slicks (Hobbs and Macrae 2012; Andréfouët et al. 2015), excess organic matter and nutrients (Albert et al. 2012; Altieri et al. 2017), and increased seawater temperatures (Hobbs and McDonald 2010; Andréfouët et al. 2015; Altieri et al. 2017). The recent mass mortality events on coral reefs associated with hypoxia point toward the critical role of oxygen in coral reef environments and underlines the importance of understanding how reef organisms respond to low-oxygen environments (Diaz 2001; Altieri and Gedan 2015; Altieri et al. 2017; Nelson and Altieri 2019). Moreover, it is expected that climate change variables, including temperature, ocean acidification, sea-level rise, precipitation, wind, and storms, will act synergistically and with other anthropogenic factors, especially eutrophication, to increase the frequency and severity of oxygen-starved coastal waters (or "dead zones") (Altieri and Gedan 2015).

Low dissolved oxygen levels do not only have an impact at large spatial scales, but can also play a key role at smaller scales by influencing the distribution and abundance of sessile marine invertebrates (Ferguson et al. 2013). For example, it can be a primarily limiting resource, used and exploited in the competition between benthic organisms, such as in interactions between corals and algae at micro- to millimeter scales (Barott and Rohwer 2012; Ferguson et al. 2013; Gregg et al. 2013; Jorissen et al. 2016). Hypoxia has been suggested as a cause of mortality in corals interacting with turf or macroalgae (Smith et al. 2006; Barott et al. 2012). In terms of life history strategy, there are only two options for organisms living on coral reefs to avoid low-oxygen environments, they must either cope with or avoid these conditions. For mobile animals such as fish and zooplankton, this means swimming up vertically in the water column to more oxygenrich environments (Ludsin et al. 2009; Teuber et al. 2013; Schlaff et al. 2014). However, the option to avoid stressful low-oxygen environments is not available for sessile marine organisms, such as adult corals, for which oxygen is a critical direct and indirect driver of many physiological processes, such as respiration, photosynthesis, and calcification (Yonge 1932; Gardella and Edmunds 1999; Finelli et al. 2006; Colombo-Pallotta et al. 2010; Wijgerde et al. 2012, 2014).

Adult corals have coping strategies when encountering periods of hypoxia, such as switching from aerobic to anaerobic metabolic pathways (Vaquer-Sunyer and Duarte 2008). However, this is only a temporarily solution and requires a recovery period afterward (Murphy and Richmond 2016). The mobile larval phase of corals might be the only life-history stage during which corals can actively avoid low-oxygen environments. Coral larvae are weak swimmers and depend mostly on ocean currents for dispersal over long distances (Szmant and Meadows 2006; Hata et al. 2017). However, they have the ability to change their vertical position in the water, to swim down to explore the substrate and eventually settle, or to swim up to try to avoid unfavorable conditions by being transported away by ocean currents (Szmant and Meadows 2006). Indeed many larvae of marine invertebrates, including coral larvae, have been shown to use chemical and physical cues to direct their vertical swimming behavior and increase the probability that they encounter suitable settlement substrata (Raimondi and Morse 2000; Gleason et al. 2006, 2009; Szmant and Meadows 2006; Ritson-Williams et al. 2009; Lagos et al. 2015; Da-Anoy et al. 2017). Under hypoxic conditions, larvae of various sessile marine invertebrate taxa, including bryozoans, barnacles and oysters, can swim to avoid hypoxic waters, reduce their time spent in habitat exploration, or can delay or cease settlement altogether (Baker and Mann 1992; Lagos et al. 2015; Campanati et al. 2016). However, the effects of oxygen on coral larval behavior and settlement have never been studied.

In this study, the swimming behavior and settlement preferences of two species of spawning corals (*Acropora cytherea* and *A. pulchra*) to a range of oxygen conditions were tested in two laboratory experiments. The hypothesis was that coral larvae would respond by avoiding downward substratum exploration and settlement in hypoxic conditions.

