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Impacts of marine heatwaves on rhodolith productivity: Potential interactions with nutrient enrichment

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Impacts of marine heatwaves on rhodolith productivity: Potential interactions with nutrient enrichment

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The 30Th June 2022,

Alexandre Paumier

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Resumo

Os bancos de rodólitos/maerl são formados por algas vermelhas coralinas, não-geniculadas, de vida livre, que criam estruturas tridimensionais calcificadas, através da precipitação de carbonato de cálcio sob a forma de calcite. São conhecidos como importantes engenheiros de ecossistemas em águas costeiras polares, temperadas e tropicais em todo o mundo, fornecendo *habitat*, refúgio e viveiro para as comunidades bentónicas associadas. A grande extensão destas comunidades cria ecossistemas bentónicos costeiros que contribuem grandemente para a produção de carbonato marinho e são frequentemente considerados como sumidouros de carbono. Apesar da grande extensão destes campos no Atlântico, poucos estudos abordam a sua produtividade global e o impacto que sobre eles exercem as alterações climáticas.

O objectivo deste estudo é abordar o potencial impacto combinado do *stress* térmico e nutricional sobre os rodólitos do género *Phymatolithon* sp., distribuídos por todo o arquipélago da Madeira (Portugal). Estas algas e as suas comunidades associadas são afectadas pelas alterações climáticas globais, entre elas o aumento das temperaturas a nível mundial. Contudo, este fenómeno global é também marcado pelo aumento da magnitude e frequência de eventos de temperaturas extremamente elevadas (Ondas de Calor Marinhas, dito Marine Heatwaves - MHWs). As ondas de calor marinhas, tal como definidas por Hobday et al. (2016), são eventos anómalos de altas temperaturas, quando as temperaturas da água do mar excedem os limiares sazonais e/ou regionais estabelecidos durante um período de pelo menos 5 dias. Estas tendências de variações de factores ambientais, definidos como factores de *stress* global, aumentaram em mais de metade ao longo do século XX, e prevê-se que a sua frequência continue a multiplicar-se nas próximas décadas. A termotolerância ao aumento da temperatura e a eventos extremos é vital para estas espécies e as comunidades que a elas estão associadas, dado que a temperatura é um factor limitativo a todas as reacções bioquímicas e, através da actividade enzimática e celular alterada, influindo diretamente sobre o crescimento e a sobrevivência. Varias espécies de rodólitos têm mostrado perdas diretas em populações, assim como competição indireta e pressão de predação por mudanças bioticas, tanto no Oeste e Norte da Austrália, como no Atlântico Norte. Infelizmente, este fenómeno não constitui o único perigo, uma vez que também estão expostos a eventos de *stress*

localizados, ou a factores locais de *stress*. A urbanização costeira em conjunção com padrões de precipitação alterados pode aumentar a entrada de nutrientes de origem terrestre, criando eventos localizados de enriquecimento de nutrientes. As regiões densamente povoadas estão, portanto, sujeitas a uma maior entrada antropogénica indirecta e directa de nitratos, amoníaco e fosfatos, aumentando o risco de eutrofização das águas costeiras. O aumento da entrada de nutrientes traduz-se no crescimento oportunista de certas algas, nomeadamente macroalgas verdes e fitoplâncton, afectando os estados tróficos dos ecossistemas locais. Fisiologicamente, o pequeno número de estudos existentes sobre as algas coralinas mostra uma variedade de respostas ao enriquecimento de nutrientes, com a produção primária e a calcificação mostrando respostas específicas das espécies a este factor de *stress* local.

A espécie *Phymatolithon* sp., uma das 4 espécies predominantes que compõem os bancos de rodolitos do Caniçal (Madeira), bem como outros bancos do arquipélago da Madeira (Portugal), demonstrou estar geneticamente ligada à espécie continental *Phymatolithon lusitanicum* e à espécie *Phymatolithon calcareum* do Atlântico Norte (Pardo et al. 2014, Neves et al. 2021). A espécie *Phymatolithon* sp. é assim representativa dos bancos de rodólitos e da biodiversidade a eles associada nas águas costeiras oligotróficas do arquipélago da Madeira. Para melhor compreender a resposta destas comunidades, é necessário considerar a resposta fisiológica aos factores de stress globais e locais das suas espécies âncora. Assim, a resposta fisiológica primária de produção e calcificação de *Phymatolithon* sp. às ondas de calor e ao enriquecimento de nutrientes, bem como o efeito combinado de ambos os factores, foram analisados para perceber o efeito que estes eventos têm sobre a fisiologia da espécie. Os dados de temperatura e irradiância dos sensores instalados no banco de rodólitos do Caniçal e do programa Marine Heatwave Tracker (Schlegel 2018) foram utilizados para calcular e simular uma réplica experimental de uma onda de calor significativa e realista para esta localização. O conceito experimental foi simular uma MHW «forte» de 2 semanas (Hobday et al. 2016), com um pico de 4 dias de 24,5°C e um aumento e diminuição linear de 0,5°C/dia. O evento simulado de MHW teve um efeito positivo na calcificação: tanto na luz como no escuro, os rodólitos responderam com um crescimento inicial da calcificação. O enriquecimento com nutrientes induziu maiores taxas de calcificação, mas praticamente não produziu alterações na fotossíntese e na respiração. A calcificação foi significativamente sensível ao factor temperatura extrema, uma vez que tanto a calcificação na luz como no escuro tiveram grandes aumentos comparativamente com os valores iniciais.

A resposta ao evento combinado de MHW e enriquecimento em nutrientes mostrou aumentos nas taxas de fotossíntese e calcificação, bem como nos integrais diários do volume destes processos. A calcificação foi particularmente reactiva: como a precipitação de $CaCO_3$ seguiu as mesmas tendências que quando exposto ao enriquecimento de nutrientes sob temperatura de controlo, o tratamento MHWxNE obteve taxas mais elevadas de calcificação à luz do que o tratamento MHW. A interacção encontrada significa que a resposta fisiológica ao evento MHW é ampliada pela resposta positiva ao enriquecimento de nutrientes. Isto permite inferir a potencial resposta da produtividade dos bancos madeirenses e a produção de carbonatos face à crescente frequência e intensidade dos eventos de anomalias térmicas e os potenciais efeitos interactivos com a eutrofização costeira. De notar que o estudo que aqui se apresenta traduz resultados ligados apenas a dois factores de *stress* durante uma época específica do ano. Para compreender plenamente o padrão de resposta fisiológica de *Phymatolithon* sp. seriam necessários estudos semelhantes que tivessem em consideração a sazonalidade e o aumento da exposição ao *stress*.

Abstract

Rhodolith/maerl beds are formed by free-living coralline algae that create three-dimensional calcified structures and thus, are important ecosystem engineers, providing habitat and refuge for associated benthic communities and contributing significantly to marine carbonate production, by building often extensive coastal benthic ecosystems. Though increased presence of these beds has been discovered in the Atlantic, few studies address their productivity and the impact of environmental changes. Rhodolith beds and their associated communities are affected by climate change, such as the increase in the magnitude and frequency of extreme high temperature events (marine heatwaves - MHWs). Unfortunately, this does not represent their sole peril, as coastal urbanization in conjunction with altered precipitation patterns can increase terrestrial-derived nutrient input. The individual and combined effects of increases in temperature, such as associated to MHWs, and nutrient concentration on the physiological performance of rhodoliths was assessed through an experimental mesocosm approach of the species *Phymatolithon* sp., one of the four prevalent species composing the rhodolith beds of the Madeira archipelago (Portugal). Nutrient enrichment induced little to no responses in the rates of photosynthesis and respiration, but increased calcification rates. Similarly, photosynthesis and respiration responded only slightly to the simulated MHW event, while the increase in temperature induced an increase in calcification. When combined, the MHW event and nutrient enrichment induced higher rates in gross photosynthesis, daily integrated net primary production, light calcification and daily integrated net calcification. This provides insight on the resilience of the Madeiran beds' productivity and carbonate production in the face of the increasing frequency and intensity of thermal anomaly events and the potential interactive effects with coastal eutrophication.

Keywords: Heatwave; Coralline Algae; Photosynthesis; Calcification; Mesocosm

All field, mesocosm and laboratory work will be conducted using the resources and the facilities of the Centro de Cîencias do Mar (CCMAR).

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i. List of Abbreviations

DLE	Daily Light Exposure (in mol photons meter^{-2} day^{-1})
DNP	Daily Net Photosynthesis (in µmol O_2 gram^{-1} Dry Weight day^{-1})
DNC	Daily Net Calcification (in µmol $CaCO_3$ gram^{-1} Dry Weight day^{-1})
GPP	Gross Primary Production (in µmol O_2 gram^{-1} Dry Weight hour^{-1})
MHW	Marine Heatwave (Hobday et al. 2016)
MHW x NE	Combined marine heatwave and nutrient enrichment treatment
NE	Nutrient enrichment
NPP	Net Primary Production (in µmol O_2 gram^{-1} Dry Weight hour^{-1})
SST	Sea Surface Temperature
TPC	Thermal Performance Curve

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1. Introduction

Temperature is the primary factor determining physiological and metabolical functioning, as it modulates all cellular and enzymatic activity (Eggert 2012a). Macroalgal communities are known to be largely influenced by temperature, as cellular responses usually show diminished performance when thermal optimum is passed (Straub et al. 2019). Vulnerability to thermal extremes relies on the intensity, frequency, and duration of the thermal stress events and temperature increases that extend over an organism's threshold, past its thermotolerance, tend to produce more intense responses that in turn affect and shift the trophic relations of the ecosystems these organisms compose (Lünning 1990). Marine environments have biogenic limitations that define the biota and its distribution through geographic scales: light, temperature, carbon and nutrient availability are all limiting factors to micro and macroalgae growth and survival in that aspect (Turpin 1991, Lüning 1991). Ongoing climate change is a threat to all ecosystems globally. As so, marine environments are exposed to a number of alterations, with increasing temperatures, ocean acidification and changes in oceanic stability as a whole. These threats are global occurrences and are combined with local impact in coastal regions, such as nutrient runoff, sedimentation, and turbidity (IPCC, 2013). As so, increased stress exposure for marine organisms is provoked not only by general temperature, pCO2 or nutrient increase, but also by higher frequency of extreme singular events (Jentsch et al. 2007). Year by year, accompanying the trend of global warming, marine heatwave (MHW) events (Hobday et al. 2016) have been gradually increasing in frequency (54% in the 20th century alone) and will continue to do so (Oliver et al. 2018, Oliver et al. 2019). Increases in temperatures, both in mean trends and in stochastic singular events, such as MHWs, are bound to influence marine ecosystems in their density and distribution (Stenseth et al. 2002), and have been reported to cause severe impacts on marine benthic ecosystems (e.g., Garrabou et al. 2009, Marbá and Duarte 2010, Wernberg et al. 2013), driving profound ecological changes over short-time scales (Jentsch et al. 2007, 2011, Ghedini et al. 2015, Smale 2015).

