



Crustose coralline algae exhibit complex responses to breakage under current and future climate scenarios

Federico Pinna^{a,b,*}, Federica Ragazzola^a, Luigi Piazzzi^b, David Evans^{c,d}, Jacek Raddatz^{c,e}, Giulia Ceccherelli^b

^a Department of Integrative Marine Ecology, Genova Marine Centre, Stazione Zoologica Anton Dohrn, Genova, Italy

^b Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy

^c FIERCE – Frankfurt Isotope and Element Research Center, Goethe University Frankfurt, Frankfurt am Main, Germany

^d Current address: School of Ocean and Earth Science, University of Southampton, Southampton, UK

^e GEOMAR – Helmholtz Centre for Ocean Research, Germany

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ABSTRACT

Rising pressures from local and global stressors on marine benthic habitats require understanding of their effects on habitat forming species like Crustose Coralline Algae (CCA), which play a crucial role in ecosystem diversity and structure. Here, the impact of mechanical damage and warming on the CCA species *Lithophyllum stictiforme* was investigated in the Mediterranean Sea using a manipulative field transplant experiment that takes advantage of temperature variations between depths of 35 m and 15 m as proxies for current and future climate scenarios. A significant effect of mechanical damage and warming on the growth angle of the thalli was detected, while no interactive effects on the other biological traits were observed. Higher temperatures increased Mg/Ca ratio in untouched thalli, but mechanical damage disrupted this pattern in broken samples. Overall, our findings highlight the resilience of thalli to warming but demonstrate suffering from mechanical damage, underlining the need for effective benthic habitat management.

1. Introduction

Understanding the vulnerability to different stressors of benthic marine communities is a crucial goal for conservation ecologists. A growing body of literature highlights the fact that the ocean health are intimately connected to human activities on the planet (Hoegh-Guldberg and Bruno, 2010; Halpern et al., 2015; Nash et al., 2022). Indeed, anthropogenic impacts such as fishing activities (e.g., trawling), pollution from urban waste, anchoring, introduction of invasive species, or activities related to tourism, have increasingly resulted in irreversible trajectories of change in the oceans (Halpern et al., 2008; Butchart et al., 2010). In addition to these local-scale stressors, anthropogenic climate change has become the most ubiquitous threat to global marine biodiversity (Halpern et al., 2008; Hoegh-Guldberg and Bruno, 2010). Local and global-scale stressors can act singularly or interact with each other, resulting in effects that may be additive, synergistic, or antagonistic, thereby exerting significant impacts on the oceans and influencing all forms of marine communities (Kirby et al., 2009; Paerl and Scott, 2010; Gobler et al., 2014; Lefcheck et al., 2017; Ceccherelli et al., 2018; Piazzzi

and Ceccherelli, 2020). The multiple effects of local and global stressors on habitat-forming species, such as crustose coralline algae (CCA), is of particular concern.

CCA represent pivotal foundation taxa in the photic zone, encompassing a diverse group of marine species renowned globally for their ecological importance. These species offer a variety of ecosystem services, including habitat formation (Bressan et al., 2001; Ballesteros, 2006; Ingrosso et al., 2018) and carbonate production (Canals and Ballesteros, 2007; Cebrian et al., 2020). Their role as marine calcifiers is critical, characterized by the precipitation of calcium carbonate in their cell walls, primarily in the form of high-Mg calcite and, mainly in tropical species, aragonite (Nash and Adey, 2017). Furthermore, CCA play a fundamental role in the ecology of temperate and tropical reefs, as their calcified thalli contribute significantly to reef creation and stabilization, enhancing benthic diversity by offering hard substrates for the settlement of various organisms (Dunham et al., 2018; de la Torriente et al., 2020; Piazzzi et al., 2022).

In the Mediterranean Sea, CCA are the main builders of coralligenous concretions which represent one of the most important habitats of

* Corresponding author at: Department of Integrative Marine Ecology, Genova Marine Centre, Stazione Zoologica Anton Dohrn, Genova, Italy.

E-mail address: federico.pinna@szn.it (F. Pinna).

interest to the European Community due to the ecological services they provide, from fishing to their crucial role in the CO₂ balance (Ballesteros, 2006; Martin and Gattuso, 2009; Thierry de Ville d'Avray et al., 2019). Coralligenous reefs are also considered among the most threatened Mediterranean habitats having been included in the Habitat 1170 "Reefs" of the Habitat Directive (E.C., 1992) and listed as "special habitat types" under the European Marine Strategy Framework Directive (MSFD, E.C., 2008). Coralligenous habitats are deemed a particularly fragile ecosystem, highly sensitive to environmental changes. Several studies have evaluated their vulnerability to both warming (Garrabou et al., 2009; Gómez-Gras et al., 2019; Verdura et al., 2019; Ceccherelli et al., 2020; Garrabou et al., 2022) and local human impacts such as pollution (Piazzi et al., 2011) and mechanical damage due to recreational diving (Betti et al., 2019) and fishing activities (Ferrigno et al., 2018; Betti et al., 2020). Mechanical damage poses a significant threat to coralligenous reef, primarily due to the slow growth rate of most species (Ballesteros, 2006) which results in a limited capacity for restoration. Furthermore, damaged reefs may exhibit increased sensitivity to other forms of human impact (Piazzi et al., 2012), suggesting potential synergistic effects between local mechanical stress and global climate changes.

Coralline algae, along with numerous other marine species, are currently facing significant challenges due to the rise in global temperature and ocean acidification and recent research has focused on their response to such stressors (Martin and Gattuso, 2009; Diaz-Pulido et al., 2012; Ragazzola et al., 2012; Martin et al., 2013a, 2013b; Martin and Hall-Spencer, 2017; Pinna et al., 2022). Notably, recent meta-analyses (Cornwall et al., 2019; Rindi et al., 2019) concur that the calcification of both temperate and tropical coralline algae might be relatively robust to ocean warming (Cornwall et al., 2019; Rindi et al., 2019), compared to other taxa like corals (Kornder et al., 2018), and that negative effects would occur well above the maximum summer temperatures, exceeding the +3 °C threshold by 2100, considering the most severe emission scenario (i.e. RCP 8.5; IPCC, 2019). This could lead to an increased frequency of necrosis and mortality, along with a net decrease in calcification and further dissolution (Martin and Gattuso, 2009; Ragazzola et al., 2012).

Recently, CCA have received renewed attention in the context of global change, particularly due to the perceived vulnerability of their high-Mg calcite skeleton (McCoy and Kamenos, 2015) and the ability to modulate the geochemistry of their thallus based on the surrounding environment (Chave and Wheeler, 1965; Moberly, 1968; Chan et al., 2017; Ulrich et al., 2021). Mg/Ca ratios have been repeatedly shown to record seawater temperature in CCA (Kamenos et al., 2008; Hetzinger et al., 2023; Caragnano et al., 2014; Diaz-Pulido et al., 2014; Ragazzola et al., 2020) as the substitution of Mg into calcite is an endothermic reaction, the Mg/Ca ratio is expected to increase with increasing temperature (Mucci and Morse, 1985; Rosenthal et al., 1997). In biogenic calcite, the mechanical properties of calcite are strongly related to its Mg content (Long et al., 2014; Kunitake et al., 2012), consequently, the dependence of Mg uptake to temperature in CCA can have serious consequences on their structural integrity. Therefore, the future of CCA as habitat formers may depend on the ability of its physical structure to withstand changes within the environment, since changes in the growth structure and the mineralogy can affect this performance. Nonetheless, the collective response of this algae to the combined effect of global and local stressors remains an area of uncertainty. Given these looming threats, evaluating the impacts of both global and local stressors, as well as their potential interactions on ecosystem engineer species such as CCA, is of great importance and studies on the direct effects of mechanical impact on *L. stictiforme* performance, whether as an isolated factor or in combination with changes in other factors (i.e. rising temperature), are notably scarce.

