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RESEARCH ARTICLE

Increasing acidification does not affect sexual reproduction of a solitary zooxanthellate coral transplanted at a carbon dioxide vent

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Abstract

The absorption of atmospheric carbon dioxide is causing significant changes to the carbonate chemistry of the ocean, in a phenomenon called ocean acidification. The latter makes it potentially more difficult for marine calcifiers like corals, to build their calcium carbonate structures, thus affecting their ability to survive and reproduce. Research on how ocean acidification impacts coral sexual reproduction has focused on tropical species investigated under controlled conditions in aquaria, lacking insights into the intricate natural environment. Here we show that the sexual reproduction of the zooxanthellate solitary scleractinian *Balanophyllia europaea* transplanted at a CO_2 vent off the Island of Panarea (Tyrrhenian Sea, Italy) for up to 5 months is unaffected by decreasing pH (pH range 8.1–7.4). These findings reinforce earlier evidence, suggesting that zooxanthellate corals may exhibit a certain degree of short-term resilience to ocean acidification. However, the interplay between ocean acidification and additional environmental stressors, including warming, will ultimately define the boundaries that distinguish winners and losers amid swift climatic changes.

Since the onset of industrialization, atmospheric concentrations of carbon dioxide (CO_2) have increased exponentially. Approximately 40% of the CO_2 emitted into the atmosphere by human activities has been absorbed by the ocean (Feely et al. 2004; Gruber et al. 2019; Terhaar et al. 2022), causing significant changes to the carbonate chemistry (Feely et al. 2004). Surface ocean pH has decreased by 0.1 units in comparison to preindustrial values and the Intergovernmental Panel on Climate Change predicts a further decrease by 0.06–0.32 units by the end of the century, causing a further increase by more than 100% in hydrogen ions concentration in global oceans (Cubasch et al. 2014).

Documenting and projecting the response of marine ecosystems to changing ocean chemistry has been of increasing concern in the scientific community (Duarte 2014). Ocean acidification makes it difficult for marine calcifiers, like corals, to build their calcium carbonate structures (Kleypas and Yates 2009). Weakening these structures affects their ability to survive and reproduce. The recovery and persistence of a population and of a species requires that reproduction and

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Chiara Marchini and Fiorella Prada contributed equally to this work (co-first authors).

This paper is dedicated to the memory of Prof. Zvy Dubinsky who led a key role in this research, but is no longer with us.

recruitment keep pace with the loss of adult individuals (Santiago-Valentín et al. 2019). However, environmental factors can disrupt these processes, resulting in compromised recruitment or recruitment failure, profoundly affecting marine population dynamics (Gaines and Roughgarden 1985; Riegl et al. 2009).

Reproduction and early life history stages of different marine invertebrates are affected by acidification (Ross et al. 2011) in terms of: larval availability (gamete production), fertilization (Kurihara et al. 2004; Reuter et al. 2011; Swiezak et al. 2018), larval development (Byrne 2011; Kurihara 2008; Lenz et al. 2019), larval growth (Kurihara et al. 2004; Lenz et al. 2019), larval settlement (Kurihara 2008), post-settlement growth, and survival (Byrne 2011). Nonetheless, limited information is available on the effects of ocean acidification on sexual reproduction in corals (Albright 2011). Gametogenesis can extend over 9–11 months for some species (Babcock et al. 1986; Vargas-Angel et al. 2006), making studies on coral gamete development challenging when performed under controlled conditions. The aquaria and mesocosm experiments that have been conducted so far on the impact of ocean acidification on coral reproduction have shown negative effects on sperm motility (Morita et al. 2010; Nakamura and Morita 2012), fertilization process (Albright and Mason 2013) and early life stages, including larval development and settlement (Albright and Langdon 2011; Albright et al. 2010; Chua et al. 2013; Doropoulos et al. 2012).

Underwater carbon dioxide (CO_2) vents are areas in the ocean where CO_2, generally of volcanic origin, is naturally released from the seafloor (Aiuppa et al. 2021). Volcanic CO vents in regions such as Italy (e.g., Goffredo et al. 2014; Hall-Spencer et al. 2008; Johnson et al. 2013), Japan (Agostini et al. 2018; Inoue et al. 2013), Mexico (Crook et al. 2013, 2016), and Papua New Guinea (Fabricius et al. 2011; Priest et al. 2024) have maintained low pH levels and altered carbonate chemistry for prolonged periods, sometimes spanning years or decades. Consequently, these naturally occurring sites offer a rare chance to investigate the impacts of ocean acidification on marine life under environmental conditions that cannot be fully replicated in laboratory aquaria (e.g., dynamic currents, nutrient availability, and predator–prey interactions) (Meron et al. 2013; Strahl et al. 2015). Research at these locations has revealed declines in species richness, habitat complexity, and functional diversity with decreasing pH, with calcifying species being particularly affected (Fabricius et al. 2014; Linares et al. 2015; Teixidó et al. 2018). Specifically, the CO_2 vent off Panarea Island (Sicily, Italy) has been extensively studied in the past decade (Capaccioni et al. 2007; Caroselli et al. 2019; Fantazzini et al. 2015; Goffredo et al. 2014; Prada et al. 2017, 2023; Sani et al. 2024). The solitary non-zooxanthellate coral *Leptopsammia pruvoti*, transplanted for 3–4 months at the Panarea CO_2 vent, experienced a slight postponement in sperm release, fertilization, and embryo formation with decreasing pH (Gizzi et al. 2017). Likewise, spermary development and the fertilization process were delayed by decreasing pH in the colonial non-zooxanthellate coral *Astroides calycularis* exposed for 3 months under the same experimental conditions (Marchini et al. 2021).

