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Withstanding the heat: Resilience of free-living coralline algae to marine heatwaves

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ABSTRACT

Marine heatwaves (MHWs) can severely impact benthic ecosystems, driving major ecological shifts. As they become longer, more frequent, and intense, MHWs are emerging as a dominant threat to marine biodiversity. Yet, their potential effects on coralline algal beds (CABs, including rhodolith beds), habitats of critical ecological importance, remain largely unknown. This study investigated the physiological responses of three rhodolith species (*Lithothamnion* cf. *valens, Lithothamnion* sp., *Lithophyllum incrustans*), which dominate the community of a Mediterranean CAB, to an experimentally simulated ecologically relevant scenario of two consecutive summer MHWs. Species responses in key physiological processes, photosynthesis, respiration, and calcification, were assessed before, during, and after the heatwave events. Overall, temperature increases had minor effects on photosynthesis and respiration, while calcification responses to MHWs were more pronounced and species-specific. Significant declines were observed in light calcification of *L. incrustans* during the first MHW, and in dark calcification of all three species, with *L. incrustans* and *L.* cf. *valens* responding during the first and second MHWs, respectively. *Lithothamnion* sp. exhibited some minor effects on light calcification and a significant shift toward carbonate dissolution in darkness during the first MHW, which reversed over time. Despite these effects, all species fully recovered by the end of the experiment, and the stronger impact of the first MHW suggests a potential priming effect that may enhance tolerance to subsequent heat events. These findings demonstrate rhodoliths' overall capacity to withstand MHWs, while highlighting the role of species-specific responses in shaping the net carbonate balance of CABs under future climate scenarios.

1. Introduction

Ongoing climate change poses a significant threat to ecosystems worldwide, with marine environments being especially vulnerable to the related increasing temperatures, ocean acidification, and disruptions to overall oceanic stability (Doney et al., 2012). The profound and increasing impacts of marine heatwaves (MHWs) are particularly concerning. MHWs are defined as sudden, discrete periods (>5 days) of anomalously warm water above the ninetieth percentile for a given location and time of year (Hobday et al., 2016). Climate change is causing these events to become more frequent, prolonged, and intense (Meehl and Tebaldi, 2004; Frölicher et al., 2018; Oliver et al., 2018; Thoral et al., 2022). As a result, their impacts on marine benthic environments are increasingly documented, ranging from changes in

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ecosystem structure and function to widespread mortality (Smale et al., 2019; Straub et al., 2019; Smith et al., 2023).

Vulnerability and resilience to MHWs vary among species. Habitat-forming foundation species are often among the most affected, and their loss can trigger cascading effects that compromise ecosystem functioning, biodiversity, and the services these systems provide to humans (Wernberg et al., 2025). In particular, due to the increasing frequency of these extreme climatic events, the likelihood of marine ecosystems experiencing multiple events concurrently rises, with the potential to exacerbate biological impacts (e.g., Gómez-Gras et al., 2021; Garrabou et al., 2022; Hatum et al., 2024; Ramirez-Calero et al., 2024). Predicting biological responses to MHW events, whether singular or recurrent, remains challenging due to limited experimental data across a broad range of taxa. Moreover, responses often differ among species and populations, and are further modulated by local environmental conditions (King et al., 2018; Starko et al., 2024).

Coralline algae are among the groups that have received little attention regarding their responses to and the potential impacts of rising temperatures. Across available studies, the majority focused on responses to gradual warming (Martin and Hall-Spencer, 2017; Cornwall et al., 2019). A recent meta-analysis showed highly variable thermal responses among species, but also a consistently negative impact on coralline algal calcification at temperatures >5 °C above ambient conditions (Cornwall et al., 2019). In contrast, few experimental studies to date have determined the potential impacts of discrete and sudden warming events (i.e., MHWs) on coralline algae (Rendina et al., 2019; Page et al., 2021; Schubert et al., 2019, 2021a; Ragazzola et al., 2021; Krieger et al., 2023a, 2023b), making it difficult to identify general trends. This is further hindered by differences in experimental designs (e.g., rate of onset/decline, intensity, frequency and duration of MHWs, time points at which measurements were performed), as well as by limited incorporation of ecological context in some studies (see Hemraj and Russell, 2025).

So far, available evidence showed no MHW effects on the physiological performance of temperate crustose and articulated coralline algae (Rendina et al., 2019; Ragazzola et al., 2021; Krieger et al., 2023a, 2023b). Yet, different responses were found in temperate and subtropical free-living coralline algal species (rhodoliths *sensu lato*; Jardim et al., 2025), including significant negative effects of simulated MHW scenarios in photosynthesis and a pronounced impact on calcification (Schubert et al., 2019, 2021a). These studies also demonstrated that rhodoliths can express a fast and complete recovery after MHWs (i.e., high resilience) and that their responses to MHWs vary depending on species identity. The latter is consistent with species-specific seasonal responses (Qui-Minet et al., 2021), though evidence also showed the influence of the local environment on rhodolith responses to MHWs (Schubert et al., 2021a), as well as on their general physiological performance (Schubert et al., 2021b, 2022). Similarly, few field observations regarding impacts of MHWs on coralline algae reported variable ecological responses, ranging from mortality (Short et al., 2015) to increased cover and abundance (Murray and Horn, 1989; reviewed in Straub et al., 2019). Hence, the scarce information regarding potential MHW responses of coralline algae, together with their generally wide range of thermal responses (Martin and Hall-Spencer, 2017; Cornwall et al., 2019), makes accurate prediction of potential impacts of these increasingly more frequent events challenging. This is concerning, in view of the significant importance of coralline algae in many marine ecosystems, like coral and rocky reefs (Cornwall et al., 2023), and their role as foundation species for the widely distributed coralline algal beds (Riosmena-Rodríguez et al., 2017; Bulleri et al., 2025).

Coralline algal beds (CABs; *sensu lato,* Jardim et al., 2025), built by rhodoliths, are globally distributed throughout all climate zones and this, together with their large areal extension (Fragkopoulou et al., 2021), makes them one of the main coastal shelf habitats (Riosmena-Rodríguez et al., 2017; Tuya et al., 2023). They provide habitat, shelter and resources for a high diversity of other species, including many endemic, red-listed, and commercial species (Tuya et al., 2023; Bulleri et al., 2025; Schubert et al., 2025). Moreover, the high productivity and carbonate production, and substantial carbonate deposits of CABs, as well as their potentially important role for organic carbon storage, makes them highly relevant in the marine carbon cycle (van der Heijden and Kamenos, 2015; Mao et al., 2020; James et al., 2024; Schubert et al., 2024). Therefore, impacts on rhodolith populations, such as caused by ongoing and future warming trends (e.g., MHWs), can have far-reaching ecological and economic implications.

