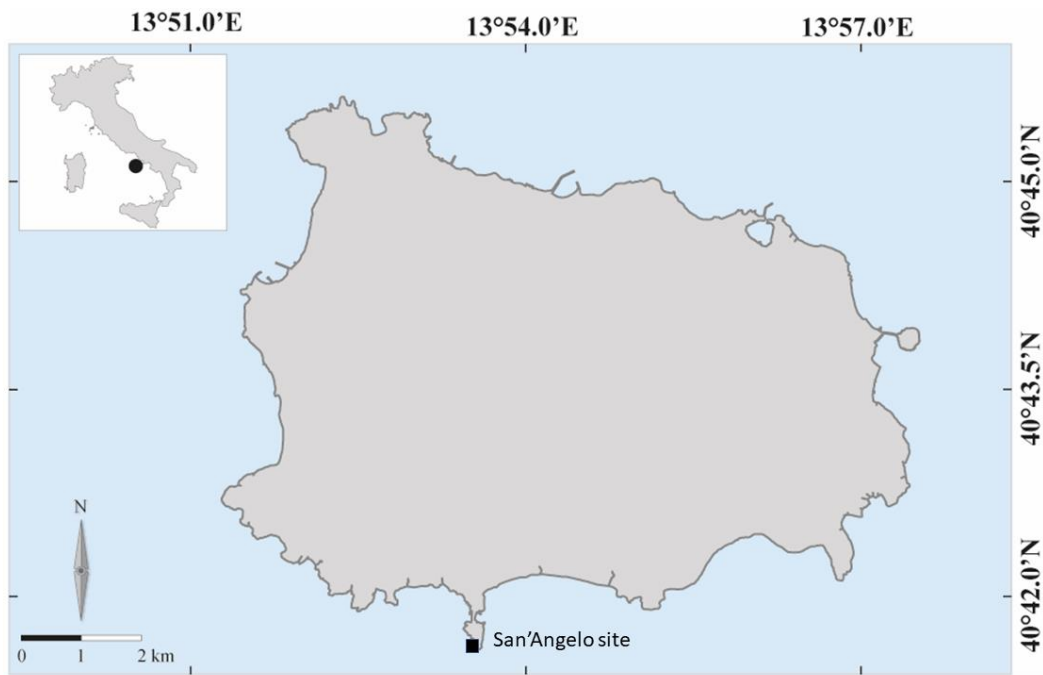


# **Early life stages of a Mediterranean coral are vulnerable to ocean warming and acidification**

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## **Supplementary materials**



**Figure S1 Map of the study site along the Southern coast of Ischia Island (Italy).** *Astroides calycularis* adult colonies were sampled at 10m depth at the site of Sant'Angelo (40°41'31.1"N 13°53'35.0"E) on the 1st of July 2020.

