Ocean warming and acidification detrimentally affect coral tissue regeneration at a Mediterranean CO_2 vent

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HIGHLIGHTS

- This is the first transplant experiment investigating coral tissue regeneration with decreasing pH and increasing temperature
- Increasing temperature and decreasing pH could detrimentally impact coral tissue regeneration following injury
- Zooxanthellate corals might be particularly impacted by ocean warming and acidification in terms of lesion recovery

GRAPHICAL ABSTRACT
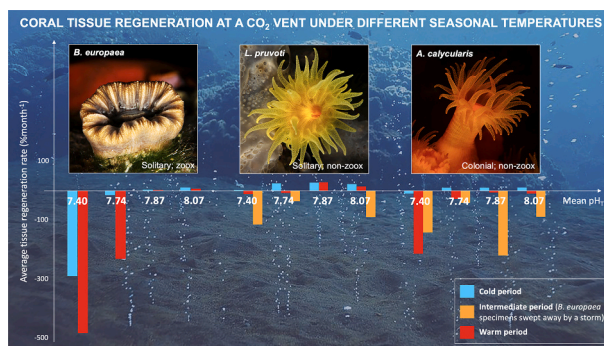
ABSTRACT

Among the main phenomena that are causing significant changes in ocean waters are warming and acidification, largely due to anthropogenic activities. Growing evidence suggests that climate change is having more substantial and rapid effects on marine communities than on terrestrial ones, triggering several physiological responses in these organisms, including in corals. Here we investigated, for first time in the field, the combined effect of increasing seawater acidification and warming on tissue regeneration rate of three Mediterranean scleractinian coral species characterized by different trophic strategies and growth modes. *Balanophyllia europaea* (solitary, zooxanthellate), *Leptopsammia pruvoti* (solitary, non-zooxanthellate) and *Astroides calycularis* (colonial, non-zooxanthellate) specimens were transplanted, during a cold, intermediate, and warm period, along a natural pH gradient generated by an underwater volcanic crater at Panarea Island (Mediterranean Sea, Italy), characterized by continuous and localized CO_2 emissions at ambient temperature. Our results show a decrease in regenerative capacity, especially in the zooxanthellate species, with increasing seawater temperature and

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acidification, with demonstrated species-specific differences. This finding suggests that increasing seawater temperature and acidification could have a compounding effect on coral regeneration following injury, potentially hindering the capacity of corals to recover following physical disturbance under predicted climate change.

1. Introduction

Climate change is one of the most pervasive threats that natural systems are currently facing. Since the 18th century, rising atmospheric carbon dioxide (CO_2) concentration via fossil fuel burning, deforestation and many other anthropogenic activities is responsible for the increase in global average surface temperatures and is leading to a decrease in surface oceanic pH, a phenomenon known as ocean acidification (Figuerola et al., 2021). When atmospheric carbon dioxide is absorbed by the oceans it reacts with seawater to form carbonic acid which rapidly dissociates into hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). The increase in H^+ ions leads to a reduction in pH and carbonate ion (CO_3^{2-}) availability (Gattuso and Hansson, 2011). Hydrogen ion concentrations have increased by 25–30 % since the industrial revolution and over the 21st century are projected to further increase by 70–90 % under a business-as-usual scenario (Kwiatkowski and Orr, 2018). As for ocean temperatures, each of the last four decades has been successively warmer. From 1850 to 1900 to 2010–2019, global surface temperatures have increases by 0.8 °C and 1.3 °C, respectively (IPCC, 2021). Thus, unless deep reductions in CO_2 and other greenhouse gas emissions occur in the coming decades, global warming is projected to exceed 1.5–2 °C during the 21st century (IPCC, 2021).