Nutrients affect primary producers through their availability, as their presence directly and indirectly affects metabolic efficiency. What is defined as nutrient availability is generally non-limiting concentrations of nitrate, ammonia, and phosphate, which all have direct influence on photosynthetic productivity, and in turn calcification in coral and coralline species. Eutrophic conditions are defined not by this concentration but rather by the production rate of a specific organic matter (Nixon 1995), from both anthropogenic and natural sources, affecting organisms directly (i.e., physiologically) and indirectly (i.e., competition, opportunistic invasion). (Andersen et al. 2006). On a global scale, coastal waters are considered to be increasingly threatened by events of increased nutrient input of both anthropogenic and natural causes. Response of coastal environments to eutrophic condition is much more severe in warmer subtropical and tropical waters, whereas temperate waters are more productive and susceptible to be exposed to anthropogenic nutrient run-off (Rothäusler and Jormalainen 2016).

Rhodolith beds are known for their importance an ecosystem engineer. These free-living, non-geniculate, coralline red algae create life-holding substrate for benthic communities, providing habitat for number of species in large depth range in coastal waters, from polar, temperate, and tropical waters (Bosence 1983, Foster 2001, Riosmena-Rodriguez et al. 2017). Their presence is beneficial to the creation of biodiversity hotspots, as living and dead rhodoliths make up for settlement and nursery sites for many invertebrate species (i.e., arthropods, annelids, bivalve, cnidaria) and seaweed species, making these beds ecological hotspots (Kamenos et al. 2004, 2013, Nelson 2009, Steller et al. 2003). Their calcifying nature, continuously precipitating CaCO3 to produce their calcite skeleton, adds to their importance as coastal benthic community creators in the global carbon and calcium carbonate cycles (Nelson 2009, Amado-Filho et al. 2012, van der Heijden and Kamenos 2015). The carbon storage made by these beds and their associated communities provides overlooked appreciation to the potential ecological utility as carbon sinks (Hill et al. 2015, van der Heijden and Kamenos 2015). Rhodoliths are often highly abundant throughout the benthic photic zone, as their ability to thrive on any substrates and/or in zones of hydrodynamic stress, gives them a competitive advantage over other algal species; this spatial distribution makes these species often locally limited by light, temperature, and sedimentation (Foster 2001, Wilson et al. 2004, Littler and Littler 2013). The importance of these species, as much as all calcifying algae, should be pushed for better recognition and relevance as biogenic habitats (Riosmena-Rodriguez et al. 2017).

Rhodoliths and coralline algae in general are impacted by temperature variations: distribution, growth and overall metabolic responses are affected by thermal increases out of optimal ranges, usually summer-high temperatures (Wilson et al. 2004, Martin and Hall-Spencer 2017). Thermal stress has been shown to alter physiological responses that can be positive or negative, depending on if they are within or outside the species' optimal thermal spectrum, respectively (Martin and Hall-Spencer 2017, Cornwall et al. 2019). The credibility of the effect produced by MHW events has been documented on many macroalgal species, giving advantage to opportunistic generalist species and increasing competition and mortality in other algal populations, such as coralline algae. (Wernberg et al., 2013, 2016). These indirect effects of MHWs on key primary producer and biota shifts have been occuring in both subtropical and temperate rhodolith beds (Straub et al. 2019, Smale et al. 2019). The direct effects of extreme and precise events such as MHW has had limited experimental development: North-Atlantic articulated coralline algae *Corallina officinalis* and Mediterranean *Elisolandia elongata* both showed little to no physiological response, with evidence of higher growth in summer periods (Rendina et al. 2019, Ragazzola et al. 2021). In contrast, Schubert et al. (2019) shows that subtropical Brazilian rhodolith species *Lithothamnion crispatum* and *Melyvonnea erubescens* were negatively impacted both in calcification and the latter also in primary production.

Indirect effects of nutrient enrichment on rhodolith performance, through competition, phytoplankton growth coverage and sedimentation have also been reported (Wilson et al. 2004, Villas-Boas et al. 2014), but direct effects in rhodoliths and other coralline algae, e.g. on growth and physiological performance, have been scarcely reported: *in situ* Tanzanian and Australian coast studies found negative (Björk et al. 1995) to no changes in calcification and growth (Koop et al. 2001, Steven 2000). Brazilian subtropical species were reported having negative responses in calcification (Schubert et al. 2019). Growth, net photosynthesis, and overall biomass decreases were reported in South Australia (Brown et al. 1977, Russel et al. 2009, Lewis 2016). Studies focused on temperate species showed all positive or no effects on growth, photosynthesis, respiration, calcification, or pigment concentration (Ichiki et al. 2000, Russel et al. 2009, Tanaka et al. 2017, Johnson and Carpenter 2018). The complexity of the responses is indicative for the differences in experimental methods, as well as in the species-specific physiological effects within the coralline algal group.

To produce an accurate assessment of the response to a stressor, one would have to understand the relation between said stressor and other environmental limiting variables. A positive or negative co-effect can create an antagonistic (combination of stressors results in a lesser effect than the sum of their individual effects) or synergistic relationship between stressors (combined effects are greater than the sum of the individual effects of each stressor) (Folt et al. 1999, Gunderson et al. 2015, Cabral et al. 2019), which can in turn make a stressor dominant on the physiological response (Brown et al. 2013). Studies on the combined effects of nutrient enrichment with another stressor are almost non-existent, as most studies focus on stress response to acidification or temperature increase. Only Schubert et al. (2019) assessed the response of *L. crispatum* and *M. erubescens* to combined MHW and nutrient enrichment, with no interactive effects found, but cumulative negative effects on both daily net primary production and calcification.

The Madeiran volcanic archipelago is comprised of four islands: Madeira and Porto Santo, which are inhabited, and Desertas and Selvagens, which are not. Situated in the North Atlantic subtropical region, their coastal oligotrophic waters are dominated by rocky reefs (Ribeiro 2008), Madeira's rhodolith beds are exposed to the increasing presence of human nutrient output, from tourism and aquaculture, and continuous increase in temperature and MHW events. Rhodoliths in the Madeiran archipelago have been reported at large depth ranges (from 8 to 129 m) and are formed of various genera: older studies reported presences of *Lithothamnion corallioides* and *Phymatolithon calcareum*, species found along the North Atlantic, as well *L. fruticulosum* (Cabioch 1974, Levring 1974, Weisscher 1983). More recently, Neves et al. (2021), in accordance with Pardo et al. (2014), reported three new species of *Lithothamnion* and one of *Phymatolithon*, the latter of which was used in this study. The new *Phymatolithon* species was found to be a sistering species of *Phymatolithon lusitanicum* (Pardo et al. 2014) and *P. calcareum*, a species already reported in the archipelago (Levring 1974, Neves et al. 2021). These latter two species' physiological responses to temperature increases and/or nutrient enrichment have been previously assessed (Qui-Minet et al. 2019, 2021, Schubert et al. 2021) and due to the genetic link between them and *Phymatolithon* sp., it would have expected that the latter might respond similarly. The aim of this study was therefore to determine the physiological response of *Phymatolithon* sp., to a simulated marine heatwave with ambient and enriched nutrient concentrations. Estimated responses of several physiological parameters, indicative of both net primary production and calcification, provided insights on overall rhodolith-associated productivity under variable environmental conditions.

2. Material and Methods

2.1. Study site and sampling

The species studied in this experiment, *Phymatolithon* sp., was collected from the rhodolith bed found in Caniçal, on the coastline of Madeira Island, Portugal (32,625N; -16,875W) (Figure 2.1.1).

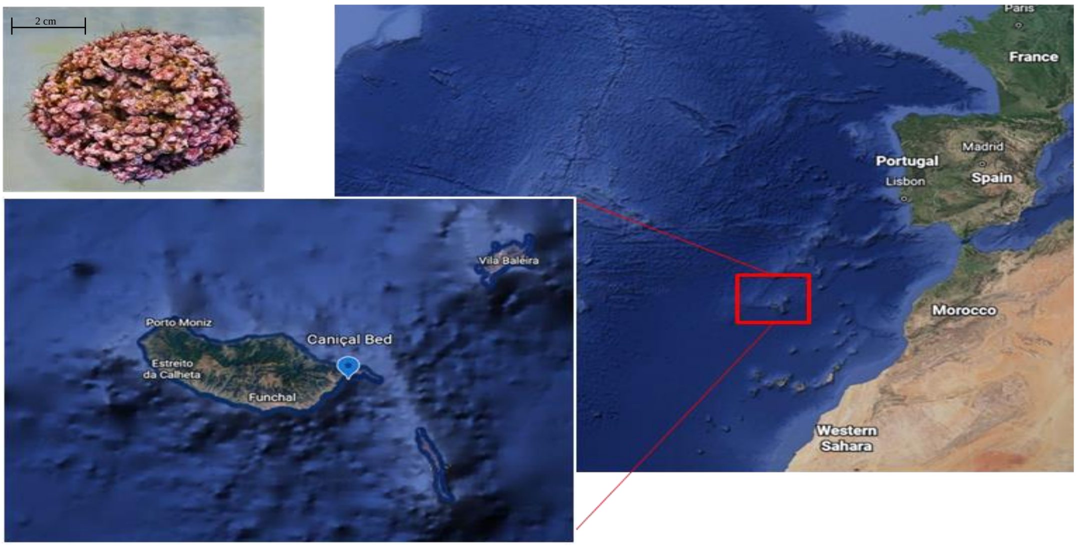


Figure 2.1.1.. Madeira archipelago in the North Atlantic (right), off the coast of North Africa (Google Earth Pro) and the Caniçal rhodolith bed at Madeira Island (blue pinpoint), dominated by *Phymatolithon* sp. (upper left).