The present study investigated the short-term influence of decreasing seawater pH on the reproductive output of the solitary zooxanthellate coral *Balanophyllia europaea* transplanted for up to 5 months at the Panarea CO_2 vent site. We hypothesize that the symbiotic relationship with photosynthetic algae, which utilize CO_2 as a substrate for photosynthesis and energy production, might allow this zooxanthellate coral to reproduce under acidified conditions.

Materials and methods

Study site

The experimental field is located near Panarea Island (Mediterranean Sea, Italy, 38°38′16.98″N; 15°6′37.26″E) and is part of an active volcanic system (De Astis et al. 2003). An underwater crater (20 × 14 m wide) characterized by continuous and localized emissions of almost pure CO_2 (98–99% of CO_2), generates a natural pH gradient (Goffredo et al. 2014). Along this gradient, four sites, whose seawater physicochemical parameters have been extensively described (Goffredo et al. 2014; Prada et al. 2017), include: a control site (site 1, mean pH in total scale [pH_T] 8.07) located ~ 34 m away from the center of the crater, representing the current condition, two intermediate pH sites (site 2, pH_T 7.87 and site 3, pH_T 7.74), that align with Intergovernmental Panel on Climate Change's mean pH prediction of a conservative CO_2 emissions scenario (Representative Concentration Pathway, RCP6.0), and a "business-as-usual" CO_2 emissions scenario (RCP8.5), respectively, and an extreme pH site (site 4, pH_T 7.40) closer to the vents, representing the projection for 2300 (Fig. 1) (Collins et al. 2014; Intergovernmental Panel on Climate Change 2021). Previous work at these sites indicates no significant differences in, for example, food availability, nutrients, or light exposure, as site 1 (control) is located only 34 m from site 4 (lowest pH) at roughly the same depth, and strong local currents facilitate rapid water exchange. The study presented here is part of a larger transplantation experiment detailed by (Prada et al. 2017), which provided a comprehensive environmental characterization of the experimental site. Subsequent research on the reproduction of transplanted species, specifically *L. pruvoti* (Gizzi et al. 2017), *A. calycularis* (Marchini et al. 2021), and *Balanophyllia europaea* (this study), rely on the foundational environmental data presented in (Prada et al. 2017).

Field transplantation

Balanophyllia europaea specimens were collected by SCUBA diving at Pietra Nave, ~ 2 km away from the experimental field, where the species grows naturally at 3–6 m depth. Upon sampling, corals were placed in a container with seawater and transported within 30 min by boat to the wet lab that was temporarily set up at the Eolo Sub Diving Center. Upon arrival at the diving center, *B. europaea* polyps were randomly assigned to each of the four sites (n = 4–6 polyps per site, per