Given the current large knowledge gaps, the goal of this study is to improve our understanding of rhodolith responses to MHWs and their potential impacts on CABs. For this, different rhodolith species, dominating a Mediterranean CAB, were exposed to a realistic and ecologically relevant scenario of strong recurrent summer MHWs. Rhodolith physiological responses, i.e. photosynthetic, respiratory, and calcification rates, were measured before, during and after two consecutive MHW events, to assess (a) their vulnerability or resilience to thermal stress and (b) potential interspecific differences among species.

2. Material and methods

2.1. Sampling location and environmental conditions

The sampling location, Punta de la Mona (36.6869°N, −3.7275°W), is located at the southern coast of Spain, in the Mediterranean Sea (Fig. 1a). Because recent *in situ* temperature records were unavailable, we retrieved daily mean sea surface temperatures for the sampling region in 2024 from the CMEMS MED-Physics (EAS7 system) product provided by the Copernicus Marine Service (Clementi et al., 2021). Temperatures ranged from 27.4 °C in summer to 14.7 °C in winter. Daily fluctuations reached up to 2 °C, especially in summer (Fig. 1b). Earlier *in situ* measurements (2010–2011) using temperature loggers (HOBO, Onset, USA) deployed at the CAB (16 m depth) indicated a seasonal range of 25.6 °C–15.8 °C and suggest markedly higher daily fluctuations - ranging from 6.0 °C to 8.5 °C during summer months (Jul–Sept) - reflecting higher nearshore variability not captured by satellite-derived data (Fig. S1).

The CAB at Punta de la Mona extends over an area of 16,000 m^2, between 9 and 24 m depth, and is composed of multiple species (25 identified morphospecies) (Del Río et al., 2022, Fig. 1c and d). The highest living-rhodolith cover has been recorded at 18 m depth, with a mean and maximum cover of ~16 % and 35 %, respectively. *Lithothamnion* cf. *valens* Foslie (1909) and *Lithophyllum incrustans* Philippi (1837) have been identified as the two predominant rhodolith species, making up ~40 % of the rhodolith community (del Río et al., 2022).

The characteristics of MHWs at this location were assessed using climatological sea surface temperature (SST) time series and records of identified MHW events provided by the Marine Heatwave Tracker (Schlegel, 2018). MHWs were defined using the shifting baseline approach (1991–2020), which accounts for long-term warming trends by applying a moving reference temperature (Smith et al., 2025). The data indicate that the region of the study has experienced 77 MHWs over the last 30 years. Our analysis shows that frequency and intensity of these events have undergone an increasing trend over time, with 49 % of those MHW events occurring during the last decade and 19 % of them identified as strong events (category II: 2-3x above local seasonal threshold; Hobday et al., 2018) (Fig. 2a). Also, the frequency of MHW events during the last 30 years exhibited a seasonal variability, with events more common during spring and summer and less frequent in autumn and winter (Fig. 2b). During summer months, corresponding to the timing of this study, most MHWs had an intensity of 2–3 °C above the climatological threshold, with a recorded maximum intensity of 4.4 °C (Fig. 2c). Most of these summer heatwaves persisted for 1–2 weeks, commonly separated by time periods of 1 to >4 weeks (Fig. 2d).

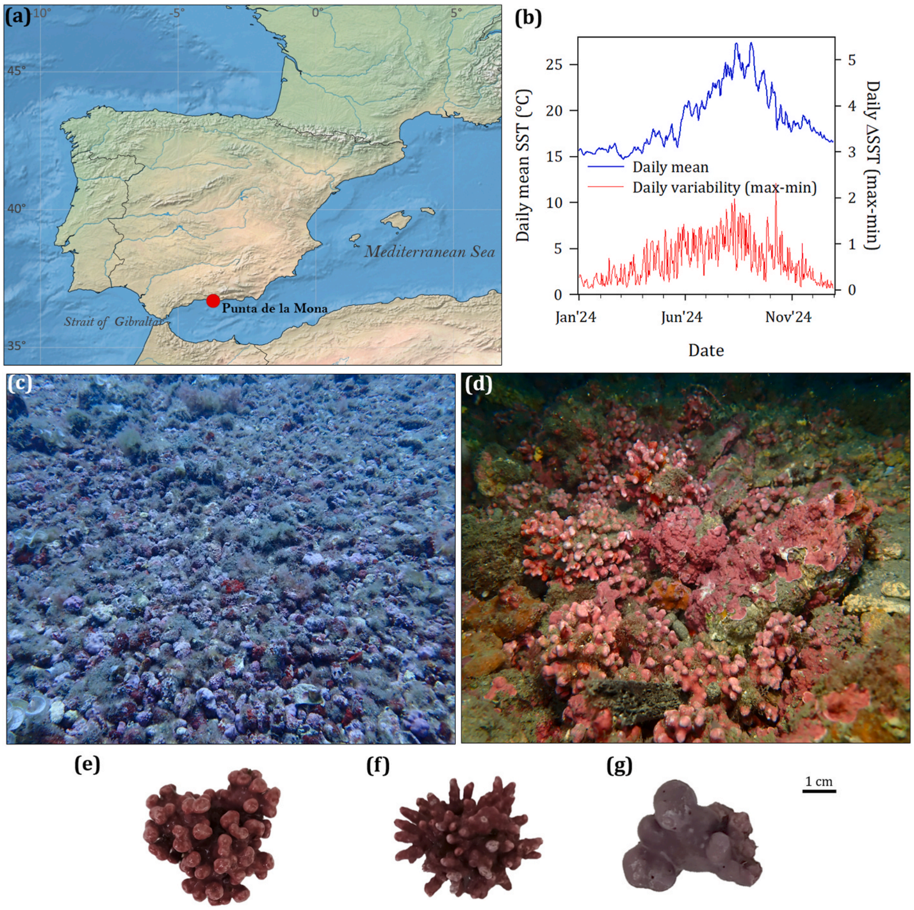

Fig. 1. Mediterranean coralline algal bed at Punta de la Mona. (a) Location of the studied bed, (b) mean daily sea surface temperature data for 2024 and daily temperature variability (obtained from the Copernicus Marine Service), and photos of (c, d) the coralline algal bed (provided by J. del Rio and J. Peñas) and the studied rhodolith species: (e) *Lithothamnion* cf. *valens*, (f) *Lithothamnion* sp., and (g) *Lithothyllum incrustans.*

2.2. Sample collection and maintenance

In June 2024, specimens of the three most visually dominant CAB morphospecies at Punta de la Mona were collected by SCUBA diving at 17 m depth (Fig. 1e). Also, *in situ* light profiles in the water column ($n = 4$) were taken, using an underwater quantum sensor (LI-192, LI-COR Environmental, USA), attached to a LI-250 A Light Meter (LI-COR Environmental, USA). Based on these measurements, the mean light attenuation coefficient (Kd_{PAR}) was 0.181 m^{-1}, indicating that 4.6 % of the surface irradiance reaches the bed depth.