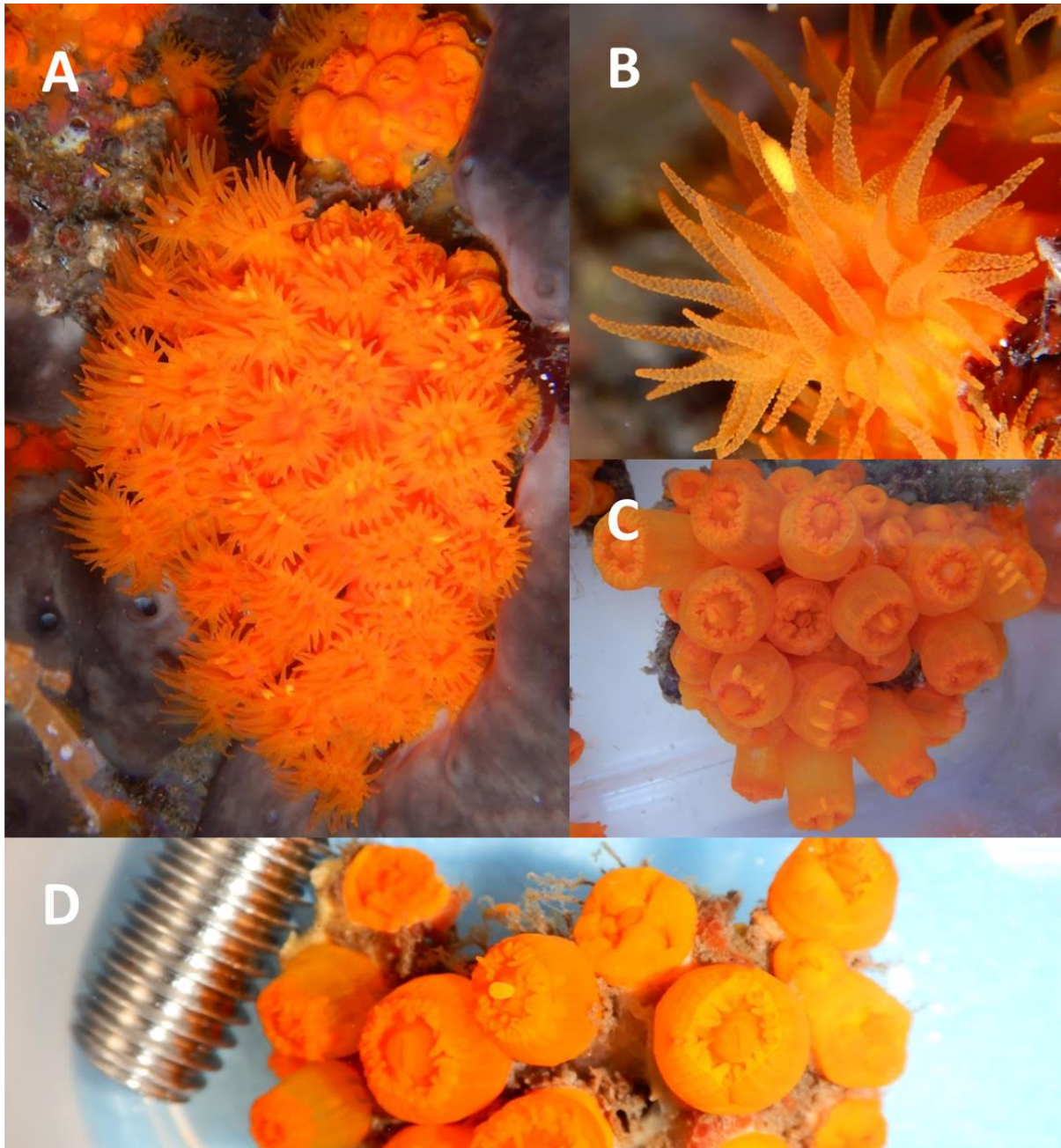


Figure S2 **Picture of the internal brooding reproduction last processes of *Astroides calycularis*** A) *In situ* Female colony brooding larvae, where the larvae are the yellow oval structures in the tentacles of the polyps B) Close up on a larva inside the tentacle of a female polyp C) Close up of larvae inside the gastrovascular cavity of a female polype and, D) Spawning of a larva from the mouth of a female polyp.

### Micro-CT scanner setup

Six skeletons of each condition (total of 24) were scanned in a micro-CT scanner (GE Healthcare, eXplore Locus RS) at a peak x-ray energy of 80 kVp and tube current of 450 uA at a resolution of 20  $\mu\text{m}$ . 900 x-ray projections or views (image matrix size 2010 x 1920 pixels) were collected at 0.4-degree angular increments over a single 360-degree rotation. Each projection was 4500 ms in duration and 2 image frames were acquired at each view angle and averaged to reduce image noise resulting in a 2.75 hourlong scan. Each averaged x-ray projection was corrected for brightfield, darkfield, and geometric distortions and then a 3D volumetric image was re-constructed from the x-ray projection data using a filtered-back projection algorithm with isotropic 20.171  $\mu\text{m}$  voxels. Sample vials containing air, water, Hydroxyapatite SB3 cortical bone mimic  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ , (Gammex RMI, density = 1.83  $\text{g}/\text{cm}^3$ ), calcite crystal (density = 2.71  $\text{g}/\text{cm}^3$ ), and aragonite crystal (density = 2.95  $\text{g}/\text{cm}^3$ ) were included in the scanner field of view (FOV) during acquisition and used for calibration purposes. The 3D data set was linearly rescaled into Hounsfield units (HU) so that air was represented by voxel greyscale values near -1000 and water by values near 0.

