

# Growth rate rather than temperature affects the B/Ca ratio in the calcareous red alga *Lithothamnion coralliooides*

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**Abstract.** The B/Ca ratio in calcareous marine species is informative of past seawater  $\text{CO}_3^{2-}$  concentrations, but scarce data exist on B/Ca in coralline algae. Recent studies suggest influences of temperature and growth rates on B/Ca, the effect of which could be critical for the reconstructions of surface ocean pH and atmospheric  $p\text{CO}_2$ . In this paper, we present the first LA-ICP-MS analyses of Mg, Sr, Li and B in the coralline alga *Lithothamnion coralliooides* collected from different geographic settings and depths across the Mediterranean Sea and in the Atlantic Ocean. We produced the first data on putative temperature proxies (Mg/Ca, Li/Ca, Sr/Ca, Mg/Li) and B/Ca in a coralline algal species grown in different Basins, from across the photic zone (12 m, 40 m, 45 m, and 66 m depth). We tested the B/Ca correlation with temperature proxies and growth rates, in order to evaluate their possible effect on B incorporation. Our results suggested a growth rate influence on B/Ca, evident especially in the deepest sample with the lowest growth rate of 0.10 mm/yr (Pontian Isl., Italy; 66 m depth) and in Elba the shallowest sample (Italy) Morlaix, Atlantic coast of France; 45±2 m depth, where the algal growth rate was the highest (0.14 mm/yr). In these two sites, where the growth rates were respectively 0.11 mm/yr and 0.13 mm/yr and the measured B/Ca was respectively the lowest,  $462.8 \pm 49.2 \mu\text{mol/mol}$ , and the highest,  $757.7 \pm 75.5 \mu\text{mol/mol}$ . A positive correlation between B/Ca and temperature proxies was found only in the shallowest sample from Morlaix (Atlantic coast of France; 12 m depth), where the amplitude of seasonal temperature variation ( $\Delta T$ ) was the highest ( $8.9^\circ\text{C}$ ). Still, fluctuations in B/Ca did not mirror yearly seasonal temperature oscillations as for Mg/Ca, Li/Ca, and Sr/Ca. We concluded that growth rates, triggered by the different  $\Delta T$  and light availability across depth, affect the B incorporation in *L. coralliooides*.

## 1 Introduction

Warming and acidification are major anthropogenic perturbations of present-day oceans (Callendar, 1938; Fairhall, 1973; Brewer, 1997; Gattuso, 1999; Caldeira, 2005; Hönisch et al., 2012; Masson-Delmotte et al., 2021). Ocean acidification reduces the saturation state of calcite and aragonite, lowering the dissolution threshold of biominerals and threatening habitat-forming species of critical ecological importance such as coralline red algae and corals (Morse et al., 2006; Hoegh-Guldberg et al.,

2007; Andersson et al., 2008, 2011; Basso, 2012; Ragazzola et al., 2012; Ries et al., 2016). Coralline algae, which precipitate high Mg-calcite (>8-12 mol% MgCO<sub>3</sub>) (Morse et al., 2006), are particularly suitable as proxy archives for paleoclimate reconstruction because of their worldwide distribution and longevity. Importantly, they show indeterminate growth, with no ontogenetic trend (Halfar et al., 2008), that is the growth trend of coralline algae does not slow down asymptotically with age, 35 as in bivalves, thus preserving the resolution of the geochemical signals in all stages of growth (Adey, 1965; Frantz et al., 2005; Halfar et al., 2008). Moreover, coralline algae thin sections under optical microscopy reveal bands that reflect the growth pattern (Cabioch, 1966; Basso, 1995a, b; Foster, 2001), similarly to tree rings (Ragazzola et al., 2016) that can be targeted for high-resolution geochemical analyses. Seasonal growth bands, indeed, consist of the perithallial alternation of dark and light bands that together constitute the annual growth patterns (Freiwald and Henrich, 1994; Basso, 1995a, b; Kamenos et al., 2009). 40 Dark bands correspond to slow-growing cells produced in the cold season, which are shorter, thick-walled and with lower Mg contents; while light bands are fast-growing cells produced in the warm season, which are longer, less calcified and with higher Mg concentrations (Kamenos et al., 2009; Ragazzola et al., 2016). The high Mg-calcite of calcareous red algae records ambient seawater temperature (Halfar et al., 2000; Kamenos et al., 2008; Nash et al., 2016; Hetzinger et al., 2018), primary productivity (Chan et al., 2017; Hou et al., 2019) and salinity (Kamenos, 2012), proving to be a suitable paleoclimate archive. Most of the data were collected from high-latitude (Kamenos et al., 2008; Anagnostou et al., 2019) and tropical species (Caragnano et al., 45 2014; Darrenougue et al., 2014), whereas less attention has been given to coralline algae from mid-latitudes.

Trace element variations in marine calcareous species inform the reconstruction of changes in the environmental parameters, which characterized the seawater during their growth (Hetzinger et al., 2011; Montagna and Douville, 2017). Boron is incorporated into the mineral lattice of calcareous marine species during calcite precipitation. In the ocean, B occurs in two 50 molecular species: boric acid B(OH)<sub>3</sub> and borate ion B(OH)<sub>4</sub><sup>-</sup> (Dickson, 1990), which are related by the following acid-base equilibrium reaction:



that shows the dependence of the two species concentration on pH. ~~Boron has two stable isotopes: the heavier <sup>11</sup>B (80.1% of the total abundance) and the lighter <sup>10</sup>B (19.9%) with an enrichment factor  $\epsilon^{11}\text{B}$  ( $\delta^{11}\text{B(OH)}_3 - \delta^{11}\text{B(OH)}_4^-$ ) equal to  $1.0272 \pm 0.0006$  (Klochko et al., 2006). The isotopic composition of  $\text{B(OH)}_3$  and  $\text{B(OH)}_4^-$  changes with pH, being both enriched in  $\delta^{11}\text{B}$  with increasing pH (Dickson, 1990; Foster et al., 2010).~~ The first analyses of the isotopic signal of marine carbonates evidenced a strong similarity with the isotopic composition of B(OH)<sub>4</sub><sup>-</sup> in solution, suggesting that borate would preferentially be incorporated into marine carbonates (Vengosh et al., 1991; Hemming and Hanson, 1992; Zeebe and Wolf-Gladrow, 2001; DeCarlo et al., 2018). ~~Therefore, the  $\delta^{11}\text{B}$  of marine carbonates should increase with pH according to the  $\delta^{11}\text{B}$  of  $\text{B(OH)}_4^-$ . The  $\delta^{11}\text{B}$  of borate is closely related to  $\text{pCO}_2$ , seawater  $[\text{CO}_3^{2-}]$ , pH and  $[\text{B(OH)}_4^-]$ , which in turn are mainly controlled by the balance between the total alkalinity (TA) and the dissolved inorganic carbon (DIC) (Zeebe and Wolf-Gladrow, 2001; Dickson et al., 2007; Foster and Rae, 2016).~~ The ~~above described theoretical overview frames the role of the~~ B content and its isotopic signature ( $\delta^{11}\text{B}$ ) in calcareous marine species, ~~as recorders of~~ information about the seawater carbonate system. The  $\delta^{11}\text{B}$  is used to reconstruct past seawater pH (Hönisch and Hemming, 2005; Foster, 2008; Douville et al., 2010; Paris et al., 2010; Rae

65 et al., 2011). The boron-to-calcium ratio (B/Ca) proved to be informative about past seawater  $\text{CO}_3^{2-}$  concentrations in different empirical studies on benthic foraminifera (Yu and Elderfield, 2007; Yu et al., 2007; Rae et al., 2011) and in synthetic aragonite (Holcomb et al., 2016). Most of the literature on boron studies is focused on its isotopic composition (Hemming and Höönsch, 2007; Klochko et al., 2009; Henehan et al., 2013; Fietzke et al., 2015; Cornwall et al., 2017; Ragazzola et al., 2020), whereas less attention has been given to B/Ca records, especially in coralline algae. Recent studies suggest that B/Ca is function of 70 seawater pH, as well as of other environmental variables such as temperature, whose effect should be considered in the attempt to reconstruct surface ocean pH and atmospheric  $p\text{CO}_2$  (Wara et al., 2003; Allen et al., 2012; Kaczmarek et al., 2016).

To achieve the best reliability of geochemical proxies for climate reconstructions, ~~a growing interest has arisen on multiple approaches, by considering multi proxies for a single environmental factor (D'Olivo et al., 2018; Cuny Guirrec et al., 2019; Zinke et al., 2019). Moreover,~~ it is important to recognize the influence of multiple factors on a single proxy (Kaczmarek et 75 al., 2016; Donald et al., 2017). For instance, more recently the effects of temperature and growth rate on B incorporation have been investigated through experiments on both synthetic and biogenic carbonates (Wara et al., 2003; Yu et al., 2007; Gabitov et al., 2014; Mavromatis et al., 2015; Uchikawa et al., 2015; Kaczmarek et al., 2016; Donald et al., 2017). In particular, culture experiment on the coralline alga *Neogoniolithon* sp. ~~(Donald et al., 2017)~~ showed a positive correlation of B/Ca with growth rate, and a negative correlation with Sr/Ca, which was proposed ~~by the authors~~ as proxy of DIC ~~(Donald et al., 2017)~~. Moreover, 80 a culture experiment on the high latitude species *Clathromorphum compactum* (Kjellman) Foslie 1898 revealed non-significant temperature influences on B/Ca and a significant inverse relationship with growth rate (Anagnostou et al., 2019). The factors which influence the B incorporation in calcareous red algae are therefore still debated. Recent experiments also suggest that coralline algae can control the calcifying fluid pH ( $\text{pH}_{\text{cf}}$ ) (Cornwall et al., 2017), as already observed in corals (Comeau et al., 2017). Both organisms have a species-specific capability to elevate pH at calcification sites in response to variations of ambient 85 pH, also influencing precipitation rates (Cornwall et al., 2017). Differences between carbonate polymorphs were also highlighted (McCulloch et al., 2012; Cornwall et al., 2018), showing more elevated  $\text{pH}_{\text{cf}}$  in aragonitic corals than calcites, pointing to the relevance of the mineralogical control on biological up-regulation. So far, no investigations on  $\text{pH}_{\text{cf}}$  modifications in natural systems have been performed on calcareous red algae.