Individuals were collected on May 6th, 2022, at a depth of 20 m and transported in the dark, wrapped in seawater-soaked paper towels to the CCMAR marine station of Ramalhete (Faro, Portugal) (arrived the following day) and placed in seawater flow-through tanks for recovery from transport. Rhodoliths were maintained at 20.5°C and 80 µmol photons m^{-2} s^{-1} (14 h light: 10h dark cycle, corresponding to a daily light exposure of 4 mol photons m^{-2} day^{-1}) for a week prior to the beginning of the experimental phase. These conditions were chosen to simulate the *in situ* environmental parameters of the Caniçal bed, which had been analysed previous to the experiment as described below.

Information regarding *in situ* environmental conditions and marine heatwave events recorded for the sampling location were obtained from sensors installed at the rhodolith bed and data downloaded from the Marine Heatwave Tracker, respectively. *In situ* temperature and light data (May-June) from 2019-2021 were recorded with HOBO (Onset, United States) and PAR data loggers (Odyssey, Dataflow Systems Pty Ltd, New Zealand), respectively the data show that the mean maximum light intensity ranges from 53 ± 16 µmol photons m^{-2} s^{-1} in winter to 379 ± 26 µmol photons m^{-2} s^{-1} in summer (Figure 2.1.2. A). Summer mean and maximum temperatures are 21,9 ± 0,5°C and 23,5 ± 0,3°C, respectively, and annual mean maximum values are 21,2 ± 1,9°C (Figure 2.1.2. C).

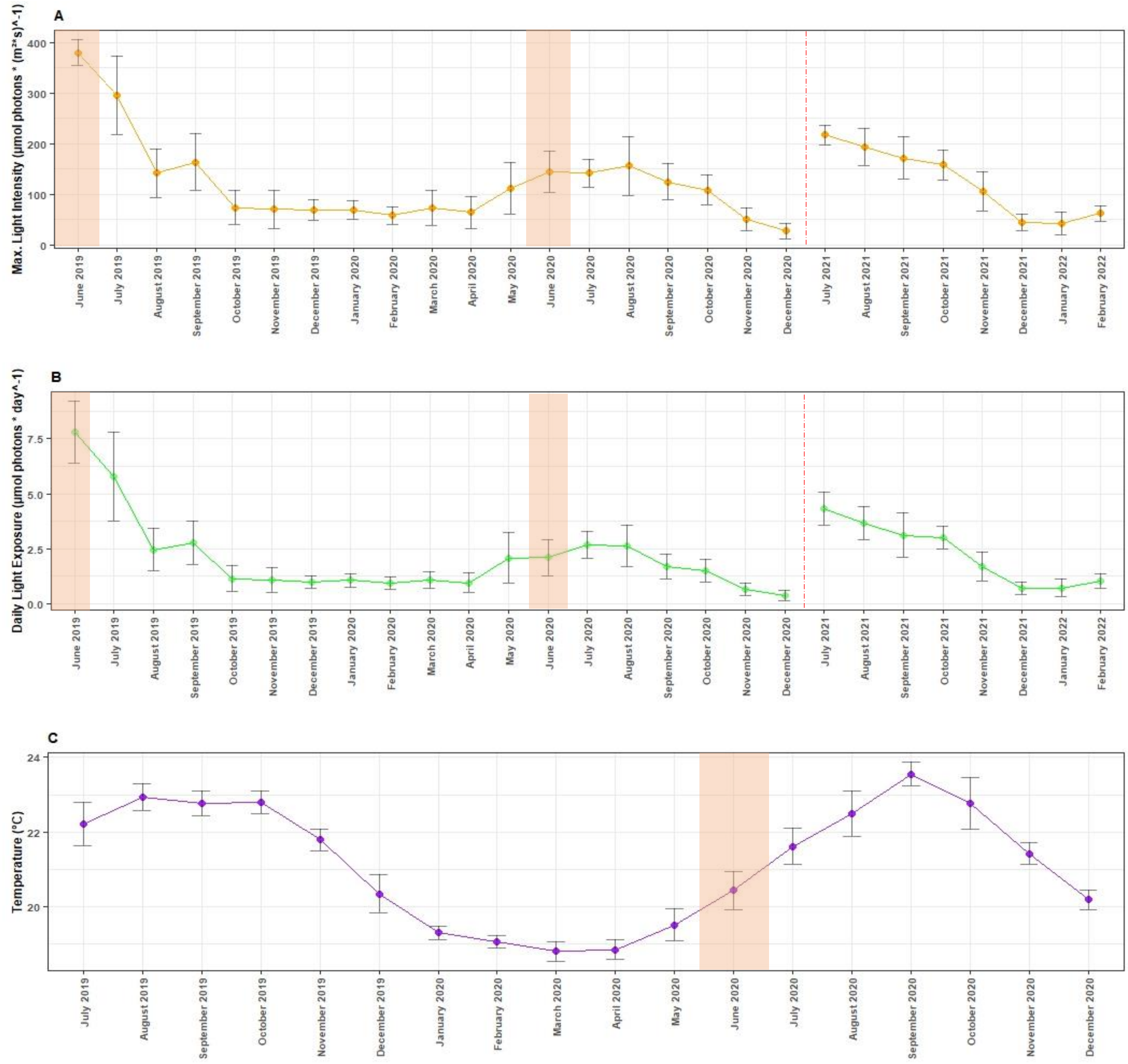


Figure 2.1.2. *In situ* environmental conditions at the Caniçal rhodolith bed (20 m depth, Jun 2019-Feb 2022). (A) Mean and SE of monthly *in situ* maximum light intensity (yellow) (B) Mean and SE of monthly *in situ* daily light exposure (green). (C) Mean and SE monthly *in situ* temperatures (purple). Period that corresponds to the experimental month (June) is highlighted in orange. Separation in data continuum is marked by a red dotted line. Data recorded continuously, with data logging every 15 minutes.

Furthermore, the data show that the mean maximum light intensity in June 2021 was 262 ± 34 µmol photons m^{-2} s^{-1}, giving a mean daily light exposure (DLE) of 4,9 ± 1,1 mol photons m^{-2} day^{-1} (Figure 2.1.2. A, B). Mean temperatures in June are 20,4 ± 0,5°C (Figure 2.1.2. C).

Data downloaded from the Marine Heatwave Tracker (http://www.marineheatwaves.org/tracker.html, Schlegel 2018) showed that MHWs have increased in both frequency and severity of events over the last decades at the sampling location (Figure 2.1.3. A).

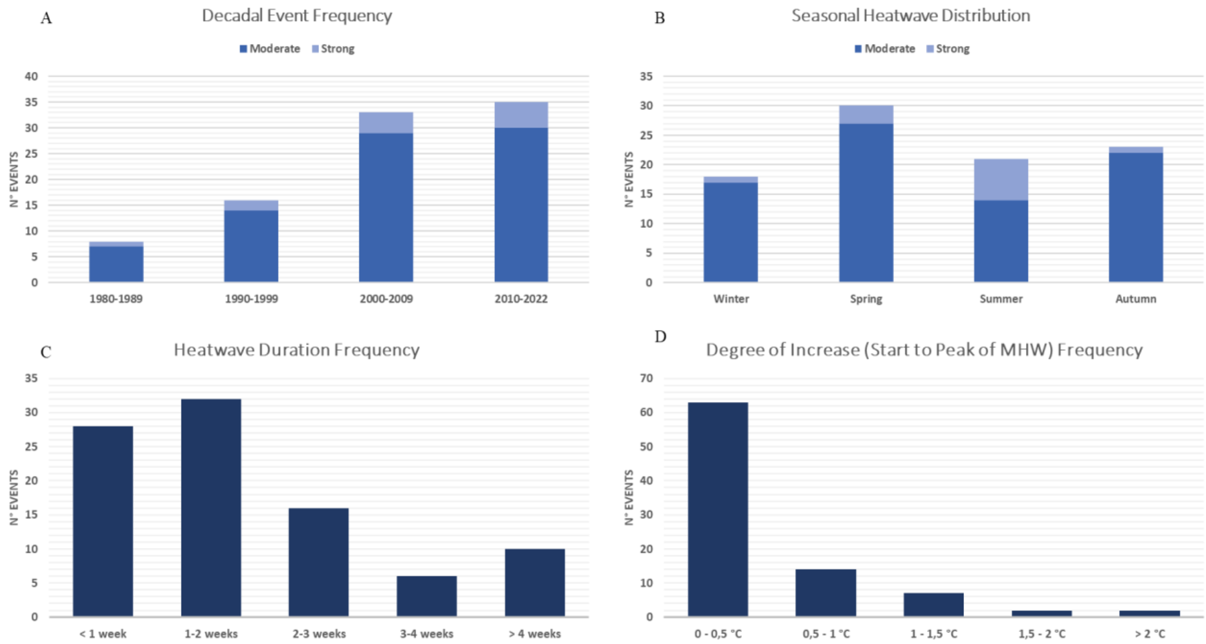


Figure 2.1.3. Characteristics of marine heatwaves at the location of the Caniçal (Madeira, Portugal) rhodolith bed (1982 to 2022), obtained from the Marine Heatwave Tracker program (http://www.marineheatwaves.org/tracker.html, Schlegel 2018). (A) Decadal heatwave frequency and severity and (B) seasonal heatwave frequency (moderate events: 1-2 times above threshold) and strong events: 2-3 times above threshold, are counted as defined by Hobday et al. 2016). (C) MHW duration (in weeks) and (D) daily temperature increase during MHW (in °C ranges).

In the last decade, 35 MHW events were recorded, with 5 of them defined as “strong”. Seasonally, the occurrence of MHWs was higher in spring and more severe (“strong”) MHWs were recorded during spring and summer (Figure 2.1.3. B). Most of the recorded MHWs exhibited a maximal daily increase of temperature of 0,5°C and usually lasted for 1-2 weeks (Figure 2.1.3. C, D).