This experimental manipulative study aimed at evaluating for the first time in the field the effect of thallus breakage (such as that due to trawling or anchoring) at two climate scenarios on the encrusting red

algae *L. stictiforme*, an important foundation species of the Mediterranean coralligenous reefs that span depths of 15 to 40 m. *L. stictiforme* is a non-geniculated crustose coralline alga and thalli consist of lobed lamellae, often overlaid, up to 1.5 mm thick, with a smooth to undulating surface which is violet to dark pink in colour. The experiment took advantage of temperature differences during the warm season between two depths at a Mediterranean site due to the presence of a thermocline (a transition layer between the warmer water at the surface and the cooler deep water below).

The experiment consisted of transplantation of broken and untouched thalli from 35 m depth to 15 m (above the thermocline) in a natural cavity (to resemble the 35 m irradiance conditions, see results), resembling the worst case future climate scenarios. Morphological (marginal growth, growth in thickness, number of conceptacles, growth angle variation, and number of reproductive structures i.e. conceptacles) and geochemical temperature-dependent (Mg/Ca, Li/Ca and Sr/Ca) variables were used to assess the performance of the *L. stictiforme* algae. The hypothesis was that temperature increase and mechanical damage will have combined effects on *L. stictiforme*: specifically, we tested if the damage due to thallus margin breakage can have consequences for the morphological characteristics of the thallus structure and reproductive features, if the higher temperature can facilitate the thallus resilience, and if the calcite geochemistry is affected by both the mechanical damage and changing temperature.

2. Materials and methods

2.1. Study system

The experiment was carried out during the warm season (from June to November 2021) at Costa Paradiso (CP, 41°04'N, 08°57'E), located on the North coast of Sardinia (Western Mediterranean, Fig. 1). Costa Paradiso is far from any anthropogenic source of influence such as urbanization, industries, aquaculture and harbors and it is known for its high level of oligotrophy (Barisiello et al., 2002; Regione Autonoma della Sardegna, 2016). Furthermore, at CP large temperature variations through depth due to seasonal stratification have been measured (Ceccherelli et al., 2020). The coralligenous reef at CP extends from 15 to 40 m in depth and is predominantly composed of massive calcareous bioconstruction, mainly consisting of red crustose coralline algae (Pinna et al., 2021). This coralligenous cliff exhibits distinct zonation, likely related to biogeographical features (Piazzi et al., 2021) and temperature variations, especially during summer (Ceccherelli et al., 2020).

2.2. Experimental set up and design

Healthy thalli of *L. stictiforme* (ranging 5 to 8 cm in diameter) with no signs of damage and bleaching were collected using a hammer and chisel from the CP coralligenous cliff at 35 m of depth. Then, they were transported in insulated aquaria (supplied with oxygenators) to the laboratory facilities at the University of Sassari within 1 h where they were cleaned by epiphytes. Algae were randomly assigned to treatments and to simulate mechanical damage due to human activities, the entire edge of the crust was manually broken in those assigned to the breakage treatment. Thereafter, a random sample of broken and untouched thalli ($n = 4$) was attributed to 35 m of depth and to a natural cavity in the bioconstruction at 15 m of depth (above the thermocline), resembling the irradiance at 35 m depth. Then, both broken and untouched thalli, were stained using Alizarine-Red stain (Sigma-Aldrich, Steinheim, Germany) 0.25 g·l⁻¹ for 24 h (Blake and Maggs, 2003; Rivera et al., 2004). Once stained, *L. stictiforme* thalli were transported back to CP and glued with a two-component epoxy underwater filler (Sub Coat Veneziani) on granite tiles which were attached on horizontal substrates. The experimental design consisted of two factors: mechanical damage (broken and untouched) and warming scenario (current and future, corresponding to 35 and 15 m of depth, respectively Fig. 2). Hereafter the treatments will

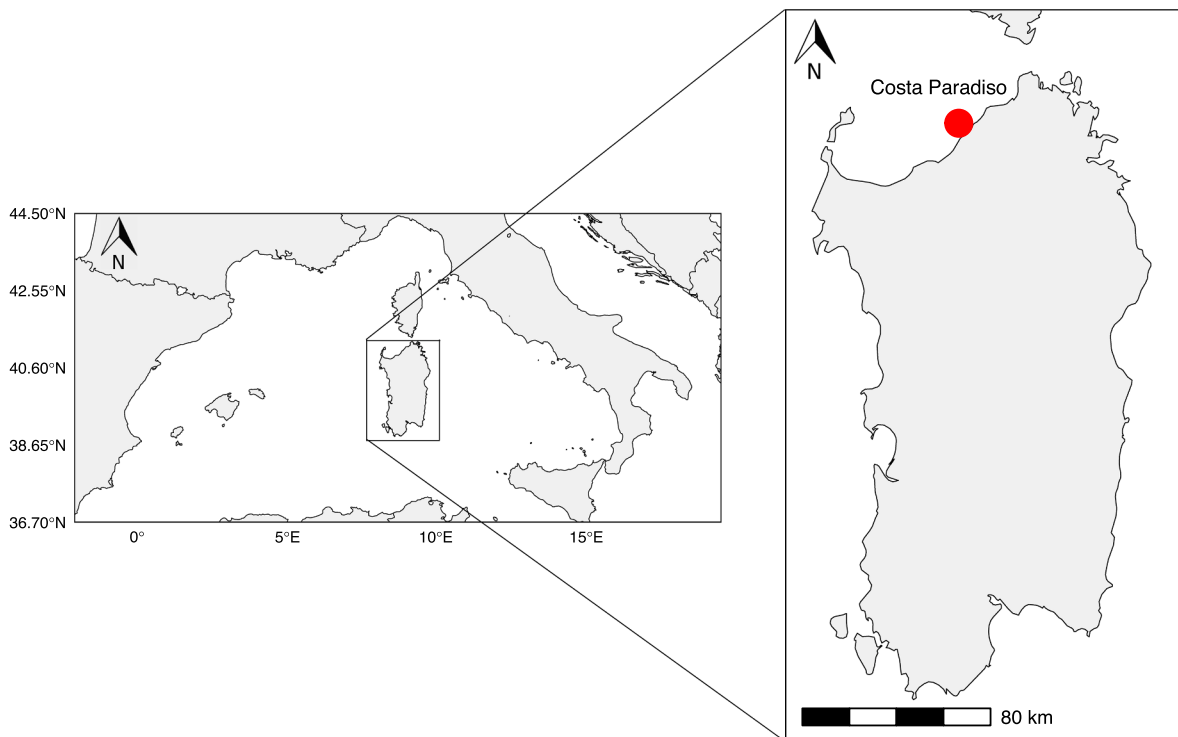


Fig. 1. Map of the study area. The red spot represents Costa Paradiso, site of the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

