# Two temperate corals are tolerant to low pH regardless of previous exposure to natural CO2 vents

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Running head: Coral tolerance to ocean acidification

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## Abstract

Ocean acidification is perceived to be a major threat for many calcifying organisms, including scleractinian corals. Here we investigate (1) whether past exposure to low pH environments associated with $CO_2$ vents could increase corals tolerance to low pH and (2) whether zooxanthellate corals are more tolerant to low pH than azooxanthellate corals. To test these hypotheses, two Mediterranean colonial corals *Cladocora caespitosa* (zooxanthellate) and *Astroides calycularis* (azooxanthellate) were collected from $CO_2$ vents and reference sites and incubated in the laboratory under present-day (pH on the total scale, $pH_T$ 8.07) and low pH conditions ($pH_T$ 7.70). Rates of net calcification, dark respiration and photosynthesis were monitored during a six-month experiment. Monthly net calcification was assessed every 27 to 35 d using the buoyant weight technique, whereas light and dark net calcification was estimated using the alkalinity anomaly technique during 1 h incubations. Neither species showed any change in net calcification rates, respiration, and photosynthesis regardless of their environmental history, pH treatment and trophic strategy. Our results indicate that *C. caespitosa* and *A. calycularis* could tolerate future ocean acidification conditions for at least 6 months. These results will aid in predicting species' future responses to ocean acidification, and thus improve the management and conservation of Mediterranean corals.

## Introduction

Ocean acidification describes the shift in carbonate chemistry caused by the uptake of anthropogenic $CO_2$ by the ocean (Caldeira and Wickett 2003, Gruber et al. 2019). It causes an increase in dissolved inorganic carbon and bicarbonate ion concentrations, along with a concurrent decrease in pH, carbonate ion concentration and its associated saturation state ($\Omega$; Orr et al. 2005). Calcifying species are perceived to be particularly vulnerable to ocean acidification (Kroeker et al. 2013a). For example, corals are particularly sensitive to changes in carbonate chemistry as their skeleton is made of aragonite, a metastable form of calcium carbonate that is less stable than calcite (Erez et al. 2011, Foster and Clode 2016). As a result, coral calcification is expected to decrease as seawater acidity increases (Gattuso et al. 1999). Coral calcification decreases by on average 22% under pH values expected by the end of the century under the high-$CO_2$ emissions scenario (RCP 8.5 from AR5 of ICPP; Chan and Connolly 2013). However, a range of contradictory species- and experience-specific responses have been described (e.g. Kornder et al. 2018), with some species such as massive *Porites* spp. being tolerant to ocean acidification (Fabricius et al. 2011). This resistance to ocean acidification was also described in several temperate and cold-water corals (e.g. Rodolfo-Metalpa et al. 2010). Such tolerance is seen in many studied parameters such as net calcification rate, respiration, photosynthetic physiology, calcifying fluid pH (McCulloch et al. 2012a, Wang et al. 2020). According to Varnerin et al. (2020) even juveniles from the temperate *Oculina arbuscula* were only slightly affected by ocean acidification.

A central question when assessing the future of corals in a high-$CO_2$ world is whether they have the capacity to acclimatize and further adapt to ocean acidification. This question remains unanswered as corals are generally long-lived organisms for which it is extremely challenging to study acclimatization and adaptation processes over several generations. Acclimatization refers to reversible phenotypic changes limited by genotype, occurring under

naturally varying conditions by a physiological readjustment of the organism's tolerance levels (Edmunds and Gates 2008). In contrast, adaptation refers to changes in the genetic composition of a population by natural selection, in which traits will be passed on to the next generation (Savolainen et al. 2013). In a recent study, Cornwall et al. (2020) conducted a one-year experiment showing that coralline algae have the capacity to acclimatize to low pH after seven generations of exposure. Replicating such a study on corals is virtually impossible as they are long-lived organisms which only reach sexual maturity after several years. $CO_2$ vents therefore offer a unique opportunity to investigate the response of corals to ocean acidification over long periods of time. In such systems, volcanic $CO_2$ bubbles from the seafloor which acidifies the surrounding seawater and creates an environment, with carbonate chemistry conditions that mimic those expected in the future (Hall-Spencer et al., 2008, Camp et al. 2018, González-Delgado and Hernández 2018). By investigating individuals, species, and communities along transects radiating from the sources of $CO_2$, it is possible to substitute time for space. It must be pointed out, however, that $CO_2$ vents are imperfect windows into the future because they generally do not mimic other climate-related changes such as ocean warming. Furthermore, it is not possible to disentangle any changes in the mean versus variability of seawater pH (Hall-Spencer et al. 2008). Overall, these systems exhibit significant decreases in trophic complexity and biodiversity, as well as a major decrease in the abundance of calcifying organisms (Kroeker et al. 2013b, Linares et al. 2015, Teixidó et al. 2018). Corals living in the vicinity of $CO_2$ vents have been exposed to low and high variability pH conditions for an extended period of time (years to decades). They are therefore ideal models to investigate acclimatization and adaptation to ocean acidification (Fantazzini et al. 2015, Foo et al. 2018). Populations or individuals living in these variable and more extreme environments are hypothesized to be more tolerant to suboptimal environmental conditions such as acidification (e.g. Cornwall et al. 2018).

$CO_2$ vents have been used for transplant experiments between acidified and nearby reference sites with ambient pH and no vent activity. For example, a decline in calcification was described in the zooxanthellate corals *Cladocora caespitosa* and *Balanophyllia europaea* transplanted to a $CO_2$ vent site in Ischia (Rodolfo-Metalpa et al. 2011). The skeletal growth of *Porites astreoides* living in a natural pH gradient showed no acclimatization to ocean acidification despite their life-long exposure to low pH (Crook et al. 2013). Few laboratory studies have focused on temperate scleractinian corals from natural $CO_2$ vents despite the relevance of studying the tolerance of temperate coral populations to ocean acidification (Trotter et al. 2011, Teixidó et al. 2020). Such studies are important for understanding and elucidating how these species will respond to the suboptimal pH conditions projected for the end of this century.

The symbiosis between scleractinian corals and endosymbiotic algae, commonly referred to as zooxanthellae, has been studied thoroughly (Davy et al. 2012). Photosynthetic products from zooxanthellae contribute significantly to the energy budget of the scleractinian host (Allemand et al. 2011, Muller-Parker et al. 2015) and can play an important role in calcification (Erez et al. 2011, Inoue et al. 2018). However, not all corals harbor zooxanthellae (azooxanthellate corals). Gibbin et al. (2014) demonstrated that zooxanthellae regulate intracellular pH under acidified conditions, whereas azooxanthellate corals can suffer from reduced intracellular pH which can lead to intracellular acidosis. It can thus be expected that photosynthesis may enhance calcification by (1) providing energy for this energetically costly process and (2) by increasing pH at the site of calcification (Allemand et al. 2011). In a transplant experiment in the $CO_2$ vents of Panarea, Prada et al. (2017) showed that the calcification of two azooxanthellate corals *Leptopsammia pruvoti* and *Astroides calycularis* decreased at low pH, whereas calcification of the zooxanthellate coral *B. europaea* was unaffected. A similar result was observed in pH-controlled aquaria by Ohki et al. (2013) as

polyps of *Acropora digitifera* with symbionts showed higher calcification rates than polyps without symbionts under low pH, suggesting that symbionts confer tolerance to ocean acidification. However, in a study led by McCulloch et al. (2012b), azooxanthellate cold-water corals presented higher intracellular pH regulation than shallow-water zooxanthellate corals under acidification. As it is not truly known whether or not symbionts promote the maintenance of elevated pH at the site of calcification, it is interesting to compare zooxanthellate and azooxanthellate corals as they may display contrasting responses to ocean acidification.

The present study focuses on two Mediterranean long-lived corals of key relevance for conservation, the zooxanthellate species *C. caespitosa* (Linnaeus, 1767) and the azooxanthellate species *A. calycularis* (Pallas, 1766). *Cladocora caespitosa* is a unique zooxanthellate reef-building coral that provides a valuable ecosystem service in the Mediterranean Sea which is lacking large bioconstructions (Kersting and Linares 2012). *Astroides calycularis* is commonly found in the southwestern Mediterranean Sea and can be highly abundant in low light, shallow rocky habitats (Zibrowius 1995). These two scleractinian corals naturally occur at two newly discovered $CO_2$ vent systems along the coast of Ischia, Italy. In situ data obtained by Teixidó et al. (2020) showed variable responses in skeletal structure and growth patterns of *A. calycularis,* with colonies from the $CO_2$ vent site characterized by smaller size, fewer polyps, and less porous, denser skeletons. Thus, these two scleractinian coral species grown under elevated $pCO_2$ environments are great model systems for assessing tolerance to acidification. Here, two hypotheses are tested: (1) past exposure to low pH conditions confers tolerance to ocean acidification and (2) the coral-algal symbiosis modulates this tolerance. To test these hypotheses, corals from natural $CO_2$ vent systems and reference sites were exposed to present-day (pH on the total scale, $pH_T \sim 8.07$)

147 and low pH conditions ($pH_T$ ~7.70) during a six-month long laboratory experiment, in which
148 calcification, respiration and photosynthesis rates were assessed.