Ocean warming and acidification are two of the main stressors causing significant changes in marine environments, posing a major threat to species that generate and accumulate calcium carbonate structures, potentially reducing the services provided by ecosystems reliant on calcifying organisms (Wilson et al., 2020). Many biological processes and key life functions, including sexual reproduction, growth, regeneration, and physiological functions can be influenced by seawater warming (Gagliano et al., 2007) and acidification (Poloczanska et al., 2016) in different marine organisms, including molluscs (Gooding et al., 2009), annelids (Vinn et al., 2008), echinoderms (Byrne et al., 2009), and corals (Fine and Tchernov, 2007; Hoegh-Guldberg et al., 2007; Jokiel et al., 2008; De'ath et al., 2009; Teixidó et al., 2020). Among vital functions, tissue regeneration is among those with the highest priority as natural selection should favor regenerative processes above other requirements (Karlson, 1988). In corals, the speed of this process is vital, since rapid healing of lesions restores the functional use of polyps (Hughes and Jackson, 1985; Sebens, 1987; D'Angelo et al., 2012) and limits the risk of invasion by competitors, pathogens, and bioeroders (Jackson and Palumbi, 1979; Hughes and Jackson, 1985; Titlyanov et al., 2005; Titlyanov and Titlyanova, 2008; Katz et al., 2014). Indeed, once a lesion is colonized by fouling organisms, it is unlikely that coral tissue will outgrow these organisms (Meesters et al., 1997; Ruiz-Diaz et al., 2016). Tissue regeneration depends on intrinsic factors: e.g., colony/polyp morphology and size, energy intake strategy, and supply of interstitial cells (Meesters et al., 1996; Henry and Hart, 2005; Counsell et al., 2019). Extrinsic factors, including seawater temperature, seawater pH, degree of damage, can also significantly affect tissue recovery (Meesters et al., 1997; Nagelkerken et al., 1999; Rempel et al., 2020). Thus, determining how ocean warming and acidification influence tissue repair in corals characterized by different energy intake strategies (zooxanthellate - i.e., mixotrophic, able to fix inorganic carbon through the activity of their Symbiodiniaceae endosymbionts and heterotrophic, able to gain nutrients from predation of plankton- *vs* non-zooxanthellate) and different growth forms (colonial *vs* solitary) is crucial to understanding how these organisms will respond to damage under predicted climate change scenarios. Although several studies have shown that exposure to increasing seawater temperatures and/or acidification may reduce or completely prevent coral tissue regeneration

(Meesters and Bak, 1993; Meesters et al., 1997; Fine et al., 2002; Horwitz and Fine, 2014; Hall et al., 2015) only a few of these were conducted in the field (Meesters and Bak, 1993; Meesters et al., 1997; Fine et al., 2002) and none considered the combined effect of increasing seawater temperature and acidification. For instance, tissue regeneration rates were mostly inhibited by low pH in slow-growing compared to fast-growing coral species incubated in aquaria at pH 7.3–8.1 for 60–120 days (Horwitz and Fine, 2014). Likewise, a study conducted on small and large fragments from *Porites porites* and *P. astreoides* incubated at pH 7.6 and 8.1 for almost 3 months revealed lower recovery rates under low pH for both fragments sizes in the former species and only in larger fragments for the latter species (Hall et al., 2015).

Compared to laboratory experiments, transplant experiments can more aptly represent real-world conditions, as organisms are maintained in a natural setting under environmental conditions difficult or impossible to simulate *ex situ* (e.g. nutrients, currents, irradiance). Here we investigated, for the first time, the effects of *in situ* exposure to different pH levels (range pH_{TS} 7.4–8.1) and seasonal temperatures (range 16–24 °C) on the tissue regeneration rate of the Symbiodiniaceae bearing solitary scleractinian (i.e., stony) coral *Balanophyllia europaea* (Risso 1826), the solitary asymbiotic stony coral *Leptopsammia pruvoti* (Lacaze-Duthiers 1897), and the colonial asymbiotic stony coral *Astroides calycularis* (Pallas 1766) transplanted along a natural pH gradient off Panarea Island (Aeolian Islands, southern Italy).

2. Materials and methods

2.1. Study site and observation periods

The experimental site is located near Panarea, the smallest island of the Aeolian Archipelago (Mediterranean Sea, Italy, 38°38′16″ N 15°06′37″ E). In this area, at 12 m depth, a crater with a conical shape (20 × 14 m) generates a stable and continuous column of bubbles at ambient temperature (98–99 % CO_2; Capaccioni et al., 2007), creating a natural pH gradient, stable throughout the year, extending for ca. 34 m from the center to the periphery of the crater (Fig. 1). Patches of sulphur-oxidizing bacterial mats are visible within the crater due to the presence of hydrogen sulphide in the vent gaseous emissions (Maugeri et al., 2009). However, water dissolved H_2S is below detection limit (Goffredo et al., 2014), thus resulting in negligible effects on the transplanted corals. Along this gradient, four experimental sites (indicated hereafter as Sites), whose seawater physicochemical parameters have been previously characterized (Goffredo et al., 2014; Fantazzini et al., 2015; Prada et al., 2017), were selected: a control Site (Site 1: mean pH total scale [TS] pH_{TS} 8.07), with normal pH conditions; two intermediate pH Sites (Sites 2 and 3: mean pH_{TS} respectively 7.87 and 7.74), with pH values matching conservative (SSP2–4.5) and worst-case Intergovernmental Panel on Climate Change (IPCC) scenarios (SSP3–7.0), respectively, and an extreme pH Site (Site 4: mean pH_{TS} 7.40) situated at the rim of the crater, that exceeds the most pessimistic scenario (IPCC, 2021). Data were collected in the following experimental periods (duration; average temperature (95 % CI) from Prada et al., 2017): 4 March – 6 June 2011 (94 days; 16.2 (16.0–16.3) °C), 31 July – 2 December 2011 (124 days; 22.8 (22.6–23.0) °C), 6 June – 31 July 2011 (55 days; 23.5 (23.4–23.7) °C).