Nevertheless, no studies have been conducted so far on the correlation between temperature proxies (Mg, Sr, Li /Ca) and 90 B/Ca. The Mg/Ca ratio is extensively used as a temperature proxy in ~~calcifying species (Barker et al., 2005), including~~ coralline algae (Halfar et al., 2008; Kamenos et al., 2008; Fietzke et al., 2015; Ragazzola et al., 2020), since the substitution of  $\text{Mg}^{2+}$  with  $\text{Ca}^{2+}$  ions in the calcite lattice is an endothermic reaction. Accordingly, Mg incorporation increases with temperature (Moberly, 1968; Berner, 1975; Ries, 2006; Caragnano et al., 2014; 2017). Sr/Ca and Li/Ca ratios in calcareous red algae have also been investigated as climate proxies, showing significant positive correlations with temperature in different species, e.g. 95 *Lithothamnion* spp. (Kamenos et al., 2008; Hetzinger et al., 2011; Caragnano et al., 2014; Darrenougue et al., 2014). The Mg/Li ratio showed a strong correlation with seawater temperature in cultured *C. Clathromorphum compactum* (Anagnostou et al., 2019), and in empirical studies on high-Mg calcites, including coralline algae (Stewart et al. 2020). Conversely, the Mg/Li calibration did not reveal improvements in the Mg/Ca or Li/Ca proxies in *Lithophyllum* spp. (Caragnano et al., 2014;~~;~~ 2017).

Here, we present a laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) conducted on a wild grown  
100 coralline alga with a wide geographic scope. This technique, which allows high-resolution analysis of a broad range of trace  
elements in solid-state samples, has been widely used in biogenic carbonates to extract records of seawater temperature,  
salinity, and water chemistry (Schöne et al., 2005; Corrège, 2006; Hetzinger et al., 2009, 2011; Fietzke et al., 2015; Ragazzola  
et al., 2020). Measurements were made on the non-geniculate coralline alga *Lithothamnion coralliooides* (P. Crouan & H.  
Crouan) P. Crouan & H. Crouan 1867, which is widely distributed in the Mediterranean Sea and in the north-eastern Atlantic  
105 Ocean, from Scotland to Canary Islands (Irvine and Chamberlain, 1999; Wilson et al., 2004; Carro et al., 2014), usually  
constituting maerl beds (Potin et al., 1990; Foster, 2001; Martin et al., 2006; Savini et al., 2012; Basso et al., 2017). It forms  
rhodoliths as unattached branches (Basso et al., 2016) with obvious banding in longitudinal sections (Basso, 1995b). These  
characteristics combine to make this species a suitable model for the measurement of geochemical proxies, by comparing  
different environmental settings.

110 In this paper, we provide the first LA-ICP-MS data of putative temperature proxies (Mg/Ca, Sr/Ca, Li/Ca, Mg/Li) and B/Ca  
measured on *L. coralliooides* collected from different geographic settings and depths across the Mediterranean Sea and in the  
Atlantic Ocean. We test the influence of temperature and growth rate on the B/Ca ratio, which could be crucial in assessing  
the reliability of B/Ca as a proxy of the seawater carbonate system.

## 2 Materials and methods

### 115 2.1 Sampling sites and collection of *Lithothamnion coralliooides*

Samples of the coralline alga *L. coralliooides* were collected in the Western Mediterranean Sea and in the Atlantic Ocean (Fig.  
1). In the Mediterranean Sea, the samples collected in Pontian Islands (Italy) at 66 m depth were gathered by grab during the  
cruises of the R/V Minerva Uno, in the framework of the Marine Strategy Campaigns 2017 (Table 1). The last two  
120 Mediterranean samples were collected by one of the authors (DB) by SCUBA diving during local surveys at 45 m off the  
coasts of Pomonte (Elba Island, Italy) (Basso and Brusoni, 2004) and at 40 m depth in the Aegadian Islands (Marettimo, Italy).  
The Atlantic sample was collected by grab at 12 m depth in Morlaix Bay (Brittany, France) (Table 1).

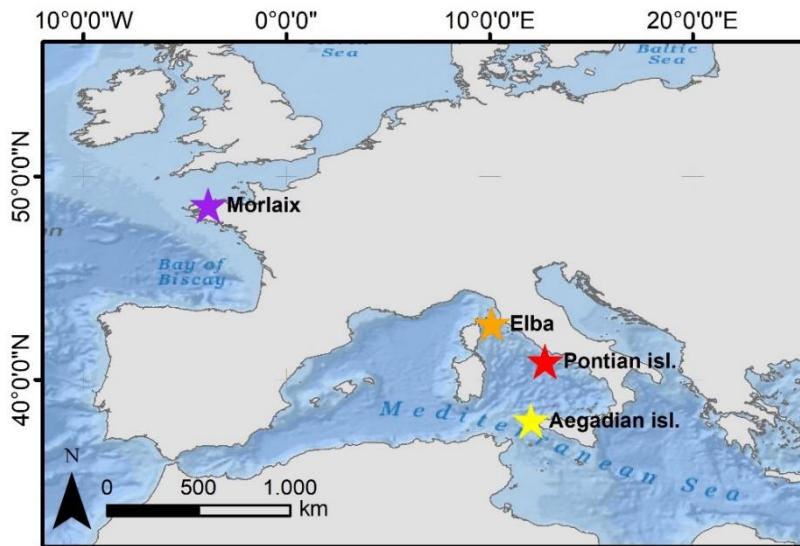


Figure 1: Map showing the distribution of sampling sites where *Lithothamnion coralliooides* samples were collected: Morlaix Bay (48°34'42"N, 3°49'36"W); Aegadian Islands (37°97'36"N, 12°14'12"E); Elba (42°44'56.4"N, 10°07'08.4"E) and Pontian Islands (40°54'N, 12°45'E). Service layer credits: Source Esri, GEBCO, NOAA, National Geographic, Garmin, geonames.org and other contributors.

The identification of the algal samples was achieved by morphological analyses of epithallial and perithallial cells using a Field Emission Gun Scanning Electron Microscope (SEM-FEG) Geomini 500 Zeiss. Samples were prepared for SEM according to Basso (1995a). Morphological identification was based on Adey and McKibbin (1970) and Irvine and Chamberlain (1994). Other information about maerl species distribution in Morlaix was provided by Carro et al. (2014) and Melbourne et al. (2017). *L. coralliooides* was selected as the target species because of its presence in both Mediterranean and Atlantic waters. The Atlantic sample (Morlaix) was used as voucher specimen for the subsequent identification of the Mediterranean samples, since *Phymatolithon* spp. and *L. coralliooides* are the only components of the Morlaix maerl (Carro et al., 2014; Melbourne et al., 2017). Hence, once its inclusion under the genus *Phymatolithon* was excluded, the Morlaix sample identified as *L. coralliooides* was used as a reference for the most reliable identification of the other Mediterranean samples.

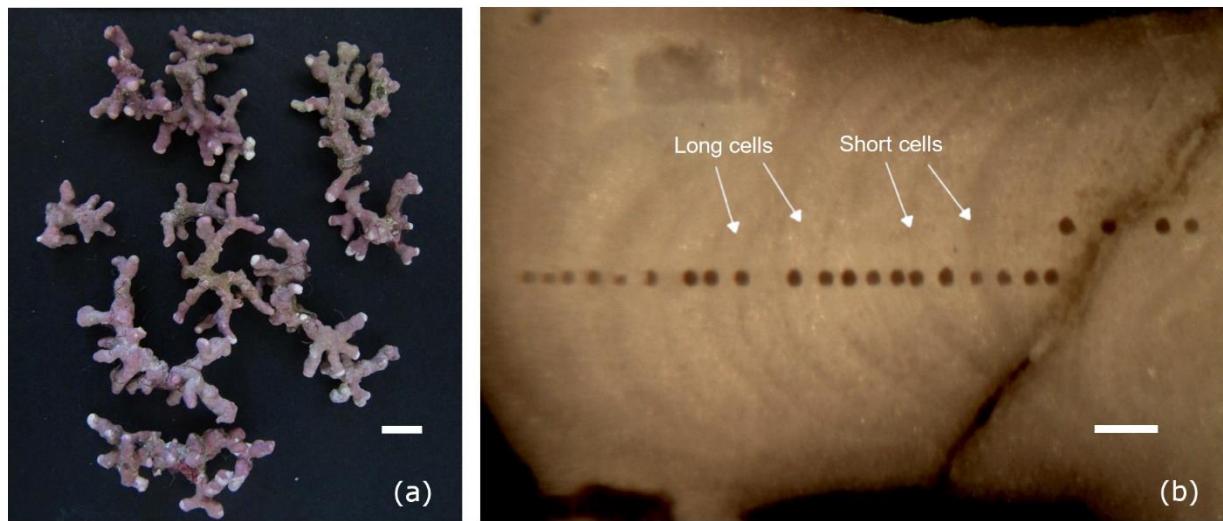
## 2.2 Sample preparation

The selected algal branches were embedded in Epo-Fix resin, which was stirred for 2 minutes with a hardener (13% w/w); they were then left to dry at room temperature for 24 hours. Afterwards, the treated branches were cut by an IsoMet diamond wafering blade 15HC along the direction of growth. In the laboratory of the Institute of Geosciences and Earth Resources (IGG) of the National Research Council (CNR) in Pavia (Italy), the sections were polished with a MetaServ Grinder-Polisher (400 RPM) using a diamond paste solution, finally cleaned ultrasonically in distilled water for 10 minutes and dried at 30°C for 24 hours.