149 **Material and Methods**

150 Study sites

151 We studied populations of the corals *Cladocora caespitosa* and *Astroides calycularis* that
152 naturally occur at two distinct $CO_2$ vent sites with low pH, and two reference sites with
153 ambient pH and no vent activity at the island of Ischia, Italy (Fig. S1 Supplementary
154 materials). *Cladocora caespitosa* occurs near a $CO_2$ vent site adjacent to rocky reefs at about
155 10 m depth (Chiane del Lume, hereafter Vent 1), whereas *A. calycularis* occurs in another
156 $CO_2$ vent site between 1 - 2 m depth (Grotta del Mago, a semi-submerged cave hereafter Vent
157 2, where $CO_2$ vents occur at 5 m depth). Vent gas compositions at Vent 1 and Vent 2 were
158 predominantly $CO_2$ (mean ± SE: 93.9 ± 0.6 for Vent 1, 93.4 ± 1.0 for Vent 2), with
159 undetectable levels of hydrogen sulfide (< 0.0002 %), and did not elevate the temperature (for
160 more information on gas measurements, see Teixidó et al. 2020). The reference sites without
161 any visible vent activity and exhibiting ambient pH were located: i) approximately 100 m
162 west from Vent 1 for *C.caespitosa* (hereafter Ambient 1, 10 m depth) and ii) 1.5 km away
163 from Vent 2 for *A. calycularis* (San Pancrazio, an overhang, hereafter Ambient 2, 1-2 m
164 depth) (Fig. S1 Supplementary materials).

165 Coral field surveys

166 The *C. caespitosa* abundance was quantified using 12 quadrats of 50 cm x 50 cm, which were
167 randomly placed along the rocky reef at 10 m depth at the two sites (n = 24 quadrats in total).
168 Abundance was assessed by counting the number of colonies found in each quadrat. In
169 addition, size of colonies was determined by measuring the major colony diameter with a

plastic ruler (with an error of ± 1 cm) (Peirano et al. 2001). Major colony diameter was selected as the best colony size descriptor (Kersting 2012). The *A. calycularis* abundance was quantified using 12 photo-quadrats (25 x 25 cm) between 1 and 2 m depth in the two other sites (n = 24 quadrats in total, Vent 2, Ambient 2). Size frequency-distribution was calculated by counting the number of polyps of each colony and each colony was then pooled into one of five size classes (I: 1-5 polyps: II: 6-10 polyps; III: 11-15 polyps; IV: 16-20 polyps; V: > 20 polyps). The survey's depth for *C. caespitosa* and *A. calycularis* corresponded to the depth range at which colonies were sampled for the experiment (see below). Abundance and size-frequency data for *A. calycularis* have been described previously in Teixidó et al. (2020).

In situ $pH_T$ and carbonate chemistry associated with $CO_2$ vents and ambient pH sites

SeaFET$^{TM}$ Ocean pH sensors (Satlantic) were deployed to quantify variation in pH at the two $CO_2$ vent and the two ambient pH sites. Measurements were taken every 15 minutes. One sensor was deployed at the Vent 1 site from May 7 to 21, 2019, and one sensor was deployed at the Ambient 1 site during the same period. At the semi-submerged cave (Vent 2), sensors were deployed in May-June 2019 (before summer) and in September 2018 (after summer). Dates of deployment for Vent 2 were from September 8 to September 24, 2018 and from May 30 to June 18, 2019 at Vent 2. One sensor was deployed in the reference area Ambient 2 during the same period. Before deployment, the SeaFETs were calibrated with ambient pH water in the aquarium facilities at the Center Villa Dohrn (Ischia, Italy). The mean offset between calibration samples and calibrated SeaFET pH was ± 0.002 units, indicating high quality pH dataset. Discrete water samples were collected using Niskin bottles to measure the carbonate system parameters during the pH sensor deployment. Samples for total alkalinity ($A_T$) were collected using standard operating protocols. The HCl (0.1 M) titrant solution was calibrated against certified reference materials distributed by A.G. Dickson (CRM, Batches #153, #171, and #177). Precision of the $A_T$ measurements of CRMs was < 2 and < 1 μmol $kg^{-1}$ from nominal

values. $A_T$ and $pH_T$ were used to determine the remaining carbonate system parameters at in situ temperature and depth of each sampling period in the R package seacarb v3.2.12. For full details of pH sensors deployment, calibration, and total alkalinity standard operating protocols, see Teixidó et al. (2020).

Coral collection

A total of 81 colonies of *C. caespitosa* and *A. calycularis* were randomly sampled for the aquarium experiment. Fourteen (Vent 1) and sixteen (Ambient 1) colonies of *C. caespitosa* were collected at 10 m depth; twenty-seven (Vent 2) and fourteen (Ambient 2) colonies of *A. calycularis* were collected from 1 to 2 m depth. A portion of approximately 3 x 3 cm was gently dislodged from each colony using a hammer and chisel. They were placed in coolers filled with seawater changed regularly and maintained in Ischia for two days prior to transportation to the aquarium facilities of the *Laboratoire d'Océanographie de Villefranche*, France. The duration of the transfer was less than 10 h; colonies were wrapped in seawater-soaked paper tissues. The colonies were cleaned, tagged with a number stuck under it using water-resistant epoxy glue, and maintained at an ambient $pH_T$ of ~8.05 during 2 weeks.

Experimental set-up and treatments

Colonies of *C.caespitosa* and *A. calycularis* were maintained in two constant pH treatments during six months (202 days), one "present-day" treatment with a pHT = 8.08 ± 0.01 (mean ± SE, n = 348, total number of weekly pHT measures in each 12 present-day experimental tank) and one "low pH" treatment with a pHT = 7.72 ± 0.01 (mean ± SE, n = 348, total number of weekly pHT measures in each 12 low pH experimental tank) corresponding to the pH values expected by the end of the century under the RCP 8.5 $CO_2$ emissions scenario. More specifically, 14 colonies from Vent 1 and 16 colonies from Ambient 1 of *C. caespitosa* and 27 from Vent 2 and 14 from Ambient 2 of *A.calycularis* were used for this experiment. The vent-

low pH condition was used to test the acclimation or long-term tolerance of the colonies to low pH in the lab, after a life-time of exposure to in situ low pH. The ambient-low pH condition was used to test the short-term tolerance to low pH of colonies that have not been exposed to low pH before. The vent-present day condition tested the response to ambient pH of colonies exposed in the long-term to in situ low pH. The ambient-present day condition was the control. There were six independent 5 L experimental tanks for each of the four conditions (origin x pH treatment), for a total of 24 experimental tanks. Each experimental tank contained one to three colonies of each species, depending on their size, to get approximately the same total amount of polyps per tank (Tab. S1 Supplementary materials). Seawater pumped from Villefranche Bay at 5 m depth was continuously flowing into six 25 L header tanks, used as water storage and to maintain gravity pressure for water flow into the experimental tanks. Three header tanks were maintained at $pH_T$ ~ 8.07 (present-day treatment) and the other three at $pH_T$ ~ 7.7 (low pH treatment). pH was controlled in the header tanks using pH controllers (APEX, Neptune Systems) which regulated the delivery of pure $CO_2$. Each header tank gravity-fed four experimental four-liter tanks at a rate of 100 ml $min^{-1}$ (Fig. 1).

Light was provided by 89 W LED light bars (Aquaristik, Aqualumix). Irradiance gradually increased from 0 at 06:00 to a maximum irradiance of 180 µmol photons $m^{-2}$ $s^{-1}$ between 11:00 and 14:00, and gradually decreased to 0 at 18:00. According to the attenuation coefficients of PAR in Villefranche Bay calculated in Martin and Gattuso (2009), the maximum irradiance levels used in the study (180 µmol photons $m^{-2}$ $s^{-1}$) is within the irradiance range observed at 10 m, where *Cladocora caespitosa* can be found in the Mediterranean Sea (154 to 449 µmol photons $m^{-2}$ $s^{-1}$). As *A. calycularis* were collected in a semi-submerged cave and an overhang, dark plastic bags covered one side of the experimental tanks to shade colonies from direct light. The 24 experimental tanks were kept in 6 water

baths under controlled temperature following Ischia's natural changes (Fig. S2 Supplementary materials, APEX, Neptune Systems). Submersible pumps (NEWA) provided water motion in each experimental tank. Three times a week, each tank was provided with a 30 mL solution of freshly hatched brine shrimps (*Artemia* sp.) for feeding.