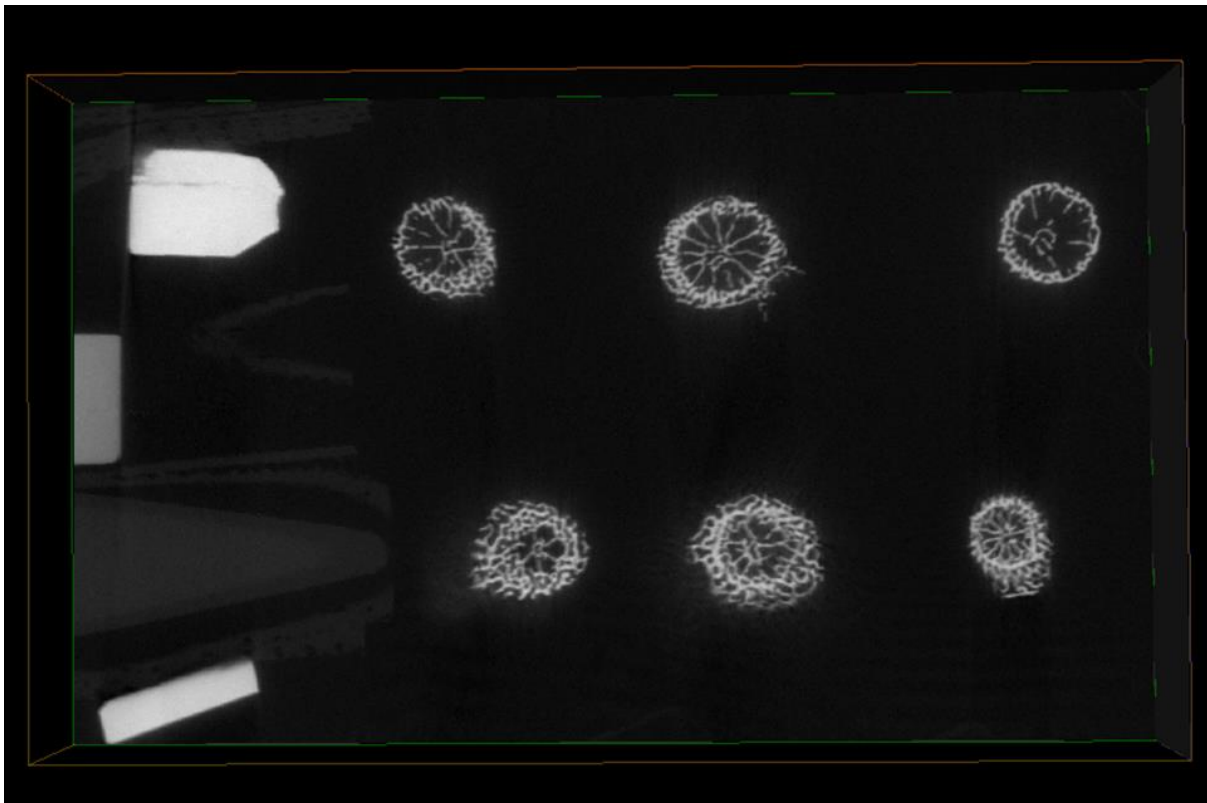


Figure S3 **Picture of the micro-CT scan set up** with six nine-month old recruits skeletons (on the right), and calcite, aragonite crystals and water for calibration (on the left).

**Table S1 Comparison among hierarchical linear mixed-effects models assessing the different measured parameters.** *The structure of the random term was selected by comparing models with different error structures using the Akaike information criterion (AIC).*

<b>Model</b>	<b>df</b>	<b>AIC</b>
<i>Proportions of planula</i>		
Planula~pH_condition*temperature_condition*date + (1 date)	10	1707.0
Planula~pH_condition*temperature_condition*date + (1 cristallyser)	10	2325.8
Planula~pH_condition*temperature_condition*date + (1 date) + (1 cristallyser)	11	1693.4
<i>Proportion of settled polyps</i>		
Settled~pH_condition*temperature_condition*date + (1 date)	10	1996.0
Settled~pH_condition*temperature_condition*date + (1 cristallyser)	10	2126.4
Settled~pH_condition*temperature_condition*date + (1 date) + (1 cristallyser)	11	1950.5
<i>Proportion of non-settled polyps</i>		
Non-settled~pH_condition*temperature_condition*date + (1 date)	10	1530.2
Non-settled ~pH_condition*temperature_condition*date + (1 cristallyser)	10	1433.6
Non-settled ~pH_condition*temperature_condition*date + (1 date) + (1 cristallyser)	11	1395.3
<i>Mortality</i>		
Mortality~pH_condition*temperature_condition*date + (1 date)	10	1496.8
Mortality ~pH_condition*temperature_condition*date + (1 cristallyser)	10	1682.8
Mortality ~pH_condition*temperature_condition*date + (1 date) + (1 cristallyser)	11	1398.3