Materials and methods

Coral colony collection and larval rearing

Colonies of *A. cytherea* (n = 12) and *A. pulchra* (n = 8) were collected one day after the new moon in September and October 2018, respectively, on the back reef at ca. 2 m depth on the West coast of the island of Moorea, French Polynesia (17° 33′ 14.4″ S 149° 53′ 07.5″ W) and transported to the CRIOBE research station. Colonies were kept in aquaria with flow-through sand-filtered water and constant aeration and checked each evening at 19 h. When signs of imminent sperm-egg bundle release were observed, colonies were isolated in 10 L buckets. Bundles from several colonies (n = 10 and 7 for *A. cytherea* and *A. pulchra*) were collected and mixed, and the mixture was distributed over several small plastic containers (500 ml), containing filtered seawater (0.45 µm, FSW) and left to fertilize for 2 h. After rinsing the sperm, embryos were

kept on an agitator at slow speed, in 12:12 light/dark regime and within a temperature range of 26–28 °C. This temperature range falls within the typical temperature range (26–30 °C) reported year around at ~ 2 m depth in the back reef of Moorea (Edmunds et al. 2010). Filtered seawater was changed every 12 h by partially draining the old containers and then pipetting the larvae into new containers with freshly filtered seawater. Dead larvae were simultaneously removed with the water changes. After 5 days, larvae were actively swimming in all directions and were assumed to have reached competency. They were used in experiments within the following 4 days, starting with Experiment 1 on Day 5 to 6 and Experiment 2 on Day 6 to 8. Experiments were conducted in the same temperature-controlled room and thus water temperature in all experiments stayed between 26–28 °C.

Experiment 1: Larval vertical swimming behavior assay

The effect of oxygen concentration on larval downward substratum exploration was tested in 500-mL graduated cylinders with a water column depth of 27 cm. Treatments were filtered seawater with three different oxygen concentrations: 45–60 µmol l^{-1}, 130–150 µmol l^{-1} and 190–200 µmol l^{-1}, thereafter called low, intermediate, and ambient oxygen treatments, respectively. The concentrations of the low and intermediate treatments were obtained by slowly diffusing nitrogen gas into the seawater, while continuously stirring the water and measuring the oxygen content (OXROB3 oxygen probe and FireSting O_2 oxygen meter, PyroScience, Germany) until they reached the desired concentration. No nitrogen was added to the ambient oxygen treatment (i.e., ambient oxygen concentration was used). The oxygen concentrations were chosen to represent oxygen regimes which have been measured at night at the edges of benthic competitors of corals: 45–60 µmol l^{-1} (cyanobacteria, macroalgae, thick ungrazed turf), 130–150 µmol l^{-1} (well grazed/short turf algae) and 190–200 µmol l^{-1} (corals, crustose coralline algae) (Barott and Rohwer 2012; Wangpraseurt et al. 2012; Gregg et al. 2013; Haas et al. 2013; Jorissen et al. 2016). Concentrations of 45–60 µmol l^{-1} correspond to 1.44–1.92 mg O_2 l^{-1} which is well below the conventional threshold value of 2 mg O_2 l^{-1} used to designate waters as hypoxic (Diaz and Rosenberg 1995; Steckbauer et al. 2011). However, many organisms can experience hypoxia impacts at higher oxygen concentrations (Vaquer-Sunyer and Duarte 2008). Concentrations of 190–200 µmol l^{-1} correspond to 6.08–6.40 mg O_2 l^{-1} which are below the limit of 6.8 mg O_2 l^{-1} used for hyperoxia and thus lie within the concentration range for normoxia (Nelson and Altieri 2019). Although we matched those oxygen concentrations, it should be noted that adding nitrogen also lowers CO_2 concentrations. While the present study chose to focus on the effects of reduced O_2 concentrations, it is not known how the joint lowering of CO_2 could impact larval behavior and settlement.