After sampling, the rhodoliths were placed in a cooler box and transported to the Ramalhete marine station of the Center for Marine Sciences in Faro, southern Portugal, within 6 h. Here, the samples were maintained in a mesocosm system at a temperature of 20 °C (measured temperature during collection), a photoperiod of 14 h:10 h (Light:Dark) and an irradiance of 50 μmol photons m^{-2} s^{-1} (corresponding to a daily light exposure of 2.5 mol photons m^{-2}), provided by LED lamps (LEDVANCE 50 W, LEDVANCE GmbH, Germany). The experimental light level was chosen based on local incident irradiance data (due to lack of data from Punta de la Mona), and considering the measured light availability at the collection depth. After ten days of acclimation, the temperature was increased by 1 °C every four days until reaching 22 °C (mean temperature for July, Fig. 3a), at which the rhodoliths were kept for a week before the start of the experiments.

The indoor mesocosm system consisted of ten 30-L aquaria, connected to a seawater flow-through system (water flow 200 mL min^{-1}). Incoming seawater was filtered through two mechanical filters (50 μm and 5 μm in series) and sterilised with a UV lamp (UV Steriliser P1 55 W, Tropical Marine Centre Ltd, United Kingdom). It was then cooled to 16 °C in a temperature-controlled 2000-L header tank using two inverter heat pumps (I-Komfort RC 900, Kripsol, Spain). From the header tank, seawater was delivered to the aquaria and adjusted to the experimental target temperature. Aquarium temperatures were regulated and logged by the Aquatronica system (version 9.1.0) through a central controller (Aquatronica Controller Evolution, ACQ115) connected to interfaces (Aquatronica Interface, ACQ210N-TL), thermal sensors (Aquatronica Temperature Probe, ACQ-001 S), and aquarium heaters. Each aquarium was equipped with a submersible air pump to maintain water circulation.

2.3. Species identification

To identify the species, molecular analyses were conducted on individuals from the three morphospecies used in the MHW experiment. Genomic DNA was extracted using a E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, United States) following the manufacturer protocol. The *psb*A locus was amplified, using primer pairs *psb*A-F1/*psb*A-R1 (Yoon et al., 2002) and eventually with psbA21-350F/psbA22-350 R (Anglés d'Auriac et al., 2019). The PCR reaction was made by using the Supreme NZYTaq II 2x Colourless Master Mix (NZYtech) and following the thermal profile used by Peña et al. (2015). The PCR products were purified and sequenced by the SAI-UBM department of the University of Coruña, Spain. Sequences were assembled with the assistance of CodonCode Aligner® (CodonCode Corporation, USA), adjusted by eye using SeaView version 4 (Gouy et al., 2010), and submitted to the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert, 2007) and GenBank.

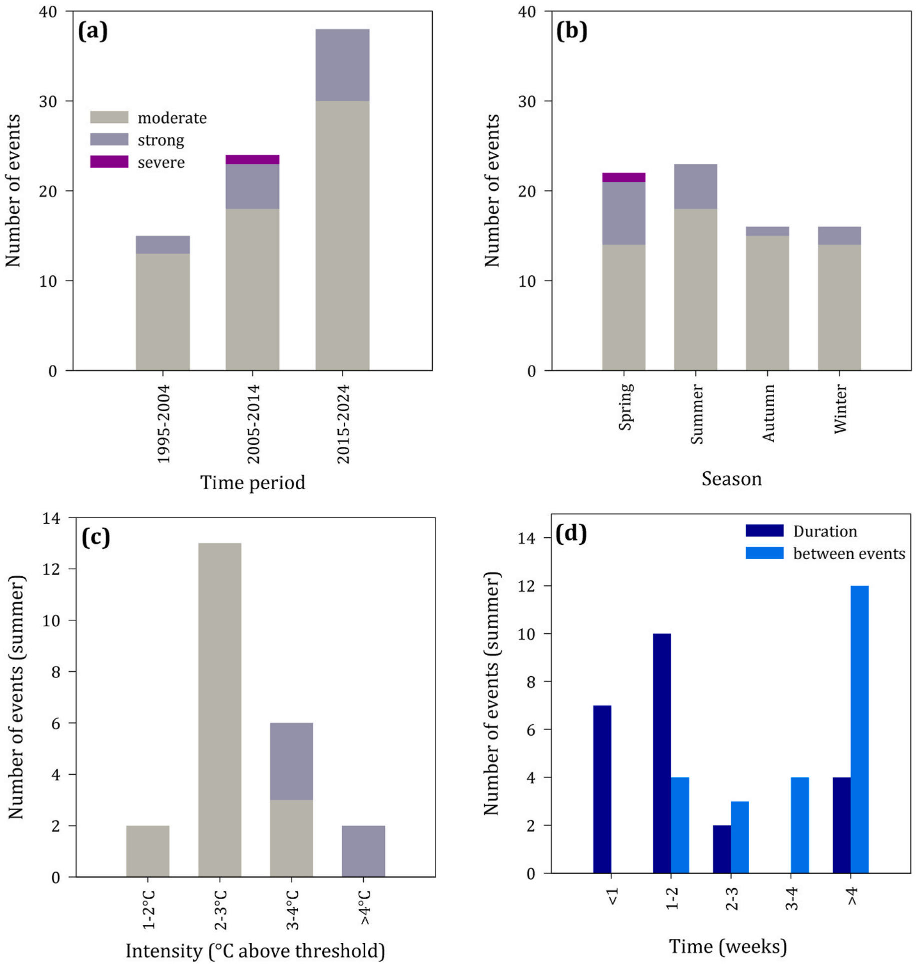

Fig. 2. Characteristics of marine heatwave (MHW) events at Punta de la Mona from 1995 to 2024. (a) Decadal occurrence of MHWs, categorized by intensity following Hobday et al. (2016, 2018): moderate (1–2 x the threshold-to-mean difference), strong (2–3 x), and severe (3–4 x), and (b) the seasonal variability in MHW frequency throughout the year; (c) and (d) focus on MHW characteristics during summer - the season in which the study was conducted. Specifically, (c) shows the intensity distribution of summer MHW events, distinguishing between moderate and strong events, while (d) illustrates the duration of these events and the time intervals between them. Data were obtained from the Marine Heatwave Tracker (Schlegel, 2018) and are based on a shifting baseline climatology (1991–2020).