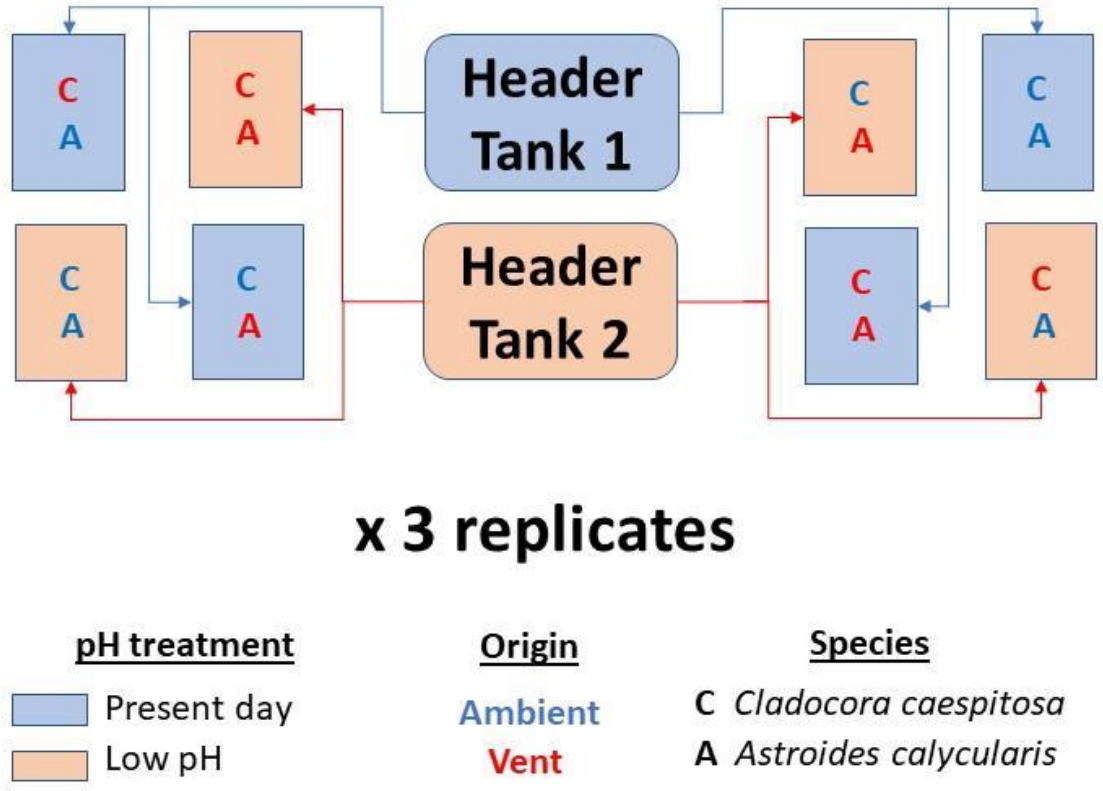



**Figure 1. Experimental set-up used to test the effects of origin (ambient vs vent site) and pH treatment (present day, $pH_T$ = 8.07 ± 0.01 and low pH, $pH_T$ = 7.72 ± 0.01) on the coral *C. caespitosa* and *A. calycularis*.** The experimental set-up was repeated 3 times, resulting in 6 header tanks, and 24 experimental tanks in which corals were randomly assigned.

Health status of experimental colonies

With pictures at the start of the experiment, each colony has been classified into two categories: colonies with loss of coenosarc (tissue between polyps) and colonies with tissue connecting their polyps. The number of dead polyps per colony (complete disappearance of tissue in the calice) was counted by comparing the pictures of the same colony at the start and the end of the experiment.

Carbonate chemistry in experimental tanks

pH in the header and experimental tanks was measured weekly using a handheld pH-meter (826 pH mobile, Metrohm) calibrated with a TRIS buffer (batch #T33 provided by A. Dickson, Scripps Institution of Oceanography, USA) before each set of measurements. Total alkalinity ($A_T$) of each experimental and header tank was measured weekly during the first four weeks in order to ascertain whether conditions were altered by metabolic activity. Subsequently, and for the remainder of the experiment, $A_T$ was measured every month in four randomly selected tanks and one header. $A_T$ was determined by potentiometric titration using a Metrohm 888 Titrando following the method of (Dickson et al. 2007). Titrations of certified reference material (Batch #186) provided by A. Dickson were used to assess the accuracy of the measurements and were within 6.5 µmol $kg^{-1}$ of the reference value. Ischia's temperatures are between 0.87 to 2.67°C higher than in the Bay of Villefranche during the studied period. Water pumped on the Bay of Villefranche was heated to be as close as possible to Ischia's temperatures. Salinity at Ischia and in the Bay of Villefranche-sur-Mer are similar. Salinity data were gathered from the salinity recorded weekly in the Bay of Villefranche by the *Service d'Observation Rade de Villefranche*, *SO-Rade*, of the *Observatoire Océanologique and the Service d'Observation en Milieu Littoral, SOMLIT/CNRS-INSU*. $pH_T$, temperature, $A_T$ and salinity were used to calculate the other carbonate chemistry parameters using the R package seacarb (Gattuso et al. 2020) (Tab. 1, Tab. S2 Supplementary materials).

Six-month and monthly calcification

Calcification rate was assessed using the buoyant weight technique (Davies 1989). Weighing was done every 27 to 35 days. Changes in wet weight were converted to dry weight using the following equation:

$$dry\ weight = \frac{wet\ weight}{(1 - \frac{water\ density}{aragonite\ density})}$$

with an aragonite density of 2.93 g $cm^3$. Six-month calcification rates corresponding to the entire experiment duration were determined as the change in dry weight between the first and the last weighing, normalized by the corals living tissue surface at the end of the experiment and the number of days of the experiment (202 days). Regular rates of calcification over time were normalized to the surface of the corals and to the number of days between weighing (~30 d). Surfaces were determined by the aluminum foil technique for *C. caespitosa* (Marsh 1970) because these 3D colonies were impossible to analyze with 2D pictures. *A. calycularis* surfaces were measured by photographic analysis with the software ImageJ for (IMAGEJ, NIH US Department of Health and Human Services) as they had flat shape. Surface measurements were completed at the start and end of the experiment. Marginal changes in surface area were observed between the beginning and end of the experiment.

Dark respiration, gross photosynthesis and short-term calcification

Dark respiration, net photosynthesis and short-term calcification were determined during less than 1 h incubations in 500 ml transparent perspex chambers, placed in a temperature-controlled water bath to maintain a constant temperature. Incubations were carried out a month after the start of the experiment (hereafter "start") and a month before the end of the experiment (hereafter "end") at the same temperature of 20°C. Magnetic stirrers were used to provide water motion in the incubation chambers. Each colony was transferred to an individual chamber filled with water from the experimental tanks from which the corals were taken for the incubation. A blank incubation with no coral colony and filled with water from the same header tank was performed for each four experimental tanks. Incubations lasted 30 to 60 min depending on the magnitude of the metabolic activity. To measure oxygen uptake

during respiration, colonies were maintained in darkness by covering the chamber with an opaque plastic bag and incubations started after a 10 min acclimation period. Production of oxygen by photosynthesis was only measured on colonies of *C. caespitosa* under an irradiance of 180 µmol photons $m^2$ $s^{-1}$. Oxygen saturation within the chambers was measured every 5 s using a fiber optic oxygen sensor (PreSens, OXY-4 mini), which was calibrated at 0% saturation in an oxygen-free solution created by saturating seawater with sodium sulfite, and at 100% saturation in an oxygen-saturated seawater obtained by bubbling ambient air during 5 min. The difference in oxygen saturation between the beginning and end of the incubation was converted to $O_2$ mg $L^{-1}$ using the gas_O2sat function of the marelac R package (Soetaert et al. 2020). Oxygen consumption was then normalized by coral surface area, incubation time and chamber volume to obtain the rates of respiration (negative in dark conditions) and net photosynthesis (oxygen production in excess of the respiratory demand under light conditions). The rate of gross photosynthesis was calculated by subtracting the respiration rate in dark to the rate of net photosynthesis, with the assumption that respiration rate is the same under light and dark conditions.

Water samples were collected at the beginning and end of the incubation for each colony for calcification determination using the alkalinity anomaly technique (Smith and Key 1975). This method relies on the stoichiometric relationship between total alkalinity and calcification, where the precipitation of one mole of $CaCO_3$ lowers the total alkalinity by two moles. Alkalinity anomalies were normalized by the coral surface area, incubation time, and chamber volume to calculate light and dark calcification rates. The alkalinity anomaly method was only applied to the zooxanthellate *C. caespitosa*.