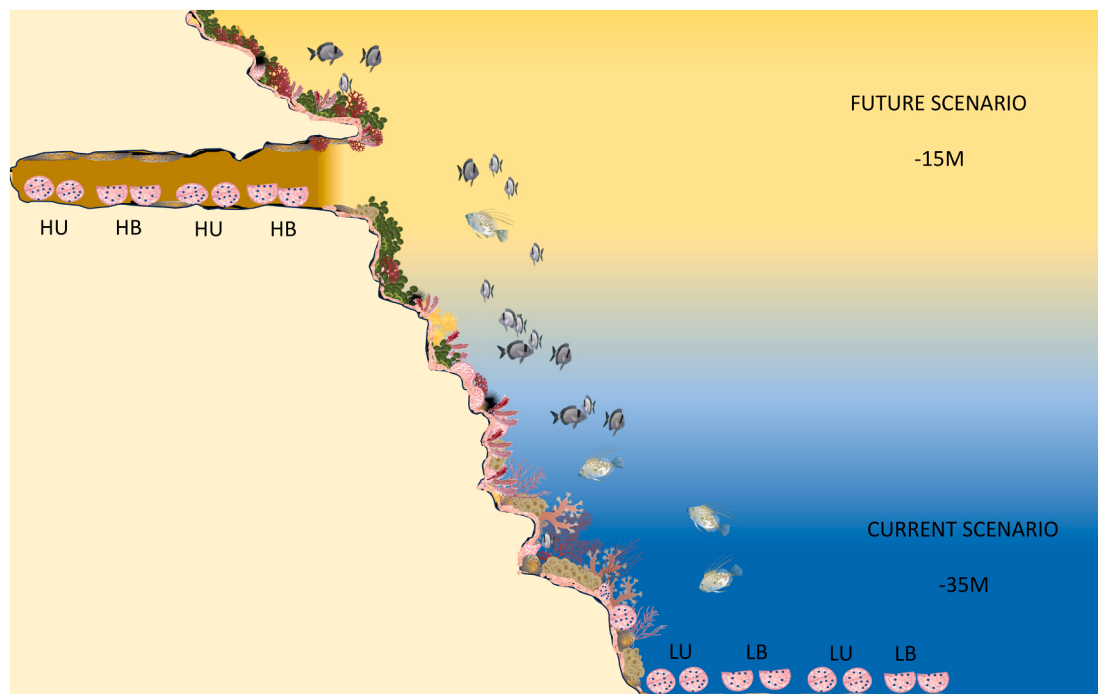


Fig. 2. Conceptual model showing experimental design using depths as a proxy of current and future climate scenarios. LU (Low temperature untouched) = untouched *L. stictiforme* thalli translocated to 35 m depth; LB (Low temperature with breakage) = broken *L. stictiforme* thalli translocated to 35 m depth; HU (High temperature untouched) = untouched *L. stictiforme* thalli transplanted to 15 m depth placed in a natural cavity (to mimic the approximate irradiance at 35 m); HB (High temperature with breakage) = broken *L. stictiforme* thalli transplanted to 15 m depth placed in a natural cavity.

be referred to as: LB (Low temperature with breakage) = broken *L. stictiforme* thalli translocated to 35 m of depth; LU (Low temperature untouched thalli) = untouched *L. stictiforme* thalli translocated to 35 m of depth; HB (High temperature with breakage) = broken *L. stictiforme*

thalli transplanted to 15 m of depth placed in a natural cavity; HU (High temperature untouched thalli) = untouched *L. stictiforme* thalli transplanted to 15 m depth placed in a natural cavity. In November, after 6 months since transplantation, *L. stictiforme* thalli were recollected for the

analysis.

2.3. Monitoring in situ temperature and irradiance fluctuations

For each treatment, two data loggers (HOBO Pendant Temp/Light MX2202, Onset Computer Corporation, USA) were fixed next to the thalli to record water temperature and light irradiance during the whole experimental time. The loggers were checked and cleaned from epiphytes every two months.

2.4. Morphological response

Once recollected from the field, thalli were rinsed with fresh water, air dried and individually embedded into epoxy resin mold (Kit EpoFix Struers). Specifically, from the mold of each thallus, five slices (about 1 cm wide) were obtained using a circular rock saw. Then each slice was polished using a machine with abrasive papers of different grain size (i.e. from 30 to 15 μm) on the longitudinal axes and photographed under the binocular microscope. Both marginal growth and growth in thickness were obtained by measuring the distance from the alizarin mark to the growing edge of the crust and thallus marginal growth and growth in thickness were estimated on the images through ImageJ software (<https://imagej.nih.gov>). A total of five measurements at equal distance (approximately 3 mm) were taken along the cross-section of each slide and then averaged to obtain the growth in thickness measurement in each thallus. Furthermore, the alteration in the growth angle was determined via image analysis by estimating the breadth of the thallus angle formed subsequent to the marking (Fig. S1).

Image analysis also allowed us to estimate the number of conceptacles in the portion of the alga produced during the experiment: conceptacles were only counted if the entire conceptacle could be seen in the area of analysis, and they were undamaged. Then the number of conceptacles of each slice was measured and averaged for each thallus. Each thallus was considered a replicate.

2.5. Geochemical response

The polished mounts with embedded CCA were cleaned in an ultrasonic bath with ethanol. Elemental compositions were measured by using laser ablation using a Resolution M50 193 nm ArF Excimer Laser system (Resonetics LLC, now Applied Spectra) coupled to a Thermo-Scientific Element XR sector field ICP-MS at the Frankfurt Isotope and Element Research Center (FIERCE), Goethe University Frankfurt. The ICPMS was tuned for maximum sensitivity ($>6 \text{ M cps } ^{238}\text{U}$, NIST SRM612, 60 μm beam diameter, 6 Hz, $\sim 6 \text{ J/cm}^2$) while ensuring robust plasma conditions ($\text{Th/U} = 1 \pm 0.1$, $m/z \text{ } 44/22 < 2\%$, $\text{ThO}^+/\text{Th}^+ < 0.5\%$).

Elemental compositions were determined with a 40 μm beam diameter, a pulse rate of 10 Hz and 3 $\mu\text{m/s}$ scan speed, under a He atmosphere, with Ar admixed into the top of the inner volume ablation cell, and N_2 added downstream to improve sensitivity. Prior to the measurement a fast precleaning pass was conducted at 0.2 mm/s, 10 Hz and 104 μm laser spot size.