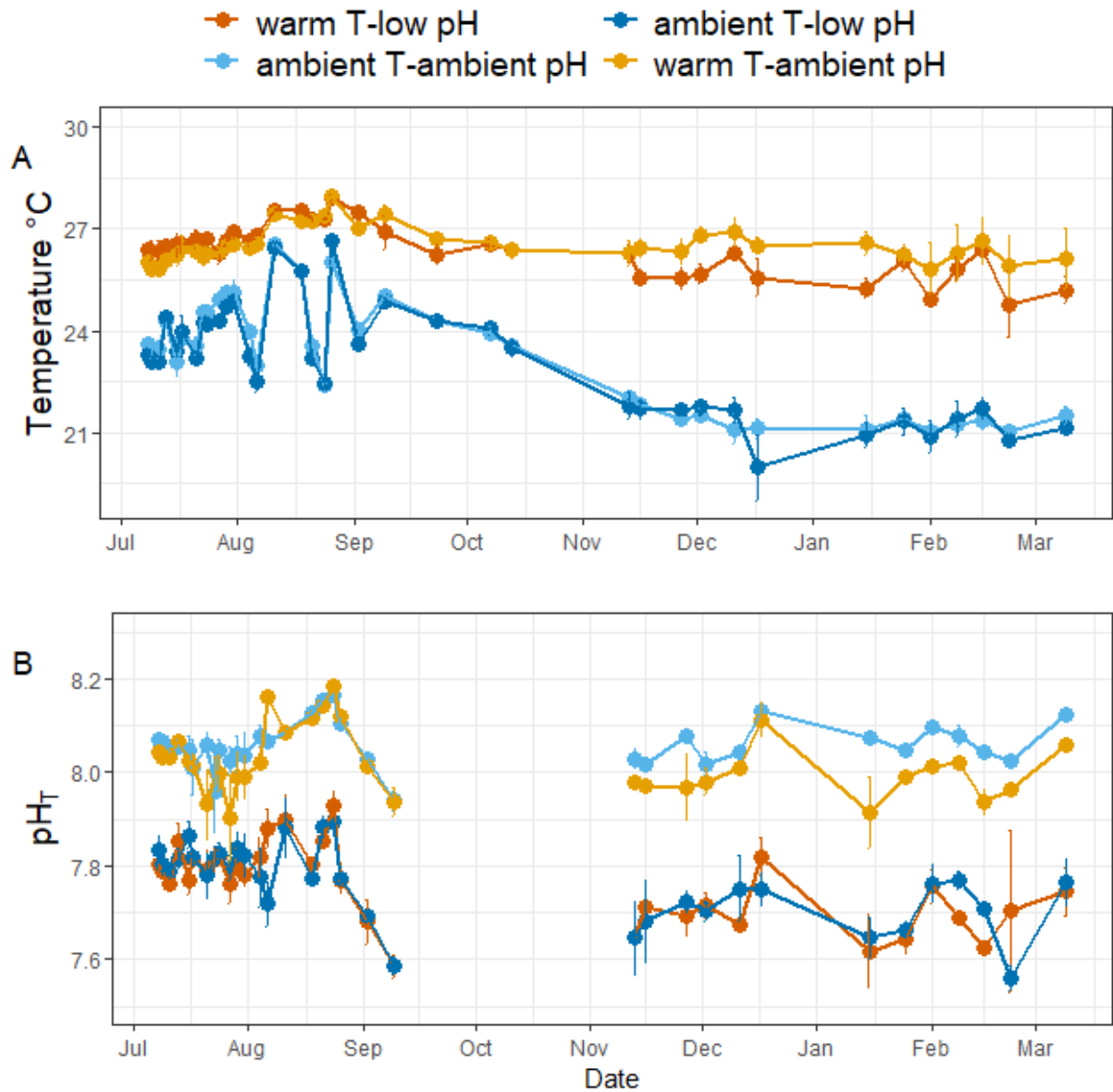


Figure S4 **Evolution of the mean temperature and pH<sub>r</sub> in the experimental tanks under the two temperature treatments (23°C for ambient Temperature and 26°C for warm Temperature) and the two pH treatments (pH<sub>r</sub> = 8.05 ± 0.09 for ambient pH and pH<sub>r</sub> = 7.78 ± 0.10 for low pH) n=36. Mean ± SE**