329 Data analysis

330 *Biological surveys:* A generalized linear mixed model was used to test for differences in
331 abundance (poisson family) as a function of site (fixed factor, 2 levels) and quadrat as a
332 random effect. Chi-square contingency tables were used to compare the size–frequency
333 distributions between sites. *Laboratory experiment*: The assumptions of normality and
334 equality of variance were evaluated through graphical analyses of residuals using QQ plot
335 functions in R software. Mean values of the colonies in the experimental tank were used as a
336 replicate for six-month calcification. Each incubation was treated as a statistical replica
337 because they were done in individual chambers on single individuals. The effect of header
338 tanks was first tested with an ANOVA and since its significance was negligible ($p > 0.05$), it
339 was not considered in subsequent analyses. A two way-ANOVA was then used to test for
340 differences in net calcification caused by the origin (vent or ambient pH sites) and the pH
341 treatments (present-day or low pH) for both *C. caespitosa* and *A. calycularis*. For net
342 calcification as a function of time, a three-way ANOVA was applied, with origin, pH
343 treatment, and time as fixed factors. Tukey's post hoc tests were conducted when significant
344 differences were detected. Three-way repeated measure ANOVA was used for dark
345 respiration, gross photosynthesis and short-term calcification since the same colonies of *C.*
346 *caespitosa* and *A. calycularis* were used at the start and end of the incubations. Results have
347 been reported as means ± standard error of the mean (SE) hereafter, unless otherwise noted.

348 **Results**

349 In situ environmental conditions and populations of corals

350 Water carbonate chemistry and in situ monitoring of seawater $pH_T$ (pH on the total scale) at
351 the $CO_2$ vent and reference sites with ambient pH showed mean $pH_T = 7.91$ at Vent 1, $pH_T =$
352 7.91 at Vent 2, $pH_T = 8.05$ at Ambient 1, and $pH_T = 7.97$ at Ambient 2, respectively (Tab. 1).

353 The field surveys showed significant lower abundance of *C. caespitosa* at Vent 1 compared to
354 Ambient 1 (1.8 ± 0.16 colonies versus 3.3 ± 0.35, $Z = 2.2$, $p < 0.02$, Fig. S3 Supplementary
355 materials). No large colonies of more than 21 cm were found at Vent 1 (Fig. S1,
356 Supplementary materials). This reduction of size was also observed for *A.calycularis* at Vent
357 2 (92 % had up to 10 polyps *versus* 72 % in Ambient 2), which differed significantly between
358 sites ($\chi^2 = 76.7$, $p<0.0001$). Abundance of *A. calycularis* was lower in Vent 2 (67.1 ± 10.3 at
359 Vent 2 *versus* 80.3 ± 14.5 at Ambient 2, $Z = 2.65$, $p < 0.08$).
360

**TABLE 1. Measured and estimated seawater physiochemical parameters at the $CO_2$ vent sites, reference areas with ambient pH and both pH treatment in the experimental tanks for salinity (S), temperature (T), total alkalinity (AT), dissolved inorganic carbon ($C_T$), $pH_T$, $pCO_2$, calcite (Ωc) and aragonite (Ωa) saturation.** Values are means ± SD with 25th and 75th percentiles. Calculated concentrations of $C_T$, $pCO_2$, Ωc and Ωa are shown. 1: Parameters measured from discrete water samples; 2: parameters measured with in situ sensors. pH conditions: vent system (low pH); ambient pH; ambient pH experimental treatment (present day); acidified pH experimental treatment (low pH).

| Local name | pH conditions | S | T (°C) | $A_T$ (µmol $kg^{-1}$) | $C_T$ (µmol $kg^{-1}$) | $pH_T$ | $pCO_2$ (µatm) | Ωc | Ωa |
|---|---|---|---|---|---|---|---|---|---|
| Vent1 | Low pH | 37.9[1] ± 0 (37.9, 37.9), n=3 | 16.8[2] ± 0.4 (16.6, 17.1), n=1326 | 2532[1] ± 16 (2522, 2545), n=25 | 2324 ± 47 (2292, 2341), n=1326 | 7.91[2] (7.89, 7.99), n=1326 | 626 ± 311 (497, 643), n=1326 | 3.69 ± 0.61 (3.43, 4.13), n=1326 | 2.39 ± 0.39 (2.22, 2.67), n=1326 |
| Ambient 1 | Ambient pH | 37.3[1] ± 0 (37.2, 37.3), n=3 | 17.3[2] ± 0.4 (17.0, 17.6), n=1331 | 2618[1] ± 15 (2607, 2633), n=14 | 2338 ± 20 (2324, 2350), n=1331 | 8.05[2] (8.03, 8.07), n=1331 | 448 ± 42 (417, 471), n=1331 | 4.80 ± 0.30 (4.61, 5.01), n=1331 | 3.11 ± 0.19 (2.98, 3.24), n=1331 |
| Vent 2 (Sept 2018) | Low pH | 37.3[1] ± 0.2 (37.2, 37.5), n=9 | 25.9[2] ± 0.2 (25.8, 26.0), n=1530 | 2564[1] ± 7 (2561, 2566), n=9 | 2542 ± 79 (2477, 2585), n=1530 | 7.65[2] (7.58, 7.90), n=1530 | 2905 ± 1664 (1724, 3438), n=1530 | 1.68 ± 0.59 (1.21, 2.21), n=1530 | 1.11 ± 0.39 (0.80, 1.47), n=1530 |
| Vent 2 (June 2019) | Low pH | 37.8[1] ± 0 (37.8, 37.8), n=7 | 21.8[2] ± 2.1 (19.8, 23.8), n=1841 | 2541[1] ± 20 (2533, 2550), n=7 | 2352 ± 89 (2289, 2389), n=1841 | 7.74[2] (7.74, 7.93), n=1841 | 983 ± 868 (590, 978), n=1841 | 3.56 ± 1.05 (2.96, 4.39), n=1841 | 2.33 ± 0.69 (1.94, 2.88), n=1841 |
| Ambient 2 | Ambient pH | 37.9[1] ± 0 (37.9, 37.9), n=7 | 26.4[2] ± 1 (25.9, 27.0), n=1691 | 2642[1] ± 17 (2629, 2659), n=17 | 2324 ± 21 (2310, 2338), n=1691 | 7.97[2] (7.94, 8.00), n=1691 | 556 ± 57 (513, 597), n=1691 | 5.53 ± 0.32 (5.31, 5.75), n=1691 | 3.67 ± 0.22 (3.53, 3.82), n=1691 |
| Experimental tanks | Present day | 37.8 ± 0.5 (37.3, 38.3), n=25 | 22.6 ± 1.9 (20.7, 24.5), n=347 | 2542 ± 54 (2488, 2596), n=347 | 2206 ± 63 (2143, 2269), n=347 | 8.06 ± 0.06 (8.00, 8.12), n=347 | 415 ± 70 (345, 485), n=347 | 5.65 ± 0.69 (4.96, 6.34), n=347 | 3.71 ± 0.46 (3.25, 4.17), n=347 |
| Experimental tanks | Low pH | 37.8 ± 0.5 (37.3, 38.3), n=25 | 22.7±1.9 (20.8, 24.6), n=348 | 2542 ± 54 (2488, 2596), n=348 | 2389 ± 65 (2324, 2454), n=348 | 7.73 ± 0.09 (7.64, 7.82), n=348 | 1048 ± 237 (811, 1285), n=348 | 2.99 ± 0.56 (2.43,3.55), n=348 | 1.97 ± 0.37 (1.60, 2.34), n=348 |

366 Experimental conditions

367 Weekly temperature in the tanks varied according to natural changes in the summer
368 temperature profile at 10 m depth in Ischia, from 19.08 ± 0.06 °C at the end of May 2019
369 (mean ± SE, n = 24) to 21.51 ± 0.07 °C at the beginning of December (mean ± SE, n = 24)
370 with a maximum value of 25.17 ± 0.15 °C in August (mean ± SE, n = 24, Fig. S2
371 Supplementary materials). The "present-day" treatment was maintained at a mean $pH_T$ of 8.06
372 ± 0.06 (n = 347) whereas "low pH" treatment had a $pH_T$ of 7.73 ± 0.09 (n = 348). Salinity was
373 37.8 ± 0.5 (n = 25).

374 Colonies of *Astroides calycularis* had physical differences depending on their site of origin.
375 85% (23/27 colonies) of vent and 14% (2/14 colonies) of ambient colonies did not present
376 coenosarc (i.e. tissue between polyps). Furthermore, by comparing photos from the beginning
377 and end of the experiment, it was observed that none of the *A. calycularis* colonies from
378 ambient suffered polyp death, while three vent colonies showed 2/5, 1/7 and 4/9 dead polyps
379 (number of dead polyps / total number of polyps in the colony). No polyp loss was observed
380 in *Cladocora caespitosa*.