Standardisation was performed using NIST SRM610 glass as the primary calibration standard, with the MACS-3 nanopellet (Garbe-Schönberg and Müller, 2014) used to assess accuracy and precision. Quantification was achieved using a sample-standard bracketing approach, with MACS-3np measured at random intervals throughout the sequence under identical conditions to the samples. We used the NIST SRM610 reference values of 465 $\mu\text{g/g}$ for Mg (Evans and Müller, 2018), 515.5 $\mu\text{g/g}$ for Sr, and 468 $\mu\text{g/g}$ for Li (Jochum et al., 2011). Standards were ablated in an identical manner to the samples. The monitored isotopes (m/z) were ^7Li , ^{24}Mg , ^{25}Mg and ^{88}Sr . ^{43}Ca was used as the internal standard and for E/Ca calculation.

Accuracy and precision, assessed via repeat measurements of the MACS-3np standard ($n = 6$), resulted in a measured Mg/Ca ratio of 7.8

$\pm 0.06 \text{ mmol/mol}$ (reference value = $7.8 \pm 0.4 \text{ mmol/mol}$), Li/Ca of $928 \pm 18 \text{ } \mu\text{mol/mol}$ (reference value = $953 \pm 64 \text{ } \mu\text{mol/mol}$), and Sr/Ca of $8.01 \pm 0.04 \text{ mmol/mol}$ (reference value = $8.2 \pm 0.4 \text{ mmol/mol}$, Jochum et al., 2012). We note that MACS-3 has a substantially higher [Li] than the coralline red algae carbonate sampled analyzed here (~ 950 versus $\sim 50 \text{ } \mu\text{mol/mol}$), such that the Li/Ca data quality quoted above may be more favorable than that which is truly applicable to our samples.

For the analysis, transects were divided into before (B) and after (A) the Alizarin staining (which mark the start of the experiment, Fig. S2) which was divided in two sections: the first (marked with W = warm period) includes the part of the thallus that was grown in summer and therefore with very distinct temperature difference between 35 m deep and the cavity at 15 m of depth, while the second section (marked with C = cold period in Fig. S2) includes the part of the thallus that was grown in winter where there was a temperature overlapping between the conditions (Fig. 3). Both morphological and geochemical analyses were conducted exclusively on the portion of the thallus grown between June and September, where the environmental data showed a clear temperature difference between the two depths. For further details on the analyzed thallus section, please refer to Fig. S2.

2.6. Data analysis

The effects of temperature scenario and mechanical damage on the morphological responses were assessed by two-way ANOVAs when normal distribution (Shapiro–Wilks test) and homogeneity of variance (Levene test) were verified. Univariate two-way permutational analyses of variance (PERMANOVA, Anderson, 2001) based on Euclidean distance were performed on the number of conceptacles, angle variation, and the elemental composition (Mg/Ca, Li/Ca and Sr/Ca). Sampling design included scenario (high and low temperature, corresponding to the future and current scenario) and mechanical impact (broken and untouched) as fixed factors ($n = 4$). A posteriori comparison of the means was done using Pair-Wise tests (Anderson, 2001). Pearson Correlation was used to analyse the different type of growth (thickness versus marginal extension). The Spearman's rank correlation was used to provide the statistical comparisons between Mg/Ca, Sr/Ca and Li/Ca. PERMANOVA and Pair-Wise tests were performed with PRIMER v7, all other analyses and graphics were performed in R using version 4.2.2 (R Core Team, 2022).

3. Results

Differences in the thermal regime between depths were found by graphical inspection (Fig. 3a): the temperature experienced by *L. stictiforme* at 15 m (HB and HU treatments) was about 4°C warmer than at 35 m (LB and LU treatments), being on average respectively $22.80^\circ\text{C} \pm 2.52 \text{ (SD)}$ and $18.60^\circ\text{C} \pm 2.08 \text{ (SD)}$ and indicating that the chosen depths were adequate to reproduce the future warming scenarios in according with RCP.8.5 climate model (IPCC, 2019). Furthermore, light irradiance between the 35 m (LU and LB) and 15 m depth (HB and HU) was similar, indicating that the natural cavity used for the experiment was adequate to reproduce the 35 m irradiance (Fig. 3b), with the highest (summer) irradiance of $\sim 30\text{--}35 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ at both treatments.

Overall, no bleached or unhealthy (discolored tissue) samples were found for the whole experiment. The marginal growth and growth in thickness were both affected by the mechanical damage, regardless the climate scenario considered (Table 1 and Fig. 4). In fact, the effect of breakage on the thalli of *L. stictiforme* resulted in a lower marginal growth and growth in thickness compared to the untouched thalli (LU and HU) in both scenarios considered (Fig. 4). The number of conceptacles was neither affected by the mechanical damage and temperature scenario, while a significant effect of both mechanical damage and

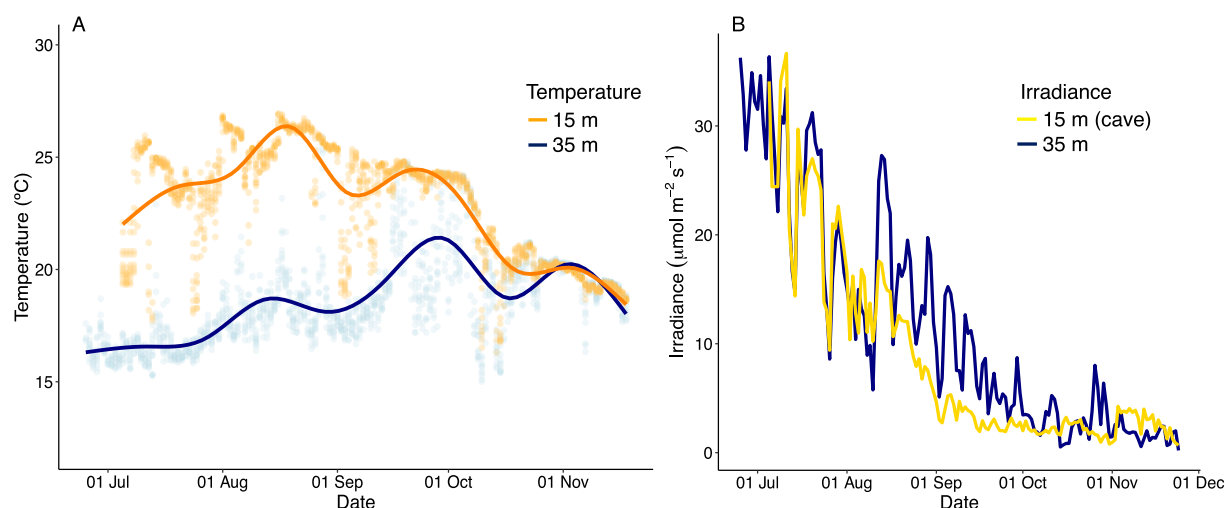


Fig. 3. Temporal variation of A) temperature and B) irradiance at Costa Paradiso from June to November at the two experimental conditions (35 m and 15 m inside the cave, the current and future scenario, respectively). Data were recorded with HOBO Pendant Temp/Light MX2202 Temperature Loggers (Onset Computer Corporation, Pocasset, MA).

Table 1

Results of ANOVAs performed on the effect of Scenario (High and Low temperature) and Mechanical damage (Broken and Untouched) on the thallus marginal growth and growth in thickness. Bold values indicate statistical significance ($p < 0.05$).