2.2. Field transplantation and regeneration measurements

Similar-sized *Balanophyllia europaea* and *Leptopsammia pruvoti* polyps and *Astroides calycularis* nubbins (2–4 polyps each) were collected by

SCUBA diving from Pietra Nave, about 2 km away from the CO_2 vent, where the species grow naturally. Sampled corals were placed in a container with seawater and transported within 30 min by boat to the Eolo Sub Diving Center, where a temporary wet lab was set up. Upon arrival to the diving center, *B. europaea* and *L. pruvoti* polyps and *A. calycularis* nubbins were randomly assigned to each of the four Sites and glued with a non-toxic bicomponent epoxy coral glue (Milliput, Wales, UK) onto ceramic tiles. The number of corals/nubbins (i.e., replicates) per Site/tile per species were 10. However, during the experimental periods, some of the transplanted polyps/nubbins went missing, possibly due to strong currents that detached them from the tiles. Corals were maintained at ambient temperature in aerated containers with use of a bubbler and no source of artificial light for 1d prior to transplantation. *L. pruvoti* polyps were placed upside-down under plastic cages to mimic their natural orientation at the sampling Sites, in agreement with a parallel experiment on coral mortality and net calcification performed on uninjured corals of the same species along the same gradient (Prada et al., 2017). Since the aim of the study was to assess how tissue regeneration rate was affected by decreasing pH under different ambient temperatures (i.e., seasonal periods), corals were collected and replaced with new ones at the beginning of each experimental period. The duration of these periods ranged from 2 to 4 months due to logistic reasons. However, this did not affect the results, as the lowest tissue regeneration rates were observed in the warmest period, which was the shortest one. Tissue lesions were inflicted on the side/upper side of the coral by gently using a metal bristle brush exposing the axial skeleton (Fig. 2). For the colonial species, injuries were always inflicted on the side of a single polyp within the nubbin, to keep the comparison with the other two solitary species as consistent as possible. Tissue removal by water picking is probably a more efficient technique than using a brush, due to tissue remnants that can remain attached to the skeleton when using the latter. However, the same procedure was applied to all corals in all treatments, so any potential tissue remnants were comparable across species and treatments. Tissue regeneration rates were then quantified by subtracting the lesion area (i.e., the area without soft tissue) measured at the beginning of the experiment by the lesion area measured at the end of the experiment, divided by the former and expressed as percentage of injury regenerated as a function of time (per month, in this case; $((A_{t0}-A_{t1})/A_{t0}\times100)/Dt_{month}$; as per Meesters et al., 1994; Horwitz and Fine, 2014). When specimens were found dead, tissue loss was calculated applying the same formula but using as A_{t1} the surface area of the coral (i.e., the entire surface of the coral would be without tissue), obtained as the sum of the surface of the oral disc, using the formula $\pi \times (\frac{L}{2}) \times (\frac{W}{2})$ (*L* is the length, i.e., maximum axis of the oral disc, *W* is the width, i.e., the minor axis of the oral disc) and the lateral surface of the coral, using the formula $\pi \times [3 \times (\frac{L}{2}+\frac{W}{2})-\sqrt{(3 \times \frac{L}{2}+\frac{W}{2}) \times (\frac{L}{2}+3 \times \frac{W}{2})}] \times h$ (*h* is the height, i.e., oral-aboral axis), excluding the base in contact with the substratum (Ramanujan, 1914; Caroselli et al., 2015b; Palazzo et al., 2021). Photographs were taken at the beginning and at the end of each of the three transplant periods and subsequently analyzed with the ImageJ software package (ImageJ 1.53 k, National Institutes of Health, USA).