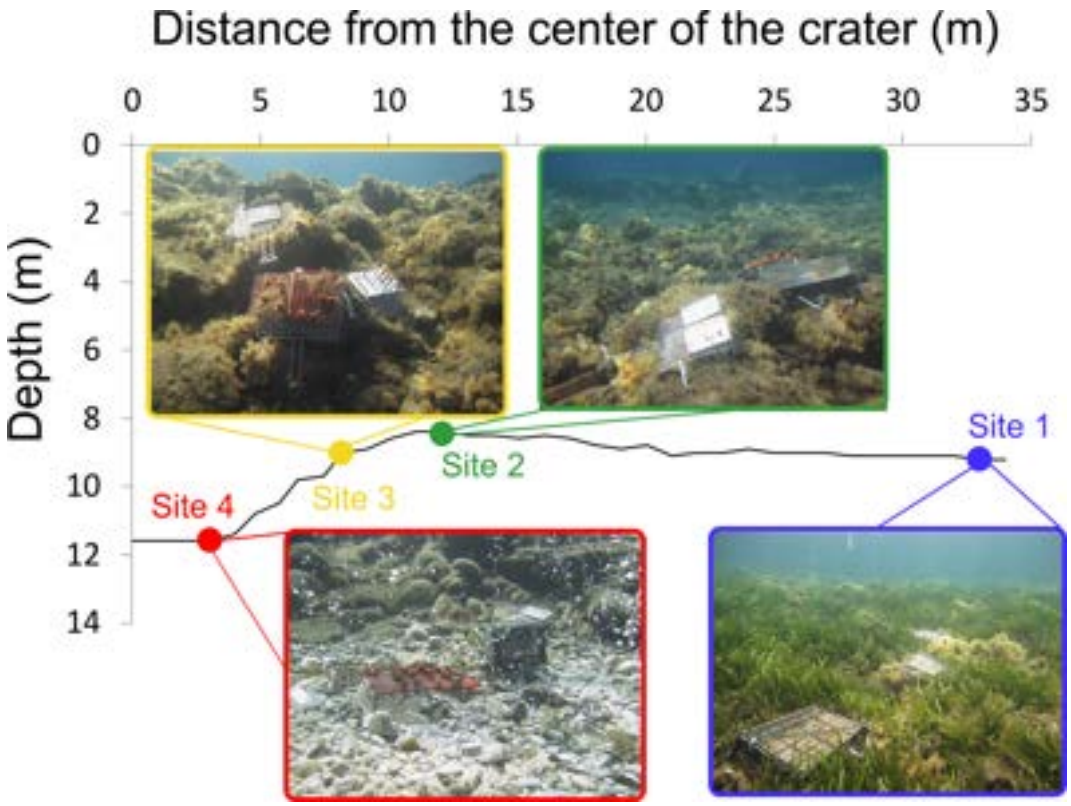

Fig. 1. Bathymetric profile of the four sites. The pictures show representative images of the four sites and transplanted corals.

experimental period) and glued with a non-toxic bicomponent epoxy coral glue (Milliput) onto ceramic tiles (Fig. 1). Corals were maintained at ambient temperature in containers aerated with a bubbler and no source of artificial light for 1 d prior to transplantation. Corals were subjected to experimental conditions in different moments of their reproductive cycle: (i) gonadal development period, characterized by the presence of small immature oocytes (< 400 μm), spermaries in the early stages of development, and embryos in the coelenteric cavity of female polyps. This period includes polyps from the following two sampling campaigns that were pooled together: transplant at the beginning of June 2011 and collection at the end of July 2011 (Supporting Information Fig. S1a), and transplant at the beginning of May 2012 and collection at the end of June 2012; (ii) maturity period, characterized by the presence of two distinct oocyte stocks, one made of smaller immature cells (< 400 μm) and the other made of big mature cells (> 400 μm). Spermaries are present at all the development stages in the same specimen (I, II, III, IV, V). Some females show the first embryos in the coelenteric cavity. This period includes polyps from the following two sampling campaigns that were pooled together: transplant in mid-November 2010 and collection in the beginning of March 2011, and transplant at the beginning of December 2011 and collection at the end of April 2012 (Supporting Information Fig. S1b). There were no specimens collected in April 2012 from site 3 due to a storm that swept away part of the experiment.

Once collected, specimens were then fixed in a formaldehyde fixative solution (90% seawater and 10% formaldehyde buffered with calcium carbonate) and transferred to the Department of Biological, Geological and Environmental Sciences at the University of Bologna for biometric, histological, and cyto-histometric analyses.

Biometric measurements

To calculate the reproductive parameters, biometric analyses were performed on 68 polyps by measuring length (L, maximum axis of the oral disc), width (W, minimum axis of the oral disc), and height (H, distance between the oral and aboral disc) of each polyp using a caliper (± 0.05 mm). These parameters allowed us to estimate the body volume (V) of the polyp using the following equation: $V = H * (L/2) * (W/2) * \pi$ (Goffredo et al. 2002).

Histological analysis

Histological analyses were performed after the polyps had been thoroughly rinsed under running tap water overnight, followed by a 24-h post-fixation in Bouin's solution. After decalcification with ethylenediaminetetraacetic acid and dehydration in increasing concentration of ethanol (from 70% to 100%), polyps were clarified in histolemon and embedded in

paraffin. Serial transverse sections were cut using a microtome at 7-μm intervals along the oral-aboral axis, from the oral to the aboral pole of the polyp. Because the average polyp measured about 7 mm in height, we typically cut around 1000 sections per polyp. Tissues were then stained with Mayer's hematoxylin and eosin (Goffredo et al. 2002).

Cyto-histometric analysis

The perimeter and area of each reproductive element were recorded using an optical NIKON eclipse 80i microscope with the image analysis software NIKON NIS-Elements D 3.1. From these two parameters, we obtained the maximum and minimum diameters of all spermaries observed in the section every seven slides and all oocytes observed in nucleate sections. Spermaries were also classified into five maturation stages distinguishable by morphological traits described in previous studies on coral gametogenesis (Beauchamp 1993; Glynn et al. 2000; Kruger and Schleyer 1998; Rinkevich and Loya 1979; Supporting Information Fig. S2). The presence of all embryos observed in the coelenteric cavity was recorded, and their maturation stage was identified (Goffredo and Telò 1998). The size of each reproductive element and each embryo was determined as the mean of the two diameters (Goffredo et al. 2002). The polyps without germ cells were classified as sexually inactive individuals.

Definitions

Reproductive output was defined through six reproductive parameters: (i) oocyte and spermary abundance, defined as the number of reproductive elements per body volume unit (100 mm^3); (ii) gonadal index, defined as the percentage of the polyp body volume occupied by reproductive elements (the volume of each reproductive element was measured as described in the subsection "Gonadal index" of Goffredo et al. 2002); (iii) reproductive element diameter, defined as the average of the maximum and minimum diameter of spermaries and oocytes in nucleate sections; (iv) fertility, defined as the number of embryos per body volume unit (100 mm^3) (Marchini et al. 2015); (v) Embryonal index, defined as the percentage of the polyp body volume occupied by embryos; (vi) embryo diameter, defined as the average of the maximum and minimum diameter of embryos.