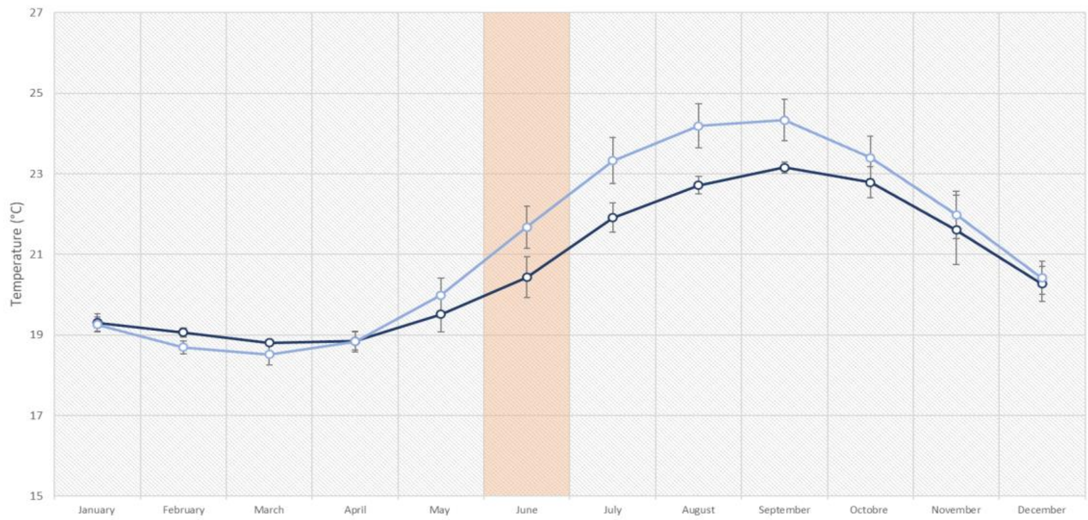


Figure 2.1.4.. Annual mean (dark blue) and threshold (light blue) temperatures obtained from Marine Heatwave Tracker, based on 30-year time series of SST data for the location of the rhodolith bed. Period that corresponds to the experimental month (June) is highlighted in orange.

Furthermore, the data available from the Marine Heatwave Tracker showed that the mean monthly temperature at Caniçal (based on SST data) ranges from 18.4 to 23.3°C (Figure 2.1.4.). Threshold temperatures, above which a temperature increase is considered a MHW (when lasting 5 days or more), range from 0.7 to 1.1°C above the mean temperature (1°C in June).

2.2. Experimental set-up and design

The experiment was performed in a seawater flow-through system at the Ramalhete Marine Station, using water input coming from the Ria Formosa (Figure 2.2.1., in blue). The incoming water went through a preliminary mechanical filtration system (Sand filter), two in-line cartridge filters (10-20 µm and 5 µm) and two UV filters (16 and 8 W). A header tank of 2000 L, equipped with a heat-pump (i-Komfort, Krispo, Hayvard), kept at constant temperature of 19.5°C allowed for the entry of water into the system. This tank fed the experimental tank setup of the 20 experimental 22-L tanks (n=5 per nutrient and temperature treatments), equipped with submersible air pumps (SICCE, Voyager Nano 2000) and aquarium heaters to control temperature for different treatments. The thermal conditions were maintained and controlled through the use of the AquaTronica program (version 9.1.0.), a central controller (Aquatronica Controller Evolution, ACQ115), connected to several interfaces (Aquatronica Interface, ACQ210N-TL) and linked to thermal sensors (Aquatronica Temperature Probe, ACQ-001S), positioned in each tank. These retrieved data every 30 minutes, allowing for the monitoring of the temperature in the tank set-up and controlling all heaters simultaneously through the centralised program (Figure 2.2.1. in orange).

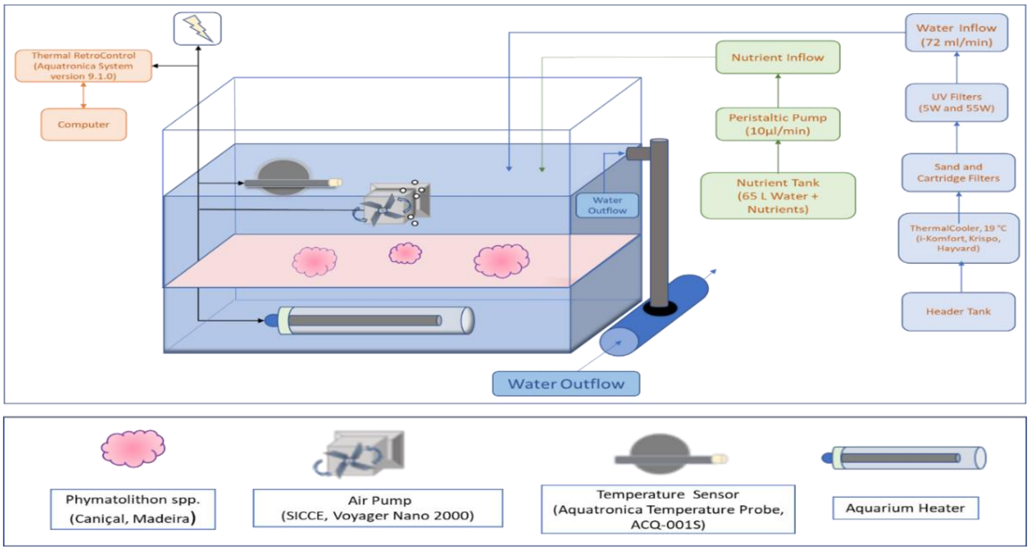


Figure 2.2.1. Experimental set-up of a flow-through mesocosm tank (a total of n=20 tanks were used in the experiment), including input and output of all components

Nutrient-enriched conditions were obtained through input of nutrient enriched water from a stock tank of 65 L, using a peristaltic pump (Figure 2.2.1., in green). To maintain target nutrient levels (15 µM of NH_4CL, 40 µM of KNO_3, 10 µM of K_2HPO_4), the pump rate and the water inflow to the individual tanks was adjusted (complete 22-L tank turnover in 5 h). Treatments with lower (ambient) nutrient concentrations were those receiving the water from Ria Formosa, without nutrient addition, which according to previous measurements exhibit oligotrophic conditions.

The experimental conditions (light, temperature) were chosen based on the above-detailed analysis of *in situ* environmental conditions and MHW characteristics for the sampling location and experimental period (June). The 35-day experiment consisted in a pre-acclimation period (1 week), during which the rhodoliths in the nutrient-enriched treatments were acclimated to the higher nutrient levels, at a stable temperature of 20.5°C, after which in some treatments temperature was changed to simulate a realistic MHW (14 days above threshold, max. temperature of 24.5°C), while in others the temperature was kept stable at 20.5°C during the experiment. For the MHW simulation, temperature was increased/decreased by 0.5°C on a day-to-day basis. Afterwards, the temperature was set back to the initial level (20.5°C) and maintained for another week (Figure 2.2.2.). These temperature treatments were combined with two different nutrient levels, resulting in a total of four treatments:

1) Control-treatment (primary control): Ambient nutrient concentration and constant temperature (as recorded during sample collection and obtained from Marine Heatwave tracker). Baseline temperature during the month of June is defined as 20,5°C.

2) NE-treatment (+ NE): Exposure to increased nutrient concentration at a constant temperature of 20.5°C (as in Control-treatment).

3) MHW-treatment (+ MHW): Simulating a heatwave event (14 days above threshold temperature), characteristic for the location of the rhodolith bed, with max. temperature at 24.5°C for 4 days.

4) MHW+NE-treatment: Combined exposure to MHW and nutrient-enriched conditions.

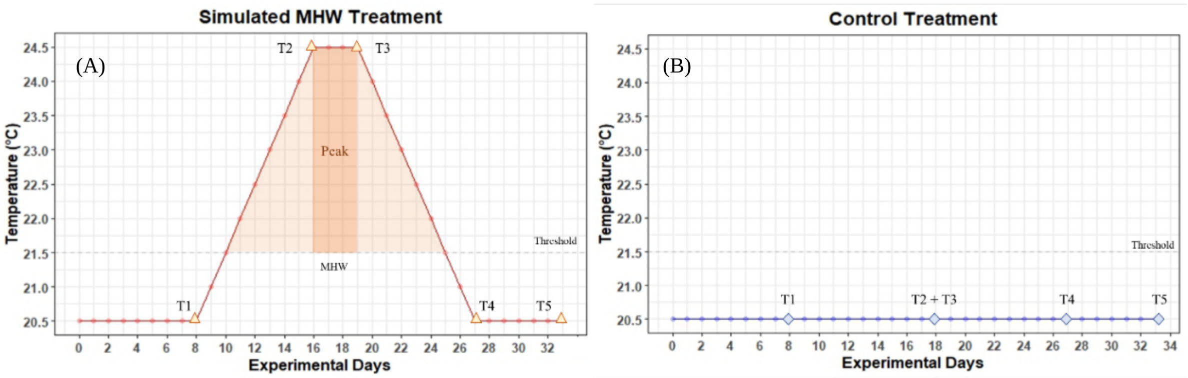


Figure 2.2.2. Schematic overview of the daily temperature profile of the different treatments. (A) MHW treatment, orange zone represents area at which temperature was above the recorded threshold, and (B) Control treatment, kept at a stable temperature. T1 to 5 represent sampling days, during which incubations were done to obtain physiological data.

2.3. General Physiological Characterization

To obtain information regarding the physiological status of the organisms at the time of collection, a photosynthesis-irradiance curve (P-I curve) was measured before starting the main experiment. Based on the P-I curve, a number of parameters were obtained: photosynthetic efficiency (initial slope of the curve, named α), maximum photosynthetic rate at saturating irradiance (Pmax), minimum saturating light intensity (Ik)) and light compensation point, where Net Photosynthesis equals zero (Ic) (Gilbert, Wilhelm and Richter, 2000). Incubations took place, using an individual incubation set-up, allowing for 5 individuals to be measured at a time under constant temperature conditions, provided by an outer chamber jacket, connected to a thermocirculator (Figure 2.3.1.). Using filtered seawater (GFF filter, 0,47 µm), each rhodolith was put inside a sealed chamber (150 mL volume) mounted on a magnetic stirrer for internal circulation of the water volume, positioned under light filters to control full exposure to the LED lights of the set-up (Lexman LED, 20W, 6500K). Using an oxygen meter (Fibox 4, PreSens, Germany), oxygen concentrations were measured under both light and dark incubating conditions.

Figure 2.3.1. (Left) Set-up of 5 incubation chambers and regulating lights. (Right) Acrylic incubation chamber, set on magnetic stirrer, holding a rhodolith. Light intensity is set by height of light bulb and covering filter (above chamber)

For the P-I curves, rhodolith individuals (n=5) were exposed to a range of increasing light intensities (6, 11, 24, 46, 128, 260 and 525 µmol photons m^{-2} s^{-1}), where initial and final oxygen measurements were taken. At the beginning and the end of the light response curve, the rhodoliths were incubated in darkness, to determine the dark (RD) and post-illuminatory (or light) respiration rate (RL), respectively. Gross photosynthetic (GPP) rate was calculated by adding respiration rates (average of RD and RL) to the net photosynthetic rates (NPP) (Annex Table 6). The maximum photosynthetic rates (Pmax) were obtained from the average maximum values above saturating irradiance. The photosynthetic quantum efficiency (α) was estimated from the initial slope of the light response curve by linear least-squares regression analysis. Irradiance of compensation (Ic) was estimated from the ratio RD/α and the saturation irradiance (Ik) was estimated as the ratio of Pmax/α.