381 Six-month, monthly and short-term calcification rates

382 The rates of six-month calcification of *C. caespitosa* and *A. calycularis* were not affected by
383 the origin and the pH treatment (Fig. 2; Tab. S3 Supplementary materials; Two-way ANOVA,
384 $F_{1,20}$= 1.778, $P$ = 0.2 for *C. caespitosa*, $F_{1,20}$= 0.13, $P$ = 0.72 for *A. calycularis*).

385 No statistically significant effect of origin and pH treatment on calcification rate was found in
386 *C. caespitosa* over time (Fig. 3A; $F_{1,110}$= 1.919, $P$ = 0.17). However, different calcification
387 rates with time were found for *A. calycularis* (Fig. 3B, $F_{1,112}$= 26.2, $P$ < 0.001), with
388 significantly higher calcification measured during the first month of the experiment (1.0 ± 0.2

mg $CaCO_3$ $cm^{-2}$ $d^{-1}$). The mean monthly calcification rate was 0.2 ± 0.1 mg $CaCO_3$ $cm^{-2}$ $d^{-1}$ during the following months of experiment.

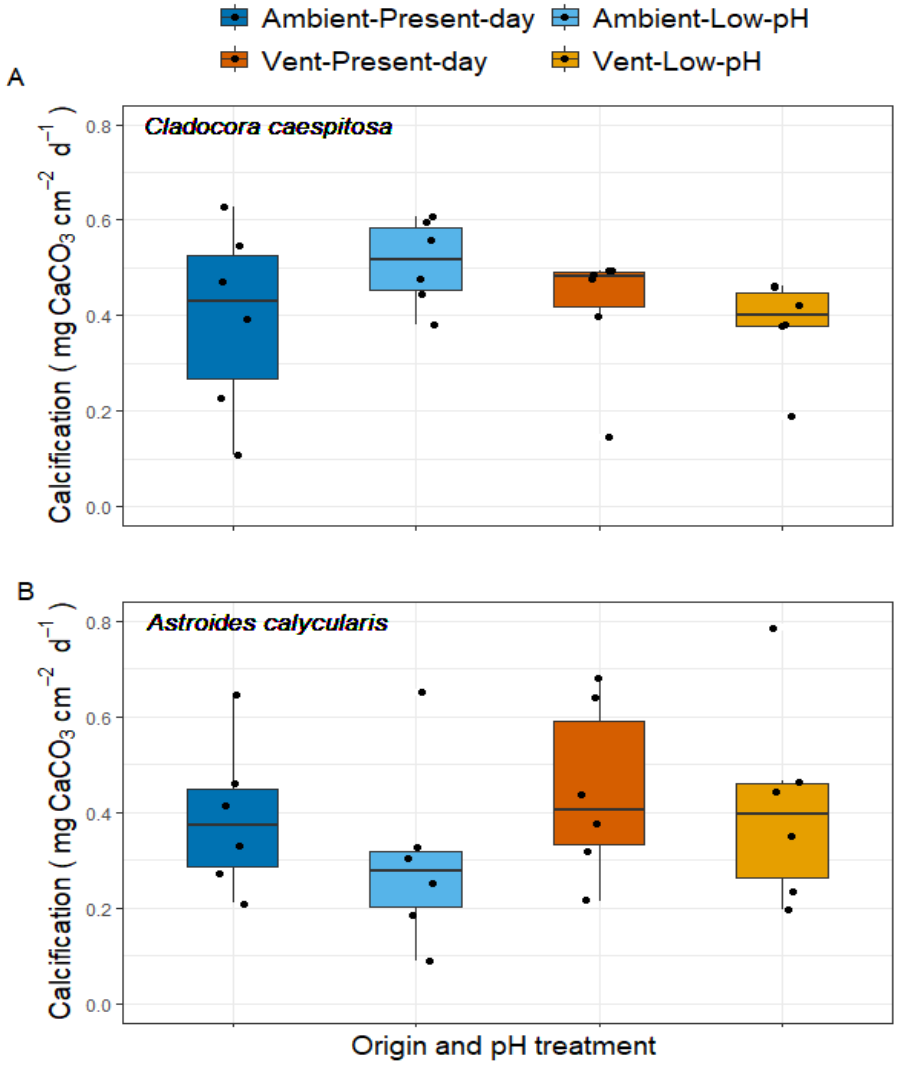
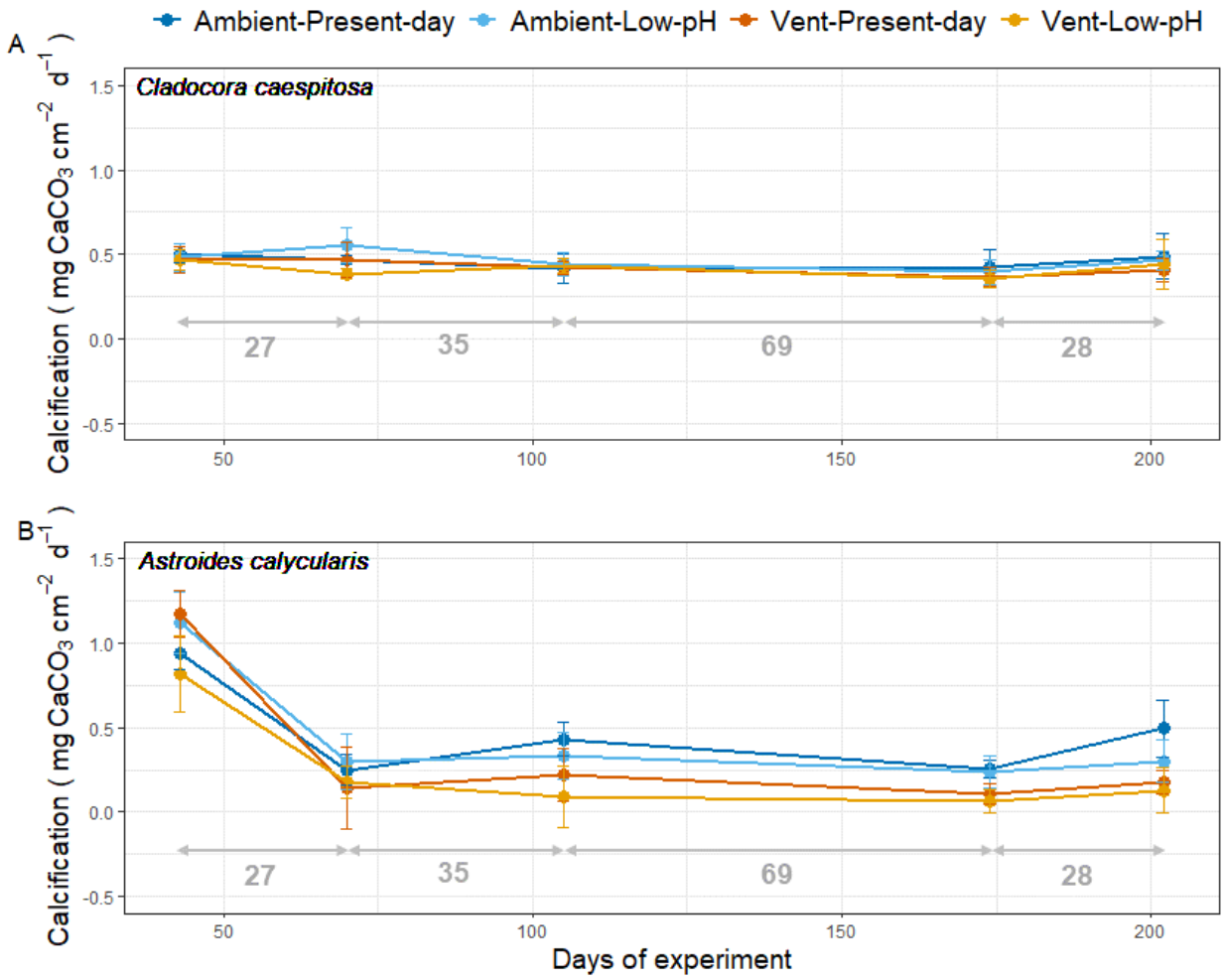



**Figure 2. Six-month calcification rates of *C. caespistosa* and *A. calycularis* collected from vent and ambient sites exposed to the two pH treatments (pHT = 8.08 ± 0.01, present day; pHT = 7.72 ± 0.01, low pH) during the entire duration of the experiment (202 days).** Panel A) shows *Cladocora caespitosa* and panel B) shows *Astroides calycularis*. Dots represent the mean calcification rates of colonies per experimental tank and boxes represent the median and the 25 and 75% quartiles. The color of the box indicates the origin and the treatment. n = 6 per pH treatment and origin.