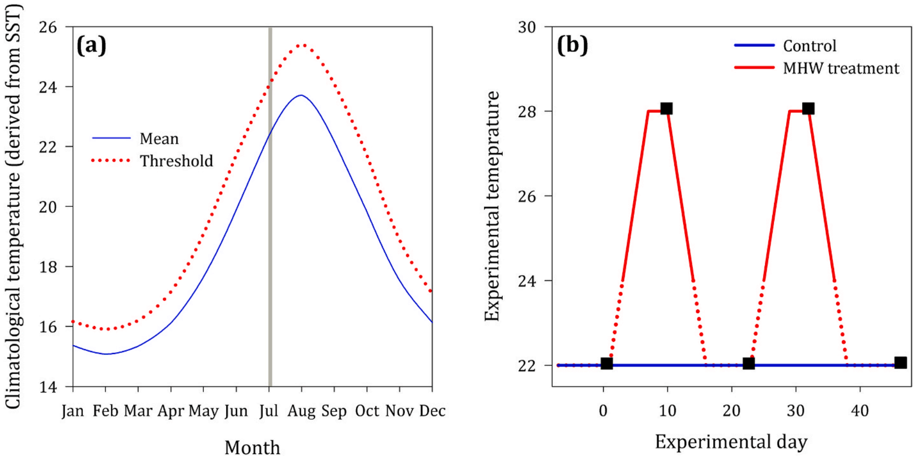

Fig. 3. Environmental and experimental temperature conditions. (a) Annual variation of long-term climatological daily mean and threshold temperatures in Punta de la Mona (southern Spain), retrieved from the Marine Heatwave Tracker, using the shifting baseline (1991–2020) (Schlegel, 2018). The starting point of the experiment is indicated by the grey line. (b) Experimental treatments (target temperatures), including a Control at 22 °C and a MHW treatment, simulating two consecutive summer MHWs (highlighted by a solid red line that indicate temperature above long-term climatological threshold), with a maximum intensity of 4 °C above threshold (i.e., strong event), a duration of 10 days each, and 12 days between MHW events. Black squares indicate time points at which physiological measurements were taken. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Sequences generated were compared with publicly available sequences in GenBank (~8900 sequences for this group of red algae). Estimates of genetic distance (uncorrected p-distances and number of base pair differences) were calculated in MEGA v. 6 (Tamura et al., 2013).

The rhodoliths studied were molecularly identified as *Lithothamnion* cf. *valens*, *Lithothamnion* sp. and *Lithophyllum incrustans*, each one corresponding to one of the morphospecies collected (Fig. 1e–g). Sequences obtained for rhodoliths of each species showed 0 bp of differences (and 0 % uncorrected p-distance) between them. Our sequences showed relevant matches with GenBank sequences of *L. incrustans* (100 % identity) and *L.* cf. *valens* (99.89 % identity). The type material of *L. valens* has not been sequenced yet, therefore identifications for this species cannot be confirmed. *Lithothamnion valens* and *L. incrustans* are reported as rhodolith species in the Mediterranean (Basso et al., 2017), and our results confirm their abundance previously reported for both species in the study area (del Río et al., 2022). The third species was tentatively identified as *Lithothamnion* sp., as it showed 99.69 % sequence identity with GenBank entries also labeled as *Lithothamnion* sp. (accession numbers PQ593850 and PQ593854) from the same study area. Among the 10 individuals of this morphospecies used in the experiment, one individual from the MHW treatment was molecularly identified as *Boreolithothamnion corallioides* (P.Crouan & H.Crouan) V. Peña & P.W.Gabrielson (100 % identity with GenBank records for this rhodolith species, previously known as *Lithothamnion corallioides*, Peña et al., 2025) and the respective data were therefore excluded from the data analysis.

2.4. Photosynthesis- and calcification-light response curves

Initial physiological characterization was conducted using photosynthesis and calcification light-response curves to assess inherent species differences and similarities under natural conditions, which could influence their responses to the MHW treatment. For the light-response curves, rhodolith specimens (n = 5 per species) were incubated with filtered seawater (45 μm) in custom-made acrylic chambers (V = 170 mL), fitted with an outer jacket that was connected to a thermocirculator (Julabo HC, JULABO GmbH, Germany) to maintain a stable temperature of 22 °C during incubations. Stir bars were used in combination with magnetic stirrers (RSLAB-11NCD RS-LAB, France) to provide water mixing within the chambers. A series of incubations at increasing light intensities (3, 9, 25, 44, 90, 180, 390 and 800 μmol photons m^{-2} s^{-1}), plus an initial and final dark incubation, were performed. Light intensities were adjusted using LED light bulbs (PAR38-7.8 W-E27, 4000 K, LEXMAN, Germany), positioned above each chamber and mounted in a set-up that allowed raising and lowering the bulb as needed, combined with the use of neutral density filters.

Rhodoliths were incubated for 1 h at each light intensity (e.g., Schubert et al., 2022) and at the beginning and end of each incubation, oxygen concentration was measured with an oxygen meter (Microx 4, PreSens, Germany). In addition, water samples were collected at the beginning of the series of incubations, and at the end of each incubation, poisoned with $HgCl_2$, and stored in borosilicate vials (2 sub-replicates, V = 25 mL each) for later estimation of calcification through total alkalinity measurements. After the incubations, the rhodolith samples were dried for 48 h at 60 °C and their dry weight was determined to normalize the physiological rates. Respiration rates were measured in darkness at the beginning (i.e., dark respiration, R_D) and immediately after each light incubation series (i.e., post-illumination or light respiration). Gross photosynthesis (GP) was calculated by adding the average of R_D and post-illumination respiration to the measured net photosynthesis (NP). The maximum GP (GP_{max}) and NP (NP_{max}) were obtained by averaging the respective values of the curve above the saturation irradiance (E_k). The photosynthetic efficiency (α) was estimated from the initial slope of the light response curve by linear least-squares regression. The compensation (E_c) and saturation (E_k) irradiances were calculated as the ratio R_D/α and E_k by NP_{max}/α, respectively.

Calcification rates were determined, using the alkalinity anomaly technique (Smith and Kinsey, 1978). Total alkalinity (TA) analyses were performed using the Gran titration method (Hansson and Jagner, 1973; Bradshaw et al., 1981). The samples were titrated with 0.1 M HCl, using an automatic titration system (Titroline 7000, SI Analytics, Mainz, Germany), coupled to an autosampler (TW alpha plus, SI Analytics, Mainz, Germany). Data were captured and processed with a computer, using the Titrisoft 3.2 software (SI Analytics, Mainz, Germany). For quality control, a certified reference material of known total alkalinity was used to calibrate the method (supplied by the Marine Physical Laboratory, Scripps Institution of Oceanography, USA). Similar to photosynthesis, maximum calcification rates for each species (G_{max}) were obtained by averaging the light calcification rates (G_{Light}) above E_k, calcification quantum efficiency (α_G) was estimated from the initial slope of the light response curve by a linear least-squares regression, and E_c and E_k were calculated through G_{Dark}/α_G and G_{Light}/α_G.