To determine the general thermal physiology of the rhodoliths, thermal-performance curves (TPC) were measured prior to the main experiment, to assess the organisms' responses to short-term changes in temperature. The temperatures used for the TPC ranged from the experimental baseline and above (20.5-26.5°C), increasing the experimental temperature every 24 hours by 1,5°C and monitoring the physiological response in photosynthesis, respiration, and calcification through daily incubations at the respective temperature. Photosynthesis and respiration measurements were performed for 1 hour each, as described above, at the holding light intensity of 80 µmol photons m^{-2} s^{-1} (Annex Table 6). Light and dark calcification rates were determined through alkalinity measurements of water samples that were taken before and after each 1-hour incubation in light and dark, respectively, and poisoned with $HgCl_2$ to estimate calcification rates (Annex Table 7). These were determined by using the alkalinity anomaly principle (Smith and Kinsey, 1978), which states the ratio of two equivalents of total alkalinity for one mole of $CaCO_3$. Using the Gran titration method, duplicate analyses were done for each sample (Hansson and Jagner 1973). The samples were titrated with HCl 0.1 M, using an automated titration system (Titroline 7000, SI Analytics, Mainz, Germany), coupled to an autosampler (TW alpha plus, SI Analytics, Mainz, Germany). Data was captured and processed with a computer, using 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 (CRMs, supplied by the Marine Physical Laboratory, Scripps Institution of Oceanography, United States). The oven-dried weight of the rhodoliths (48 h at 60°C) was used to normalize the physiological rates.

2.4. Photosynthesis, respiration and calcification measurements during the mesocosm experiment

Rhodolith physiological responses to the different treatments were monitored by incubating the same individuals at different time points during the experiment (see Figure 2.2.1.), as described above. The 1-hour incubations were performed at the respective experimental temperature and at 80 µmol photons m^{-2} s^{-1} and the physiological parameters were calculated from initial and final O_2 and alkalinity measurements (see Annex Table 6 and 7) According to Gazeau et al. (2015), it is assumed that total alkalinity in calcifying organisms is impacted also by processes other than calcification: assimilation and remineralization of 1 mol of ammonia, nitrate or phosphate ions respectively leads to an increase or decrease of 1 mol in total alkalinity. As so, calcification rates assuming that only net calcification affects alkalinity have to be corrected by including variations of NH_4^+, NO_3^-, and PO_4^{3-} concentrations. Thus, to correct the changes due to nutrient assimilation, samples of the incubation water (before and after incubation) of the nutrient-enriched treatments were also collected for nutrient analysis.

2.5. Nutrient analysis

Water samples for nutrient analysis were collected on a weekly basis from the treatment tanks and the incoming seawater, filtered through cellulose acetate filters, and stored at -20°C. The concentrations of ammonium, nitrate, and phosphate in the seawater were determined in a loop-flow analyser (µMac-1000; Systea, Anagni, Italy). Ammonium concentration was determined using the hypochlorite method and nitrate concentration was determined using the Cd-Cu column reduction method. Phosphate was determined using the molybdate and ascorbic acid colorimetric method. The detection limit for ammonium was 0,10 ± 0,03 µM, 0,04 ± 0,01 for nitrate µM, and 0,07 ± 0,02 µM for phosphate.

Table 2.5.1. Mean ± SE nutrient concentrations (in µM/L) of weekly sampling (n=5 per nutrient treatment and week) during the experimental period. Incoming represents the nutrient values from the seawater input coming from Ria Formosa, feeding all tanks. NE- nutrient enriched treatment.

	Incoming	**Control**	**NE**	**MHW**	**MHW x NE**
[NH_4^+]	0	0	6,01 ± 6,69	0	0
[PO_4^{3-}]	0,77 ± 0,28	1,64 ± 2,88	8,04 ± 2,20	0,58 ± 0,30	6,86 ± 2,56
[NO_3^-]	3,53 ± 0,92	1,67 ± 1,44	38,33 ± 11,1	1,08 ± 0,75	33,28 ± 7,89

2.6. Statistical analysis

Thermal performance data was processed through a Gaussian model fit, as:

$$f(x) = y = a \times \exp\left(-0{,}5 \times \left(\frac{x - x_0}{b}\right)^2\right)$$

With x_0 being the thermal optimum, a being the maximum value and b being the thermal range/breadth. Both, thermal optimums and ranges obtained were analysed through a One-way ANOVA statistical test, to identify potential differences in thermal performance between the different physiological processes.

The experimental design of this study was made to follow physiological response over time of two independent stressors and their combination, with sampling done one the same rhodolith individuals in a repeated way over the experimental period. A two-way repeated measures ANOVA was used, with time and nutrient concentration as fixed parameters to evaluate interactive and isolated effects. These analyses were done separately for the Control treatments (+/- NE) and the MHW-treatments (+/- NE), as the former time has 4 levels and was kept at a constant temperature, while the latter time had 5 levels and the temperature was changed (increased/decreased) during the experiment. All data was tested for heteroscedasticity, using a Levene's test and normality using a Shapiro-Wilk's test. Tukey's HSD test was used in significantly different data to determine significant groupings.

3. Results

3.1. General Physiological Characterization

The parameters derived from the P-I curves showed that *Phymatolithon* sp. from Caniçal reached a photosynthetic maximum of 0,61 µmol O_2 g^{-1} DW h^{-1} and that its photosynthesis saturated at 46 µmol photons m^{-2} s^{-1} (Table 3.1.1).

Table 3.1.1. Photosynthesis-Irradiance- Curve parameters of *Phymatolithon* sp..

Maximum Gross Photosynthesis (µmol O_2 g^{-1} h^{-1})	**Photosynthetic Quantum Efficiency** (α, in µmol O_2 g^{-1} h^{-1})	**Respiration** (µmol O_2 g^{-1} h^{-})	**Ik** (Saturation Irradiance) (µmol photons m^{-2} s^{-1})	**Ic** (Compensation Irradiance) (µmol photons m^{-2} s^{-1})
0,605 ± 0,097	0,068 ± 0,014	0,157 ± 0,081	46 ± 3	14 ± 1

.

Furthermore, the species' thermal physiology indicated a thermal optimum of ~24°C with NPP having the lowest thermal optimum, and with a thermal breadth/range of ~5°C for photosynthesis and respiration, while calcification exhibited a narrower range of ~2°C (Table 3.1.2., Annex Figure 2).

Table 3.1.2. Thermal performance of *Phymatolithon* sp. physiological parameters, with predicted Gaussian fitted values of mean optimal temperatures (T° Opt), and mean temperature ranges. Post-hoc results of the One-way ANOVA on the Thermal optimum (T°-Opt.) and Thermal Range, showing differences between physiological parameters are represented by uppercase letters (data are shown as mean±SE).

	GPP	NPP	Respiration	Light Calcification	Dark Calcification
Thermal Opt. (°C)	23,71 ± 0,30 ^{AB}	23,10 ± 0,74 ^{B}	24,56 ± 0,21 ^{A}	24,38 ± 0,50 ^{A}	24,40 ± 0,37 ^{A}
Thermal Range (°C)	5,13 ± 1,55 ^{A}	4,88 ± 1,61 ^{A}	4,52 ± 0,54 ^{A}	2,53 ± 0,30 ^{B}	1,49 ± 0,42 ^{B}

Only the NPP showed a difference in thermal optimum with respiration and calcification- However, a clear difference in thermal range was found between primary production and calcification parameters, with a smaller range of the latter (Table 3.1.2.).

3.2. Rhodolith physiological responses to MHWs and nutrient enrichment

Most measured parameters in the control group varied significantly during the experiment, with exception of NPP (Table 3.2.1.). In this group, nutrient enrichment did not exert significant effects on any of the measured parameters, except for the daily net calcification (DNC). Moreover, the variability in respiration and calcification responses differed between nutrient conditions. Similarly, in the MHW treatments, all measured parameters showed a significant variability during the experiment, in relation to the temperature changes during MHW simulation. Here, nutrient condition affected the response to the MHW for GPP, DNP, as well as light and daily net calcification (Table 3.2.1.).

		Days		Nutrients		Days x Nutrients	
		F	**p-value**	**F**	**p-value**	**F**	**p-value**
Control	GPP	**11,013**	**<0,001**	2,029	0,192	1,791	0,176
	NPP	2,732	0,066	2,829	0,131	0,788	0,512
	Respiration	**31,539**	**<0,001**	0,564	0,474	**5,47**	**0,005**
	DNP	**7,796**	**<0,001**	2,199	0,176	1,37	0,276
	Light Calcif.	**23,607**	**<0,001**	2,052	0,19	**11,683**	**<0,001**
	Dark Calcif.	**17,812**	**<0,001**	0,14	0,718	**11,708**	**<0,001**
	DNC	**9,485**	**<0,001**	**6,165**	**0,038**	**4,057**	**0,019**
MHW	GPP	**6,587**	**<0,001**	1,266	0,293	**3,01**	**0,032**
	NPP	**3,009**	**0,032**	0,826	0,39	1,629	0,191
	Respiration	**6,865**	**<0,001**	1,952	0,2	2,191	0,092
	DNP	**5,738**	**0,001**	1,169	0,311	**2,777**	**0,044**
	Light Calcif.	**12,51**	**<0,001**	0,00231	0,963	**3,102**	**0,031**
	Dark Calcif.	**13**	**<0,001**	0,411	0,542	0,767	0,556
	DNC	**6,936**	**<0,001**	0,0834	0,78	**3,72**	**0,015**

Table 3.2.1. Results of repeated measures two-way ANOVA of physiological parameters, showing effect of time and nutrient concentration for Control treatments (4 levels of time) and MHW treatments (5 levels of time). P-values < 0,05 are highlighted in blue.

Under stable temperature conditions (Control), net photosynthesis remained stable, showing no variation in trend through time, with response rates never increasing more than 30% from the initial values (Figure 3.2.1.). GPP response across the experimental period followed the same trend in both control and NE treatment, as both had a ~1 µmol O_2 g^{-1} h^{-1} increase from beginning to end of experimental time in both treatments (Annex Table 2). Similar trend was found the case for NPP in both treatments (Figure 3.2.1., Annex Table 2).