Source of variation	Df	Mean sq	F value	Pr(>F)
Marginal growth				
Scenario (S)	1	1.306	2.472	0.14190
Mechanical damage (M)	1	3.841	7.272	0.01940
S $\times$ M	1	0.046	0.088	0.77200
Residuals	12	0.528		
Growth in thickness				
Scenario (S)	1	0.000	0.130	0.72510
Mechanical damage (M)	1	0.022	23.102	0.00043
S $\times$ M	1	0.003	2.654	0.12926
Residuals	12	0.001		

temperature on the growth angle of the thalli was detected (Table 2 and Fig. 4): specifically, the growth angle was lower in broken thalli only at high temperature, while no differences were found at low temperature conditions and in untouched thalli at both temperature scenarios.

A strong positive correlation was found in LU and LB samples between marginal vs vertical growth rates ($R^2 = 0.89$, $p = 0.0003$), while a very poor correlation was found in HB and HU samples ($R^2 = 0.32$, $p = 0.44$), showing a disruption in the growth relation due to warming

(Fig. 5a and b).

An interactive effect of temperature scenario and mechanical damage was found on the overall elemental composition (Fig. 6, Table 3). Particularly, the breakage of the thallus changed the Mg/Ca ratio in different directions depending on the temperature scenario: a significant increase in the Mg/Ca ratio was found in broken thallus at low temperature, while at high temperature the untouched thalli had higher Mg/Ca ratios. Conversely, the effects of treatments on Li/Ca and Sr/Ca were essentially the opposite: both ratios increased and decreased in broken thalli at high temperature and low temperature, respectively (Fig. 6, Table 3). A different trend was found in the broken samples (LB and HB) compared to the untouched samples (LU and HU) with no significant difference in the Mg/Ca ratio on the vertical growth (Fig. 6).

Comparisons between the proxies Mg/Ca, Sr/Ca and Li/Ca were carried out and weak correlations were evidenced (Fig. 7). Interestingly, in the LU samples correlations between the measured elemental ratios were stronger (Mg/Ca_{vs}Sr/Ca; $\rho = 0.5$, p -value < 0.001 ; Mg/Ca_{vs}Li/Ca; $\rho = 0.34$, p -value < 0.001) than in the HU samples (Mg/Ca_{vs}Sr/Ca; $\rho = 0.24$, p -value < 0.001 ; Mg/Ca_{vs}Li/Ca; $\rho = 0.2$, p -value < 0.001), likely related to the higher variability of the overall Sr/Ca and Li/Ca in the transplanted samples (HU and HB).

4. Discussion

Variation in the morphological features and geochemical responses

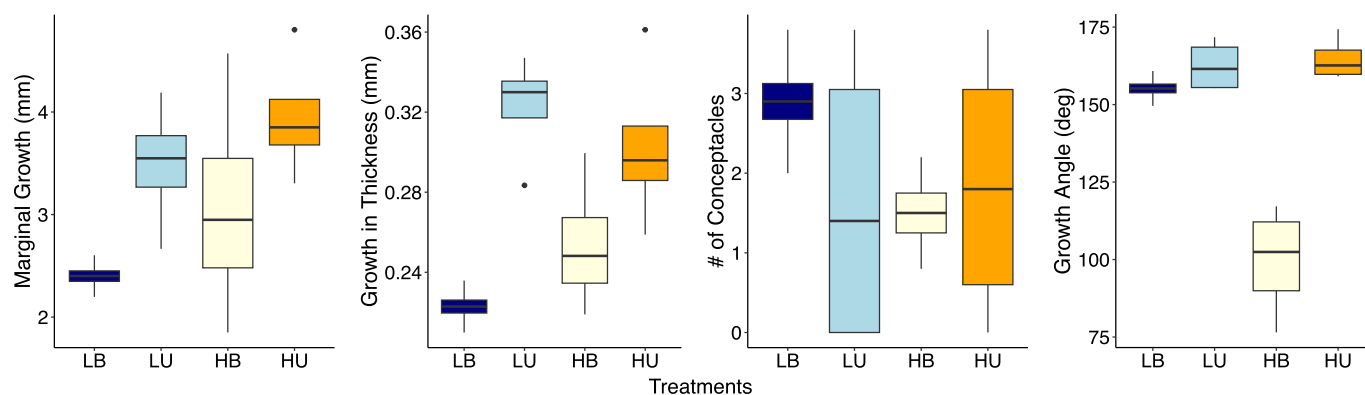


Fig. 4. Box plots of the morphological variables (marginal growth, growth in thickness, number of conceptacles and angle growth) of *L. stictiforme* among treatments: LU (Low temperature untouched), LB (Low temperature with breakage), HU (High temperature untouched) and HB (High temperature with breakage).

Table 2
Results of PERMANOVAs testing the effects of Scenario (High and Low temperature, H and L) and Mechanical damage (Broken and Untouched, B and U) on the number of conceptacles and angle variation, and pair-wise test on their interaction. Bold values indicate statistical significance ($p < 0.05$).

Source of variation	Df	Mean sq	Pseudo-F	P(per)
# of conceptacles				
Scenario (S)	1	0.748	0.354	0.54990
Mechanical damage (M)	1	1.329	0.629	0.42940
S $\times$ M	1	2.363	1.119	0.30680
Residuals	12	2.113		
Angle variation				
Scenario (S)	1	4110.200	28.303	0.00040
Mechanical damage (M)	1	3216.900	22.152	0.00100
S $\times$ M	1	3707.200	25.527	0.00090
Residuals	12	145.220		

Angle variation: pair-wise test on Scenario x Mechanical damage	
High temperature U > B	Broken L > H
Low temperature U = B	Untouched L = H

of *L. stictiforme* due to the temperature increase and mechanical damage were explored by assessing their growth performance and geochemical composition in relation to stressors. Results highlight a significant effect

of mechanical damage and warming on the growth angle of the thalli, but no evidence of interacting effects on other biological traits was found. Nevertheless, thallus breakage has a negative effect independently of the scenarios considered: at the current temperature scenario (LU and LB treatments) both marginal growth and growth in thickness were reduced by breakage (Fig. 2a and b), while no significant difference concerning the formation of new reproductive structures was found. Performance of *L. stictiforme* thalli in the warmer future scenario was consistent to that observed in the current climate conditions.