Eight replicate cylinders were randomly assigned to each of three oxygen treatments. In each container, fifteen actively swimming larvae were introduced in the middle of the cylinder. Every 30 min for a period of 2 h, the number of larvae that were swimming in the bottom 3 cm (equivalent to 50 mL), the top 3 cm and the middle of the cylinder were counted. Oxygen content of the cylinders was measured at the beginning and the end of each experimental run with the oxygen probe submerged at 5 cm from the surface. There was no significant difference between the oxygen concentration at the beginning and the end of each experiment in the graduated cylinders (ESM Table S1; Paired *t* test: *A. cytherea* $t_{23} = 1.963$, $p = 0.062$; *A. pulchra* $t_{23} = 1.834$, $p = 0.080$). Preliminary measurements in the bottom 3 cm, the middle and the top 3 cm of 4 randomly selected cylinders over a 4-h duration at two oxygen concentrations (~ 50 and ~ 150 µmol l^{-1}) showed no differences in oxygen concentrations within the water column of the cylinders (Repeated measures ANOVA; 50 µmol l^{-1}: $F_{2,18} = 1.28$, $p = 0.301$; 150 µmol l^{-1}: $F_{2,18} = 0.60$, $p = 0.563$).

To assess the effects of species and oxygen treatment on larval downward substratum exploration, the percent of larvae found in the bottom 3 cm of the cylinders was analyzed using a linear mixed effects model to approximate a repeated-measures ANOVA (*lmer* function, *lme4* package; Bates et al. 2015), with species (2 levels), oxygen treatment (3 levels) and time point (4 levels) as fixed factors, and day as random factor. Model selection was performed by generating a set of models with all possible combinations of the terms in the global model using the *dredge* function (*MuMIn* package). The best fit model was selected using corrected Akaike Information Criterion (AIC) scores. A type III analysis of variance with Satterthwaite's method on the main effects was produced (*survey* package). Tukey's honestly significant difference (HSD) posthoc tests were conducted to test for significant differences within oxygen treatments for each species (*emmeans* package). All analyses were performed using R (v.3.3.5).

Experiment 2: Larval settlement assay

The effect of oxygen concentration on coral larval settlement was tested in four 40-cm-long hypoxitron tubes with a diameter of 4.8 cm and a volume of 700 ml (Fig. 2). The tubes were adapted from the design of the "hypoxitron 2" by Bodamer and Bridgeman (2014). The oxygen gradient

in each hypoxitron was created by pumping ambient air on one end and nitrogen on the other end in smaller tubes with microscopic holes during the entire duration of the experiment. The smaller tubes were attached and sealed at the bottom of the bigger plastic tube. The microscopic holes created micro-bubbles, barely visible to the naked eye, which generated very little water movement. During the testing of the hypoxitron, a coral larva was pipetted in the middle of the tube and its position and swimming behavior were followed for a period of 5 min. The test was repeated 20 times with new larvae. The micro-bubbles did not disturb the swimming trajectories of the larvae and the larvae could equally access all areas of the hypoxitron.

The experiment was run over 3 consecutive days. The end of the hypoxitron receiving ambient air and the end receiving nitrogen were initially assigned randomly and switched the next day. Fragments of the CCA *Titanoderma prototypum* were collected daily in the backreef of Moorea (17° 28′ 51.5″ S 149° 50′ 52.6″ W). In the Pacific, the genus *Titanoderma* has a remarkable capacity to induce coral recruitment (Harrington et al. 2004; Price 2010; Doropoulos et al. 2012). In each hypoxitron, 6 pieces, cut to a 1 cm^2 size, were placed every 5 cm. One single piece was cut out from one CCA fragment. Therefore, each piece represented one CCA individual. Pieces were haphazardly allocated across the tubes. At the start of the experiment at 1900 h, 30 actively swimming larvae were introduced in the middle of each hypoxitron and at 0900 h the next morning the number of coral larvae that had successfully settled on each CCA chip or still swimming were counted under a binocular microscope. Oxygen was measured above each CCA piece with OXROB3 oxygen probes and FireSting O2 oxygen meter (PyroScience, Germany) to assess oxygen concentrations along the gradient at the beginning and end of each experimental day. There was no significant difference in oxygen concentrations at the beginning and end of the experiments (ESM Table S2; Paired *t* test: *A. cytherea* $t_{71} = -0.086$, $p = 0.932$; *A. pulchra* $t_{71} = -0.559$, $p = 0.578$). There were no visible differences in the physical appearance of the *Titanoderma* fragments at the end of the experiments, notably in the oxygen-poor vs. oxygen-rich ends of the hypoxitrons, suggesting that the short hypoxia had no effect on the settlement substrate. All larvae that had not settled at the end of the experiments were still swimming and were not dead, indicating that they did not find a suitable substrate or were not ready to settle.