Statistical analysis

To compare reproductive parameters (i.e., oocyte and spermary abundance, gonadal index, reproductive element diameter, fertility, embryonal index, and embryo diameter) among the four sites, within each reproductive period (i.e., gonadal development period and maturity period), assumptions for parametric statistics were first tested. Specifically, Levene's test was used to assess homogeneity of variance. To test for normality, the Shapiro–Wilk test (Shapiro and Francia 1972) was applied when the sample size was below 2000, and the Kolmogorov–Smirnov test was used when the sample size exceeded 2000. One-way ANOVA was performed for spermary diameter in the gonadal development period and for oocyte and spermary diameter in the maturity period. Assumptions for parametric statistics were not fulfilled for oocyte and spermary abundance and gonadal index in both gonadal development and maturity periods, for oocyte and embryo diameter in the gonadal development period, and for fertility and embryonal index, thus the non-parametric Kruskal–Wallis equality-of-populations rank test (Kruskal and Wallis 1952) was used. When the Kruskal–Wallis test was significant, Spearman's rank correlation coefficient (rho) was used to calculate the significance of correlation between reproductive parameters and pH_T and pairwise comparisons between sites were performed using the Mann–Whitney post hoc test. The above-mentioned analyses were computed using SPSS v21.0 (Apache Computer Software Foundation). A one-way permutation multivariate ANOVA (PERMANOVA) (Anderson 2001), based on Euclidean distances, was performed with 999 permutations to compare the oocyte size distribution and spermary maturation stage distribution among sites within each reproductive period. For a small sample size, the Monte Carlo (MC) correction of p-value was used.

Results

Both the gonadal development period and the maturity period were characterized by gametogenic and embryogenic polyps, while only the maturity period showed inactive polyps due to the lack of reproductive elements (Table 1). All gametogenic polyps analyzed were hermaphrodite (Table 1). Polyps sampled in June contained only oocytes, since spermatogenesis had yet to begin after the release of spermatozoa for fertilization.

Gonadal development period

Size-frequency distribution of oocytes showed no significant differences among sites (PERMANOVA, df = 3, pseudo-$F = 0.631$, p [MC] = 0.725; Fig. 2a). Polyps from all sites were characterized almost entirely by a stock of small and immature oocytes (diameter < 400 μm; Fig. 2a). While oocyte abundance and gonadal index were homogeneous among sites (Kruskal–Wallis test, abundance p [MC] = 0.881 and gonadal index p (MC) = 0.377; Supporting Information Table S1; Fig. 3a), oocyte diameter significantly differed (Kruskal–Wallis test, $p = 0.037$; Supporting Information Table S1; Fig. 3a) but did not correlate with pH_T (Spearman's correlation test, $r = 0.182$, $p = 0.242$). Oocyte diameter was significantly different only between sites 1 and 3 (Mann–Whitney test, $p = 0.014$) and between sites 2 and 3 (Mann–Whitney test, $p = 0.013$) and homogeneous otherwise.

Maturation stage/frequency distribution of spermaries did not show significant differences among sites (PERMANOVA, df = 3, pseudo-$F = 1.350$, p [MC] = 0.261; Fig. 2b) and was characterized by early maturation stages (I and II) in all sites (Fig. 2b). Spermary abundance, gonadal index, and diameters were homogeneous among sites (Kruskal–Wallis/ANOVA test, abundance p [MC] = 0.216; gonadal index p [MC] = 0.078; diameters p [MC] = 0.493; Supporting Information Table S2; Fig. 3).

Table 1. Number of *Balanophyllia europaea* polyps analyzed at the four sites in the gonadal development and maturity periods. The reproductive state (hermaphrodite or sexually inactive), embryogenic polyps, and pH_T values are reported for each site. For June 2012 (gonadal development period) only oocytes were detected. N_p: number of polyps.

Gonadal development period

Site	pH_T	N_p	Hermaphrodite	Sexually inactive	Embryogenic
1	8.07	12	12	0	6
2	7.87	12	12	0	7
3	7.74	12	12	0	6
4	7.40	9	9	0	7
Total		45	45	0	26

Maturity period

Site	pH_T	N_p	Hermaphrodite	Sexually inactive	Embryogenic
1	8.07	8	7	1	0
2	7.87	6	6	0	2
3	7.74	2	2	0	0
4	7.40	7	6	1	1
Total		23	21	2	3

Embryos were found in the coelenteric cavity in all sites of the gonadal development period (June 2011 and July 2012). Fertility, embryonal index, and diameter did not show differences among sites (Kruskal–Wallis test, fertility p [MC] = 0.815, embryonal index p [MC] = 0.895 and diameter p [MC] = 0.707; Supporting Information Table S3; Fig. 4).