## 2.3 Trace elements analyses and environmental data

LA-ICP-MS analyses were carried out at the IGG-CNR laboratory of Pavia (Italy), on one algal branch per sampling site.  $^{43}\text{Ca}$ , 145  $^7\text{Li}$ ,  $^{25}\text{Mg}$ ,  $^{88}\text{Sr}$  and  $^{11}\text{B}$  contents were measured using an Agilent ICP-QQQ 8900 quadrupole ICP-MS coupled to an Excimer laser ablation system (193 nm wavelength, MicroLas with GeoLas optics). Element/Ca ratios were calculated for these isotopes, as well as Mg/Li ratio. Measurements were performed with laser energy densities of 4 J/cm<sup>2</sup> and helium as carrier gas.

The laser transects crossed the algal growth direction with a spot size of 50  $\mu\text{m}$ , attempting to target each growth band change 150 which marked the transition between the cells usually produced in the warm season and those usually produced in the cold season, hereinafter referred to as long and short cells (Fig. 2, S1, S2, S3). NIST 612 was used as an external standard (e.g. Fietzke et al., 2010; Jochum et al., 2012), whereas Ca was adopted as an internal standard. Accuracy and precision were better than 4% for NIST 612 and 8% for Ca standard. Minimum detection limits (99% confidence) of measured elements were: Ca 155 = 16.91 ppm, Li = 0.07 ppm, Mg = 0.11 ppm, Sr = 0.004 ppm, B = 2.64 ppm. Each analysis was carried out in MS/MS mode for 3 minutes by acquiring 60 seconds of background before and after the sampling period by the laser on the polished surface. The first part of the signal was not used for the integration to avoid surface contamination. The Glitter software (v. 4.4.4) was used for data reduction.



160 **Figure 2: (a) Thalli of *Lithothamnion coralliooides* collected in Morlaix (scale bar = 5 mm). (b) Longitudinal section through the *L. coralliooides* branch sampled in Morlaix showing the LA-ICP-MS transects targeting each growth bands (scale bar = 200  $\mu\text{m}$ ).**

In the absence of in-situ environmental data, the seawater temperature has been extracted by 11 years of monthly reanalysis spanning 1979-2017 from ORAS5 (Ocean ReAnalysis System 5), at 0.25-degree horizontal resolution (Zuo et al., 2019). The time interval of extraction in each site corresponded to 11 years before sample collection. Minimum, maximum, and mean values, as reported in Table 1, refer to the temperature at sampling depth and have been measured on the entire time interval 165 of extraction.

For the purpose of this work, we considered temperature data in terms of a) the amplitude of temperature variation ( $\Delta T$ ), and b) the temperature maxima and minima.  $\Delta T$  represents the temperature fluctuations during the algal growth and has been measured as the difference between the maximum and minimum temperature registered in the site over 11 years. We used  $\Delta T$  when comparing the sampling sites, given their differences in depth and geographical regions. The temperature peaks 170 (maximum and minimum) have rather been used when considering data corresponding to long and short cells, since they are related to warm and cold periods of growth, respectively. We used the temperature peaks over the entire time interval of extraction (11 years) when comparing the mean elemental ratios of long and short cells per sampling sites. The maximum and minimum temperature within each year have been used instead for the reconstruction of the algal age model. In the sample from Morlaix Bay, indeed, the good visibility of the growth bands allowed us to relate the temperature record with the algal 175 growth at annual resolution. We therefore plotted all the element ratios against the average seawater temperature values of the coldest and warmest months of the year to reconstruct the temperature variations during the algal growth (Moberly, 1970; Corrège, 2006; Williams et al., 2014; Ragazzola et al., 2020; Caragnano et al., 2014). Missing element ratios possibly due to non-targeted consecutive bands were calculated as the means of known values.

Carbon system parameters for each site have also been estimated. Even if they were not available in the same time interval of 180 temperature data, the seasonal variations occurring in the extracted period have allowed the characterization of the sampling sites. Monthly mean seawater pH has been derived by the CMEMS (E.U. Copernicus Marine Service Information) global biogeochemical hindcast spanning 1999-2017, at 0.25-degree horizontal resolution. Monthly means of DIC in the time interval 1999-2017 have been extracted by CMEMS biogeochemical reanalysis for the Mediterranean Sea, at 0.042-degree horizontal resolution (Teruzzi et al., 2021). In the Atlantic site, monthly means of DIC in 1999-2017 were derived from CMEMS IBI 185 biogeochemical model, at 0.083-degree horizontal resolution. The pH and DIC data showed consistent variations among sites, despite being derived from different biogeochemical models. The mean values of DIC and pH, as reported in Table 1, refer to sampling depth and have been measured on the entire time interval of extraction. The complete timeline of temperature and carbon data used in this paper is shown in Supplement Fig. S5, S6, S7, S8.

## 2.4 Growth rate estimation

190 Growth rates were estimated under light microscope by measuring the length of the LA-ICP-MS transect and dividing it by the number of annual growth bands crossed by the transect. The obtained values have been expressed in linear extension over year (mm/yr). In the samples where the growth bands were not easily detectable under microscope, i.e. the Elba sample, we also used the Mg/Ca results in order to check for the correspondence of Mg peaks with growth bands.

## 2.5 Statistical analysis

195 Statistics were performed on both the dataset with all the spot analyses and the dataset with the records from long and short cells separately. Short cells were referred to slow-growing cells in dark bands, usually produced in the cold season; long cells corresponded to fast-growing cells in light bands, usually produced in the warm season (Kamenos et al., 2009; Ragazzola et

al., 2016). For each spot, a distinction between the cells was thus made by image analyses, except for the Elba sample, given the poor resolution of the growth bands. The Spearman's correlation was tested to provide the statistical comparisons between 200 Mg/Ca, Li/Ca, Sr/Ca and B/Ca records from the LA-ICP-MS analyses in *L. coralliooides*. The Kruskal-Wallis test followed by the Dunn's test for comparisons and the One-way ANOVA followed by the Tukey's test for post-hoc analysis were used to compare the geochemical signals among sampling sites and to evidence the differences between group medians and means. All statistical analyses were run in R 3.6.3 software.

### 3 Results

#### 205 3.1 Environmental data

The temperature data obtained by ORAS5 reanalysis revealed a lower amplitude of the seasonal temperature change in the Mediterranean samples with respect to the Atlantic site, as shown by the standard deviation and  $\Delta T$  values in Table 1. This difference is explained in terms of the different sampling depths, with the seasonal variations decreasing with increasing depth. Temperature variations in Morlaix Bay (Atlantic Ocean) were higher, registering overall a mean seawater temperature of 12.4 210  $^{\circ}\text{C}$  (Table 1). Among Mediterranean samples, mean seawater temperatures were highest in the Aegadian Isl., followed by the Elba and the Pontian Isl. (Table 1). Aegadian Isl. also registered the highest temperature variations among the Mediterranean sites (Table 1). Moderate temperature variations characterized the site in Elba, which registered the lowest monthly mean temperature among Mediterranean sites (Table 1). At the Pontian Isl., consistently with the fact that it is the deepest sampling site at 66 m depth, the lowest seawater temperature variations were found (Table 1).

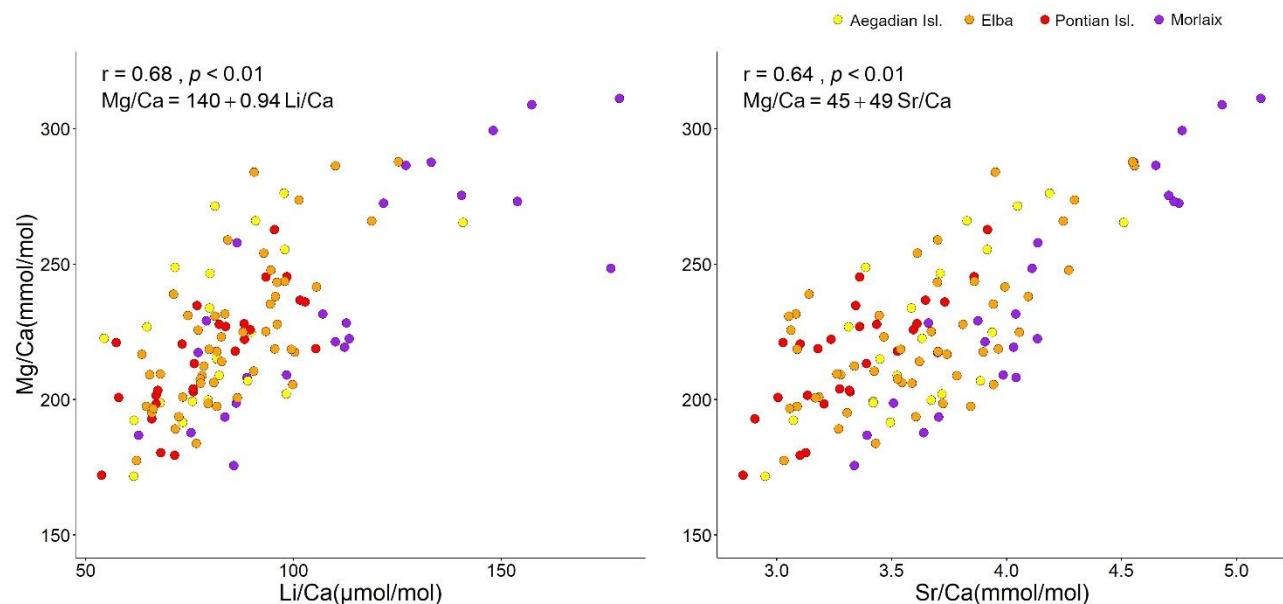
215 The pH estimates at the Mediterranean sites were all similarly high ~8.13 and less variable than the Atlantic site (8.05). The mean pH had slightly higher values in Pontian Isl. and Elba, than Aegadian Isl. (Table 1). Similarly, DIC was higher in the Mediterranean sites and decreased in Morlaix, as this is largely dictating the pH (Table 1).