MHW-exposed rhodoliths showed slight, but significant variations in both GPP and NPP in response to the temperature changes, which were affected by nutrient enrichment only in the case of GPP (Table 3.2.1., Annex Table 3). Peak heatwave temperatures induced solely minimal responses in both parameters that disappeared post-MHW (Figure 3.2.1., Annex Table 4).). GPP responses over time differed between nutrient treatments, most likely due to the pronounced increase from peak to post-MHW in the MHWxNE treatment (Annex Table 4).

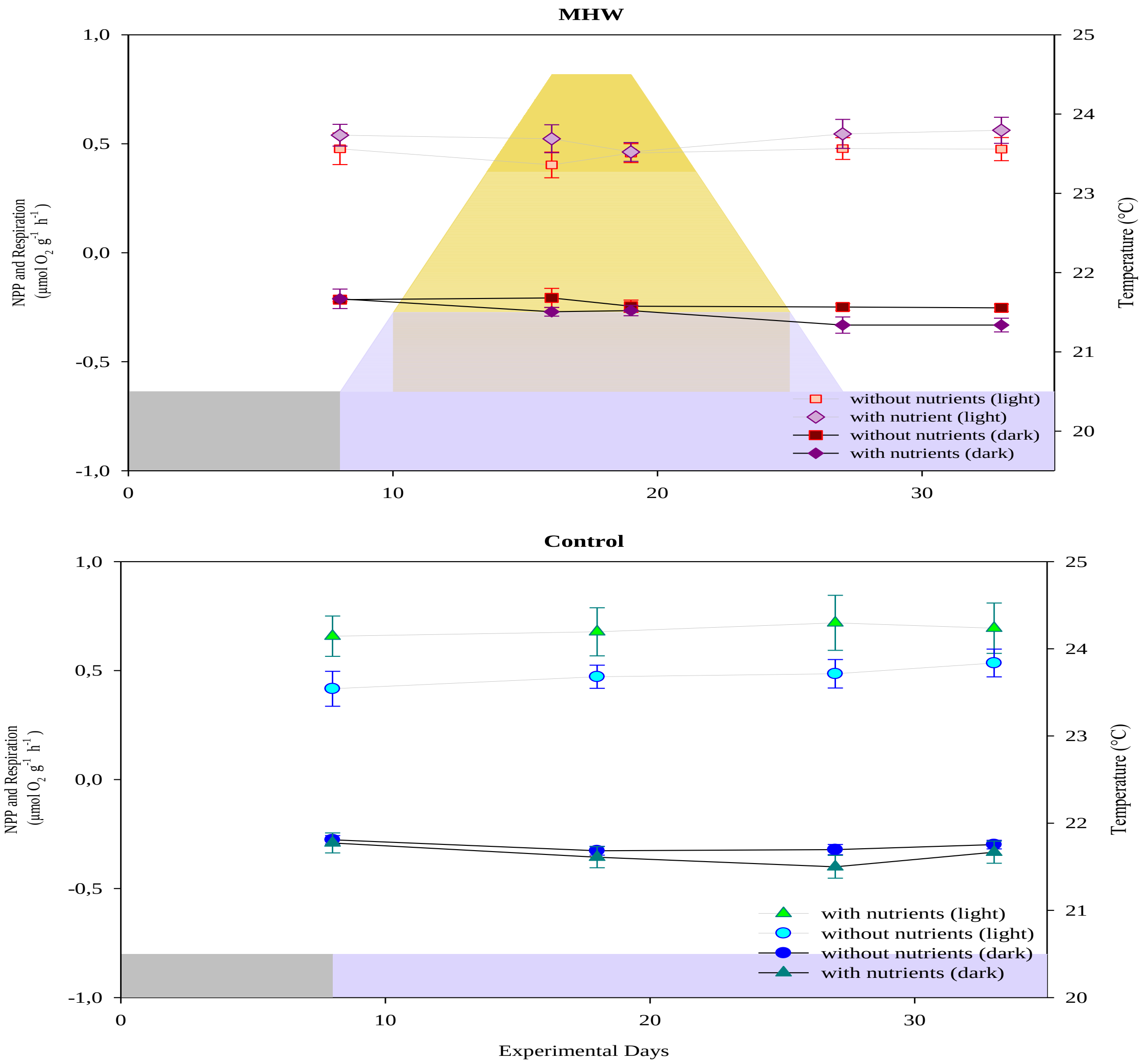


Figure 3.2.1. Metabolic net primary production (NPP) and respiration responses of *Phymatolithon* sp. to the experimental treatments. Pre-acclimation to different nutrient conditions is indicated in gray, experimental "normal" temperatures (purple) and above-threshold MHW temperatures (yellow). "MHW": heatwave temperatures-imposed group, with 5 time points; "Control": control group, with 4 time points. Data represent the mean and standard error of each group (n =5) per time point (t1 to 5 in MHW, t1 to 4 in Control).

Respiration varied significantly over time in the constant temperature treatment (Control at 20.5°C), a variation that was depending on nutrient conditions (Figure 3.2.1., Table 3.2.1., Annex Table 2).

The MHW event had significant impact on the respiration in the MHW group, a response that did not differ between nutrient conditions (Table 3.2.1., Annex Table 4).

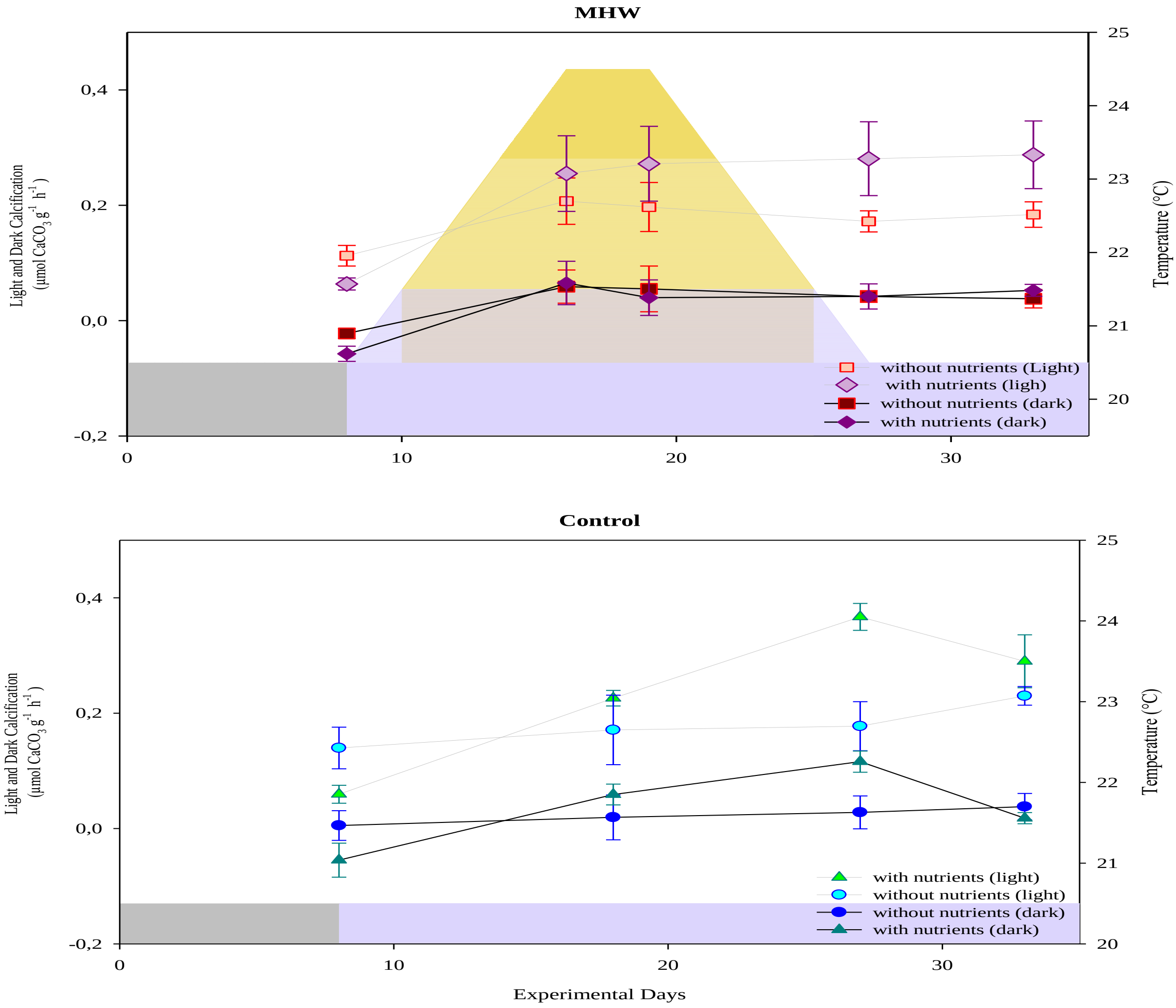


Figure 3.2.2. Responses of light and dark calcification rates of *Phymatolithon* sp. to the different treatments. Pre-acclimation to different nutrient conditions is indicated in gray, experimental "normal" temperatures (purple) and above-threshold MHW temperatures (yellow). "MHW": heatwave temperatures-imposed group, with 5 time points; "Control": control group, with 4 time points. Data represent the mean and standard error of each group (n =5) per time point (t1 to 5 in MHW, t1 to 4 in Control).

The constant temperature treatment exhibited significant variability in light calcification that differed between nutrient treatments. While under ambient nutrient conditions light calcification increased slightly, the nutrient-enriched treatment induced a significantly larger increase (~300% above initial values; Figure 3.2.2., Annex Table 2, 3).

Light and dark calcification also responded significantly to MHW temperatures, a response that was depending on nutrient condition for light calcification, but not dark calcification (Table 3.2.1.) . However, in both nutrient treatments, an increase of calcification rates in response to increased temperatures was found, which stabilised and maintained high rates even under post-MHW conditions (Figure 3.2.2.). Though, nutrient enrichment resulted in a significantly higher increase in light calcification rates (300% increase in $CaCO_3$ production from its initial values), compared to the increase under ambient nutrient concentrations (Table 3.2.1., Annex Table 5).