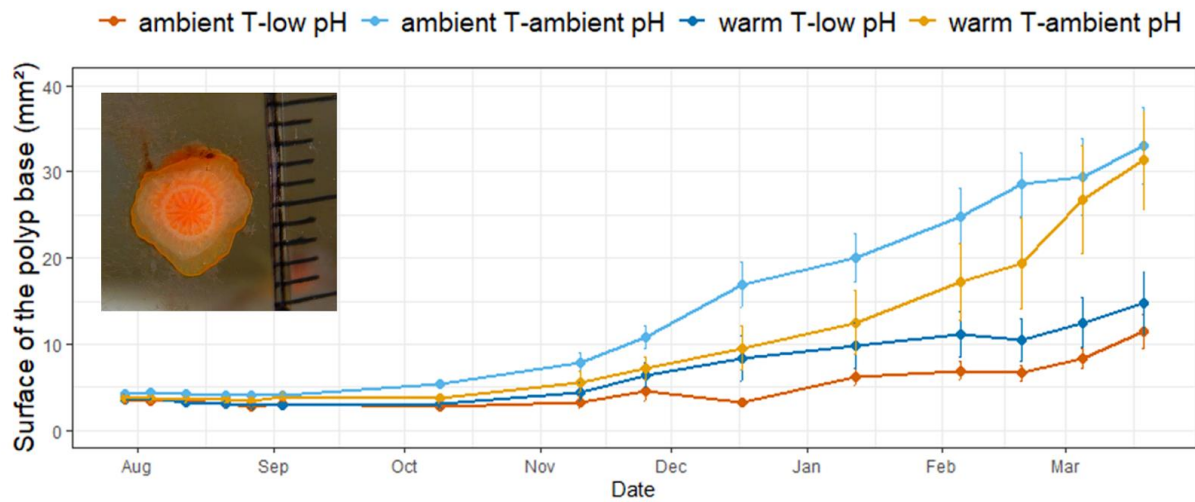


Figure S5 Mean surface of the base of the juvenile polyps of *A. calycularis* under the two temperature treatments (23°C for ambient Temperature and 26°C for warm Temperature) and the two pH treatments (  $\text{pH}_t = 8.05 \pm 0.09$  for ambient pH and  $\text{pH}_t = 7.78 \pm 0.10$  for low pH) during 9 months. Values are means  $\pm$  SE. The surfaces were obtained by using ImageJ software on bi-monthly pictures of the base of the polyps through the glass of the crystallizers. The color of the dots and lines indicates the origin and the treatment. n= 33-65 per date.



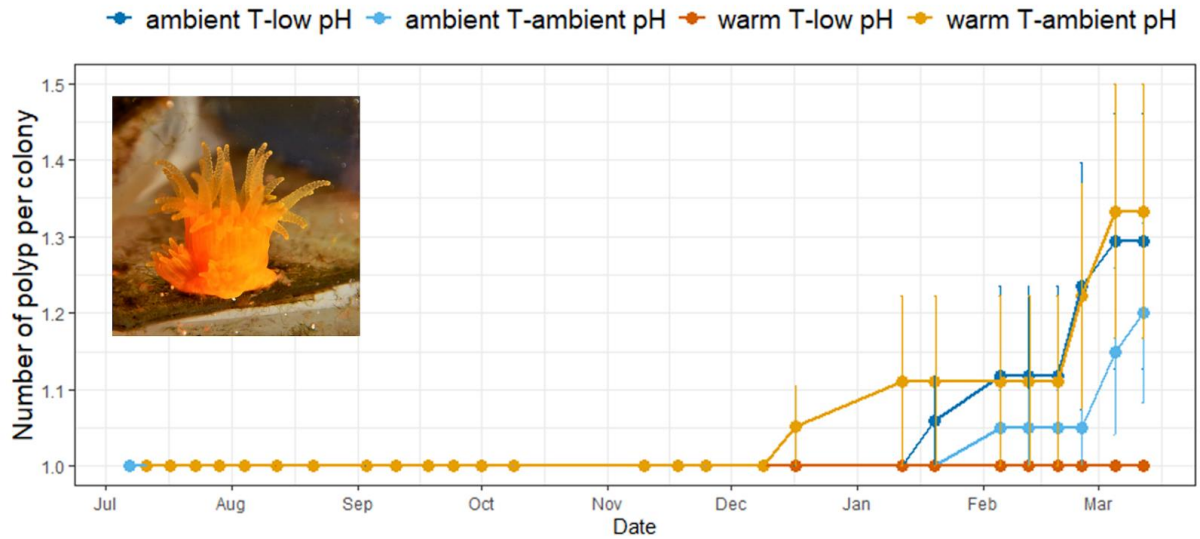


Figure S6 Mean number of polyp per juvenile colony of *A. calycularis* under the two temperature treatments (23°C for ambient Temperature and 26°C for warm Temperature) and the two pH treatments (  $pH_r = 8.05 \pm 0.09$  for ambient pH and  $pH_r = 7.78 \pm 0.10$  for low pH) during 9 months. The number of polyp was recorded by counting the apparition of new polyps from the initial polyp. The color of the dots and lines indicates the origin and the treatment. n= 7-120 per date.