2.5. Experimental design

The MHW experiment was performed in the above-described mesocosm system and consisted of a Control (n = 5 aquaria), kept at a constant temperature, and a treatment simulating two consecutive MHWs (n = 5 aquaria). The chosen experimental conditions were based on climatological data of the sampling location (Hemraj and Russell, 2025). In the Control, rhodoliths from the three species (n = 1 rhodolith per species and aquarium) were maintained at 22 °C, corresponding to the mean temperature in July (Fig. 3a–S2a). The scenario for the MHW treatment was based on the analysed MHW characteristics at the sampling location (see Fig. 2c and d). Two consecutive summer MHWs were simulated by gradually increasing or decreasing the temperature by 1 °C per day. Each MHW reached a maximum temperature of 28 °C, which was maintained for 4 days. This is 4 °C above the long-term July climatological threshold (~24 °C) and represents a strong event (Category II; 2–3 times the threshold-to-mean difference; Hobday et al., 2018). Each simulation lasted 14 days with temperatures above the control, including 10 days that exceeded the climatological threshold and thus constitute a MHW. The two events were separated by 7-day recovery periods at 22 °C (Fig. 3b–S2b).

Throughout the experiment, the rhodoliths' maximum photosynthetic and light calcification rates, along with respiration and dark calcification rates, were measured at the beginning (Day 0) and after 9, 21, 30, and 42 days (Fig. 3b), using the same individuals at each time point. For this, incubations were performed as described above, at the respective experimental temperature and under saturating light (250 μmol m^{-2} s^{-1}), corresponding to ~2.5E_k as derived from the initial photosynthesis light-response curves, and dark conditions.

2.6. Statistical analysis

Key physiological parameters derived from photosynthesis and calcification light-response curves were compared among species using one-way ANOVA to evaluate potential differences or similarities in overall physiological performance. *A priori*, data were tested for normality and heteroscedasticity, using the Shapiro-Wilk and Levene's tests, respectively. When required, ln-transformation was used to normalize the data (α of photosynthesis), but when data still did not reach normality, a Kruskal-Wallis test was used instead (E_c of photosynthesis, G_{Dark}). When significant differences were detected, pairwise comparisons were performed using Tukey's HSD post hoc test following ANOVA, or Dunn's post hoc test following Kruskal–Wallis analyses.

Data of the MHW experiment were analysed using Linear Mixed Effects Models (LMEMs) to test for the effect of "treatment" (MHW vs. control), separately for each species, on the four physiological responses (photosynthesis, respiration, light and dark calcification), by means of the R "lmerTest" package (Kuznetsova et al., 2017). We included "day", as a random factor, to account for the non-independency (i.e., repeated experimental measures) of the data. The function "check_model" from the R "performance" package (Lüdecke et al., 2021) was used to assess model assumptions. This included visualization of density plots of predicted versus observed values, residuals versus fitted values, and QQ-plots of residuals (Figs. S3–S8). When a significant effect was detected, pairwise comparisons between experimental treatments were performed, separately for each time, via the R "emmeans" package (Lenth, 2024). In addition to statistically significant effects ($p < 0.05$), we also report comparisons with p-values close to 0.05, as some of these pairwise tests revealed potentially meaningful differences between treatments.

3. Results

3.1. Characterization of species-specific physiological performance

The three species generally exhibited comparable physiological performance, with similar values for key parameters such as maximum photosynthesis and calcification rates (Table 1). Photosynthesis saturated at ~100 μmol photons m^{-2} s^{-1}, about four times higher than the E_k values estimated for calcification, and the relationship between photosynthesis and calcification was consistent across species, with ~0.2 mol $CaCO_3$ precipitated per mol of O_2 produced.

Some species-specific differences were observed. R_D in *L.* cf. *valens* was significantly lower - over 75 % lower - than in the other two species, resulting in photosynthetic E_c values approximately three times lower than those of the other species (Table 1).

Table 1

Summary of physiological parameters derived from the initial characterization of the three species using photosynthesis and calcification light-response curves. Results of one-way ANOVAs or Kruskal-Wallis test are included and significant differences among species, identified by pairwise comparisons, are indicated by different superscript letters. Data are shown as mean ± SE (n = 5 per species) and significant *p*-values are highlighted in bold.

Parameter	*Lithothamnion* cf. *valens*	*Lithothamnion* sp.	*Lithophyllum incrustans*	ANOVA/ Kruskal-Wallis
Photosynthesis				
NP_{max}	2.23 ± 0.21	3.62 ± 0.33	3.44 ± 0.68	MS = 2.534, p = 0.1411
GP_{max}	2.39 ± 0.20	4.41 ± 0.57	4.36 ± 0.88	MS = 6.089, p = 0.0715
R_D	0.16 ± 0.01^a	0.77 ± 0.22^b	0.77 ± 0.19^b	MS = 20.37, **p < 0.0001**
α	0.023 ± 0.001	0.032 ± 0.007	0.032 ± 0.006	MS = 1.326, p = 0.3085
E_k	96 ± 8	116 ± 15	108 ± 14	MS = 0.653, p = 0.5416
E_c	7 ± 1^a	23 ± 2^b	23 ± 2^b	H = 8.171, **p=0.0168**
Calcification				
G_{max}	0.64 ± 0.13	0.65 ± 0.13	0.66 ± 0.12	MS = 0.579, p = 0.5780
G_{dark}	0.09 ± 0.05	0.11 ± 0.02	0.14 ± 0.03	H = 3.342, p = 0.1880
α	0.029 ± 0.006	0.029 ± 0.005	0.034 ± 0.007	MS = 0.180, p = 0.8376
E_k	22 ± 3	25 ± 10	23 ± 5	MS = 0.450, p = 0.6497
E_c	3 ± 1	4 ± 21	7 ± 3	MS = 0.168, p = 0.8478
G_{max}: GP_{max}	0.21 ± 0.04	0.15 ± 0.02	0.16 ± 0.02	MS = 1.624, p = 0.2449

Maximum net (NP_{max}) and gross (GP_{max}) photosynthetic rate (in μmol O_2 g^{-1} DW h^{-1}), dark respiration (R_D; in μmol O_2 g^{-1} DW h^{-1}), photosynthetic efficiency [α; in (μmol O_2 g^{-1} DW h^{-1}) (μmol photons m^{-2} s^{-1})$^{-1}$], saturation (E_k) and compensation (E_c) irradiance (in μmol photons m^{-2} s^{-1}), maximum light calcification rate (G_{max}) and dark calcification rate (G_{dark}) (in μmol $CaCO_3$ g^{-1} DW h^{-1}).

3.2. Physiological responses to marine heatwaves

The experiment revealed species-specific responses to MHWs, but overall high recovery across all three species. In the overall LME analysis, *L.* cf. *valens* and *Lithothamnion* sp. showed a significant treatment effect in gross photosynthesis, while *L. incrustans* showed a significant treatment effect in respiration (Table 2). However, pairwise comparisons at individual time points revealed no significant differences between the Control and MHW treatment (Fig. 4, Table 2). Thus, the significant treatment effects reported in the models for gross photosynthesis and respiration reflect overall trends rather than detectable increases or decreases at specific time points during the experiment.