A generalized linear mixed effects model with binomial distribution (*glmer* function, *lme4* package; Bates et al. 2015) was used to model probability of settlement and to assess the relationship between coral settlement and oxygen concentration. Species (2 levels) was used as a categorical fixed factor, and oxygen concentration was added as continuous fixed factor to the model. Since the oxygen treatment was not independent within a tube and the experiment was conducted over 3 consecutive days, hypoxitron tube and experimental day were included as random factors. Oxygen concentrations were calculated for each chip by averaging beginning and end oxygen measurements. Model selection was conducted as detailed for Experiment 1 above. The model output was then plotted as a regression with individual curves and confidence intervals for each coral species and raw data points overlaid, using the R packages *sjPlot* and *ggplot2* (Wickham 2016; Lüdecke 2020).

Results

Experiment 1: Larval vertical swimming behavior

There were significant effects of coral species and oxygen treatment on the percentage of larvae in the bottom 3 cm of the graduated cylinders, as well as a significant interaction between these factors (Table 1; Fig. 1). There was no significant difference on the percentage of larvae in the bottom 3 cm of the graduated cylinders across time points. The percentage of larvae in the bottom 3 cm declined from an average of 10.83% (± 0.88 SEM) in the ambient oxygen treatment to 0.41% (± 0.29) in the low-oxygen treatment for *A. cytherea*, and from 4.38% (± 0.71) to 0% (± 0) for *A. pulchra*, representing reductions of 96% and 100% in bottom exploration between the ambient and low-oxygen treatments for each species, respectively. Post hoc tests showed that bottom exploration in *A. cytherea* differed significantly between all oxygen treatment levels. For *A. pulchra*, there was no difference between the low and intermediate oxygen treatments. In this species, almost no larvae were found exploring the bottom in these two

Table 1 ANOVA (Type III) summary with Satterthwaite's method of linear mixed effects model on the effects of coral species (*Acropora pulchra* and *A. cytherea*), oxygen treatment (low, intermediate and ambient) and time point (30 min, 60 min, 90 min, and 120 min) on the percent of larvae in the bottom 3 cm of the cylinders

Fixed factors	*F*	*df*	*p*
Species	65.92	1	**< 0.001**
Oxygen treatment	51.41	2	**< 0.001**
Time point	0.388	3	0.534
Species × oxygen treatment	14.14	2	**< 0.001**
Random factors	Variance		SD
Day	0.204		0.452