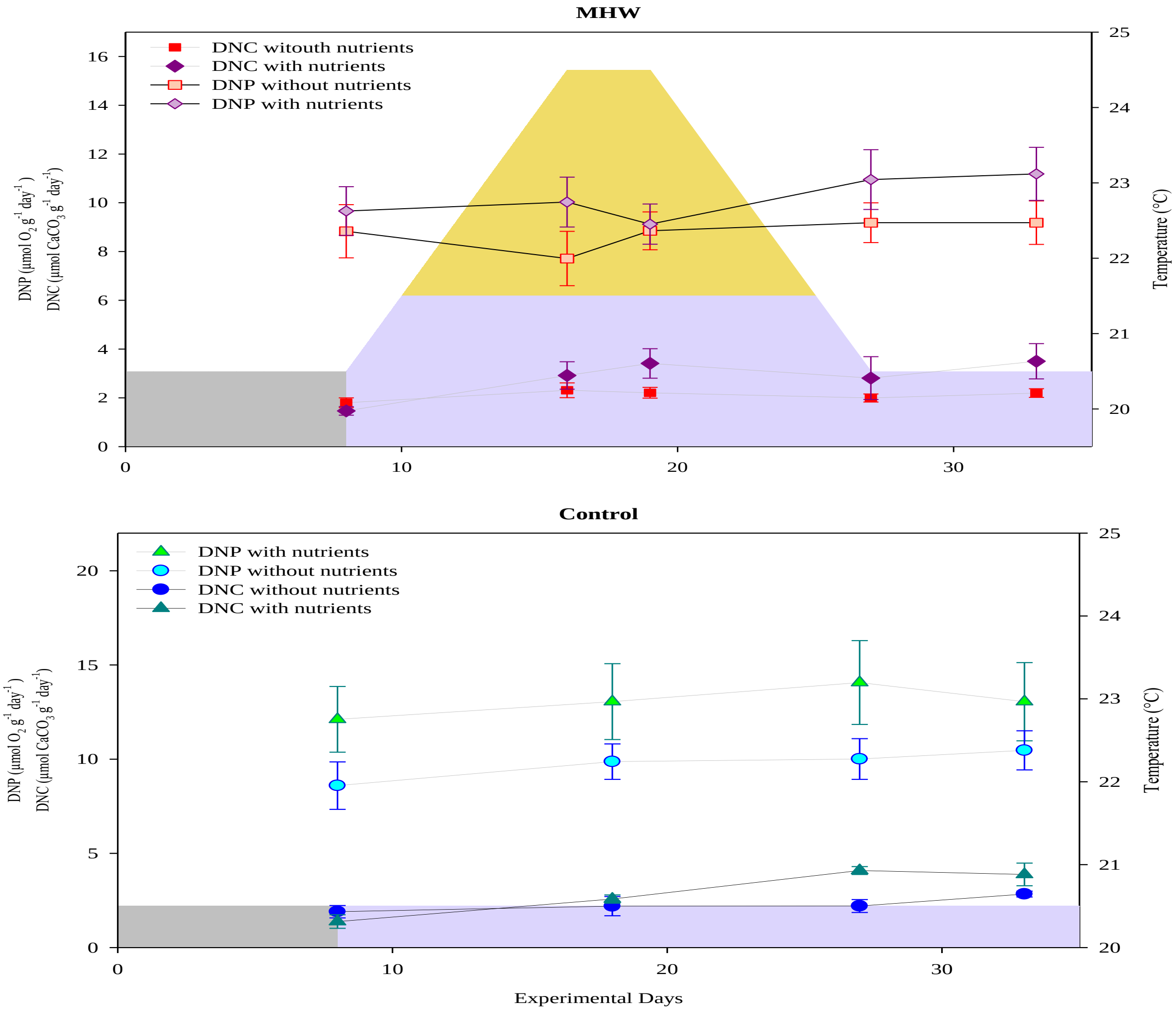


Figure 3.2.3. Responses of Daily Net primary productivity and Daily Net Calcification of *Phymatolithon* sp. to the experimental treatments. Pre-acclimation to different nutrient conditions is indicated in gray experimental "normal" temperatures (purple) and above-threshold MHW temperatures (yellow). "MHW": heatwave temperatures-imposed group, with 5 time points; "Control": control group, with 4 time points. Data represent the mean and standard error of each group (n =5) per time point (t1 to 5 in MHW, t1 to 4 in Control).

DNP varied slightly but significantly during the experiment under constant control conditions, independently from the nutrient condition (Table 3.2.1 and Figure 3.2.3.). On the other hand, DNC showed a significant variation which was depending on nutrient conditions, as well as a general significant nutrient effect (Table 3.2.1., Annex Table 2).

In the MHW group, DNP and DNC varied significantly with temperature, a variation that differed depending on nutrient condition. DNP exhibited inverse responses between the two treatments during the peak MHW temperatures, while the response pattern of DNC was similar between nutrient treatments, but with a larger increase at the MHW peak and subsequent higher post-MHW DNC values (Figure 3.2.3., Annex Table 4).

4. Discussion

The simulated marine heatwave event induced responses in all parameters, but induced a significant increase only in dark calcification and consequently, daily net calcification. The nutrient enrichment conducted under marine heatwave conditions had a synergistic effect on daily net photosynthesis, but most importantly on light calcification as well as daily net calcification. In this group, the initial increase in response rates due to increased temperatures found in the control marine heatwave treatment was amplified, resulting in significantly different rates from the initial ones throughout the experimental time. In contrast, under stable temperature conditions, nutrient enrichment induced increased respiration and calcifying responses in both light and dark condition, leading to a significant effect on the integrated daily net calcification.

Temperature is well known to be a limiting factor to most biological reactions, meaning macroalgal metabolic processes are directly linked to temperature variations (Lüning 1991, Rendina et al. 2019, 2020). However, the response of coralline algal species has been highly variable within studies: certain temperate European species were never responsive to increased temperatures (Wilson et al. 2004, Martin et al. 2013, Rendina et al. 2019), whereas others had their O_2 production reduced by increased temperatures (Tanaka et al. 2017, Page et. al. 2021). In this study, photosynthesis and respiration showed little to no increase in rate, with stable response all along the marine heatwave treatment. It is most likely that peak temperatures ranged too close to both physiological thermal optimums and maximum *in situ* temperatures recorded (Figure 2.1.2., Figure 2.1.3.). The minimal response in photosynthesis and respiration were similar to sistering species of *Phymatolithon* sp., *P. lusitanicum* (Pardo et al. 2014), which responded with only short-term effects of primary production from strong MHW event (Schubert et al. 2021). Respiration in this study showed to be less responsive to increased temperature as in other temperate coralline algae species, which were found to have increased respiration rates with increased temperatures, or in some cases, post-marine MHW (Ichiki et al. 2001, Martin et al. 2006, Rendina et al. 2019). Several studies note the effects of increased temperatures over O_2 production rates as non-significant variations (Ichiki et al. 2001, Martin et al. 2006, Steller et al. 2007, Martin et al. 2013, Vasquez-Elizondo and Enriquez 2016, Rendina et al. 2019, Schubert et al. 2021). Rises in temperature above thermal optimums can cause negative physiological responses in rhodoliths, but temperature increases within thermal range tend to produce a positive response (Rendina et al. 2019, Cornwall et al. 2019). While the MHW simulated during this experiment did not induce any negative effects, it cannot be excluded that marine heatwaves of longer duration and/or intensity (as predicted in the near future) (Oliver et al. 2018, 2019) would negatively impact rhodolith physiology and consequently rhodolith productivity.

Calcification showed to be more responsive to increased temperatures, an effect which was also found in other studies (Vasquez-Elizondo and Enriquez 2016, Schubert et al. 2019). This was expected, as the thermal performance curve results showed that calcification had a significantly smaller thermal range (~2°C) than primary production (~4°C) for similar thermal optimums (Table 3.1.2.). This implied that calcification altogether would be physiologically more responsive to variations of temperatures. Even though both light and dark calcification showed similar response pattern to the MHW, the induced increase with respect to the pre- marine heatwave (initial) values was significant only for dark calcification. Dark calcification has been known to show higher response variability, due to already minimal precipitation rates (Kolzenburg et al. 2019). However, the response in both light and dark calcification differed from other studies reporting negative MHW effects on coralline algae and rhodoliths, which were either null or negative (Rendina et al. 2019, Ragazzola et al. 2021, Schubert et al. 2021). However, it was consistent with the found positive effect in *P. lusitanicum* when exposed to a moderate temperature increase, as occurring in the natural environment of this species (Schubert et al. 2021). This, together with the measured thermal optimum for calcification from the thermal performance curves, supports the idea stated above that the intensity of the simulated MHW (i.e., max. temperature of 24.5°C) did not represent a stressful condition in relation to the thermal acclimation of *Phymatolithon* sp. from Madeira. Coralline species and particularly rhodoliths are known to have a wide response in calcification, from large ranges in light calcification to variating response from lack of irradiance (Chisholm 2000, Schubert et al. 2019, 2022). This divergence is also reflected in the variability in response to thermal stress, which has been shown to be both species- and habitat-specific (Martin and Hall-Spencer 2017, Cornwall et al. 2019, Qui-Minet et al. 2021).

Nutrient enrichment had generally no significant effect on most physiological parameters, except for the daily net calcification in control temperatures. This is related to the increase in both light and dark calcification: the same trend in increased rate and the same variability was obtained, as shown by the significant difference of in response from initial calcifying rate in all calcification parameters (Annex Table 4). Whilst photosynthesis was unchanged, respiration a slight increase due to nutrient enrichment in control temperatures. This might be related to evidence, showing that increased nitrogen assimilation is usually coupled with increased respiratory rates (Turpin 1988). Rhodoliths are considered to have low-nutrient requirements, though have been shown to respond to enrichment/pulses with increase nutrient uptake (Schubert et al. 2019). This positive response to eutrophic conditions is consistent with very few other observed responses in the small number of studies of nutrient enrichment effect on coralline algae, as only tropical Pacific *Porolithon onkodes* and *L. kotschyanum* were reported to have positive response in respiration and calcification to respectively nitrogen-based nutrient enrichment and pulsed nutrient enrichment of phosphate and nitrate (Johnson and Carpenter 2018, Shayka et al. 2018). Interestingly, in the same study, Shayka et al. (2018) reported differences in both primary production and calcification depending on the nature of nutrient enrichment.