2.3. Statistical analyses

Shapiro Wilk (*n* < 50) and Levene's tests were run to verify the underlying assumptions of normality and homogeneity of variances, respectively. When these assumptions were not met, the non-parametric Kruskal-Wallis test was used to test whether tissue regeneration rates
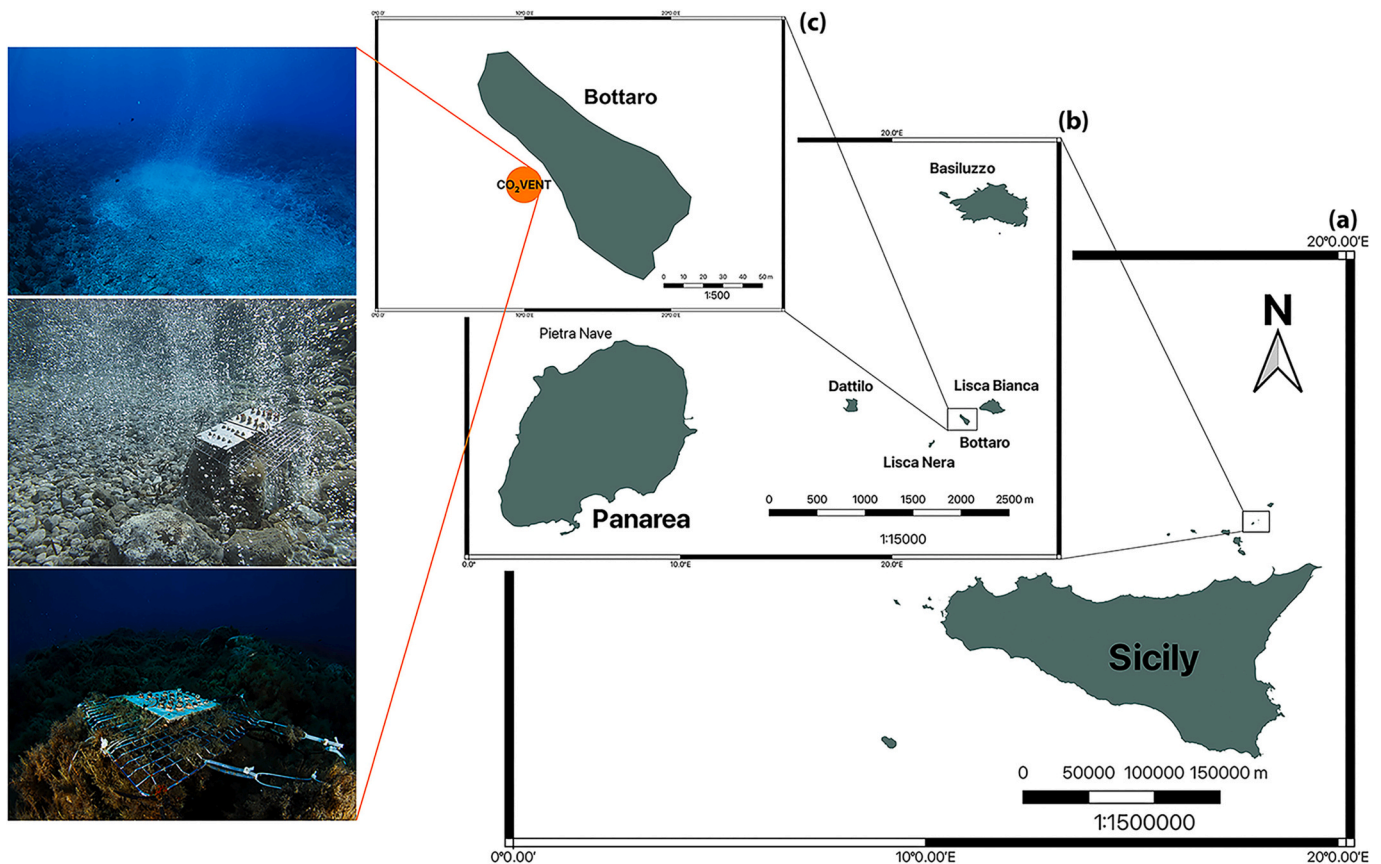

Fig. 1. Experimental site located (a) off Panarea Island (b), close to Bottaro Islet, (c) where an underwater volcanic crater releases persistent gaseous emissions (98–99 % CO_2 without instrumentally detectable toxic compounds), resulting in a stable pH gradient. Maps were created using QGIS 3.28.0 Firenze version, 2022. The pictures show representative images of the volcanic crater and transplanted corals (Photographs by Francesco Sesso).
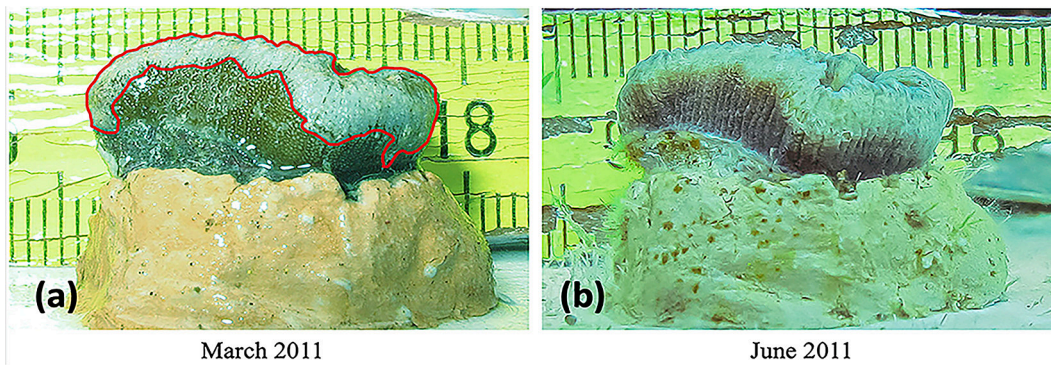
Fig. 2. Lesion recovery of a representative *B. europaea* specimen from Site 1 (control) photographed (a) at the beginning of one of the transplant periods (March 2011), thus immediately after scraping the tissue (the injured area is enclosed within the red line) and (b) at the end of the transplant period (June 2011), showing full tissue recovery. The recovered area was less pigmented (i.e., reduced Symbiodiniaceae) than uninjured areas (Photographs by co-author Erik Caroselli).