220 **Table 1: Environmental data in each sampling site. The minimum and maximum monthly means of temperature are indicated, as well as the highest temperature variation ( $\Delta T$ ), the mean and the standard deviation of the time series. Data from monthly means extracted by 11 years of ORAS5 reanalysis. pH and DIC in each sampling site are also indicated. The minimum, maximum, mean, and standard deviation values have been measured on the time interval 1999-2017. Carbonate system parameters have been extracted from monthly means biogeochemical data provided by CMEMS.**

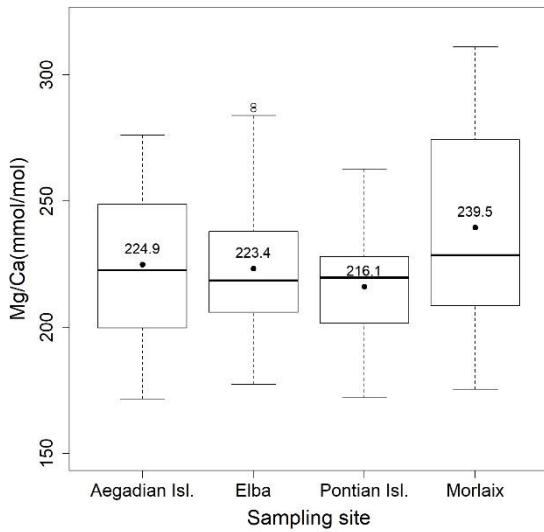
Sampling site	Depth (m)	Collection date	Temperature ( $^{\circ}\text{C}$ )				pH		DIC ( $\mu\text{mol/kg}$ )		Growth rates (mm/yr)
			min	max	$\Delta T$	mean	st. dev.	mean	st. dev.	mean	
Aegadian Isl.	40	25/08/1993	13.7	18.8	5.1	15.9	1.3	8.11	0.02	2303.72	11.58
Elba	45	01/12/1990	12.9	17.5	4.6	15.0	1.2	8.13	0.02	2322.24	7.08
Pontian Isl.	66	14/07/2017	13.9	16.7	2.8	15.0	0.6	8.13	0.01	2321.29	6.71
Morlaix	12	02/05/1991	8.3	17.2	8.9	12.4	2.5	8.05	0.04	2155.60	15.27

### 3.2 Mg/Ca, Li/Ca, Sr/Ca and Mg/Li

Both Li/Ca and Sr/Ca records had positive correlations with Mg/Ca in our samples of *L. coralliooides* (Fig. 3 and Supplement 225 Fig. S4). The overall mean Mg/Ca was  $225.3 \pm 30.4$  mmol/mol, registering the minimum value in the sample from Aegadian Isl. (171.7 mmol/mol) and the maximum value in Morlaix (311.2 mmol/mol) (Fig. 4; Table 2). The Kruskal-Wallis test did not show significant differences in Mg/Ca among samples (Table A1; Fig. 4). Among Mediterranean sites, the algal sample 230 from Aegadian Isl. had the highest Mg/Ca mean value, followed by Elba and Pontian Isl., which had the lowest Mg/Ca mean value of all sampling sites (Fig. 4). The highest mean Mg/Ca was registered in the sample from Morlaix bay, which also showed a large dispersion of data above the median Mg/Ca value (Fig. 4).



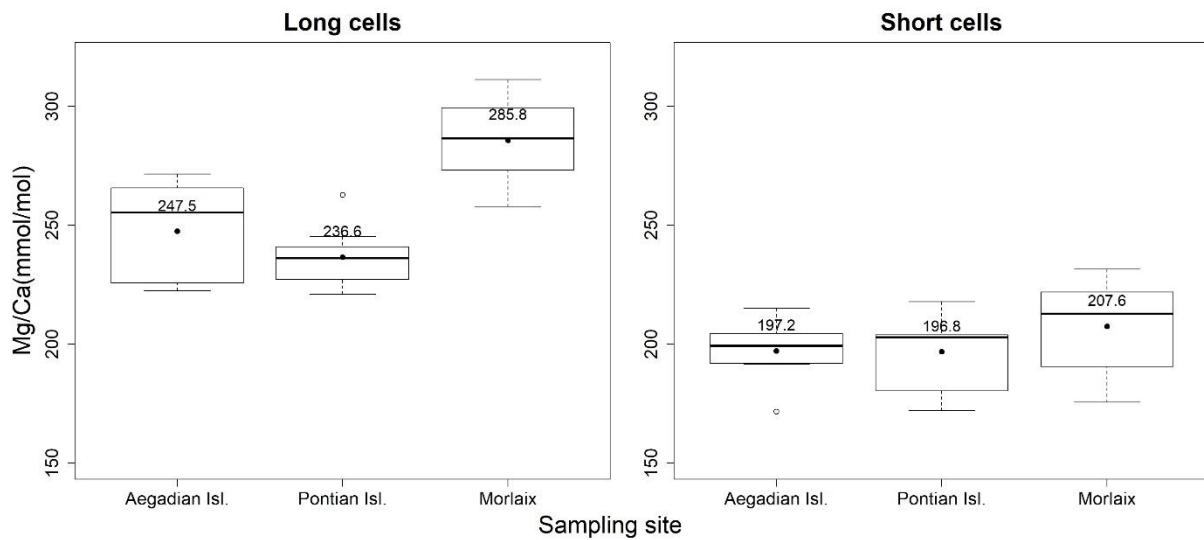
**Figure 3: Correlation plots of Mg/Ca with Li/Ca and Sr/Ca. For each analyses the Spearman's coefficient  $r$ , the  $p$ -value and the line equation are given.**



235 **Figure 4: Box plot of the statistical tests performed to evaluate the differences of Mg/Ca in *L. coralliooides* collected in different sampling sites. The horizontal black lines indicate the median values. The black filled circles and the numbers inside the plot indicate the mean values.**

Long cells had high Mg/Ca values; conversely, short cells corresponded to areas with low Mg/Ca ratio.

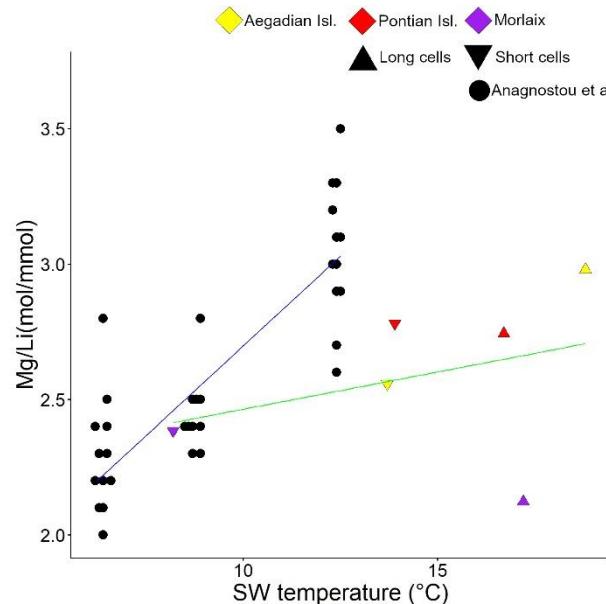
240 The ANOVA test followed by the Tukey's test for multiple comparisons evidenced a significant variability of algal Mg/Ca among 3 sites in long cells (Table A2; Fig. 5). In the long cells of *L. coralliooides* collected from Aegadian Isl. and Pontian Isl., the Mg/Ca data showed quite similar distribution (Table A2; Fig. 5). The Mg/Ca of the alga from Pontian Isl. was the lowest (Fig. 5). In Morlaix a higher Mg/Ca mean value was registered, significantly different compared to Aegadian and Pontian Isl. (Table A2; Fig. 5). In short cells, the differences in Mg/Ca among samples were not statistically significant (Table A1). The magnesium incorporation was slightly higher in Morlaix and very similar between Aegadian and Pontian Isl. samples (Fig. 5).



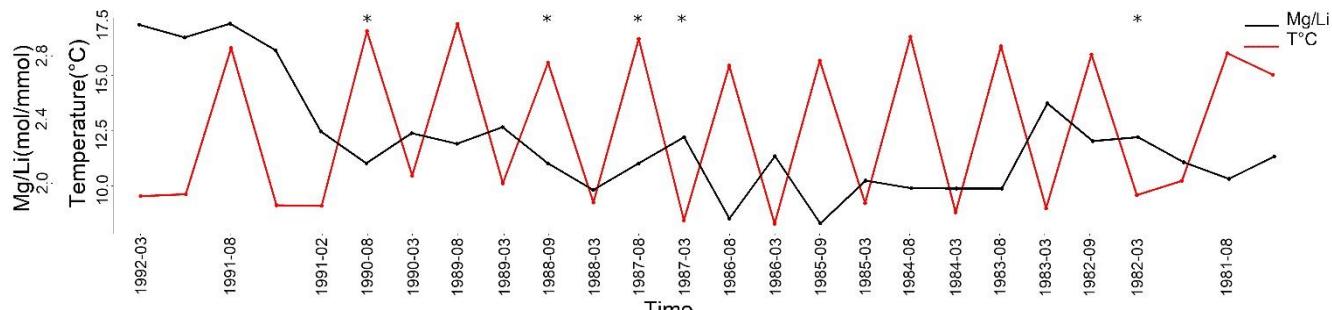
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**Figure 5:** Box plots of the statistical tests performed to evaluate the differences of Mg/Ca in the long and short cells of *L. corallioides* collected in different sampling sites. The horizontal black lines indicate the median values. The black filled circles and the numbers inside the plot indicate the mean values.