Even though, on one hand, mechanical damage negatively affected the performance of *L. stictiforme* thalli, on the other hand, a good adaptability to this stress was found as damaged thalli continued to grow in both directions, albeit slower than controls. However, linear growth data showed a disruption of the ratio between marginal and thickness growth at higher temperatures compared to the controls, which is even more evident in the HB samples. While this is a short-term study such that we cannot assess whether the thalli would have regained the initial growth rate through time, we can state that the higher temperature scenario did not buffer the reduction in growth rate. Overall, the deceleration of thallus growth due to mechanical damage is significant given that CCA make a substantial contribution to calcium carbonate production, along with other calcifying organisms such as bryozoans, corals, and other calcifying algae (Nelson, 2009; Cornwall et al., 2023). A slowdown in CCA growth could lead to a subsequent decrease in the ecosystem carbon budget. The reduction in calcium carbonate

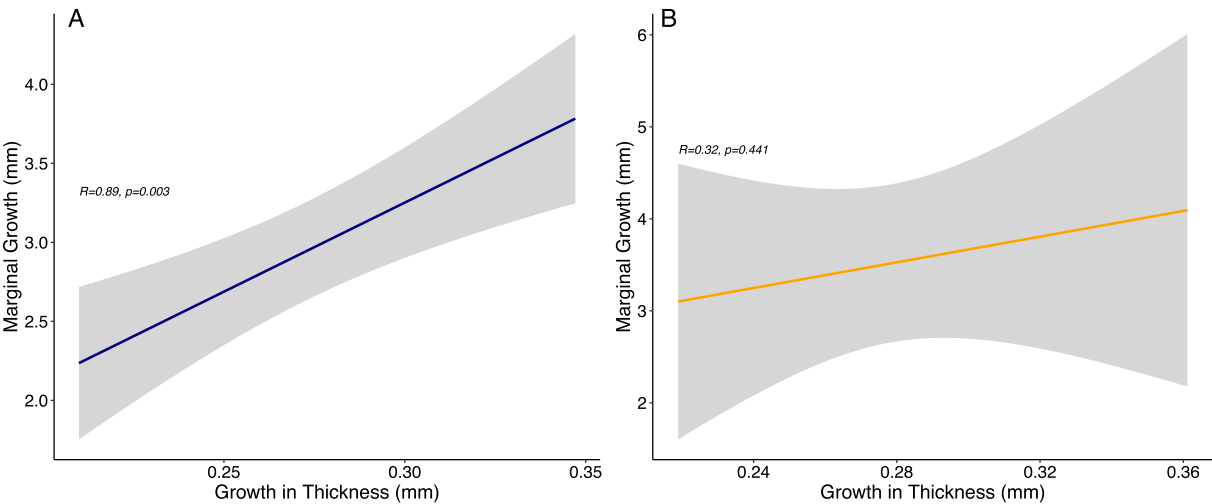


Fig. 5. Correlation between marginal and thickness growth in untouched thalli in the A) low and B) high temperature scenario.

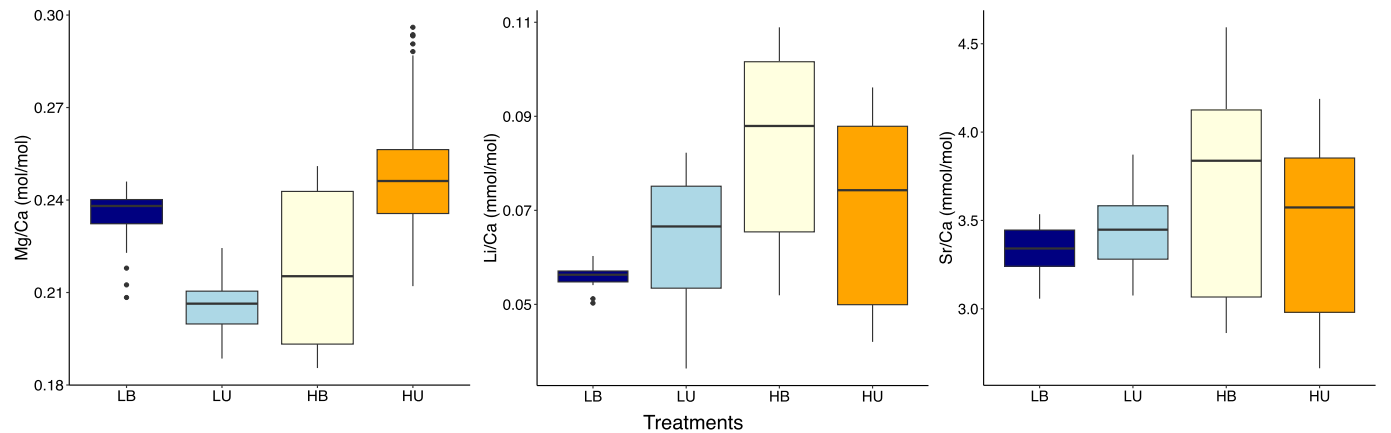


Fig. 6. Box plots of the elemental composition with 10 point moving average (Mg/Ca, Li/Ca and Sr/Ca) of *L. stictiforme* among treatments: LU (Low temperature untouched), LB (Low temperature with breakage), HU (High temperature untouched) and HB (High temperature with breakage).

Table 3

Results of PERMANOVAs testing the effects of Scenario (High and Low temperature, H and L) and Mechanical damage (Broken and Untouched, B and U) on the Mg/Ca, Li/Ca, Sr/Ca ratios, and pair-wise test on their interaction. Bold values indicate statistical significance ($p < 0.05$).

Source of variation	Df	MS	Pseudo-F	P(permanova)
Mg/Ca ratio				
Scenario (S)	1	8.9E-03	43.461	0.00010
Mechanical damage (M)	1	4.2E-03	0.002	0.96420
S × M	1	4.4E-02	214.600	0.00010
Residuals	418	2.0E-04		
Li/Ca ratio				
Scenario (S)	1	0.0174	73.089	0.00010
Mechanical damage (M)	1	0.0003	13.893	0.23750
S × M	1	0.0052	22.039	0.00010
Residuals	418	0.0002		
Sr/Ca ratio				
Scenario (S)	1	21.342	17.662	0.00020
Mechanical damage (M)	1	0.070	0.576	0.45450
S × M	1	12.353	10.224	0.00160
Residuals	418	0.121		
Mg/Ca ratio: pair-wise test on scenario × mechanical				
High Temperature U > B			Broken H < L	
Low Temperature U < B			Untouched H > L	
Li/Ca ratio: pair-wise test on scenario × mechanical				
High Temperature U < B			Broken H > L	
Low Temperature U > B			Untouched H > L	
Sr/Ca ratio: pair-wise test on scenario × mechanical				
High Temperature U < B			Broken H > L	
Low Temperature U > B			Untouched H = L	

production by CCAs would have relevant implications for the health of the marine ecosystem since calcium carbonate is crucial for the formation and stability of critical habitats, such as coralligenous habitats and coral reefs, and for the survival of numerous marine species that rely on these calcareous structures for their sustenance and protection.

The reduction in growth rates of CCA after mechanical damage should be taken into account in experiments using these algae, especially for those with the most crustose forms, where the collection of thallus samples is usually made by fragmenting portions of crusts to obtain an adequate sample size. Thus, thalli could be subjected to the same stress tested in this study, although *L. stictiforme* has a thallus of different shape (i.e. foliose) and not completely adherent to the substrate (such as in *Mesophyllum alternans*, *Porolithon onkodes*, and *Hydrolithon reinboldii*). Therefore, differences in growth found among studies on both temperate and tropical CCA could depend not only on experimental conditions (either laboratory or field experiments), but also be biased by the mechanical stress produced due to sample collection.

Furthermore, physical damage caused by the mechanical impact on the CCA community could reduce the biomass and coverage of habitat-forming species leading to changes in the structure and diversity of the associated community (Piazzi et al., 2022) and increasing the sensitivity to other impacts (Piazzi et al., 2012).