Maturity period

Size/frequency distribution of oocytes was homogeneous among sites (PERMANOVA test, df = 3, pseudo-F = 0.941, p [MC] = 0.455; Fig. 2a). All sites showed two distinct stocks of oocytes: one stock was characterized by small and immature cells (< 400 μm) and the second stock by larger and mature cells (> 400 μm; Fig. 2a). Oocyte abundance, gonadal index, and diameter did not show significant differences among sites (Kruskal–Wallis/ANOVA test, abundance p [MC] = 0.214; gonadal index p [MC] = 0.132; diameter p [MC] = 0.069; Supporting Information Table S4; Fig. 3a).

The maturation stage distribution of the spermaries was homogeneous among sites (PERMANOVA, df = 3, pseudo-F = 0.846, p [MC] = 0.509; Fig. 2b). In this period, spermatogenesis was characterized by all maturation stages, with the mode of the spermaries in maturation stage V in all sites (Fig. 2b). Site 3 showed an anomalous distribution due to the lack of polyps in April 2012 and the presence of polyps only in March 2011 (Fig. 2b). Spermary abundance, gonadal index, and diameter were homogeneous among sites (Kruskal–Wallis/ANOVA test, abundance p [MC] = 0.185; gonadal index p [MC] = 0.229; diameter p [MC] = 0.195; Supporting Information Table S5, Fig. 3b).

Embryos were found in the coelenteric cavity of two female polyps in site 2 (pH_T 7.87; Table 1) and one female polyp in site 4 (pH_T 7.40; Table 1) of the maturity period (March 2011 and April 2012). The analysis of embryogenesis was not performed since it had just begun, and few embryos were present in only 8.7% of the individuals of this reproductive period.

Discussion

The current study showed that the reproductive output of the zooxanthellate temperate coral *Balanophyllia europaea* transplanted along a natural pH gradient was unaffected by acidification. Indeed, during both reproductive periods, oocyte size/frequency, spermarial maturation stage distributions, abundance, and gonadal index of both oocytes and spermaries were not affected by decreasing pH. A similar response on coral gametogenesis was observed in a mesocosm experiment where the tropical zooxanthellate species *Montipora capitata* did not show a decrease in gamete production after 6 months of exposure to acidified conditions (treatment mesocosms exceeded control mesocosms by 365 ± 130 μatm [mean ± SD] reaching pH values up to 7.8) (Jokiel et al. 2008). This observation is consistent with previous findings reporting normal gametogenesis in two colonial zooxanthellate corals, *Oculina patagonica* and *Madracis pharencis*, when exposed to pH 7.4 for 1 year (Fine and Tchernov 2007).

The findings presented here are partially in agreement with a previous study showing unaffected gametogenesis in the non-zooxanthellate scleractinian coral, *Leptopsammia pruvoti*, transplanted along the same pH gradient considered here (Gizzi et al. 2017). While the oogenesis of *L. pruvoti* along the gradient seems unaffected by end-of-century pH levels, conditions projected for a more distant future (pH 7.4, i.e., year 2500) influenced spermary production and development with a slight