Mg/Li values in long and short cells fell in the range found by Anagnostou et al. (2019) for cultured *Clathromorphum compactum* (Fig. 8). When plotted against the extracted seawater temperature in Morlaix (Fig. 9), Mg/Li results did not reflect the seasonal oscillations in temperature.



**Figure 6:** Correlation plot between Mg/Li and seawater temperature. Data are shown for cultured *C. compactum* (Anagnostou et al., 2019) and *L. corallioides* (this paper). *L. corallioides* results are shown separately in long and short cells, per sampling site.

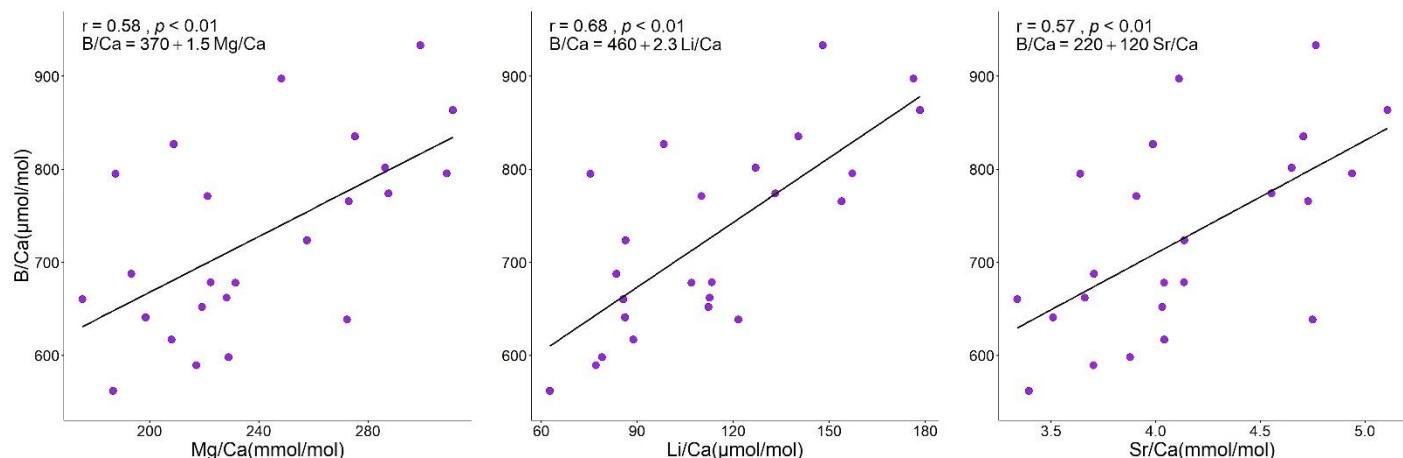


255 **Figure 7: Mg/Li ratio of *L. coralliooides* collected in Morlaix Bay. Note the lack of cyclic variations in Mg/Li results. In the timeline, the coldest and the warmest months have been reported. Mg/Li in the missing bands (asterisks) have been calculated as the means of the values measured in warm and cold periods. Monthly means of seawater temperature have been extracted by ORASS reanalysis.**

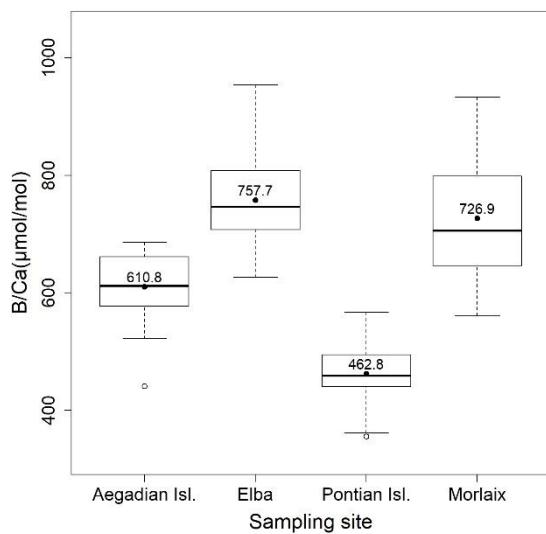
260 **3.3 B/Ca**

The B/Ca ratio in the sample collected from Morlaix showed a moderate positive correlation with all the examined temperature proxies (Mg/Ca, Li/Ca, Sr/Ca), with a more defined trend when plotted against Li/Ca ( $r=0.68$ ) and slightly less defined against Mg/Ca ( $r=0.58$ ) and Sr/Ca ( $r=0.57$ ) (Fig. 8). On the contrary, the Spearman's analyses did not evidence significant correlations between B/Ca and the temperature signals in the algae collected elsewhere ( $p>0.05$ ).

265 Overall, the B/Ca ratio in *L. coralliooides* was  $661.9 \pm 138.9 \mu\text{mol/mol}$ , registering the minimum value in the long cells of the sample from Pontian Isl. ( $356.0 \mu\text{mol/mol}$ ) and the maximum value in Elba ( $954.1 \mu\text{mol/mol}$ ) (Fig. 9; Table 2). The Kruskal-Wallis coefficient showed a highly significant difference in the B/Ca value among sites, particularly in the *L. coralliooides* from the Pontian Isl., which had the lowest boron incorporation (Table A3; Fig. 9). The algae collected in Aegadian Isl. had still significantly lower B/Ca compared to those collected in Elba and Morlaix (Table A3; Fig. 9). The highest B/Ca 270 mean value was registered in Elba, with medians comparable to Morlaix (Table A3; Fig. 9).



**Figure 8: Correlation plots of B/Ca with Mg/Ca, Li/Ca and Sr/Ca in *L. coralliooides* collected in Morlaix bay. For each analyses the Spearman's coefficient r, the p-value and the line equation are given.**

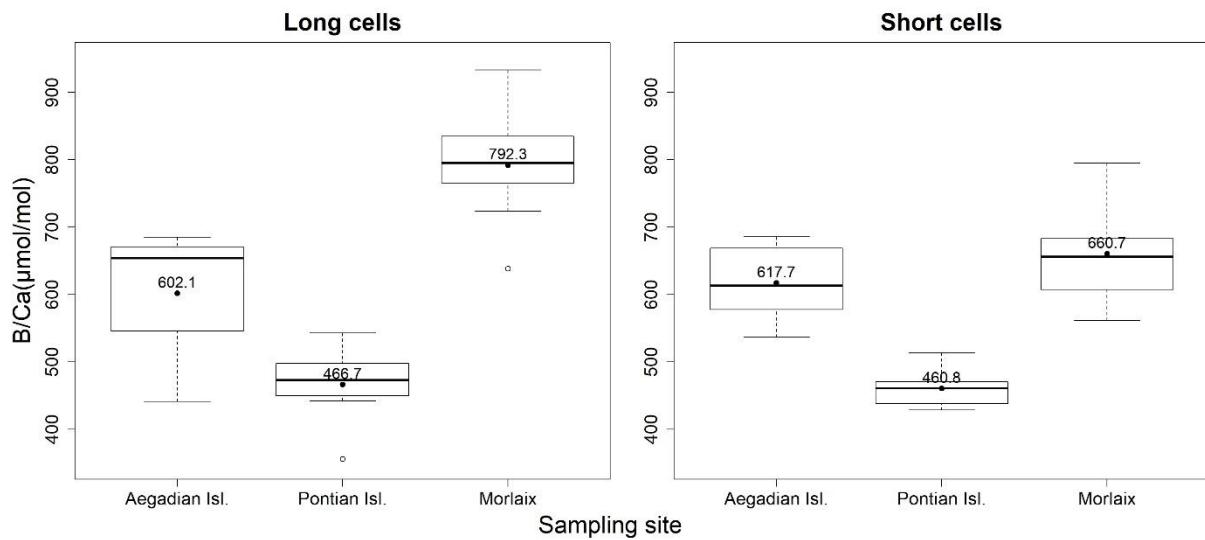


275 **Figure 9: Box plot of the statistical tests performed to evaluate the differences of B/Ca in *L. coralliooides* collected in different sampling sites. The horizontal black lines indicate the median values. The black filled circles and the numbers inside the plot indicate the mean values.**

The ANOVA test followed by the Tukey's test for multiple comparisons by site, for long (Table A4) and short cells (Table A5) separately, showed lower values in the Mediterranean sites and higher values in the Atlantic site (Fig. 10).

280 The algal cells from Pontian Isl. had the lowest mean B/Ca in both seasons, being significantly different from both the samples from Morlaix and Aegadian Isl. (Table A4, A5; Fig. 10). Morlaix had the highest mean B/Ca in both long and short cells (Table A4, A5; Fig. 10). *L. coralliooides* from Aegadian Isl. had intermediate B/Ca mean value in long cells, differing significantly from both the Morlaix and Pontian Isl. samples (Table A4; Fig. 10). In short cells, the sample from Aegadian Isl. slightly differed from the one in Morlaix (Table A5; Fig. 10).

285 Interestingly, the long cells of all samples had higher median B/Ca values compared to short cells (Fig. 10), although only in Morlaix, the differences between B/Ca measured in long and short cells were statistically significant ( $\chi^2=8.4899$ ,  $p<0.01$ ).