In contrast to other variables, calcification showed more pronounced responses to MHWs, with species-specific patterns (Fig. 5, Table 2). Light calcification generally declined during MHWs and increased during recovery in *L.* cf. *valens* and *L. incrustans* (Fig. 5a–c). However, this effect was statistically significant only in *L. incrustans*, which exhibited a 54 % decline during the first MHW (Table 2). In *Lithothamnion* sp., light calcification also declined during the first MHW, showing a marginally significant decrease followed by gradual recovery (Fig. 5b–Table 2). Dark calcification responses were more variable but significant across species. In *Lithothamnion* sp., dark calcification exhibited a statistically significant shift toward carbonate dissolution during the first MHW and the subsequent recovery period, which reversed during the second MHW, returning to initial values by the end of the experiment (Fig. 5e). Significant treatment effects on dark calcification were also observed in *L.* cf. *valens* and *L. incrustans*, with notable declines during the second and first MHWs, respectively (Fig. 5d–f). These responses may have been amplified by significantly lower initial values in the MHW treatment compared to controls (Fig. 5d–f, Table 2).

4. Discussion

Our study demonstrates considerable resilience of free-living coralline algae to MHWs, evidenced by their rapid and complete recovery and the absence of lasting effects following two consecutive strong events. While all species ultimately recovered, their ability to maintain physiological function during thermal stress varied, underscoring species-specific differences in thermal tolerance. The reduced impact of the second MHW and full post-event recovery suggest a potential priming effect induced by repeated thermal exposure.

The high resilience observed in this study aligns with previous findings. For example, another rhodolith species, *Phymatolithon lusitanicum*, exhibited significant physiological impairment during a MHW, particularly in calcification rates, yet showed a rapid and complete recovery post-event (Schubert et al., 2021a). Growing evidence suggests that local variation in temperature can mediate the impacts of MHWs, as it influences the magnitude of stress directly experienced by individuals, facilitating local adaptation and acclimatization of populations (reviewed in Starko et al., 2024). In particular, the strong influence of high-frequency temperature variability, such as daily temperature range, has been pointed out as a key mitigating factor for thermal stress impacts, such as coral bleaching (Safaie et al., 2018). Such variability may contribute to the observed resilience of the rhodolith species studied here and in previous work, as their natural habitat along the southern Iberian coast is characterized by substantial daily temperature fluctuations (Fig. 1b–S1; Schubert et al., 2021a).

Following the second MHW, all three species exhibited physiological performance comparable to initial values, even those that showed reduced calcification during the events. However, species-specific differences in tolerance to thermal stress were evident. *Lithothamnion* sp. was the most affected, with a strong decline observed in dark calcification during and after the first MHW, while light calcification showed a marginally significant decrease. *Lithophyllum incrustans* exhibited notable effects on light and dark calcification, primarily during the first MHW, whereas *L.* cf. *valens* displayed minimal responses, with only

Table 2
Linear Mixed Effects Model results for each rhodolith species, testing for differences in physiological performance between Control and MHW treatment over the course of the experiment (rec - recovery period). Significant effects ($p < 0.05$) are highlighted in bold.

Species	Physiological variable	Main effect of treatment		Pairwise comparison of Control and MHW treatment		
		t-value	p-value	Time point	t-value	p-value
Lithothamnion cf. *valens*	Gross photosynthesis	2.629	**0.0116**	Day 0	−2.228	0.0564
				Day 9 (1st MHW)	−0.978	0.3605
				Day 21 (rec)	−0.217	0.8343
				Day 30 (2nd MHW)	−1.018	0.3385
				Day 42 (rec)	−1.69	0.1296
	Respiration	0.344	0.733	–	–	–
	Light calcification	−0.893	0.377	–	–	–
	Dark Calcification	−3.313	**0.0019**	Day 0	4.328	**0.0025**
				Day 9 (1st MHW)	2.193	0.0644
				Day 21 (rec)	−0.305	0.7693
				Day 30 (2nd MHW)	2.315	**0.0493**
				Day 42 (rec)	0.929	0.3803
Lithothamnion sp.	Gross photosynthesis	2.812	**0.0074**	Day 0	−1.257	0.2489
				Day 9 (1st MHW)	−2.082	0.0758
				Day 21 (rec)	−0.264	0.7991
				Day 30 (2nd MHW)	−1.735	0.1264
				Day 42 (rec)	−0.918	0.3894
	Respiration	1.105	0.275	–	–	–
	Light calcification	−1.934	0.0604	Day 0	−0.359	0.7301
				Day 9 (1st MHW)	2.536	**0.0389**
				Day 21 (rec)	2.391	**0.0481**
				Day 30 (2nd MHW)	1.757	0.1224
				Day 42 (rec)	−1.986	0.0874
	Dark calcification	−4.155	< **0.0001**	Day 0	−0.446	0.6693
				Day 9 (1st MHW)	5.504	**0.0009**
				Day 21 (rec)	3.974	**0.0106**
				Day 30 (2nd MHW)	1.474	0.1909
				Day 42 (rec)	0.210	0.8400
Lithophyllum incrustans	Gross photosynthesis	1.34	0.187	–	–	–
	Respiration	2.152	**0.0365**	Day 0	−2.025	0.0774
				Day 9 (1st MHW)	−0.079	0.9388
				Day 21 (rec)	−0.892	0.3984
				Day 30 (2nd MHW)	−0.384	0.7110
				Day 42 (rec)	−1.713	0.1250
	Light calcification	−2.413	**0.0201**	Day 0	2.158	0.0629
				Day 9 (1st MHW)	3.180	**0.0130**
				Day 21 (rec)	−0.857	0.4166
				Day 30 (2nd MHW)	1.465	0.1811
				Day 42 (rec)	0.425	0.6818
	Dark calcification	−5.040	< **0.0001**	Day 0	3.995	**0.0040**

marginal changes in dark calcification - possibly influenced by initial differences between Control and MHW treatment.

Ample evidence on the large variability in thermal responses among coralline algal species exists (Martin et al., 2013; Cornwall et al., 2019). In rhodoliths, multi-species studies assessing responses to experimental thermal stress or seasonal variation in physiological and growth performance have consistently revealed high interspecific variability (Adey, 1970; Adey and McKibbin, 1970; Schubert et al., 2019; Qui-Minet et al., 2021). For instance, a thermal stress experiment applying a similar temperature increase as in the present study (+4 °C above the local seawater maximum) to co-occurring rhodolith species in southern Brazil showed markedly different effects on photosynthesis and calcification between species (Schubert et al., 2019). This pronounced variability within the coralline algal group is further supported by the contrasting results of the limited number of MHW studies conducted on other coralline species. In several cases, no significant effects were observed in temperate articulated and crustose coralline algae from the coasts of New Zealand and the Mediterranean (Rendina et al., 2019; Ragazzola et al., 2021; Krieger et al., 2023a, 2023b). This variability is further highlighted by a study showing differential transcriptomic responses in two coralline algal species exposed to elevated temperature and/or low pH conditions (Page et al., 2022). The differences likely arise from species-specific physiological traits - potentially shaped by evolutionary history, as suggested by Page et al. (2022) - and may also reflect local environmental conditions and variation in the intensity and duration of simulated MHWs.