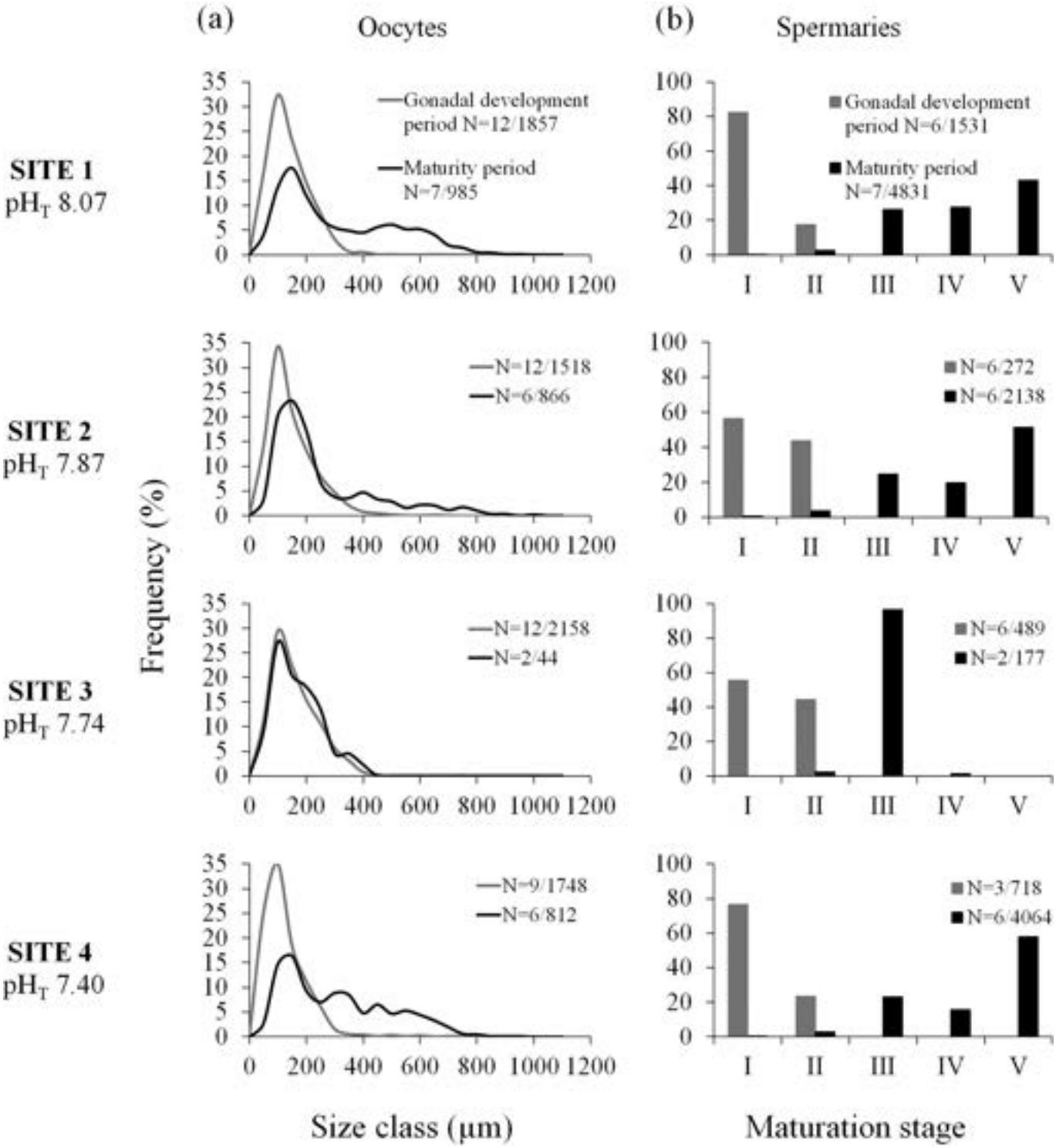

Fig. 2. Oocyte size class and spermary maturation stage distributions. (**a**) Distribution of oocyte size (diameter) during the gonadal development (gray line) and maturity (black line) periods. *N* = number of polyps/oocytes. (**b**) Distribution of the five spermary maturation stages during the gonadal development (gray bars) and maturity (black bars) periods.

delay in sperm release, in the fertilization process, and in embryo development (Gizzi et al. 2017). Also, colonies of the non-zooxanthellate coral *Astroides calycularis* transplanted along the same gradient showed a delay in spermary development at pH 7.4, which could postpone sperm release in the water column and consequently affect the fertilization process