**Figure 10:** Box plots of the statistical tests performed to evaluate the differences of B/Ca in the long and short cells of *L. coralliooides* collected in different sampling sites. The horizontal black lines indicate the median values. The black filled circles and the numbers inside the plot indicate the mean values.

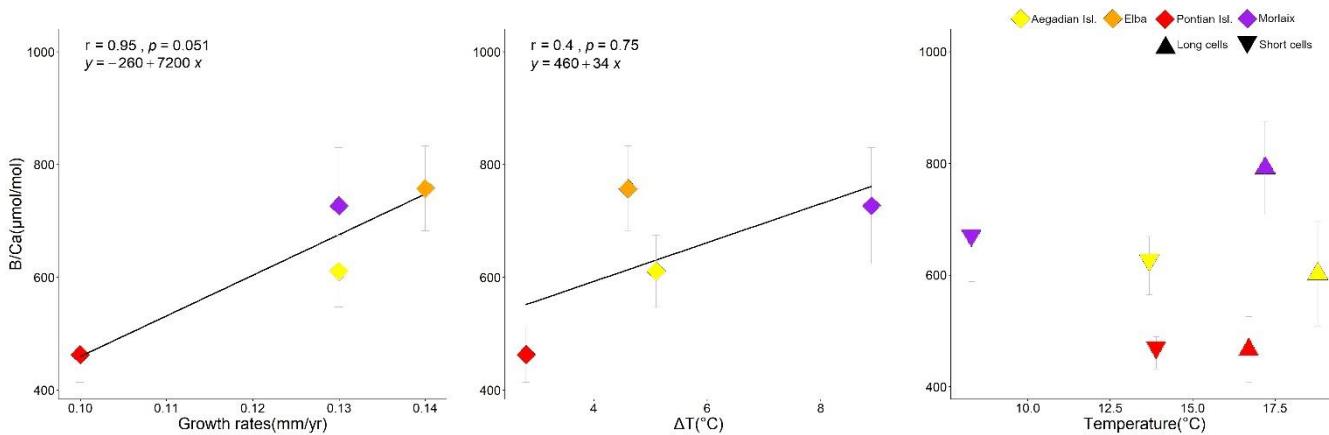
**Table 2:** Element/Ca ratio measurements in *L. coralliooides*.

Sampling site	B/Ca (μmol/mol)		Mg/Ca (mmol/mol)		Li/Ca (μmol/mol)		Sr/Ca (mmol/mol)		Mg/Li (mol/mmol)	
	mean	st. dev.	mean	st. dev.	mean	st. dev.	mean	st. dev.	mean	st. dev.
Aegadian Isl.	610.8	63.9	224.9	30.3	81.9	18.4	3.7	0.4	2.8	0.5
Elba	757.7	75.5	223.4	26.4	85.2	14.3	3.6	0.4	2.7	0.3
Pontian Isl.	462.8	49.2	216.1	21.9	79.6	14.6	3.3	0.3	2.8	0.4
Morlaix	726.9	102.8	239.5	41.2	113.1	32.9	4.1	0.5	2.2	0.4
total	<b>661.9</b>	<b>138.9</b>	<b>225.3</b>	<b>30.4</b>	<b>89.0</b>	<b>23.3</b>	<b>3.7</b>	<b>0.5</b>	<b>2.6</b>	<b>0.4</b>

### 3.4 Growth rates

In the sample from Aegadian Isl., the LA-ICP-MS transect was 1.31 mm long and 10 years of growth have been detected by coupling microscopical imaging and Mg/Ca peaks, resulting in 0.13 mm/yr of growth rate. In the Elba sample the laser transect was 1.15 mm long, crossing 8 years of growth, with a resulting growth rate of 0.14 mm/yr. The Pontian Isl. sample had 1.08 mm of transect including 11 years of growth, hence a growth rate of 0.10 mm/yr. Finally, the transect from Morlaix sample was 1.38 mm long, counting 11 years and resulting in 0.13 mm/yr of growth rate.

Growth rates did not show any linear relationship with Mg, Li and Sr/Ca, but they were positively correlated with the samples mean B/Ca values (Fig. 11).



**Figure 11: Correlation plots of growth rates and seawater temperature with B/Ca in *L. coralliooides* samples analysed in this study. Spearman's coefficient  $r$ , the  $p$ -value and the line equation are given. Temperature variations ( $\Delta T$ ) correspond to the differences between the maximum and minimum temperature registered over 11 years of monthly reanalysis (ORASS5). The B/Ca means measured in long and short cells correspond respectively to the maximum and minimum temperature.**

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#### 4 Discussion

In general, temperature variations affect many physiological processes involved in the biomineralization, and the rate of calcification, along with the preservation state of mineral structures, influences the content of trace elements in carbonates (Lorens, 1981; Rimstidt et al., 1998; Gussone et al., 2005; Noireaux et al., 2015; Kaczmarek et al., 2016). Trace elements concentrations recorded from the four *L. coralliooides* branches analysed in this study were consistent with previously published values for other calcareous red algae (Chave, 1954; Hemming and Hanson, 1992; Hetzinger et al., 2011; Darrenougue et al., 2014). Particularly, the Mg/Ca ratios recorded in this study ranged from 172 to 311 mmol/mol, comparable to previous studies on rhodoliths of *Lithothamnion glaciale* Kjellman 1883 grown at 6-15 °C (148-326 mmol/mol) (Kamenos et al., 2008). The B/Ca ratios in *L. coralliooides* from our results range from 356 to 954 μmol/mol, wider than the range measured by solution ICP-MS on bulk samples of *Neogoniolithon* sp. (352-670 μmol/mol) (Donald et al., 2017) and *C. compactum* (320-430 μmol/mol) (Anagnostou et al., 2019), both cultured with controlled  $p\text{CO}_2$  and a pH ranging from 7.2 to 8.2. The high resolution given by laser ablation should be more effective in measuring the heterogeneity of B/Ca across the thallus, thus explaining the wider range of our data.

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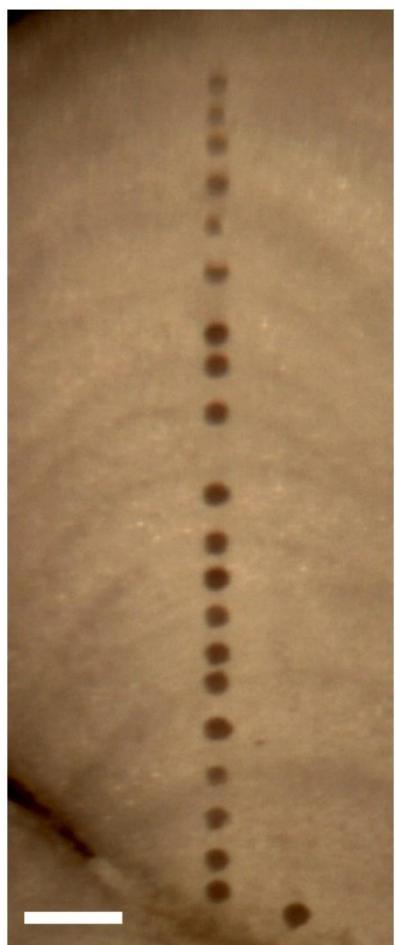
~~In the sample from Elba the growth bands were not clearly visible, preventing the analyses of trace elements in long and short cells separately (Fig. S1, S2, S3). To measure the growth rate in this sample, we considered the results from the spots where the light/dark bands resulting from the image analyses corresponded to obvious Mg/Ca peaks.~~

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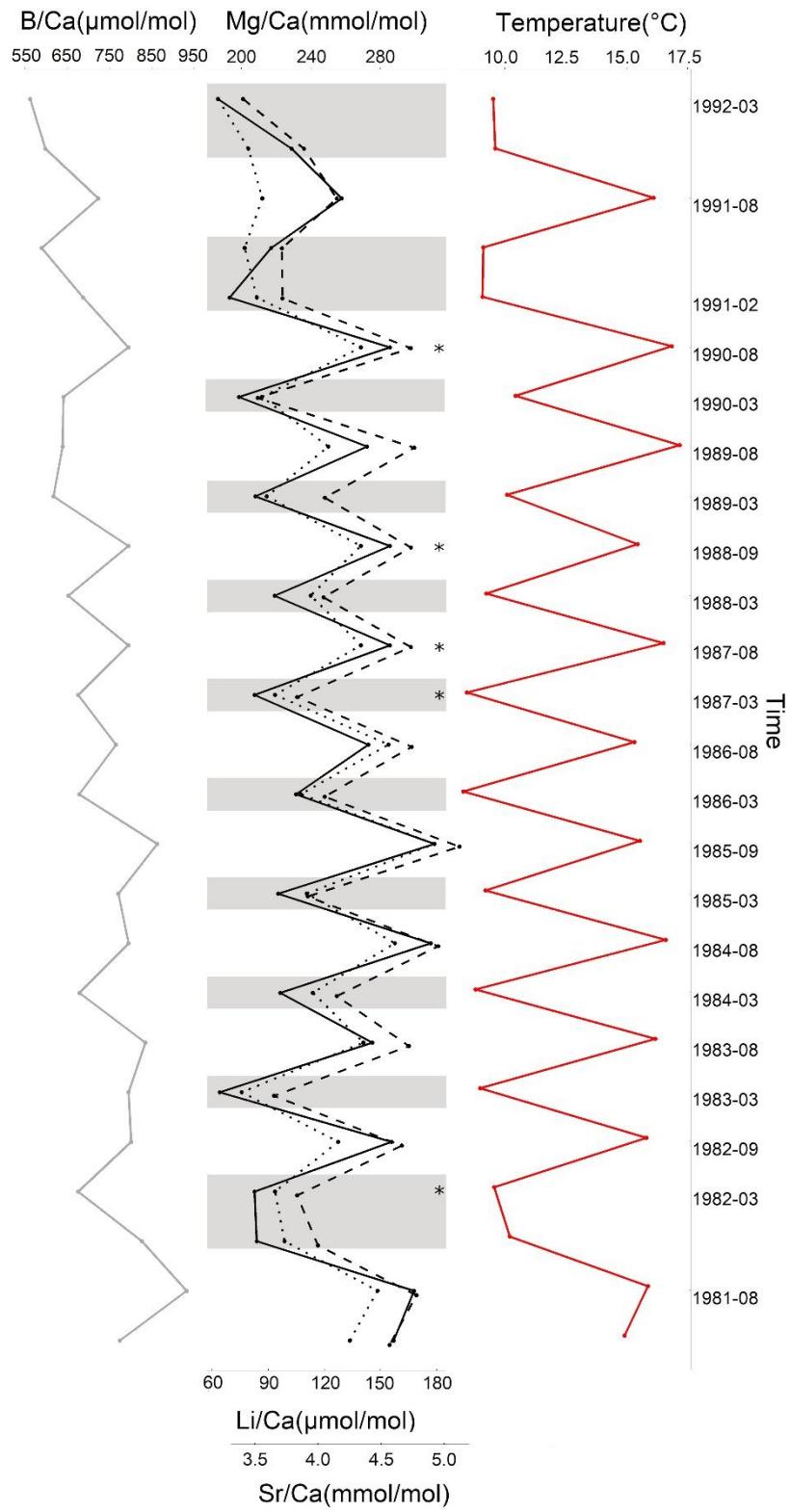
The Mg/Ca results evidenced a strong relationship with the seawater temperatures extracted from ORASS5 (Table 1; Fig. 12), as expected. *L. coralliooides* from Aegadian Isl. had slightly higher Mg/Ca values, followed by Elba and Pontian Isl. (Fig. 4). This was consistent with local temperature values in the Mediterranean (Table 1), since Pontian Isl. registered the lowest mean value and the lowest  $\Delta T$ , while Aegadian Isl. showed the highest mean temperature and  $\Delta T$ . On the contrary, the sample from