Our results also reinforce previous findings that calcification is more sensitive to thermal stress than photosynthesis (Martin et al., 2013; Schubert et al., 2019, 2021a; Qui-Minet et al., 2021). Given the strong relationship between the two physiological processes in coralline algae, i.e. photosynthesis provides energy for calcification (Comeau et al., 2013; Schubert et al., 2022; McCoy et al., 2023), this would suggest an uncoupling of those processes under non-optimal thermal conditions, as previously suggested for articulated coralline algae (Kolzenburg et al., 2019), with energy being diverted from calcification to other physiological processes. As previous studies in macrophytes showed, under thermal stress, energy resources can be re-allocated towards processes, such as antioxidant defense and upregulation of heat shock proteins (e.g., Collén et al., 2007; Eggert, 2012; Franssen et al., 2014; Gouvêa et al., 2017; Nguyen et al., 2020; Pazzaglia et al., 2022). This pattern is consistent with transcriptomic data from the articulated coralline alga *Amphiroa fragilissima*, which exhibited upregulation of genes encoding

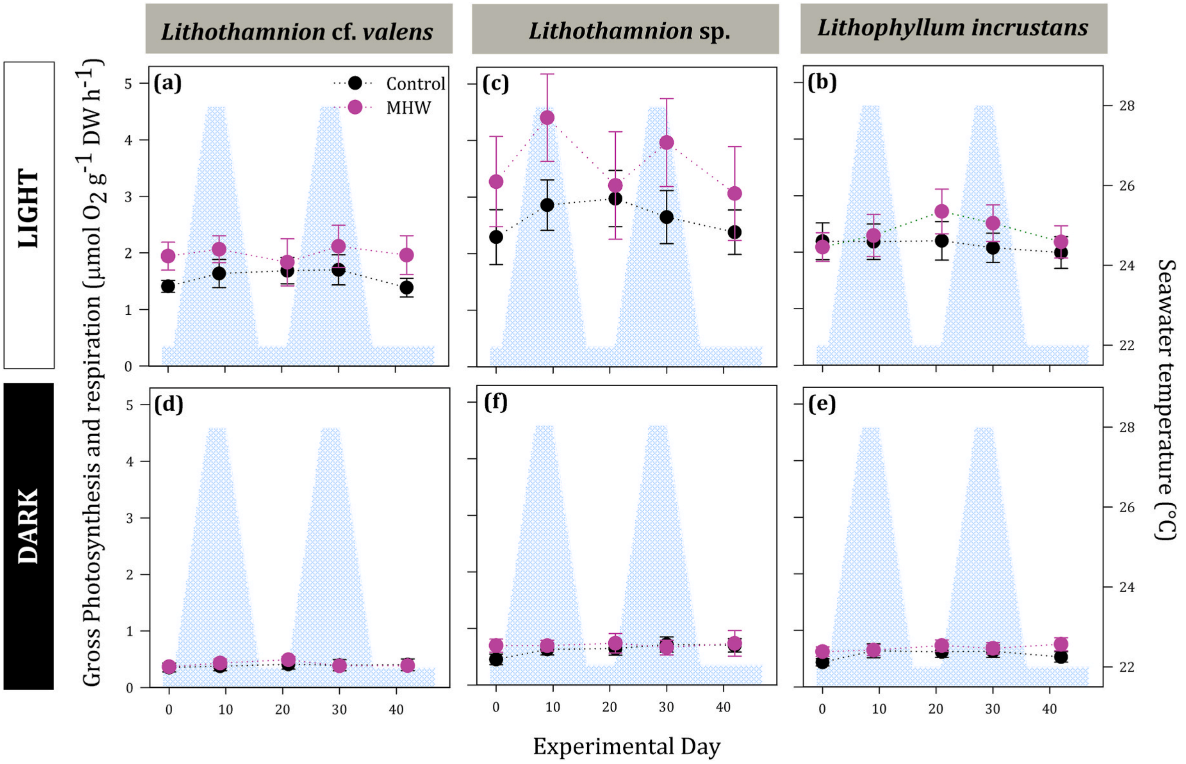

Fig. 4. Photosynthetic and respiratory responses of three free-living coralline algal species to different experimental conditions. Species' responses of (a–c) gross photosynthesis and (d–f) respiration to the two experimental conditions (Control – stable at 22 °C, MHW treatment - simulating two consecutive heatwaves, with temperature changes of the latter during the experiment highlighted by the light blue area). Data represent mean values ± SE (n = 5 per species and treatment), except for the MHW treatment of *Lithothamnion* sp., where n = 4 (see subsection 2.3. for details). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

heat shock proteins and antioxidant enzymes under thermal stress, while genes encoding photosystem reaction center proteins (e.g., D1), ATPase, nitrate reductase, and carbonic anhydrase were downregulated (Yang et al., 2021). Furthermore, a comprehensive study showed that rhodolith species differ in the strength of the relationship between photosynthesis and calcification, as well as in the associated mechanisms (e.g., reliance on transporters and/or enzymes; Schubert et al., 2022), which may account for differences in thermal sensitivity, even among species sharing the same habitat. Evidence for such mechanistic differences among the species studied here is further supported by their distinct responses during MHW events, despite an overall similar physiological performance under natural conditions as revealed by the initial light-response curves.

Interestingly, two of the studied species showed stronger physiological responses to the first MHW compared to the second. The available information regarding effects of recurrent MHWs on marine macrophytes indicates largely variable responses, ranging from positive and neutral responses, to negative and/or cumulative impacts (Kishimoto et al., 2019; Saha et al., 2020; Nguyen et al., 2020; Schubert et al., 2021a; Straub et al., 2022; Pazzaglia et al., 2022; Ito et al., 2024; Vivanco-Bercovich et al., 2024; Gillis et al., 2025). In some cases, and similar to our findings, prior thermal stress has been shown to prime organisms to cope better with subsequent stress events, as in the case of *P. lusitanicum* (Schubert et al., 2021a), and other marine macrophytes (Kishimoto et al., 2019; Nguyen et al., 2020; Pazzaglia et al., 2022). Transcriptomic analysis in seagrasses showed that repeated exposure to thermal stress led to acquisition of stress-induced traits at the cellular level, by frontloading key proteins (e.g., heat shock proteins) that allow for a more rapid response during subsequent stress events ("thermal priming"; Nguyen et al., 2020; Pazzaglia et al., 2022). While there is currently no information available regarding transcriptomic responses to repeated thermal stress in coralline algae, it is likely that the high temperature variability in their natural habitat, together with a high capacity for acclimatization via non-genetic mechanisms (e.g., changes in physiology, gene expression), could explain the thermal-stress tolerance and resilience of the rhodoliths studied here.