Significant *p*-values ($p < 0.05$) are indicated in bold

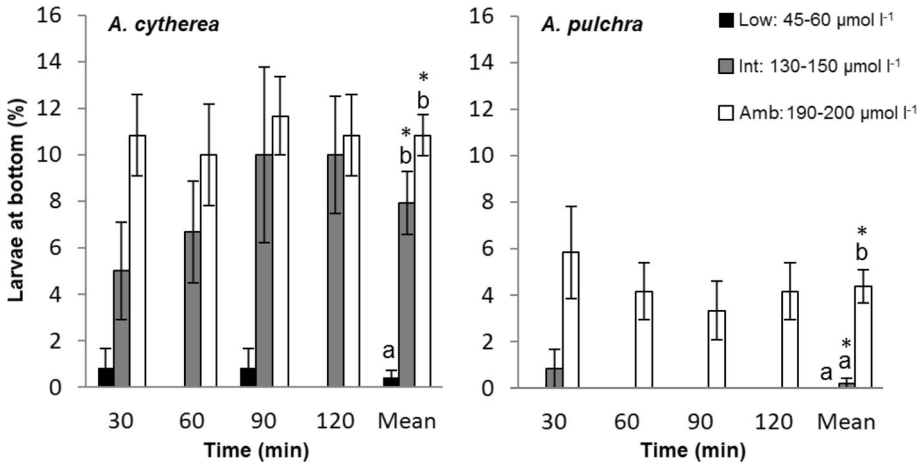

Fig. 1 Percent of coral larvae (mean ± SE, n = 8) of **a** *Acropora cytherea* and **b** *A. pulchra*, swimming in the bottom 3 cm of the water column depth of 27 cm in the graduated cylinders for the three oxygen treatments. Letters above bars indicate significant differences among oxygen treatments on data averaged across time points for each species and asterisks indicate differences between coral species for each treatment (Tukey's HSD)

treatments. Fewer *A. pulchra* larvae explored the bottom compared to *A. cytherea* larvae.

Experiment 2: Larval settlement

There were significant effects of coral species and oxygen concentrations on settlement rates, with no interaction between these factors (Table 2; Fig. 2). The random factors tube and day hardly contributed to the variation, providing confidence that the statistical results are robust. Larvae settled almost exclusively on *T. prototypum* fragments placed in well-oxygenated environments, with settlement rates increasing nonlinearly with oxygen concentrations. Only 2.1% and 0.0% of the *A. cytherea* and *A. pulchra* larvae, respectively, settled in concentrations below 100 µmol l^{-1}. The probability of settlement of *A. cytherea* was higher than that of *A. pulchra*. There was an overall settlement rate of 26.94% (± 1.66 SEM) for *A.cytherea* and 17.78% (± 0.85 SEM) for *A.pulchra*.

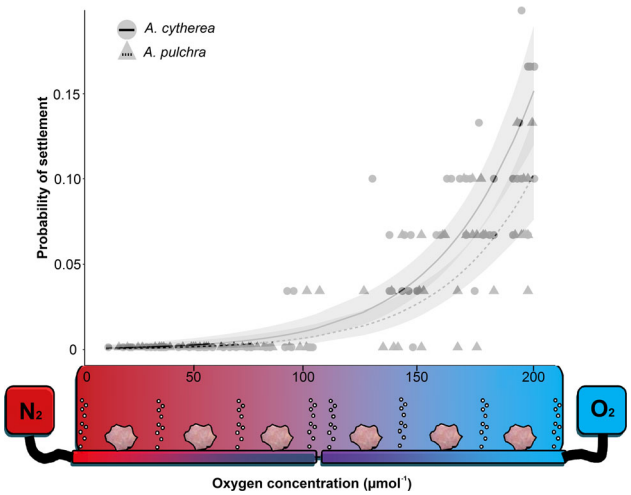

Fig. 2 GLMM model predictions for the probability of settlement for *Acropora cytherea* (solid line ± 95% confidence interval) and *A. pulchra* (dashed line ± 95% confidence interval) larvae on *Titanoderma prototypum* fragments as a function of oxygen concentration measured along the hypoxitrons. Actual settlement proportions on individual *T. prototypum* fragments are overlaid on model predictions as circles (*A. cytherea*) and triangles (*A. pulchra*)

Table 2 ANOVA summary of generalized linear mixed effects (GLMM) model on the effects of coral species (*Acropora pulchra* and *A. cytherea*) and oxygen concentration on the probability of settlement

Fixed factors	χ^2	*df*	*p*
Species	7.12	1	**0.008**
Oxygen concentration	104.49	1	**< 0.001**
Species × oxygen concentration	< 0.001	1	0.977
Random factors	**Variance**		**SD**
Day	< 0.001		< 0.001
Tube	< 0.001		< 0.001

Significant *p*-values ($p < 0.05$) are indicated in bold

Discussion

Oxygen is a very important environmental variable for adult corals, as it can directly or indirectly impact physiological processes, such as photosynthesis, respiration, and calcification. Moreover, many corals bleach as a consequence of being exposed to low-oxygen conditions (Yonge 1932; Zhu 2004; Altieri et al. 2017; Nelson and Altieri 2019). Our results suggest that corals can also be severely impacted by low-oxygen conditions during the settlement stage. Larvae of both *Acropora* species avoided bottom exploration in reduced oxygen environments. When offered the choice to settle on an otherwise preferred settlement substrate (*Titanoderma prototypum*) in oxygen-rich

or oxygen-poor environments, they settled almost exclusively in the oxygen-rich environment.