were significantly different among the four Sites; if the results were significant, pair-wise Mann-Whitney tests were run to verify which Sites differed. To account for multiple comparisons within the dataset, the conservative Bonferroni correction was used (pair-wise comparisons among Sites significant when $P < (\alpha/N = 0.05/6 = 0.008)$.

Spearman's rank correlation coefficient was used to assess whether significant differences in tissue regeneration rates among Sites were driven by changes in seawater pH_{TS}. Spearman's rank correlation coefficient is an alternative to Pearson's correlation coefficient (Altman, 1991). It is useful for data that are non-normally distributed and do not meet the assumptions of Pearson's correlation coefficient. Analysis of covariance (ANCOVA) was used to examine differences in tissue regeneration rate and mean pH_{TS} regression slopes between: 1) experimental periods/temperature, 2) trophic strategy (zooxanthellate *vs* non-zooxanthellate species), and 3) growth modes (solitary *vs* colonial species). All data analyses were performed using IBM SPSS Statistics.

3. Results

The underlying assumptions of normality and homogeneity of variances were not met, therefore the non-parametric Kruskal-Wallis and pair-wise Mann-Whitney tests were used for all comparisons. Tissue regeneration rate in *Balanophyllia europaea,* was lower at the low pH Sites in the coldest and warmest periods (Kruskal Wallis test, $p < 0.001$; Table S1) and significantly correlated with pH in both periods, with pH explaining ca. 30 % of the variation in tissue regeneration in the coldest period and ca. 50 % in the warmest one (Fig. 3). Data for the intermediate period are missing for this species because samples were swept away by a storm. The slope for the warmest period was significantly steeper than for the coldest period (ANCOVA, $F_1 = 4.347$, $n = 69$ $p < 0.01$).

Tissue regeneration rate in *Leptopsammia pruvoti* was lower at the low pH Sites in the three investigated periods (coldest period, Kruskal-Wallis test $p < 0.001$; intermediate period, Kruskal-Wallis test $p < 0.05$; warmest period, Kruskal-Wallis test $p < 0.01$; Table S1) and significantly correlated with pH in the coldest and warmest periods (Fig. 4), with pH explaining ca. 15 % of the variation in tissue regeneration in the coldest period and ca. 24 % in the warmest one. Tissue regeneration did not correlate with pH in the intermediate period. The slopes between the coldest and the warmest period were not significantly different (ANCOVA, $F_1 = 0.567$, $n = 68$, $p > 0.05$).

Tissue regeneration rate in *Astroides calycularis* was lower at the low pH Sites in the intermediate (Kruskal-Wallis test $p < 0.05$) and warmest period (Kruskal-Wallis test $p < 0.01$; Table S1) and significantly correlated with pH only in the warmest period (Fig. 5), with pH explaining ca. 60 % of the variation in tissue regeneration.

Slopes for the zooxanthellate species were steeper than for the non-zooxanthellate species in both the coldest and warmest periods
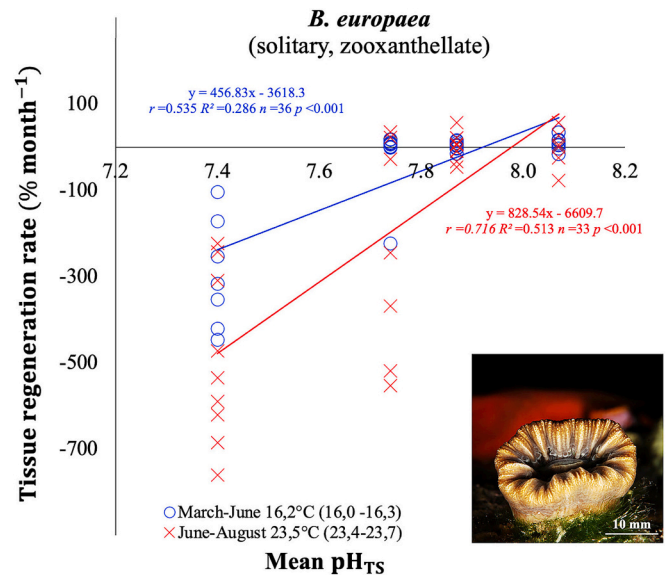

Fig. 3. Tissue regeneration rates in *Balanophyllia europaea*. Variation in tissue regeneration rates with average pH in the solitary zooxanthellate *B. europaea* analyzed at the four Sites in two experimental periods. Data for August–December 2011 (intermediate period) is missing because a storm swept away part of the experiment. *r* Spearman's correlation coefficient, *n* number of individuals. (Photograph by Francesco Sesso).