Video 1 Spawning of a larva from the mouth of *Astroides calycularis* female polyp.

Video 2 One-day old swimming larvae of *Astroides calycularis*

Video 3 Sequence of early life stages development of *Astroides calycularis*

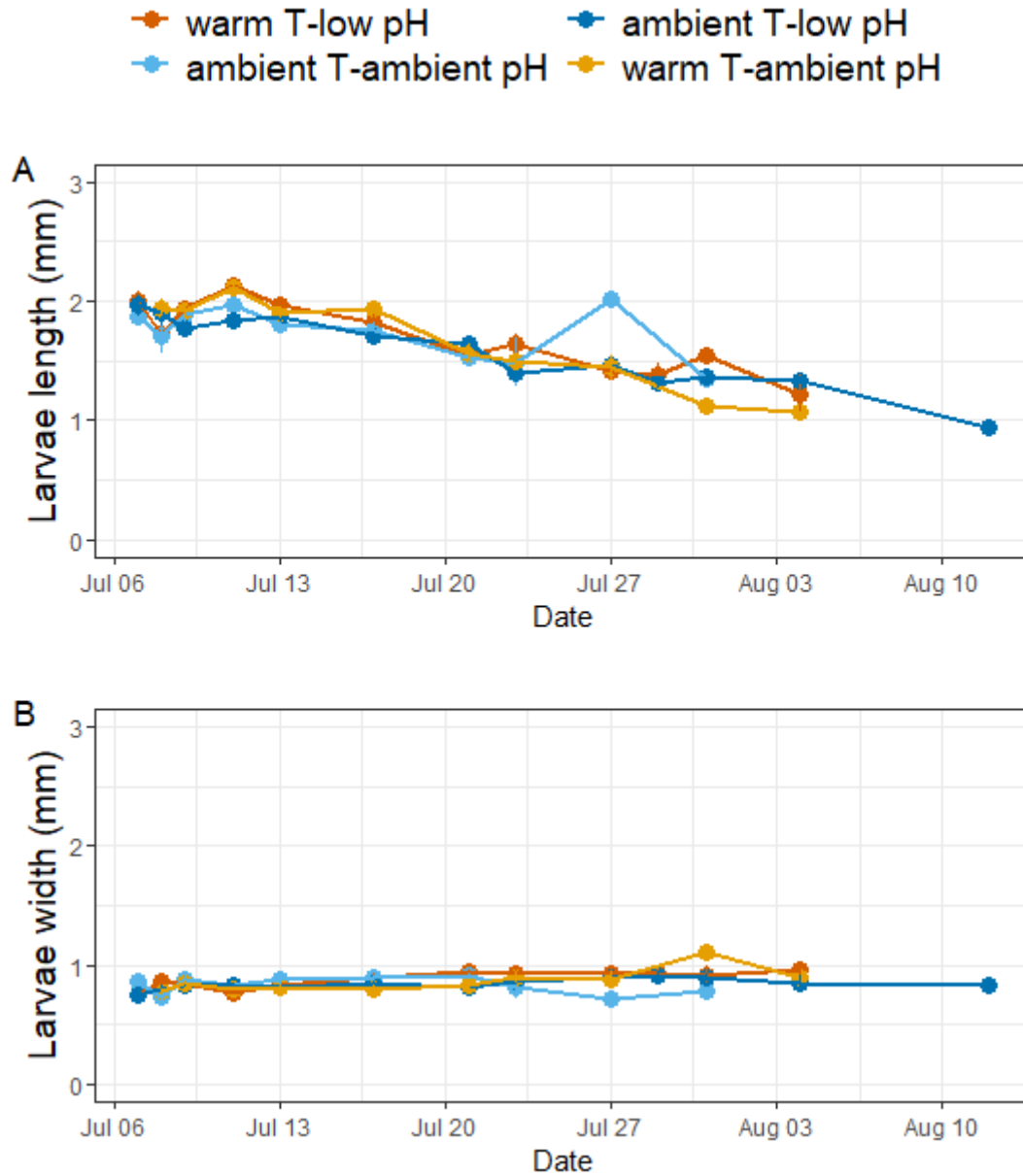


Figure S7 **Size of *Astroides calycularis* larvae monitored under the two temperature treatments (23°C for ambient Temperature and 26°C for warm Temperature) and the two pH treatments (  $pH_r = 8.05 \pm 0.09$  for ambient pH and  $pH_r = 7.78 \pm 0.10$  for low pH).** A) Length of the larvae B) Width of the larvae. Mean  $\pm$  SE

Table S2 Statistical results of the linear mixed-models with a hierarchical structure of the impact of temperature, pH and time on the larval development of *A. calycularis*. . =  $p < 0.1$ , \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Variable	Factor	df	F	p-value
Planula	pH	1,145	3.308	0.069 .
	temperature	1,145	2.215	0.137
	time	1,145	16.189	5.73 10 <sup>-5</sup> ***
	pH x temperature	1,145	0.878	0.349
	pH x time	1,145	16.999	3.75 10 <sup>-5</sup> ***
	temperature x time	1,145	11.695	6.30 10 <sup>-4</sup> ***
	pH x temperature x time	1,145	4.437	0.035 *
Settlement	pH	1,397	3.523	0.061 .
	temperature	1,397	0.091	0.763
	time	1,397	0.002	0.962
	pH x temperature	1,397	0.145	0.703
	pH x time	1,397	1.803	0.179
	temperature x time	1,397	12.833	3.41 10 <sup>-4</sup> ***
	pH x temperature x time	1,397	29.155	6.68 10 <sup>-8</sup> ***
Non-settled polyp	pH	1,397	1.236	0.266
	temperature	1,397	0.179	0.672
	time	1,397	11.802	5.91 10 <sup>-4</sup> ***
	pH x temperature	1,397	0.267	0.605
	pH x time	1,397	1.423	0.233
	temperature x time	1,397	39.293	3.65 10 <sup>-10</sup> ***
	pH x temperature x time	1,397	36.369	1.64 10 <sup>-9</sup> ***