399

400 **Figure 3. Calcification rates of *C. caespistosa* and *A. calycularis* from vent and ambient**
401 **sites under the two pH treatments (**$pH_T$ = 8.08 ± 0.01, present day; $pH_T$ = 7.72 ± 0.01, low
402 pH**) over time (every 27 to 35 days).** Values are means ± SE, Panel A) *Cladocora caespitosa*
403 and B) *Astroides calycularis*. The color of the dots and lines indicates the origin and the
404 treatment. We had a technical problem with day 139 weighing which is therefore not
405 represented here. Numbers below arrows between dots represent the number of days between
406 measurements. n = 6 per pH treatment and origin.

407 Short-term calcification of *C. caespitosa* in the dark and in the light was not significantly
408 different at the start (Fig. 4A and 4B; 10 ± 1.1 and 15.65 ± 1.85 µg $CaCO_3$ $cm^{-2}$ $h^{-1}$,
409 respectively) and the end of the experiment (Fig. 4C and 4D; 11.42 ± 1.40 and 25.21 ± 3.03
410 µg $CaCO_3$ $cm^{-2}$ $h^{-1}$, respectively). Colonies light and dark calcification were also not affected
411 by origin and pH treatment (Fig. 4; $F_{1,40}$= 0.07, $P$ = 0.795).

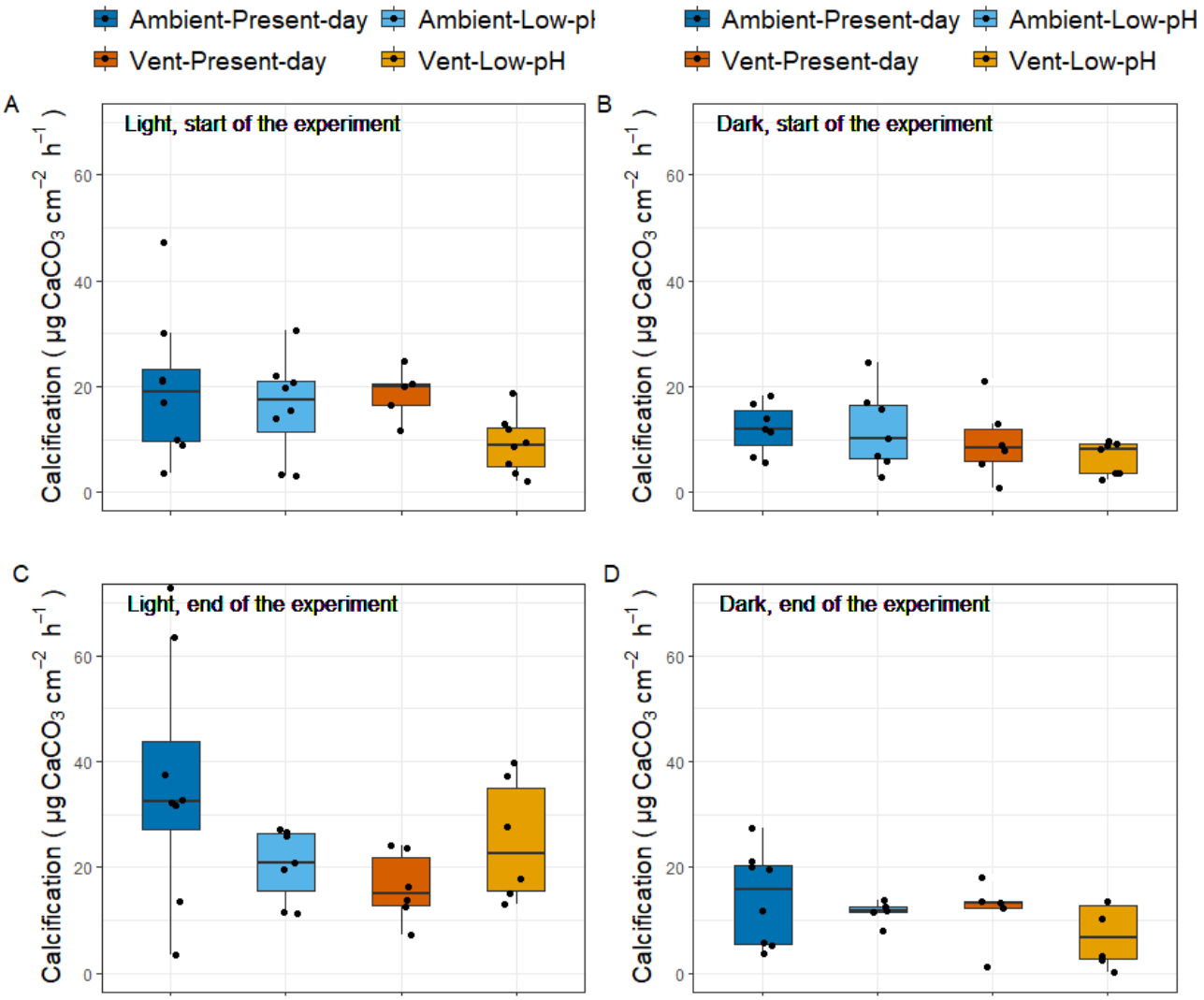



**Figure 4. Short-term calcification rates for *Cladocora caespitosa* from vent and ambient sites under the two pH treatments** ($pH_T$ = 8.08 ± 0.01, present day; $pH_T$ = 7.72 ± 0.01, low pH) **in the light and dark at the start (27th of June 2019) and end of the experiment (24th of October 2019).** Values are means ± SE. Rates were calculated using alkalinity anomaly method following short term incubations (< 1 h) at 20°C. Panel A) and C) under light conditions, B) and D) under dark conditions. Dots represent the calcification rates of the coral colonies and boxes represent median and 25 and 75% quartiles. The color of the box indicates the origin and the treatment. n = 5 to 8 per pH treatment and origin.

Dark respiration and gross and net photosynthesis

Net photosynthesis of *C. caespitosa* was not affected by origin and pH treatment (Fig. 5A; $F_{1,40}$= 2.36, $P$ = 0.134 and $F_{1,40}$= 1.24, $P$ = 0.272, respectively), but was different between the

beginning and the end of the experiment (Fig. 5A; $F_{1,40}$= 13.14, $P$ < 0.001). Gross photosynthesis of *C. caespitosa* did not exhibit any statistically significant difference between origin, pH treatment and time (Fig. 5B; $F_{1,24}$= 0.17, $P$ = 0.683). Mean gross photosynthesis for all pH treatments was similar at the start and end of the experiment (0.024 ± 0.001 vs 0.020 ± 0.002 mg $O_2$ $cm^{-2}$ $h^{-1}$, n = 30). As light and dark respiration may differ, gross photosynthesis measurements are uncertain, and most likely underestimated.

Dark respiration rate of *C. caespitosa* was not significantly different between origin and pH treatment (Fig. 5C, $F_{1,24}$= 0.008, $P$ = 0.93 and $F_{1, 24}$= 3.09, $P$ = 0.091, respectively), but was significantly different between the beginning and end of the experiment ($F_{1,24}$= 9.45, $P$ = 0.005). In June, mean dark respiration rates for all conditions (origin and pH treatment) was -0.0079 ± 0.0003 mg $O_2$ $cm^{-2}$ $h^{-1}$ (n = 30) while in November it was -0.0097 ± 0.0006 mg $O_2$ $cm^{-2}$ $h^{-1}$ (n = 28), which represents a 23% of increase in dark respiration.

At the start of the experiment, the dark respiration of *A. calycularis* did not differ between origin or pH treatment (Fig. 5D, $F_{1,37}$= 0.32, $P$ = 0.58). At the end of the experiment (Fig. 5D), a significant difference between site of origin was observed ($F_{1,36}$= 18.5, $P$ < 0.0001), with corals from the ambient site having a mean dark respiration of -0.038 ± 0.003 mg $O_2$ $cm^{-2}$ $h^{-1}$ (n = 13) whilst colonies from the $CO_2$ vent site had a rate of -0.087 ± 0.008 mg $O_2$ $cm^{-2}$ $h^{-1}$ (n = 27). The overall (all treatments) mean dark respiration rate were also different at the start and at the end of the experiment, with a lower mean dark respiration at the start (-0.026 ± 0.002 vs -0.071 ± 0.007 mg $O_2$ $cm^{-2}$ $h^{-1}$; $F_{1,36}$= 52.3, $P$ < 0.0001).