(*B. europaea* vs *L. pruvoti*, ANCOVA, $F_1 = 30.436$, $n = 71$, $p < 0.001$, $F_1 = 16.187$, $n = 66$, $p < 0.001$ respectively; Figs. 3 and 4). Slopes within the warmest period were significantly steeper for the colonial than for the solitary species (*L. pruvoti* vs *A. calycularis*, ANCOVA, $F_1 = 9.847$, $n = 55$, $p < 0.01$; Figs. 4 and 5).

4. Discussion

The current study showed a combined effect of seawater warming and acidification leading to a generalized decrease in tissue regeneration rate of the zooxanthellate solitary coral *Balanophyllia europaea*, the solitary non-zooxanthellate coral *Leptopsammia pruvoti*, and the colonial non-zooxanthellate coral *Astroides calycularis*, with differences likely driven by different trophic strategies and growth modes.

4.1. Zooxanthellate vs non-zooxanthellate corals

When comparing the regenerative capacity of the three species, the zooxanthellate coral was more sensitive to higher seawater temperature
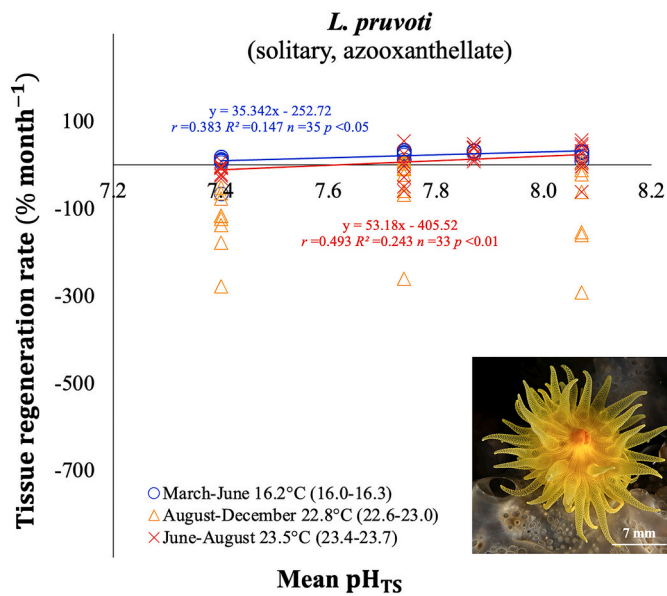
Fig. 4. Tissue regeneration rates in *Leptopsammia pruvoti*. Variation in tissue regeneration rates with average pH in the solitary non-zooxanthellate *L. pruvoti* analyzed at the four Sites in three experimental periods. r Spearman's correlation coefficient, n number of individuals. (Photograph by Francesco Sesso).

Fig. 5. Tissue regeneration rates in *Astroides calycularis*. Variation in tissue regeneration rates with average pH in the colonial non-zooxanthellate *A. calycularis* analyzed at the four Sites in three experimental periods. r Spearman's correlation coefficient, n number of individuals. (Photograph by Francesco Sesso).
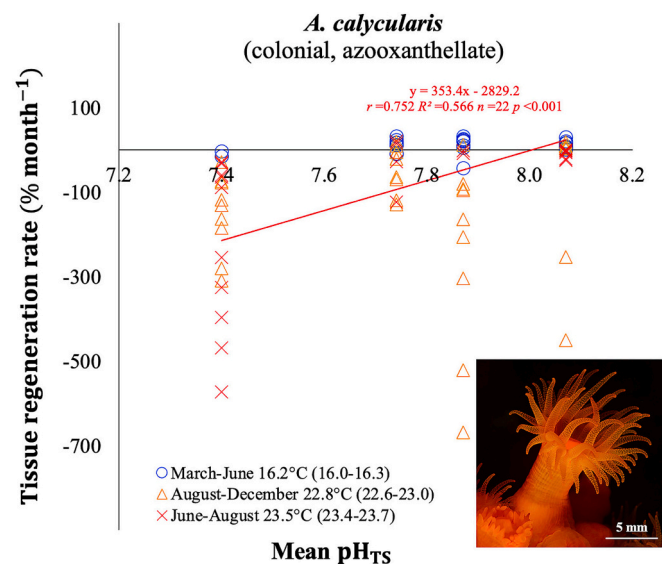
and lower seawater pH$_{TS}$ compared to the two non-zooxanthellate species. A possible explanation could be the allocation of available resources to processes such as reproduction, calcification, and/or acid-base regulation, at the expense of tissue maintenance in *B. europaea* (Horwitz and Fine, 2014). Indeed, a parallel transplant experiment conducted in the same site and on the same (uninjured) species showed that decreasing pH$_{TS}$ and increasing seawater temperature did not affect net calcification rates in *B. europaea*, while they showed a negative additive effect on net calcification rates in *L. pruvoti* and *A. calycularis* (Prada et al., 2017). Moreover, *B. europaea* naturally occurring along this gradient adjusts to decreasing pH$_{TS}$ by: i) increasing host tissue thickness (Prada et al., 2023), ii) maintaining the physiological pH at the