Nevertheless, the capacity to repair damages seems to be efficient in *L. stictiforme* and its ability to withstand injuries is probably related to its strategy for reproduction through fragmentation. In this regard, the number of new reproductive structures were unaffected by both mechanical damage and temperature. The lack of response on the formation of new conceptacles underlines that *L. stictiforme* may be able to reproduce under multiple stressors; such consistency in the production of conceptacles despite the changed environmental conditions suggests promising future performance of the alga, that if more widely applicable, could help in maintaining the genetic variability of populations, crucial for resilience and adaptability to environmental changes.

However, the presence of conceptacles does not necessarily imply thallus fertility, since the functionality of these reproductive structures was not investigated. Various stress factors, although not yet fully explored in coralline algae, can impact fertility in different ways, such as gametogenesis and post-fertilization development. Additionally, genetic variability also depends on other factors, like mating success, genetic diversity of reproductive individuals, and the dispersal of gametes or propagules. Therefore, while constant conceptacle production is a positive indicator, further studies are needed to fully assess the impact of various stressors on the reproductive effectiveness of *L. stictiforme*.

A noteworthy finding of our study is the impact of mechanical damage combined with warming on the growth angle direction of *L. stictiforme*. Specifically, it appears that these two stressors in combination significantly alter the thallus growth angle, whose linear extension shifts to a curvature of about 100°, heavily disrupting the marginal vs thickness growth ratio. The growth angle could be an important metric because it may reflect how the algae adjust the thallus structure in response to environmental stressors. A shift in the growth angle can influence the overall architecture of the thallus, which may affect its ability to resist physical disturbances and maintain structural integrity in dynamic environments. This aspect is particularly critical in the context of habitat formation, as the growth angle is linked to the internal structure of the thallus and can influence calcification processes and the overall mechanical strength of the algae (Ragazzola et al., 2012). This finding is novel and it highlights the need for further research to understand how combined warming and mechanical damage may influence habitat construction by CCA by affecting the angle growth. This discovery underscores the critical importance of such environmental interactions in shaping marine ecosystems.

The geochemical signature shows an increase in the Mg/Ca with temperature which corroborates the extensive use of this ratio as a temperature proxy in coralline algae and other calcifying organisms (Barker et al., 2005; Fietzke et al., 2015; Hetzinger et al., 2009; Kamenos et al., 2008; Raddatz et al., 2022). Mg/Ca values are in the same order of magnitude as other coralline algae (Hetzinger et al., 2023) from the Mediterranean Sea. The Mg/Ca ratio of this study was, however higher (0.21 ± 0.0006 Mg/Ca_{mol/mol}) than previously determined for the same species (0.08 ± 0.003 Mg/Ca_{mol/mol} from Ragazzola et al., 2020), also collected in the Mediterranean Sea (i.e. Pontine Islands). It is generally accepted that the elemental abundance in marine calcifying organism skeletons reflects a combination of physical and chemical properties of seawater, mineralogy and biomineralization pathways (Ulrich et al., 2021). This inconsistency can be due to different sites, depths (35 m this study and 44 m in Ragazzola et al., 2020), temperatures and physical and chemical properties of the water bodies. According to the Copernicus data set (MEDSEA_ANALYSISFORECAST_BGC_006.014) the sites where the specimen of Ragazzola et al. (2020) were collected have a lower pH, TALK (pH 8.10_{tot scale}; TALK: 2.58 mol/m³) and lower sea water alkalinity (TALK) compared to the site of this study (pH 8.11_{tot scale}; TALK: 2.65 mol/m³) while the salinity was very similar (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/nrlHycomGLBu008e912D.html>). In Coralline algae (Ragazzola et al., 2016; Ries et al., 2009; Egilsdottir et al., 2013; Teichert et al., 2020) and other calcifying organisms (Evans et al., 2016), it has been shown that co-variance between temperature and seawater carbon chemistry influence Mg/Ca-derived temperature estimates. The decrease in Mg concentration with increase in CO₂ in coralline algae has been explained as an attempt to decrease the solubility of the thallus, thereby preventing rapid dissolution (Hofmann and Bischof, 2014). This effect of CO₂ on the Mg concentration in coralline algae undermines the accuracy of Mg/Ca as a proxy for past temperatures in time intervals in which significantly different seawater chemistry may have occurred. In our study, the effect of seawater chemistry on Mg/Ca ratio in the thallus can be neglected since the experiment was carried out in only one location and in a short time interval, i.e. no long-term changes in CO₂ concentration in the seawater occurred. Light has also been shown to strongly correlate with Mg/Ca (Moberly, 1968) in