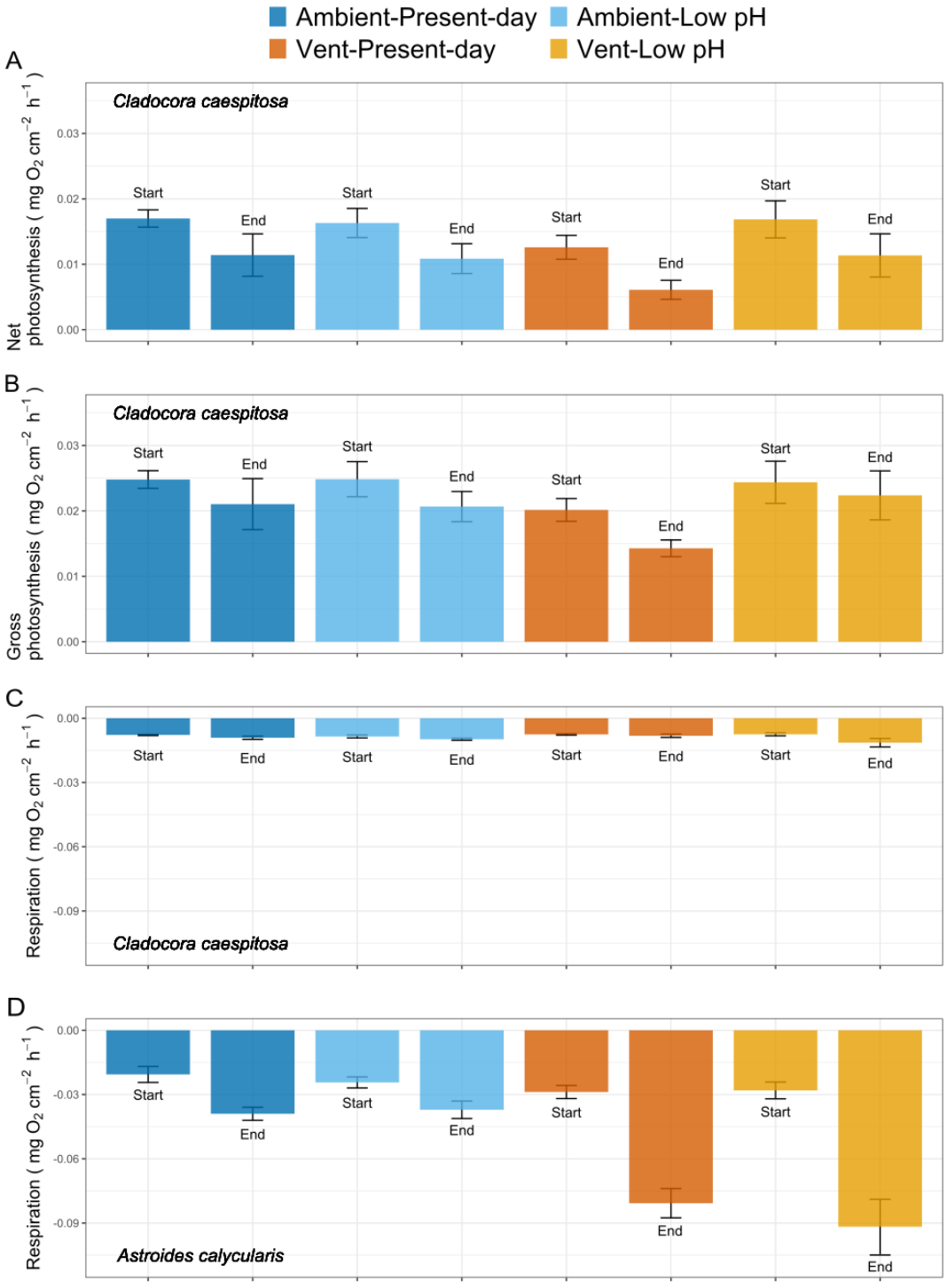



**Figure 5. Net and gross photosynthesis and dark respiration rates of *C. caespitosa* and *A. calycularis* from the vent and ambient sites under the two pH treatments (**$pH_T$ = 8.08 ± 0.01, present day; $pH_T$ = 7.72 ± 0.01, low pH**) at the start (27th of June 2019) and end of the experiment (24th of October 2019).** Values are means ± SE, Panel A) *Cladocora caespitosa*'s net photosynthesis, B) *Cladocora caespitosa*'s gross photosynthesis, C) *Cladocora caespitosa*'s dark respiration. D) *Astroides calycularis*' dark respiration. The color of the bars indicates the origin and the treatment. n = 6 to 15 per pH treatment and origin.

## Discussion

During this six-month laboratory experiment, we tested whether colonies of the zooxanthellate coral *Cladocora caespitosa* and the azooxanthellate coral *Astroides calycularis* growing in naturally acidified waters were more tolerant to low pH than colonies from ambient pH sites or acclimated. In contrast to our initial hypothesis, calcification rates and metabolic responses did not change for any coral species no matter the site of origin and pH treatment. Therefore, our results suggest that both species, independently of environmental history and trophic strategy, can tolerate low pH conditions for six months, under controlled conditions in aquaria. In contrast, in situ surveys revealed lower abundance and smaller sizes for both corals at $CO_2$ vent sites compared with ambient sites.

The hypothesis that corals from vent sites would be less impacted by low pH stems from reports that organisms experiencing highly variable or low pH in their natural habitat may be more tolerant to ocean acidification (Cornwall et al. 2018). Corals living in suboptimal environmental conditions may possess physiological and potentially genetic mechanisms that confer tolerance to such environmental conditions (Camp et al 2018, Teixidó et al 2020). For example, microhabitats experiencing periodic temperature extremes are potential habitats for heat tolerant corals (Schoepf et al. 2015), yet these habitats can exhibit a mosaic of populations, harboring species previously reported with high to low thermal tolerance (Ainsworth et al. 2016). Such physiological mechanisms and intraspecific variability may exist in *C. caespitosa* and *A. calycularis* which highlights the importance of studying the influence of suboptimal environmental conditions on tolerance to stressors.

Previous research has shown a decrease in calcification when corals were transplanted from ambient pH sites to $CO_2$ vents (Rodolfo-Metalpa et al. 2011; Prada et al. 2017). However, previous studies on temperate/cold-water coral including *C. caespitosa* and *A. calycularis* have also shown a greater tolerance to ocean acidification than most tropical corals (see Tab.

S5 Supplementary materials). Calcification rates reported in the present study agree with earlier studies. For *C. caespitosa*, Rodolfo-Metalpa et al. (2010) observed a yearly calcification rate of 0.51 mg $CaCO_3$ $cm^{-2}$ $d^{-1}$, which is similar to what we measured during this six-month experiment (0.43 ± 0.03 mg $CaCO_3$ $cm^{-2}$ $d^{-1}$). The calcification rates of *A. calycularis* measured here are also of the same order of magnitude to those reported by Movilla et al. (2016) (0.39 ± 0.04 and 0.47 ± 0.06 mg $CaCO_3$ $cm^{-2}$ $d^{-1}$, respectively). In contrast to temperate and cold-water corals, tropical corals tend to be more affected by ocean acidification, as they exhibit a mean decrease in calcification of 10-25% when exposed to the $pCO_2$ expected by the end of the century under the high-$CO_2$ emissions scenario (Chan and Connolly 2013; Cornwall et al. 2021). Most tropical corals investigated so far are fast calcifiers, growing in warm waters, therefore as seawater pH declines, they may be unable to maintain these energetically costly fast rates of calcification (Comeau et al. 2014). In contrast, temperate and cold-water corals are slower calcifiers with lower energy requirements for mineralization, thus making them more likely to maintain constant calcification rates at lower pH (Rodolfo-Metalpa et al. 2010, Comeau et al. 2014). Ries et al. (2010), also suggested that the effect of acidification on the calcification of temperate corals, such as *Oculina arbuscula*, may manifest at a lower pH threshold compared to tropical corals. The capacity of *C. caespitosa* and *A. calycularis* to control the carbonate chemistry at their site of calcification could explain this tolerance to low pH. For example, using the geochemical proxy $\delta^{11}B$ it was shown that the temperate coral *Balanophyllia europaea* at the $CO_2$ vents of Panarea Island (Italy) can maintain constant calcifying fluid pH when exposed to pH ranging from 8.07 to 7.74 (Wall et al. 2019). This confirms the capacity of some corals to maintain internal pH homeostasis and utilize this as a way of calcifying under reduced seawater pH (Comeau et al. 2019).