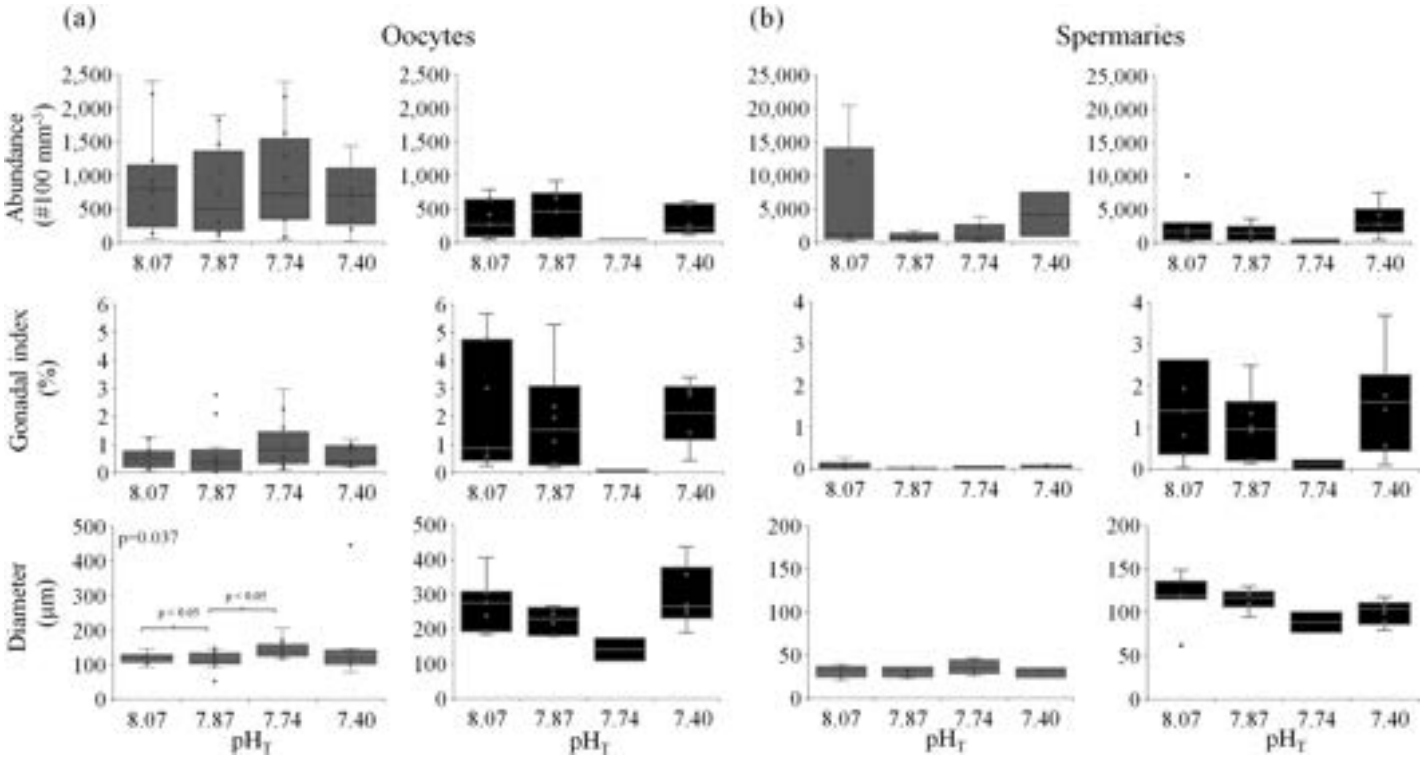

Fig. 3. Oocyte and spermary reproductive parameters. Boxplots for oocyte (**a**) and spermary (**b**) abundance, gonadal index, and diameter in the gonadal development (gray) and maturity (black) periods at the four sites. The box indicates the 25th and 75th percentiles, and the line within the box marks the median. Whisker length is equal to 1.5 × interquartile range (IQR). Dots represent inner points and outliers. Sample number and mean values for each site are listed in Supporting Information Table S1 for oocytes and Supporting Information Table S2 for spermaries.

(Marchini et al. 2021). This result is supported by the persistence of mature oocytes and lack of embryos with increasing acidification, which could be linked to a delay in the fertilization process (Marchini et al. 2021). The latter could also be affected by sperm motility, which has been shown to be negatively affected by increasing acidification in many marine organisms, including tropical corals (Albright and Langdon 2011; Byrne 2011; Morita et al. 2010; Nakamura and Morita 2012).

On the other hand, the current study showed that *B. europaea* was able to produce and develop both oocytes and spermaries even under extremely low pH conditions (pH 7.4). In general, the zooxanthellate coral *B. europaea* seems quite tolerant to increasing acidification compared to non-zooxanthellate corals. Indeed, a parallel transplant experiment conducted in the same site showed no impacts of decreasing pH and increasing seawater temperature on net calcification rates in *B. europaea*, while *L. pruvoti* and *A. calycularis* were both negatively affected (Prada et al. 2017). Moreover, *B. europaea* transplanted up to 7 months along pCO_2 gradients at volcanic vents off Ischia Island (Tyrrhenian Sea, Italy) showed increased gross calcification rates with decreasing pH (up to pH 7.4) (Rodolfo-Metalpa et al. 2011). Likewise, *B. europaea* naturally living along the Panarea pH gradient seems to acclimate to long-term acidification conditions by: (i) increasing symbiont cell density and triggering the establishment of novel dinoflagellate haplotypes potentially better adapted to lower pH conditions (Prada et al. 2023); (ii) enhancing microbial communities capable of dinitrogen fixation as well as N storage and mobilization within the coral tissue/mucus (Prada et al. 2023); (iii) maintaining gross calcification rates and pH homeostasis at the site of calcification (Wall et al. 2019); and (iv) reproducing, regardless of external seawater pH (Caroselli et al. 2019). Non-genetic, trans-generational acclimatization mechanisms (e.g., epigenetics) have the capacity to influence coral population trajectories in a rapidly changing climate and may help explain the observed responses (Putnam and Gates 2015).

Symbiotic dinoflagellates (family Symbiodiniaceae) contribute up to 95% of their photosynthetically produced carbon to the coral host (Falkowski et al. 1984). In some species, increasing pCO_2 can stimulate the photosynthetic efficiency of microalgae (Gao and Campbell 2014; Riebesell 2004; Wu et al. 2017; Yang and Gao 2003), macroalgae (Kim et al. 2016; Scherner et al. 2016; Sordo et al. 2016), seagrasses (Collier et al. 2018), and some phylotypes from the family Symbiodiniaceae (Brading et al. 2011). The zooxanthellate Mediterranean coral *Cladocora caespitosa* exposed to low pH (pH 7.9) for 1 month showed increased symbiont cell density and chlorophyll content (Rodolfo-Metalpa et al. 2010). Increased symbiont cell densities with increasing