Chemotaxis, or the use of chemical cues, is very common among marine invertebrates as a mechanism to enhance their fitness by selecting optimal settlement habitats (Morse 1990; Rodriguez et al. 1993; Hadfield and Paul 2001; Dumas et al. 2014). Since habitat selection has a strong influence on their post-settlement survival, coral larvae are known to use a plethora of physical and chemical cues to find a suitable settlement substrate (Babcock and Mundy 1996; Harrington et al. 2004; Birrell et al. 2008; Ritson-Williams et al. 2009; Da-Anoy et al. 2017). However, the use of oxygen as a chemical cue, or oxytaxis, has not been extensively studied. While it is a common mechanism used by bacteria and microalgae to avoid low-oxygen environments (Hillesdon and Pedley 1996; Porterfield 1997), there are hardly any studies on the use of oxygen as a chemical cue in marine invertebrates. Low-oxygen conditions increase both mortality and time to metamorphosis of post-larvae of brachyuran crabs, suggesting that these crabs use oxygen as a cue to delay post-larval metamorphosis (Forward et al. 2001; Tankersley et al. 2002). Another example is a recent study by Lagos et al. (2015), which showed that under low oxygen levels, larvae of the bryozoan *Bugula neritina* reduced the time spent in habitat exploration and delayed settlement. To our knowledge, the study by Lagos et al. (2015) was the only study so far demonstrating oxytaxis in a marine invertebrate with a biphasic lifecycle and our study is the first to suggest oxytaxis in coral larvae.

In the reef environment, oxygen regimes vary enormously at small scales and according to benthic organisms (Ferguson et al. 2013; Gregg et al. 2013; Jorissen et al. 2016). For example, diurnal variations in surface oxygen concentrations are much larger over macroalgae compared to corals and crustose coralline algae (Jorissen et al. 2016). Therefore, the behavioral avoidance of low-oxygen conditions, suggested by our study, might lead to the preferential settlement of coral larvae on or near environments or substrates within oxygen levels in the ambient range (i.e., 190–200 µmol l^{-1}). Such behavioral drivers of settlement preferences could increase the chance of larvae to settle in a more favorable oxygen environment and represent a short-term coping strategy for enhanced survival in a heterogeneous oxygen environment. While coral larvae can survive for a long time in the water column, the energy spent on swimming or staying in the water column cannot be used for post-settlement growth (Graham et al. 2008; Ritson-Williams et al. 2016). Therefore, if conditions are constantly suboptimal, this strategy could also result in larvae having to settle with low energy reserves, or not settling at all. To better predict the effect of hypoxic waters on coral recruitment, further research should investigate the swimming response of larvae that have been previously exposed to low-oxygen conditions and test whether their avoidance behavior declines over time or varies in relation with energy reserves. The ambient oxygen treatment was similar to values found on crustose coralline algae, while the low-oxygen treatments were typical of those found on thick (ungrazed) turf algae and macroalgae (Barott and Rohwer 2012; Haas et al. 2013; Jorissen et al. 2016). These last two functional groups are known to be hostile competitors for coral recruits and thus well worth avoiding by coral larvae (McCook et al. 2001; Ritson-Williams et al. 2009; Mumby et al. 2013; Webster et al. 2015; Elmer et al. 2018). In contrast, crustose coralline algae are commonly favored by coral recruits (Morse and Morse 1991; Morse et al. 1994; Heyward and Negri 1999; Harrington et al. 2004; Ritson-Willimas et al. 2016). It is plausible that the advantages of avoiding low-oxygen environments are more just than physiological, but also that low oxygen levels are used by coral larvae as a proxy of competition intensity. Marine invertebrate larvae can delay settlement to avoid dominant competitors (Young and Chia 1981; Birrell et al. 2008; Rius et al. 2009; von der Meden et al. 2015). It is still unclear how larvae can recognize and classify many different species and their abilities as competitors. Using a proxy such as oxygen to gauge for the presence of competitors could potentially provide coral larvae with the information they need.