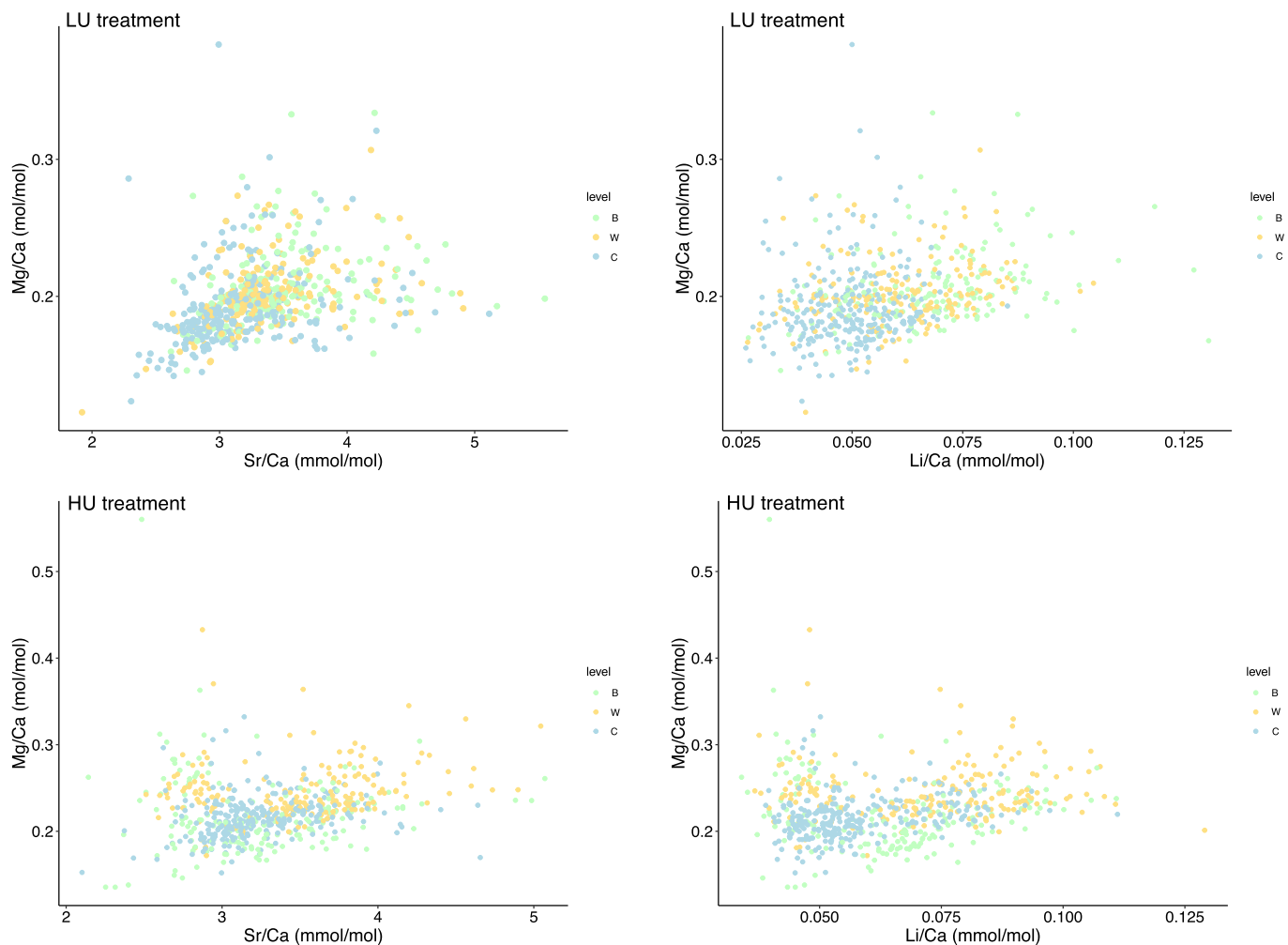


Fig. 7. Spearman's rank correlation between Mg/Ca and Sr/Ca and between Mg/Ca and Li/Ca in the LU and HU samples. Yellow dots (W) correspond to the warm period; light blue dots (C) correspond to the cold period; green dots (B) correspond to before the thallus staining (see Fig. S2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cold water coralline algae (Williams et al., 2018), especially at higher temperatures where an increase of 1.8 mol%MgCO₃ was calculated for temperatures above 10 °C. The correlation of Mg/Ca with light intensity has been used to explain the intersample variability of samples from the same site that, hence, experienced the same temperature (Williams et al., 2014). Such intersample variability can be caused, for example, by differences in orientation relative to the sea surface or differences in depths. In our study, the influence of light intensity in Mg uptake can be neglected since the light intensity was the same in both temperature scenarios (Pinna et al., 2022).

Overall, the untouched thalli showed, in accordance with expectations, a strong positive response of Mg/Ca to higher seawater temperature while, surprisingly, in the broken samples such response was not observed suggesting a possible the involvement of the thallus repair by the alga on mineralization (higher Mg, increase in hardness and elastic modulus, Long et al., 2014) subsequent to the breakage. However, more work is required due to the scarcity of the number of broken samples.

Furthermore, all thalli displayed a positive correlation between Li/Ca and Sr/Ca and temperature. Sr, together with Mg, is one of the most abundant elemental substitutions across all mineralogies (Ulrich et al., 2021), with Sr content often broadly following Mg/Ca. This is related to the incorporation of the smaller Mg²⁺ compared to the Ca²⁺ cation in the calcite lattice and the subsequent distortion of the crystal lattice, facilitating Sr²⁺ uptake (Mucci and Morse, 1983) although this factor alone cannot explain the abundance of other elements via lattice

distortion (Ulrich et al., 2021). In our study there is an increase in Li/Ca ratio with temperature in both broken and untouched thalli, and in Sr/Ca only for broken thalli. However, Mg/Ca and Sr/Ca are correlated at both temperature scenarios, while Mg/Ca and Li/Ca covary only in the sample grown under our 'future' scenario.

While we observe an increase in the concentration of the three trace elements measured here at higher temperature, broadly as expected, our results also highlight that different growth patterns can hinder the reliability of extracting temperature records (see also Ragazzola et al., 2020). This is one reason that species of this genera are not widely used for environmental reconstructions (Ragazzola et al., 2020), compared to other species belonging to the genera *Lithothamnion* (Piazza et al., 2022).

Finally, *L. stictiforme* is common in the Mediterranean Sea, where it is particularly widespread in regions including Spain, France, Italy, Malta, Slovenia, Croatia, Albania, Greece, Turkey, Syria, Israel, Egypt, Libya, Tunisia, Algeria, and Morocco (Athanasiadis, 1999; Rindi et al., 2019). Our study site is located in the northern part of Sardinia, near the center of the geographical distribution of *L. stictiforme*, as the studied population may be considered to have typical features of the species. On the contrary, populations located at the edge of the range, where organisms could experience more extreme environmental fluctuations and potentially have different adaptive traits (Pörtner, 2010), may exhibit different responses to environmental changes. Thus, further studies on marginal populations in the Mediterranean are necessary to fully understand potential differences in responses to climate change and

mechanical stress of *L. stictiforme*. Additionally, it is important to note that in a previous study (Pinna et al., 2022, Fig. S1), molecular analyses were conducted on samples collected from the same site, area and at the same depth as in the present study and these analyses confirmed that the samples corresponded to true *L. stictiforme*, as described by Pezzolesi et al. (2019) using morphological criteria validated by genetic data, including thalli with overlapping lobed lamellae, up to 1.5 mm in thickness, a smooth or wavy surface, and a colour ranging from purple to dark pink. Such molecular confirmations strengthen the reliability of the identification and the representativeness of the samples within the central Mediterranean context.

Overall, our results highlight the crucial role that local management plays in protecting benthic habitats even from mechanical impact activities. Therefore, to preserve these important ecosystem engineers and maintain the health of marine habitats, it is essential to minimize human activities where CCAs are abundant and protect them from damages so to ensure their ecological function in marine ecosystems. However, the capability of growth showed by broken *L. stictiforme* thalli in the present study suggests a potential recovery of the system to mechanic damages which does not seem to be influenced by warmer climatic conditions. However, it is of course important to bear in mind that climate change is associated with effects other than temperature increase, with ocean acidification of particular importance and which may affect the resilience of CCAs to breakage which was not a part of our experimental design but could be a focus of further investigations. Although our study primarily focused on the effects of temperature and mechanical stress, it is important to discuss the potential impact of ocean acidification on calcifying organisms such as *L. stictiforme*. Recent studies on other coralline algae species have shown that ocean acidification can negatively affect calcification, growth, and reproduction in these organisms (Anthony et al., 2008; Webster et al., 2012; Cornwall et al., 2022). On the other hand, ocean acidification does not seem to influence the mineralogical composition of the thallus in Mediterranean CCA species (Nash et al., 2016), potentially implying that geochemical proxies such as Mg/Ca are not compromised by a potential change in mineralogy (Ragazzola et al., 2016). In the context of our study, increased temperature combined with ocean acidification could alter the energetic cost of calcification (Briggs and Carpenter, 2019), further reducing the ability of the algae to repair mechanical damage. These results also provide an important knowledge regarding the choice of the thalli for eventual restoration activities: even though more experimental evidence is needed, our data show that the use of undamaged thalli would be preferable because the use of broken ones could slow down the recovery and weaken the structure of the coralligenous community, especially under future environmental conditions.

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CRediT authorship contribution statement

Federico Pinna: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Federica Ragazzola:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Luigi Piazzzi:** Writing – review & editing, Investigation. **David Evans:** Formal analysis. **Jacek Raddatz:** Formal analysis. **Giulia Ceccherelli:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence

of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2024.117219>.

Data availability

Data will be made available on request.

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