Morlaix, collected at 12 m depth, showed high Mg/Ca values in both long and short cells (Table A2; Fig. 5). The monthly mean temperatures had the highest variations during the year ( $\Delta T$  in Table 1), due to the shallow~~er~~ depth (12 m) and the geographical location. Temperature correlates to seasons, influencing primary production, respiration, and calcification in *L. coralliooides* (Payri, 2000; Martin et al., 2006) as well as other calcareous red algae (Roberts et al., 2002). The high seasonality that characterized the sample from Morlaix, represented by the high  $\Delta T$  (Table 1), was probably responsible for the highest variation of Mg/Ca values and undoubtedly accounted for most of the differences with Mediterranean samples. For the first time, we confirmed here the reliability of the temperature proxies Li/Ca and Sr/Ca on a wild-grown coralline alga collected at different depths and locations. Li/Ca and Sr/Ca records were positively correlated with Mg/Ca in *L. coralliooides* (Fig. 3), which, in turn, showed a strong relationship with seawater temperature. Moreover, both Li and Sr/Ca showed periodical oscillations in correspondence to long and short cells, related to seasonal temperature variations (Fig. 12). Therefore, Li/Ca and Sr/Ca could be regarded as temperature proxy in *L. coralliooides*, as for Mg/Ca. The coupling of the Mg/Ca ratio with Li/Ca and Sr/Ca~~represents a multi proxy approach that minimize the possible species effect and~~ can be considered a useful tool to gather information about past temperature for paleoclimate reconstructions (Halfar et al., 2011; Caragnano et al., 2014; Williams et al., 2014; Fowell et al., 2016; Cuny-Guirrieuc et al., 2019).

The B/Ca ratio in coralline algae has been rarely measured and it is not clear how the environmental factors control its incorporation. The carbonate system primarily drives the changes in B incorporation (Hemming and Hanson, 1992; Yu and Elderfield, 2007). In benthic foraminifera, B/Ca increases with  $[CO_3^{2-}]$  (Yu and Elderfield, 2007), whereas there is no consensus on the effect of  $[CO_3^{2-}]$  on Mg/Ca and Sr/Ca (Rosenthal et al., 2006; Dueñas-Bohórquez et al., 2011). Experiments with inorganic calcite showed a positive correlation between B/Ca and [DIC] (Uchikawa et al., 2015). Nevertheless, in culture experiments of the coralline algae *Neogoniolithon* sp. (Donald et al., 2017) and corals (Gagnon et al., 2021), [DIC] had a negative effect on B/Ca. DIC and B/Ca values showed a negative relationship in the samples from Morlaix, Aegadian Isl. and Pontian Isl., not observed in Elba (Fig. 9; Table 1). Significant differences among B/Ca values in the Mediterranean samples were not expected, since DIC concentrations were similar (Table 1). This evidence suggests influences others than DIC on the B signal. The occurrence of low DIC concentrations in Morlaix (Table 1), compared to the Mediterranean sites, could therefore contribute to the high B/Ca. Nevertheless, based on the extracted pH and DIC data, significantly higher values of B/Ca should be expected in the sample from Pontian Isl., similarly to the other Mediterranean sites (Table 1).



— B/Ca  
 — Mg/Ca  
 .... Li/Ca  
 - - - Sr/Ca  
 — T°C



355 **Figure 12: Elemental ratios in *L. corallioides* collected in Morlaix Bay (scale bar = 200  $\mu$ m). Mg, Li and Sr/Ca show cyclic variations mirroring the local seawater temperature. In the timeline, the coldest and the warmest months have been reported, which correspond to dark and light bands of growth. Elements/Ca in the missing bands (asterisks) have been calculated as the means of the values measured in warm or cold periods. Monthly means of temperature have been extracted by ORAS5 reanalysis.**

The mean estimated growth rate of *L. corallioides* was  $0.13 \pm 0.02$  mm/yr and it was supposed to decrease with increasing depth as a direct consequence of lower light availability (Halfar et al., 2011); indeed, the growth rate of the sample from Pontian Isl. was the lowest (0.10 mm/yr). As already suggested by previous studies on both synthetic and biogenic calcite, B incorporation is likely affected by growth rate (Gabitov et al., 2014; Mavromatis et al., 2015; Noireaux et al. 2015; Uchikawa et al., 2015; Kaczmarek et al., 2016). Indeed, in the cultured calcareous red alga *Neogoniolithon* sp. the B/Ca increases with increasing growth rate (Donald et al., 2017). In Pontian Isl., The slowest growth rate found in Pontian Isl. possibly could have contributed to the lowest B/Ca values; similarly, the highest growth rate (0.14 mm/yr) in the sample from Elba, could be responsible for the highest B/Ca (Fig. 9, 11). The mean annual growth rate of the shallowest sample (Morlaix) was equal to the one in Aegadian Isl., at 40 m depth intermediate (0.13 mm/yr), and likely not constant during the year. In Morlaix, the alga probably significantly slowed down the growth in cold months, when the monthly mean seawater temperature was the lowest of all the sampling sites (Table 1). Nevertheless, its growth rate likely speeded up in the warm season due to the abundant light availability at shallow depth and the warming of seawater (Table 1), contributing to the significantly higher B/Ca values in long cells (Fig. 10). According to this interpretation, the effect of the growth rate on B/Ca might be significant across depth and geographical regions (Fig. 11).

In Morlaix, B/Ca showed higher values in comparison with Aegadian Isl. and Pontian Isl., and a weak positive correlation with temperature proxies (Mg/Ca, Li/Ca and Sr/Ca; Fig. 8). A positive correlation between B/Ca and Mg/Ca was already observed in planktonic foraminifera (Wara et al., 2003; Yu et al., 2007). Therefore, we reconstructed the elemental variations during the algal growth in the Morlaix sample at annual resolution, in order to highlight the influence of temperature (Fig. 12). Contrary to Mg, Li, and Sr/Ca, the B/Ca did not mirror the seasonal temperature variations accurately as the other proxies.

In the sample from the Pontian Isl., the seasonal  $\Delta T$ , Mg/Ca and B/Ca values were the lowest among sites. In particular, B/Ca was significantly low (Fig. 9), differing more from the other samples than the results on Mg/Ca (Fig. 4). This suggests that in this sample the B incorporation should be influenced by factors other than those affecting Mg. In general, the poor correlation with seawater temperature (Fig. 11), and most of all the lack of distinct seasonal oscillations in B/Ca across the algal thallus (Fig. 12), excludes the suitability of B/Ca as a temperature proxy and suggests a closer relationship with growth rate rather than temperature.

Knowing the biogeochemistry and the variation of the environmental variablesparameters of seawater is crucial for a more comprehensive picture of the reliability of geochemical proxies, like the ones we investigated in this paperhere (Mg, Li, Sr/Ca and B/Ca). Boron incorporation in marine carbonates is still debated, rising questions about the boron isotopic fractionation, the mechanisms of boron incorporation into marine carbonates, the seawater isotopic composition, and the so-called “vital effects” (i.e. the metabolic activities that can bias the proxy recordisotopic signal), and the seawater isotopic composition. Vital effects include species-specific biologically controlled up-regulations of pH in the calcifying fluid in both corals and coralline

algae, in response to pH manipulations mimicking the ongoing ocean acidification (Cornwall et al., 2017, 2018). Natural marine pH undergoes pH fluctuations with characteristic ranges of about 0.3 pH units in very shallow coastal water, in areas with restricted circulation, or in shallow near-shore communities supporting high rates of benthic metabolism, such as seagrass beds (Cornwall et al., 2013; Duarte et al., 2013; Hofmann et al., 2011). However, this study analysed the trace element record in a single high Mg-calcite species grown in a natural wide bathymetric interval (from 10 to 66 m depth), characterized by normal marine pH (range 8.05 – 8.13, Table 1). Therefore, we considered up-regulation, if present, to be constant among our conspecific specimens, thus irrelevant for the measured B/Ca variations. Moreover, no significant yearly pH fluctuations is expected at each site. Thus, within a single specimen, the observed differences in B/Ca between short and long cells (i.e. cold and warm periods of growth) (Fig. 10) are unlikely to be related to changes in  $pH_{cf}$ .