Mortality	pH	1,397	0.409	0.523
	temperature	1,397	0.166	0.684
	time	1,397	71.417	<10 <sup>-16</sup> ***
	pH x temperature	1,397	18.498	1.70 10 <sup>-5</sup> ***
	pH x time	1,397	59.607	1.16 10 <sup>-14</sup> ***
	temperature x time	1,397	11.157	8.3 10 <sup>-4</sup> ***
	pH x temperature x time	1,397	110.880	< 10 <sup>-16</sup> ***
Larvae length	pH	1,933	0.108	0.947
	temperature	1,933	3.502	0.1736
	time	1,933	100.224	< 10 <sup>-16</sup> ***
	pH x temperature	1,933	0.021	0.885
	pH x time	1,933	0.038	0.845
	temperature x time	1,933	1.040	0.3079
	pH x temperature x time	1,933	0.375	0.540
Larvae width	pH	1,933	19.594	5.56 10 <sup>-5</sup> ***
	temperature	1,933	17.885	1.31 10 <sup>-4</sup> ***
	time	1,933	37.073	1.14 10 <sup>-9</sup> ***
	pH x temperature	1,933	13.756	2.08 10 <sup>-4</sup> ***
	pH x time	1,933	1.868	0.172
	temperature x time	1,933	4.870	0.027 *
	pH x temperature x time	1,933	0.734	0.392

Table S3 Statistical results of the generalised mixed-models of the impact of temperature and pH skeleton parameter of *A. calycularis* 9-month recruits. . =  $p < 0.1$ , \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Variable	Factor	df	F	p-value
Growth of the base	pH	1,35	23.631	2.4 10 <sup>-5</sup> ***
	temperature	1,35	0.381	0.541
	time	1,35	0.026	0.873
Number of polyps per colony	pH	1,55	0.307	0.582
	temperature	1,55	0.493	0.485
	time	1,55	2.298	0.135
Volume of skeleton	pH	1,46	4.051	0.044 *
	temperature	1,46	1.144	0.285
	pH x temperature	1,46	3.519	0.061
Number of septa	pH	1,48	10.549	0.002 **
	temperature	1,48	1.809	0.185
	pH x temperature	1,48	0.552	0.461
Density of skeleton	pH	1,19	0.785	0.387
	temperature	1,19	4.064	0.058
	pH x temperature	1,19	9.251	0.007 **