From this study no conclusions can be drawn as to whether colonies from the vent sites are acclimated to low pH, as colonies from both vent sites and the ambient sites were not affected by low pH in the laboratory. However, controlling the transport of calcium and inorganic carbon to the calcifying medium and producing the skeletal organic matrix are all energy-demanding processes (Allemand et al. 2011, Wall et al. 2019), therefore the maintenance of calcification rates under these treatments may have come at a cost. For example, Wall et al. (2019) suggest that the inactivation of some calicoblastic cells could compensate for the energy required to regulate internal pH and maintain calcification rates under low seawater pH, but at the cost of increased skeletal porosity. This is supported by a study that showed a constant growth rate of the temperate coral *B. europaea* in acidified conditions at the expense of skeletal density (Fantazzini et al. 2015), which in return increases the sensitivity to bioerosion and susceptibility to damage (Crook et al. 2013). Skeletal porosity was not measured in the present study. However, increased sensitivity to bioerosion can also be linked to the tissue coverture of the skeleton. Rodolfo-Metalpa et al. (2010) observed that following transplantation to low pH conditions, skeleton dissolution was observed in *Cladocora caespitosa* which had exposed skeleton, and not in *Balanophyllia europaea* which had less exposed skeleton. In our study, we observed that most of the *A. calycularis* colonies collected at the $CO_2$ vent sites did not possess coenosarc (i.e. coral tissue between polyps) and exhibited polyp death during our experiment. These phenomena were also observed in natural colonies of *A. calycularis* (Teixidó et al. 2020) and the corals *Pocillopora damicornis* and *Oculina patagonica* when incubated at low pH (Kvitt et al. 2015). The increased sensitivity to bioerosion of *A. calycularis* and *C. caespitosa* with exposed skeleton could explain in part the field observation.

Field observations confirmed the difference between the population of the $CO_2$ vent and ambient sites, as both corals were less abundant and had smaller colonies at the vent sites

527 (Fig. S3 Supplementary materials, Teixidó et al. 2020). These contrasting results between
528 field and laboratory observations, could be explained by differences in pH variability, which
529 is much greater in the field with short periods of very low pH. Alternatively, other
530 environmental parameters such as food availability or biological interactions (e.g.,
531 competition with algae) may contribute to these differences. pH variability at the $CO_2$ vent
532 sites can be very high, as $pH_T$ can range from 6.70 to 8.13 in a day (Foo et al. 2018, Teixidó et
533 al. 2020), whereas $pH_T$ was very stable during our pH-controlled laboratory experiment ($pH_T$
534 = 7.72 ± 0.01 for low pH treatment). It may be that no differences in calcification and
535 photosynthesis were observed in the laboratory, due to the fact that both corals are slow-
536 growing and long-lived species that have had a lifetime to acclimatize to their environmental
537 pH. Therefore, the six-month time scale of the experiment may not have been enough to
538 trigger any visible variation. However, even if our results did not show any statistical
539 difference between treatments during the laboratory experiment, different trends in
540 calcification between conditions can be observed for *A. calycularis*. The colonies from
541 ambient-low pH condition seem to have lower calcification rate than the control colonies of
542 ambient-present day condition (Fig. 2), suggesting a possible impact of low pH on
543 calcification in the short term. The same trend is observed with the colonies from the vent
544 site, as the vent-low pH presents reduced calcification rate compared to the vent-present day
545 condition. Moreover, calcification was similar in the vent-low pH and the control ambient-
546 present day conditions. This trend suggests a possible acclimation to low pH in corals
547 growing at the $CO_2$ vent site

548 Zooxanthellate and azooxanthellate corals were both used in the present study to assess the
549 role that photosynthesis could play in mitigating the effects of ocean acidification on
550 calcification. In zooxanthellate corals, light-enhanced calcification describes the relationship
551 between calcification, photosynthesis and irradiance (Gattuso et al. 1999; Allemand et al.

552 2011). Four main mechanisms explaining light-enhanced calcification were proposed:
553 photosynthesis could (1) supply energy to the animal through the translocation of
554 photosynthates, (2) increase pH at the site of calcification by using $CO_2$, (3) remove
555 phosphate, which is poison to carbonate crystallization, and (4) facilitate the production of the
556 organic matrix which serves as a scaffold in which $CaCO_3$ is precipitated (Allemand et al.
557 2011). Here, short-term calcification of *C. caespitosa* was higher in light than in the dark,
558 with a mean light:dark calcification ratio of 2.38 (See Tab. S4 Supplementary materials),
559 which is close to the mean ratio of 3.0 obtained by Gattuso et al. (1999). However, neither
560 light nor dark calcification rates differed with experimental pH conditions, thus we cannot
561 conclude that photosynthesis did provide better tolerance to ocean acidification. Furthermore,
562 no difference in the response of calcification to ocean acidification was observed between the
563 zooxanthellate (*C. caespitosa*) and azooxanthellate species (*A. calycularis*). The data from the
564 in situ survey at Ischia did not highlight a difference between the zooxanthellate and
565 azooxanthellate corals either, as the size and abundance of colonies from $CO_2$ vent sites were
566 lower for both species. Contrastingly, Prada et al. (2017) reported that the zooxanthellate
567 coral *B. europaea* was unaffected by low pH conditions, whereas the azooxanthellate corals
568 *Leptopsammia pruvoti* and *A. calycularis* showed a negative effect of pH on calcification.
569 However, the study of Prada et al. (2017) was conducted in situ, on corals transplanted next to
570 $CO_2$ vents in Panarea Island, whereas our study is based on a laboratory experiment and field
571 observations. Feeding could also explain this difference as it can mitigate the negative effects
572 of environmental stressors on coral physiology (Fox et al. 2018). In our experiment, corals
573 were fed freshly hatched *Artemia nauplii* three-times a week therefore the demand of energy
574 required to maintain calcification under low pH was likely sustained by heterotrophy
575 (Edmunds 2011). Kornder et al. (2018) showed that in studies where corals were fed, the
576 negative impacts of ocean acidification on corals were mitigated. Therefore, the fact that

577 Movilla et al. (2012) observed a decrease in calcification at low pH in *C. caespitosa* may be
578 explained by the fact that the colonies were only fed once per week.

579 No difference in photosynthesis was observed between site of origin or pH treatment. This
580 aligns with the observation that there is a limited impact of environmentally relevant
581 decreases in pH on photosynthesis (Comeau et al. 2017), and that low pH rarely causes
582 bleaching (Anthony et al. 2008). Moreover, Mediterranean corals harbor Clade B
583 *Symbiodinium*, that have growth rates, photosynthetic capacities and respiration rates that are
584 unaffected by acidification (Brading et al. 2011). Respiration was also not affected by the pH
585 treatment in *C. caespitosa*, confirming previous observations for this species (Rodolfo-
586 Metalpa et al. 2010), and other coral species (Comeau et al. 2017). However, colonies of *A.*
587 *calycularis* from the vent site exhibited a higher rate of respiration at the end of the
588 experiment (November) for both low and present-day pH treatments. This result contrasts
589 with studies that show a decrease in respiration at lower pH (e.g. Comeau et al. 2014), or no
590 impact on respiration (Rodolfo-Metalpa et al. 2010; Comeau et al. 2017). However, an
591 increase in respiration could be due to the increased energetic demand associated with
592 maintaining calcification rates at lower pH (Comeau et al. 2017). For example, a decrease in
593 seawater $pH_T$ of 0.7 units requires an additional 1–2 kJ of energy $mol^{-1}$ of $CaCO_3$
594 precipitated (McCulloch et al. 2012a). For zooxanthellate corals, this energy is less than 1%
595 of the energy provided by photosynthesis, however, for azooxanthellate corals such as *A.*
596 *calycularis*, respiration is the only way to up-regulate this energy demand. Moreover, the
597 increase of respiration from colonies of vent sites of *A. calycularis* could be partly due to the
598 smaller size of colonies at the vent site than at the ambient site (Edmunds and Burgess 2016).

599 Although the corals studied here showed tolerance in the laboratory to pH conditions expected
600 by 2100, it is a fragile equilibrium that can be disrupted by the inclusion of changes in other

environmental variables such as temperature that could lead to a greater impact on physiological processes and survival, as observed in the in situ survey and predicted by the negative trend on the calcification of *A. calycularis*. For example, marine heatwaves can lead to mass mortality of *C. caespitosa* and *A. calycularis* (Rodolfo-Metalpa et al. 2005) and are projected to be more frequent and severe in the future (Galli et al. 2017, Smale et al. 2019). Moreover, the impact of ocean acidification on reproduction as well as larval and juvenile survival, which can limit the persistence and dispersion of a species remains to be investigated. The present study contributes to our knowledge of *Cladocora caespitosa* and *Astroides calycularis'* ability to survive under future ocean acidification conditions. Such information will improve our ability to manage and conserve the biodiversity of Mediterranean calcifying species.

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## Author contributions

S.C., N.T., B.M, and J.-P.G designed the study. N.T., S.C., A.M. and B.M. were involved with fieldwork. C.C, B.M., T.G., S.C., and N.T performed the experiments. C.C., N.T. and A.M. analyzed the data. C.C. wrote the first draft of the manuscript which was then finalized by all co-authors.