In an ecological context, our findings and previous evidence (Schubert et al., 2019, 2021a) suggest significant effects of MHWs on rhodolith calcification, indicative for a short-term decline in net carbonate production of CABs during warming events, with the magnitude of the decline determined by their community composition. In the particular case of the CAB at Punta de la Mona, this suggests that during MHWs the carbonate production may decline at varying degrees along its depth range, due to recorded shifts in species' abundance. For example, *L.* cf. *valens*, the species least affected by thermal stress, dominates the community between 15 and 24 m depth, while in the shallower parts (9–12 m depth), *L. incrustans* is the dominant species (Del Río et al., 2022). Based on our experimental findings, this would suggest that the shallower portion of the CAB might experience a stronger decline in carbonate production, during MHWs, while the deeper portion would be less affected. While this currently might not result in a significant impact, due to the mainly short-lived nature of these events (Fig. 2d), longer-lasting events are becoming more frequent (e.g., year-long MHW in the North Atlantic in 2023; England et al., 2025). Given the importance of calcification for rhodolith growth and hence CAB structural complexity, increasingly prolonged MHWs - and the associated reductions in carbonate production - could eventually lead to significant habitat-scale impacts.

In summary, our findings add to growing evidence that free-living coralline algae generally exhibit resilience to short-term warming events, with rapid recovery and limited lasting effects (this study; Schubert et al., 2021a). While a recent meta-analysis suggested that long-lived coralline species may be less tolerant to warming (Cornwall et al., 2019), our results challenge this notion, showing that rhodoliths,

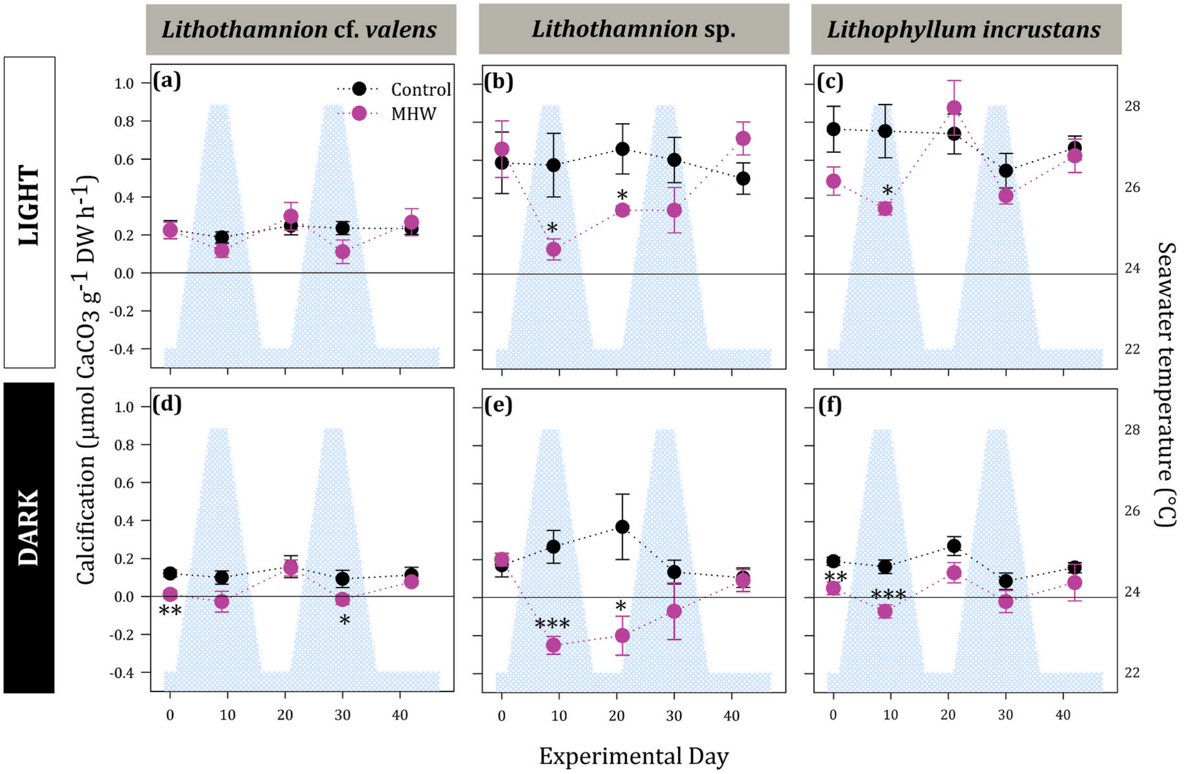

Fig. 5. Calcification responses of three free-living coralline algal species to different experimental conditions. Species' responses of (a–c) light calcification and (d–f) dark calcification to the two experimental conditions (Control – stable at 22 °C, MHW treatment - simulating two consecutive heatwaves, with temperature changes of the latter during the experiment highlighted by the light blue area). Data represent mean values ± SE (n = 5 per species and treatment), except for the MHW treatment of *Lithothamnion* sp., where n = 4 (see subsection 2.3. for details). Asterisks indicate significant differences relative to the respective control treatment at different time points during the experiment (*p < 0.05, **p < 0.01, ***p < 0.001; for details see Table 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

despite their longevity (>100 years; Foster, 2001; McConnico et al., 2014), can be remarkably resilient to short-term MHWs. However, some Arctic rhodoliths have shown reduced growth under long-term warming (Teichert et al., 2024), suggesting that resilience may be context-dependent. Further work is warranted to identify general patterns and trends and determine whether this trait is generalizable across free-living coralline algae, or if it is species-specific and influenced by local environmental conditions, stress duration, or interactions with other stressors such as ocean acidification. In this context, transcriptomic and proteomic studies will be valuable for uncovering the molecular mechanisms underlying stress response and adaptation, including potential thermal priming.

CRediT authorship contribution statement

Matteo Nannini: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Martina Cerpelloni:** Writing – review & editing, Investigation. **Tainá L. Gaspar:** Writing – review & editing, Investigation. **Viviana Peña:** Writing – review & editing, Resources, Investigation. **Fernando Tuya:** Writing – review & editing, Formal analysis. **Julio Peñas:** Writing – review & editing, Resources. **Jesús del Rio:** Writing – review & editing, Resources. **Carolina V. Mourato:** Writing – review & editing, Investigation. **João Silva:** Writing – review & editing, Resources. **Rui Santos:** Writing – review & editing, Resources. **Federica Ragazzola:** Writing – review & editing. **Irene Olivé:** Writing – review & editing. **Nadine Schubert:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Data availability statement author-disclosure

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information file. The nucleotide sequences of the psbA genes from the here identified species have been deposited in GenBank under the accession numbers PX064670-PX064675, PX064679-PX064682, PX064684-PX064687, PX064689-PX064691, PX107920, and PX107921.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107538.

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