~~T~~Moreover, the paucity of B/Ca measurements from coralline algae and, most of all, the complete absence of these data on specimens grown in nature makes it difficult to compare our B/Ca data with the literature. This observation takes stock of the significance of our results and emphasizes the importance of collecting more representative B/Ca data in coralline algae.

Further studies on *L. coralliooides* and other calcareous red algae should be carried out to clarify the environmental factors influencing the B/Ca in these organisms, and to ensure the reliability of this proxy for paleoclimate reconstructions.

## 5 Conclusion

This paper presents the first measures of trace elements (Mg, Sr, Li and B) from the coralline alga *L. coralliooides* collected across the Mediterranean Sea and in the Atlantic Ocean, at different oceanographic settings and depths (12 m, 40 m, 45 m, and 66 m depth), providing the first geochemical data of a wild-grown coralline algal species, sampled at different depths and geographical locations. LA-ICP-MS records of Mg/Ca, Sr/Ca and Li/Ca have shown a similar trend, primarily controlled by seawater temperatures in the algal habitat. On the contrary, Mg/Li did not provide a valuable temperature proxy in this species. In order to evaluate the control exerted by temperature over B incorporation, we also tested the correlation between B/Ca with Mg/Ca, Li/Ca and Sr/Ca. This led us to provide the first B/Ca data on wild grown coralline algae from across the photic zone in different Basins. The correlation between B/Ca and Mg/Ca in *L. coralliooides* was statistically significant only in the shallow Atlantic waters of Morlaix, where seasonality, hence the seasonal temperature variations, during the algal growth was the strongest among sites. Accordingly, B incorporation differences between long and short cells of *L. coralliooides* strongly depend on seasonality, being statistically significant just in Morlaix. Nevertheless, in contrast to Mg, Li and Sr/Ca, B/Ca oscillations across the algal growth showed a poor relationship with seasonal variations in seawater temperature. We found high B/Ca values in the Atlantic sample, where pH and DIC were the lowest. Carbon data did not explain the low B concentration in the Pontian Isl. sample (66 m depth), though, where pH and DIC were similar to the other Mediterranean sites. The estimation of growth rate, that was low in the Pontian Isl. sample (0.104 mm/yr) and got higher in the other Mediterranean samples and in Morlaix (~0.13 mm/yr), led us to conclude that B/Ca relates to growth rate rather than seawater temperature. B incorporation

is therefore subject to the specific algal growth patterns and rates, whose knowledge is essential in order to assess the reliability

420 of B/Ca in tracing seawater carbon variations.

## Appendix A

**Table A1:** (a) Statistically non-significant results of tests performed to evaluate (a) the differences of Mg/Ca in *L. coralliooides* and (b) the differences of Mg/Ca in the short cells of *L. coralliooides* collected in different sampling sites. Test significance at  $\alpha = 0.05$ .

(a) Kruskal-Wallis test (Mg/Ca)			
	Df	$\chi^2$	P
SITE	3	3.799	0.284

(b) One-way ANOVA test (Mg/Ca)					
Short cells					
	Df	Sum sq.	Mean sq.	F value	Pr(>F)
SITE	2	788.1	394.0	1.4647	0.2496
Residuals	26	6994.5	269.0		
Shapiro-Wilk normality test				P=0.6442	
Bartlett's K-squared				P=0.5856	

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**Table A2:** Results of statistical tests performed to evaluate the differences of Mg/Ca in the long cells of *L. coralliooides* collected in different sampling sites. Statistically significant p-values are given in bold. ANOVA test significance at  $\alpha = 0.05$ ; Tukey's test significant at  $p \leq \alpha$ .

One-way ANOVA test (Mg/Ca)					
Long cells					
	Df	Sum sq.	Mean sq.	F value	Pr(>F)
SITE	2	10897.7	5448.9	16.413	<b>0.0001</b>
Residuals	20	6639.8	332.0		
Shapiro-Wilk normality test				P=0.1440	
Bartlett's K-squared				P=0.5826	
Tukey's test					
Multiple comparisons of means					
SITE	Mean difference		95% confidence interval		P. adjusted
	SITE	lower bound	upper bound		
Morlaix-Aegadian Isl.	38.32918	15.09816	61.56019	<b>0.00130</b>	
Pontian Isl.-Aegadian Isl.	-10.84361	-35.48382	13.79661	0.51716	
Pontian Isl.-Morlaix	-49.17278	-72.40380	-25.94177	<b>0.00009</b>	

**Table A3: Results of statistical tests performed to evaluate the differences of B/Ca in *L. corallioides* collected in different sampling sites. Statistically significant p-values are given in bold. Kruskal-Wallis test significance at  $\alpha = 0.05$ ; Dunn's test significant at  $p \leq \alpha/2$ .**

Kruskal-Wallis test (B/Ca)			
	Df	$\chi^2$	P
SITE	3	79.816	<b>&lt;2.2e-16</b>
Dunn's test			
Comparisons by SITE (Bonferroni)			
Z P. adjusted	Aegadian Isl.	Elba	Morlaix
Elba	-4.64580 <b>0.00000</b>		
Morlaix	-3.07755 <b>0.00630</b>	1.17249 0.72300	
Pontian Isl.	2.80564 <b>0.01510</b>	8.38673 <b>0.00000</b>	6.15663 <b>0.00000</b>

**Table A4: Results of statistical tests performed to evaluate the differences of B/Ca in the long cells of *L. corallioides* collected in different sampling sites. Statistically significant p-values are given in bold. ANOVA test significance at  $\alpha = 0.05$ ; Tukey's test significant at  $p \leq \alpha$ .**

One-way ANOVA test (B/Ca)							
Long cells							
	Df	Sum sq.	Mean sq.	F value	Pr(>F)		
SITE	2	428364	214182	33.066	<b>0.0000</b>		
Residuals	20	129546	6477				
Shapiro-Wilk normality test				P=0.5527			
Bartlett's K-squared				P=0.5470			
Tukey's test							
Multiple comparisons of means							
SITE	Mean difference SITE	95% confidence interval lower bound	upper bound	P. adjusted			
Morlaix-Aegadian Isl.	190.11730	87.50374	292.73094	<b>0.00040</b>			
Pontian Isl.-Aegadian Isl.	-135.42490	-244.26303	-26.58672	<b>0.01342</b>			
Pontian Isl.-Morlaix	-325.54220	-428.15581	-222.92862	<b>0.00000</b>			

**Table A5: Results of statistical tests performed to evaluate the differences of B/Ca in the short cells of *L. corallioides* collected in different sampling sites. Statistically significant p-values are given in bold. ANOVA test significance at  $\alpha = 0.05$ ; Tukey's test significant at  $p \leq \alpha$ .**

One-way ANOVA test (B/Ca)			
Short cells			

	Df	Sum sq.	Mean sq.	F value	Pr(>F)
SITE	2	216232	108116	35.360	<b>0.0000</b>
Residuals	26	79497	3058		
Shapiro-Wilk normality test					P=0.1699
Bartlett's K-squared					P=0.0576
<b>Tukey's test</b>					
Multiple comparisons of means					
SITE	Mean difference SITE	95% confidence interval lower bound	upper bound	P. adjusted	
Morlaix-Aegadian Isl.	43.09640	-19.61932	105.81212	0.22146	
Pontian Isl.-Aegadian Isl.	-156.90170	-223.66771	-90.13574	<b>0.00001</b>	
Pontian Isl.-Morlaix	-199.99810	-260.58727	-139.40898	<b>0.00000</b>	

#### 440 Data availability

Data resulting from this study are available at <https://doi.org/10.1594/PANGAEA.932201>.

Environmental data were provided by E.U. Copernicus Marine Service Information.

DIC data in the Mediterranean: [https://doi.org/10.25423/CMCC/MEDSEA\\_MULTIYEAR\\_BGC\\_006\\_008\\_MEDBFM3](https://doi.org/10.25423/CMCC/MEDSEA_MULTIYEAR_BGC_006_008_MEDBFM3)

DIC data in the Atlantic Ocean:

445 [https://resources.marine.copernicus.eu/?option=com\\_csw&view=details&product\\_id=IBI\\_MULTIYEAR\\_BGC\\_005\\_003](https://resources.marine.copernicus.eu/?option=com_csw&view=details&product_id=IBI_MULTIYEAR_BGC_005_003)

pH data:

[https://resources.marine.copernicus.eu/?option=com\\_csw&view=details&product\\_id=GLOBAL\\_REANALYSIS\\_BIO\\_001\\_029](https://resources.marine.copernicus.eu/?option=com_csw&view=details&product_id=GLOBAL_REANALYSIS_BIO_001_029)

Temperature data: <https://icdc.cen.uni-hamburg.de/daten/reanalysis-ocean/easy-init-ocean/ecmwf-oras5.html>

#### 450 Author contributions

DB, VB, and GP conceptualized the research question and study design. AL, DB, and VB conducted the experimental work; AM and GP the environmental data extraction. GP performed the data analysis and prepared the draft of the paper. All authors contributed to the editing and reviewing of the paper.

#### Competing interests

455 The authors declare that they have no conflict of interest.

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