site of calcification as well as gross calcification rates (Wall et al., 2019), and ii) continuing to reproduce, regardless of external seawater pH$_{TS}$ (Caroselli et al., 2019). Reproduction, skeletal accretion, tissue thickening, and physiological pH up-regulation are energetically expensive processes (Allemand et al., 2011). Thus, it is likely that *B. europaea* may be unable to sustain the cost of tissue regeneration due to allocation of energy towards growth, physiological pH homeostasis, and reproduction, leading to decreased regeneration capacity. The stronger decrease in tissue regeneration rates in warmer compared to colder months in the zooxanthellate compared to the non-zooxanthellate species could depend on the inhibition of photosynthesis at high temperatures that can cause negative effects on growth, demography, reproduction, and skeletal structural parameters (Goffredo et al., 2007, 2008, 2009, 2015; Caroselli et al., 2011; Fantazzini et al., 2013; Airi et al., 2014). Indeed, reef-building corals are highly dependent on their relationship with endosymbiotic dinoflagellates (family Symbiodiniaceae: LaJeunesse et al., 2018), receiving much of their carbon and energy requirements from their symbionts (Falkowski et al., 1984, 1993; Muscatine et al., 1984; Tremblay et al., 2012). The loss of these endosymbionts, due to environmental stress, can impact coral energy and carbon budget, and can lead to death if stress is prolonged and severe (Lesser, 2011; Landsberg et al., 2020; Sikorskaya and Imbs, 2020). Analyses on the photosynthetic performance of *B. europaea* exposed to different temperature regimes in aquaria showed that the optimal temperature for photosynthesis in this species ranges between 20.0 and 21.6 °C. At temperatures >21.6 °C all parameters analyzed indicate a reduction in photosynthetic efficiency (Caroselli et al., 2015a). Thus, considering that the average temperature of the warmest period analyzed (23.5 °C) exceeds this physiological threshold, this could potentially explain the stronger decline in tissue regeneration rate observed in *B. europaea* in the warmest period compared to the non-zooxanthellate species. Additionally, tissue recovery occurs in stages, with an initial thin tissue layer, devoid of symbionts, covering the skeleton. As recovery continues, the symbionts appear in the tissue and the polyp details begin to emerge (DeFilippo et al., 2016; Traylor-Knowles, 2016). Pigmentation of regenerated tissue in *B. europaea* along the pH gradient often appeared less pronounced than tissue that was not injured (Fig. 2), likely due to reduced zooxanthellae in the newly formed tissue. Previous studies have shown that decreased zooxanthellae lowers the ability of corals to regenerate injured tissue (Mascarelli and Bunkley-Williams, 1999). This is likely accentuated under temperatures exceeding the physiological threshold for this species (Caroselli et al., 2015a), which could further explain the significant decrease in tissue regeneration in *B. europaea* during the warmer period. Further studies on the photosynthetic efficiency of corals transplanted in different seasons along this pH gradient are needed to validate this hypothesis.

4.2. Colonial vs solitary corals

The greater susceptibility displayed by the colonial non-zooxanthellate *A. calycularis* compared to the solitary non-zooxanthellate *L. pruvoti* could depend on different internal self-regulation mechanisms between the two species, as highlighted by previous studies (Movilla et al., 2016; Prada et al., 2017). Indeed, *A. calycularis* subjected to high temperatures (from 15.2 to 24.9 °C) in aquaria during the summer period, exhibited a decrease in calcification rates of about 25 % compared to control seawater temperature (ST; ranging from 12.4 °C in winter to 22.5 °C in summer) conditions, while calcification rates of *L. pruvoti* were unaffected by high-ST conditions (Movilla et al., 2016). Lipid and protein reserves in *L. pruvoti* under control conditions were twice as high as those in *A. calycularis*, which could provide the former species with greater energy availability to maintain energetically costly processes such as calcification rate (Movilla et al., 2016). This study also reported no additive effects of low pH and high temperature on calcification, skeletal micro-density, porosity, and biochemical composition of the tissue in either species.