Adding nitrogen gas reduces both O_2 and CO_2 concentrations and thus differs from reductions in O_2 in natural systems, which are typically coupled with increases in CO_2 due to increased metabolic activity. While we cannot rule out that low CO_2/high pH confounded our results, we believe that it is unlikely. Early life stages of marine calcifying organisms are highly vulnerable to changes in the seawater carbonate system associated with reduced pH (Kroeker et al. 2013). In contrast, to our knowledge, there is no study showing negative effects of elevated pH on the settlement of marine invertebrates. On the contrary, high pH has been linked to enhanced recruitment in several species of oysters and it has been proposed that oyster larvae use high pH as a cue to detect areas within an estuary with high phytoplankton productivity as a food source and abundant oxygen for growth (Coon et al. 1990; Anderson and Underwood 1994; Anderson 1996). Future work should explore the separate and joint effects of dissolved O_2 and CO_2 gases on coral settlement to better assess the effects of oxygen depletion in the natural environment. In particular, high CO_2 condition has been shown to negatively affect the early life stages of calcifying marine invertebrates (Byrne 2011), including corals (Albright 2011; Edmunds et al. 2013; Foster et al. 2015; Jiang et al. 2015; Olsen et al. 2015; Fabricius et al. 2017). Therefore, in situ low O_2/high CO_2 conditions are likely to

reduce settlement even further and affect the ability of coral settlers to calcify.

In the settlement assay, the low-oxygen conditions could have altered the composition of the microbial communities or the biochemistry of *T. prototypum* and the larvae could have responded to these changes instead of the changes in oxygen concentrations. Disentangling these mechanisms would require running further experiments using chemical extracts of *T. prototypum*. However, previous research has shown that short term exposure (< 24 h) of settlement substrates to stressors such as low pH or high temperatures does not cause changes in settlement patterns of coral larvae (Albright et al. 2008; Webster et al. 2013). Moreover, the results of the swimming behavior assay (i.e. without settlement substrate) support that coral larvae respond to different oxygen concentrations.

In adult corals, several case studies of hypoxic events have shown that there are differences in tolerance to hypoxia between coral genera. Guzmán et al. (1990) found that an hypoxic event following a dinoflagellate bloom in Caño island (Costa Rica) caused 90–100% mortality among *Pocillopora* spp., while other species, such as *Porites lobata, Gardineroseris planulata, Pavona clavus* and *Pavona gigantea,* were unaffected. Generally massive and encrusting corals, such as *Porites* and *Favia* spp., are the least affected by hypoxic events, while branching and solitary corals, such as *Acropora, Pocillopora, Stylophora, Fungia* spp., suffer high mortality rates (Guzmán et al. 1990; Simpson et al. 1993; Adjeroud et al. 2001). In our first experiment, we saw a difference in larval behavior in relation to oxygen conditions between coral species from the same genus. Unlike *A. cytherea*, *A. pulchra* avoided exploring the bottom at intermediate oxygen concentrations, suggesting a possible species-specific larval responses to low-oxygen environments. These differences could be important to predict recruitment success and survival in certain locations and to make informed choices on which coral species can be successful to replenish coral reef areas that experience hypoxic events.

In sum, our results support that coral larvae are able to detect and to react to variations in local oxygen environments. This sensory ability could help them avoiding to settle in unfavorable environments (e.g., surface and vicinity of thick turf algae and macroalgae). Our findings add further concerns about the prevalence of deoxygenated water on coral reefs globally (Altieri et al. 2017). Decreases in available oxygen could reduce coral recruitment and impact the resilience of coral reefs, which is highly dependent on the ability of corals to settle after disturbances (Ritson-Williams et al. 2009). If local oxygen regimes influence successful coral propagation and survival, they could represent an important factor to take into account when considering placement of coral reef nurseries, choices of reefs to protect and chances of success of reef recovery efforts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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