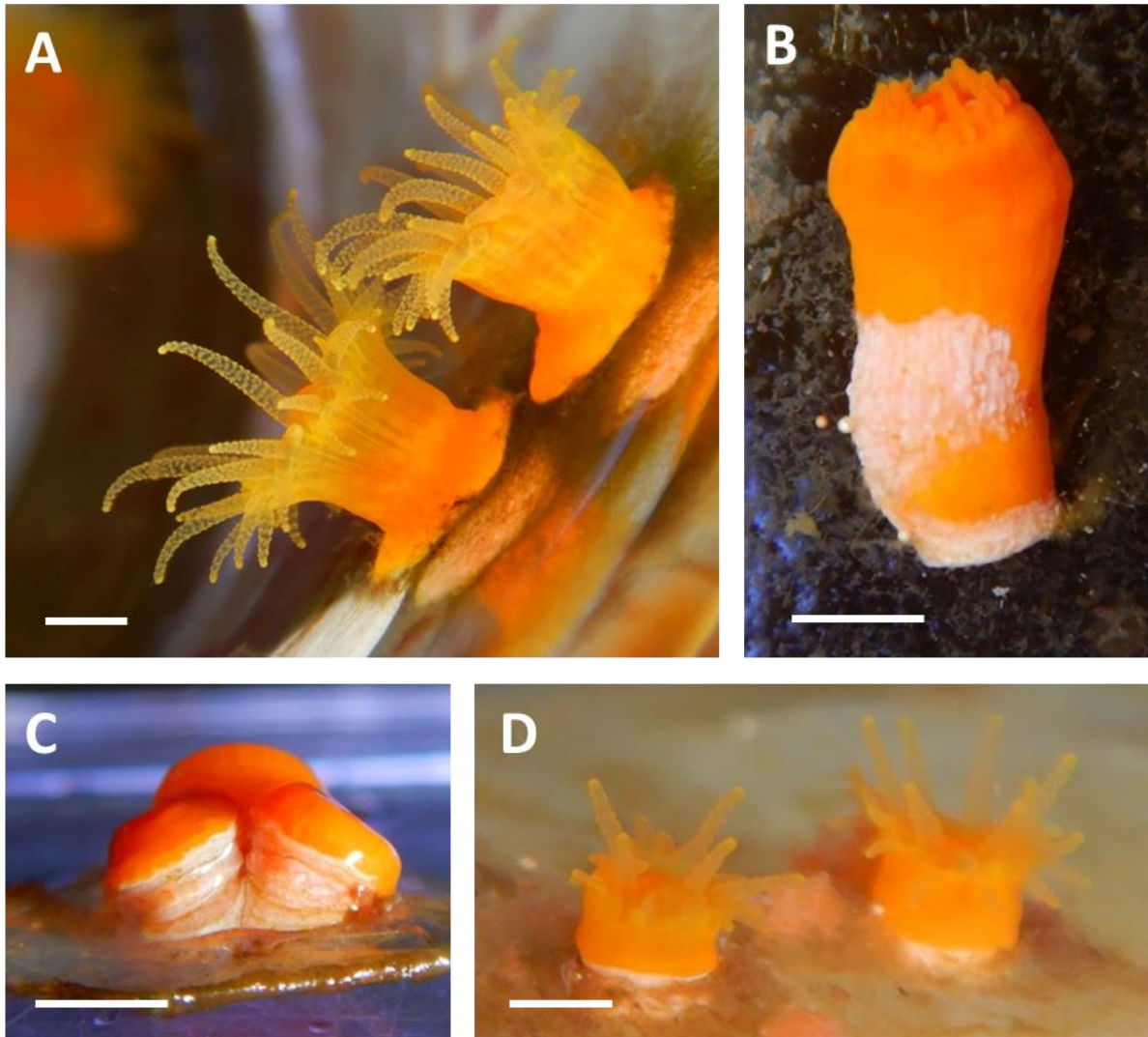


Figure S8 **Exposed skeleton of nine-month old recruits in the low pH treatment** A) Picture of two healthy recruits under ambient pH treatment, with outskirts tissue covering the skeleton and invading the substrate, B) Picture of a non-settled recruit presenting an exposed skeleton under low pH treatment, C and D) Pictures of recruits presenting an exposed skeleton and tissue retracted to the top section of the corallite under low pH treatment. Scale : 3 mm