This is partially in disagreement with Prada et al., 2017 who conducted a transplant experiment (on uninjured specimens) along the same gradient used in the current study. The transplant study showed negative net calcification rates in both species, particularly in *A. calycularis*, driven by an additive effect of high temperature and low pH. The apparent discrepancy between these findings could be due to the fact that in Prada et al., 2017, as in the current study, corals were exposed to lower pH values (pH 7.4) and higher seawater temperatures (23.5 °C) compared to Movilla et al. (2016) (pH 7.7 and 22.5 °C). Another study investigating the gene expression and induction of a 70-kDa heat shock protein (HSP70) – which plays an important role by promoting stabilization and refolding of denatured proteins (Dong et al., 2008; Tomanek, 2010; Mishra and Palai, 2014) – in *L. pruvoti* and *A. calycularis*, showed different heat stress transcriptional profiles between these two species (Franzellitti et al., 2018). Physiological performance and responses are a species' primary tool for coping with environmental variability and, as such, they may change the ability of individual corals to cope with changing conditions (Somero, 2012). During exposure to high temperatures, changes can occur damaging protein complexes and regulation, leading to subsequent activation of signaling pathways triggering a stress-related HSP70 induction (Richter et al., 2010). Heat-shock experiments conducted on *A. calycularis* and *L. pruvoti* revealed that the former is much more sensitive to heat stress than the latter in terms of HSP70 fold changes (Franzellitti et al., 2018). These studies and the results of the current investigation all suggest that *A. calycularis* is particularly sensitive to elevated temperatures, and even more so, to the combined increase in seawater temperatures and acidification, at least compared to the solitary non-zooxanthellate species. Thus, in our experimental setting, increasing temperature likely led to a significant decrease in tissue regeneration to such an extent that this in turn probably made the corals more susceptible to the detrimental effects of ocean acidification.

In the current study, contrary to other studies on injury repair, many corals showed tissue loss rather than tissue regeneration. The fact that corals were exposed to both low pH and high temperature could partially explain the significant tissue loss reported here compared to other studies which only considered pH or temperature, separately (Horwitz and Fine, 2014; Bonesso et al., 2017; Edmunds and Yarid, 2017). Moreover, tissue repair seems to be affected by coral size (Kramarsky-Winter and Loya, 2000), and most likely also growth mode (solitary *vs* colonial). It is likely that larger corals have larger energy and tissue reserves and can therefore undergo tissue repair faster than smaller corals (Chadwick and Loya, 1990; Kramarsky-Winter and Loya, 2000). Most studies on lesion regeneration in corals have used fragments from massive tropical species composed of tens of polyps (see review in Henry and Hart, 2005), which might more easily recover from injury than smaller solitary corals or coral nubbins made of 3–4 polyps (current study).

Injury may also result in infection if the temperature is high enough to enhance bacterial growth (Aeby and Santavy, 2006; Lamb et al., 2014). Other factors affecting injury recovery rates include species, morphology, wound depth and location (e.g., van Woesik, 1998; Titlyanov et al., 2005). Previous studies found that small corals with tissue loss >40 % do not recover and eventually die (Chadwick and Loya, 1990), indicating that there is a critical mass of tissue necessary for regeneration to occur, which is species-specific. Thus, we cannot exclude that some of the injuries inflicted in the current study might have approached this threshold.

5. Conclusion

This is the first study conducted along a natural pH gradient aiming to assess tissue regeneration rate of three Mediterranean scleractinian coral species that differ in terms of trophic strategies (zooxanthellate *vs* azooxanthellate) and growth modes (solitary *vs* colonial). Our study showed that zooxanthellate species may be less resilient than non-zooxanthellate corals, in terms of tissue regeneration, to ocean warming and acidification. These results contribute to a growing body of evidence showing how combined warming and acidifying conditions predicted in the coming decades will likely be detrimental to important components of shallow-water benthic ecosystems, potentially affecting the capacity of Mediterranean stony corals to recover following physical damage.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167789.

CRediT authorship contribution statement

Teresa Sani: Formal Analysis, Writing Original Draft, Writing - Review & Editing, Visualization; **Fiorella Prada**: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing Original Draft, Writing - Review & Editing, Visualization, Supervision; **Giulia Radi**: Formal Analysis, Writing Original Draft; **Erik Caroselli**: Conceptualization, Investigation; **Giuseppe Falini**: Resources, Funding acquisition; **Zvy Dubinsky**: Resources, Funding acquisition; **Stefano Goffredo**: Conceptualization, Methodology, Validation, Resources, Supervision, Project Administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Zvy Dubinsky reports financial support was provided by Bar-Ilan University.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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