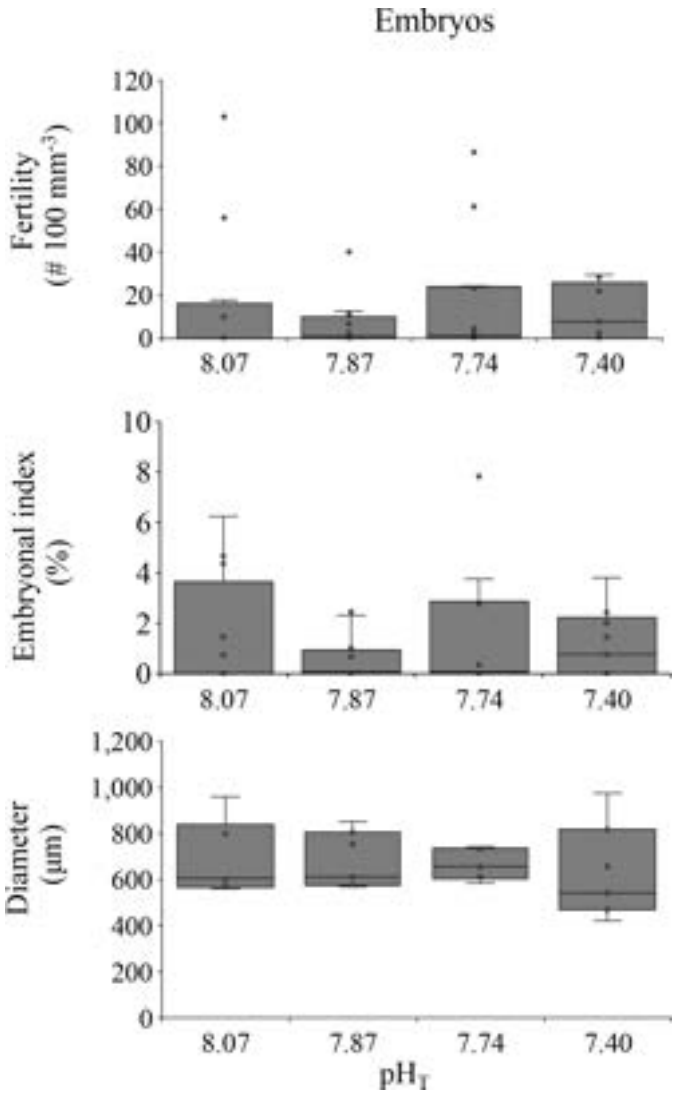

Fig. 4. Embryo reproductive parameters. Box-plots for fertility, embryonal index, and diameter at the four sites. Median (solid horizontal line), 1st and 3rd quartiles (box outline), 2nd and 4th quartiles (vertical lines). Dots represent inner points and outliers. Sample numbers and mean values for each site are listed in Supporting Information Table S3.

acidification found in *B. europaea* naturally growing along the pH gradient could lead to increased photosynthetically fixed C, as highlighted by the increase in symbiont C/N ratios at low pH (Prada et al. 2023). Moreover, $\delta^{13}C$ values of the symbionts and host tissue suggest an increased influence of autotrophy compared to heterotrophy with increasing acidification (Prada et al. 2023). Increased symbiont cell densities also in transplanted corals could potentially provide additional energy to the host under short-term acidified conditions (Biscéré et al. 2019), thus supporting processes such as calcification (Prada et al. 2017) and reproduction (this study).

Fertility, embryonal index, and embryo diameter did not change among sites, indicating that the fertilization process and embryogenesis were unaffected in *B. europaea* transplanted along the pH gradient. Even though pH did not affect gametogenesis in *B. europaea* naturally living up to pH 7.7 (site 3) along the same pH gradient, decreasing pH led to a reduction in larval production and recruitment efficiency (i.e., larvae required to obtain one settled polyp) (Caroselli et al. 2019). However, embryo reproductive parameters were not assessed in that study; thus, we cannot exclude that the fertilization process and embryogenesis could be negatively affected by acidification in the long-term in this species. Previous studies indicate that pH values up to 7.8 impair the fertilization success in *Acropora palmata* (Albright et al. 2010) and *Montastraea faveolata* (Albright and Langdon 2011), but in both studies, the effect of pH is dependent on sperm concentration. Indeed, when sperm concentration is high (3.2×10^6 sperm mL^{-1}), fertilization in *Acropora millepora* and *Acropora tenuis* is unaffected by pH values predicted for the end of this century (Albright and Mason 2013; Chua et al. 2013).

In conclusion, the zooxanthellate coral *B. europaea* seems quite tolerant, in terms of gametogenesis and embryogenesis, when exposed to pH levels predicted for the end of the century and beyond, at least in the short-term. Enhanced photosynthetically fixed C by the symbiotic algae could provide additional energy to the coral host, allowing it to acclimate to acidified conditions. However, ocean acidification will not act alone but rather in synchrony with other stressors such as warming, pollution, and habitat destruction (Allemand and Osborn 2019). Thus, an understanding of the winners and losers in the face of rapid climatic events and changes in ocean carbonate chemistry requires experiments investigating the combined effects of multiple stressors in the longterm. Further studies on the highly vulnerable early life history stages between larval development and recruit settlement and growth in a wider range of settings and species are needed to predict coral responses in an acidifying and warming ocean.

Author Contributions

Stefano Goffredo, Giuseppe Falini, and Zvy Dubinsky conceived and designed the research. Fiorella Prada, Erik Caroselli, Francesca Gizzi, Arianna Mancuso, and Stefano Goffredo collected the samples and performed the diving field work. Chiara Marchini, Valentina Airi, Francesca Gizzi, Dragana Paleček, Umberto Valdrè, and Ilaria Z. Destefani performed the measurements and analyzed the data. Chiara Marchini and Fiorella Prada wrote the manuscript. All authors contributed to revising the manuscript and participated in the scientific discussion.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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