This difference is representative of a bigger consensus that no clear general trend or effect can be described for coralline algae response to eutrophic conditions, as reported effects come from studies lacking similarity in nutrient input. As so, most species showed to have negative or no responses under either in situ (Björk et al. 1995, Steven 2000, Koop et al. 2001) and experimental conditions (Shayka et al. 2018, Schubert et al. 2019, Bélanger and Gagnon 2020), though nutrient concentrations or nutrient type differed among studies. This is supported by reported responses of *Porolithon onkodes*, which were shown to be completely different depending on the nutrient experimentally used (Tanaka et al. 2017, Johnson and Carpenter 2018). Therefore, the positive response of *Phymatolithon* sp. to nutrient enrichment, using ammonia, nitrate and phosphate, provides new information to a metabolic response yet unclear.

Under the simulated marine heatwave event, rhodoliths in both ambient and nutrient-enriched treatments had similar responses: photosynthesis and respiration showed relatively stable rates during the MHW event (Figure 3.2.1). Calcification responded positively to the initial temperature increase of the heatwave and maintained higher rates even during post-marine MHW (Figure 3.2.2.). However, nutrient input intensified the positive increase from initial rates. Furthermore, previously insignificant differences between initial, peak heatwave and post heatwave rates turned significant with nutrient input (Annex Table 4). The minimal effect of the MHW event on daily net photosynthesis and, more importantly, the positive effect on light calcification and daily net calcification, were amplified. This suggests a synergistic effect between nutrient enrichment and MHW, inducing higher response rates with increased temperatures. This response is contrary to the only other study to date that determined the relationship between a MHW and nutrient enrichment (Schubert et al. 2019). These authors observed that a combination of these treatments affected significantly the Brazilian subtropical species *M. erubescens* and *L. crispatum*, with combined additive negative effects in net productivity. Thus, the positive response obtained in response to this marine heatwave event in presence of nutrients, in both daily net photosynthesis and daily net calcification, indicates resilience of the temperate species *Phymatolithon* sp. to variable environmental conditions. It also provides evidence of the uncoupling between primary productivity and calcifying physiology, a species-specific response known to occur during thermal stress (Schubert et al. 2019, 2021, 2022). This implies that during marine heatwaves, with so far recorded characteristics at the sampling location (see Figure 2.1.3), carbonate production of the Caniçal rhodolith beds would not be negatively affected and might increase and eutrophic conditions might intensify this effect.

Exposure to both increased nutrient input and/or MHWs is set to be a reality for all coastal habitats, with alterations bringing stress responses of coralline species outside of the range of their capabilities

(Boesch 2002, Gouvéa et al. 2017, Frölicher et al. 2018). Responses observed in this study show that present realistic extreme environmental conditions could affect positively calcification of *Phymatolithon* sp., with no effect on primary production, meaning Madeiran rhodolith beds would survive and thrive if intensity and severity of these events were kept unchanged. This is consistent with responses of the sister species *Phymatolithon lusitanicum*, as well as several other temperate species that also showed resilience to singular strong marine heatwave events (Martin et al. 2006, Tanaka et al. 2017 Rendina et al. 2019, Schubert et al. 2021). Though, as models predict increased severity and frequency of marine heatwave and eutrophication events, it would be necessary to extend this study with future projections as to extend these factors outside of optimal ranges (Boesch 2002, Frölicher and Laufkötter 2018). Oligotrophic regions such as the Madeiran Coast, much like most North Atlantic shallow waters, are in constant threat of disruption from possible eutrophication events brought by anthropogenic and natural sources, creating direct physiological and indirect ecosystem impacts (Barbera et al. 2003, Grall and Hall-Spencer 2003, Hall-Spencer et al. 2006). If similar resistance to further increased severity in these factors was possible, rhodolith beds comprised of *Phymatolithon* sp. would be able to maintain their capacity as major carbonate producers; ecosystem services provided by these beds would be kept, though indirect negative effects due to environmental changes on the associated community cannot be excluded, which in turn could affect the performance of the rhodolith bioengineers.

5. Conclusion

As marine environments are increasingly exposed to threatening environmental conditions, it is necessary to understand their physiological resilience to extreme events This study provides the first analysis of combined marine heatwave and nutrient enrichment response from a temperate North Atlantic species. The positive calcification response obtained when exposed to a strong summer marine heatwave event, nutrient enrichment and combination of these factors is a sign of the adaptative resilience of *Phymatolithon* sp. The outcome of this study predicts that present extreme conditions would affect positively the Madeiran rhodolith beds, allowing for greater carbonate production. With settlement and nursery of many species, their ecological utility as carbon sinks is high and often overlooked. Their presence in coastal benthic communities is major, as they therefore contribute directly to global carbon and calcium cycles (McCoy and Kamenos 2015). The outcome of this study therefore assures that this ecosystemic role will continue to be fulfilled, in presence of present day of anthropogenic local stress and global climate change. The direct physiological effect of thermal and nutrient extreme events assessed in this study implied does not however answer to the indirect effects these factors can have. To fully understand this, analysis of response from associated biota would be necessary, as the competitive stress and shifts in trophic dynamics could influence rhodolith performance. As the frequency of eutrophic conditions and extreme thermal events increases, physiological resilience form these events would be coupled to other limiting environmental factors (i.e., light availability, pCO_2) (IPCC, 2013). As relationship between biotic and abiotic stressors is not yet fully understood, it would be necessary, in future research, to provide extensive analysis of the response from coralline algae to all limiting factors, comprising exposure to present and future abiotic stresses, as well as interaction with associated community under these conditions.

6. Bibliography

Amado-Filho, Gilberto M., Rodrigo L. Moura, Alex C. Bastos, Leonardo T. Salgado, Paulo Y. Sumida, Arthur Z. Guth, Ronaldo B. Francini-Filho, et al. 2012. 'Rhodolith Beds Are Major CaCO3 Bio-Factories in the Tropical South West Atlantic'. *PLOS ONE* 7 (4): e35171. https://doi.org/10.1371/journal.pone.0035171.

Andersen, Jesper H., Louise Schlüter, and Gunni Ærtebjerg. 2006. 'Coastal Eutrophication: Recent Developments in Definitions and Implications for Monitoring Strategies'. *Journal of Plankton Research* 28 (7): 621–28. https://doi.org/10.1093/plankt/fbl001.

'AR5 Climate Change 2013: The Physical Science Basis — IPCC'. n.d. Accessed 16 December 2022. https://www.ipcc.ch/report/ar5/wg1/.

Barbera, C., C. Bordehore, J.a. Borg, M. Glémarec, J. Grall, J. M. Hall-Spencer, Ch. de la Huz, et al. 2003. 'Conservation and Management of Northeast Atlantic and Mediterranean Maerl Beds'. *Aquatic Conservation: Marine and Freshwater Ecosystems* 13 (S1): S65–76. https://doi.org/10.1002/aqc.569.

Bélanger, David, and Patrick Gagnon. 2020. 'Low Growth Resilience of Subarctic Rhodoliths (Lithothamnion Glaciale) to Coastal Eutrophication'. Marine Ecology Progress Series 642 (May): 117–32 https://doi.org/10.3354/meps13312.

Björk, Mats, Salim Mohammed, Marie Bjorklund, and Adelaida Semesi. 1995. 'Coralline Algae, Important Coral-Reef Builders Threatened by Pollution'. *AMBIO A Journal of the Human Environment* 24 (December): 502–5.

Boesch, Donald F. 2002. 'Challenges and Opportunities for Science in Reducing Nutrient Over-Enrichment of Coastal Ecosystems'. *Estuaries* 25 (4): 886–900. https://doi.org/10.1007/BF02804914.

Bosence, Daniel W. J. 1983. 'The Occurrence and Ecology of Recent Rhodoliths — A Review'. In *Coated Grains*, edited by Tadeusz M. Peryt, 225–42. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-68869-0_20.

Brown, Christopher J., Megan I. Saunders, Hugh P. Possingham, and Anthony J. Richardson. 2013. 'Managing for Interactions between Local and Global Stressors of Ecosystems'. *PLOS ONE* 8 (6): e65765. https://doi.org/10.1371/journal.pone.0065765.

Brown, V., S. C. Ducker, and K. S. Rowan. 1977. 'The Effect of Orthophosphate Concentration on the Growth of Articulated Coralline Algae (Rhodophyta)'. *Phycologia* 16 (2): 125–31. https://doi.org/10.2216/i0031-8884-16-2-125.1.

Cabioch, J. 1974. 'Un Fond de Maerl de l'Archipel de Madère et Son Peuplement Végétal.' *Bull Soc Phycol Fr 19:74–82*, 1974.

Cabral, Henrique, Vanessa Fonseca, Tânia Sousa, and Miguel Costa Leal. 2019. 'Synergistic Effects of Climate Change and Marine Pollution: An Overlooked Interaction in Coastal and Estuarine Areas'. *International Journal of Environmental Research and Public Health* 16 (15): 2737. https://doi.org/10.3390/ijerph16152737.

Chisholm, John R. M. 2000. 'Calcification by Crustose Coralline Algae on the Northern Great Barrier Reef, Australia'. *Limnology and Oceanography* 45 (7): 1476–84. https://doi.org/10.4319/lo.2000.45.7.1476.

Cornwall, Christopher E., Guillermo Diaz-Pulido, and Steeve Comeau. 2019. 'Impacts of Ocean Warming on Coralline Algal Calcification: Meta-Analysis, Knowledge Gaps, and Key Recommendations for Future Research'. *Frontiers in Marine Science* 6. https://www.frontiersin.org/article/10.3389/fmars.2019.00186.

Eggert, Anja. 2012a. ‘Seaweed Responses to Temperature’. In *Seaweed Biology: Novel Insights into Ecophysiology, Ecology and Utilization*, edited by Christian Wiencke and Kai Bischof, 47–66. Ecological Studies. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-28451-9_3.

Folt, C.L., C.Y. Chen, Marianne Moore, and J. Burnaford. 1999. ‘Synergism and Antagonism among Multiple Stressors’. *Limnology and Oceanography* 44 (May). https://doi.org/10.4319/lo.1999.44.3_part_2.0864.

Foster, Michael S. 2001. ‘Rhodoliths: Between Rocks and Soft Places’. *Journal of Phycology* 37 (5): 659–67. https://doi.org/10.1046/j.1529-8817.2001.00195.x.

Frölicher, Thomas L., and Charlotte Laufkötter. 2018. ‘Emerging Risks from Marine Heat Waves’. *Nature Communications* 9 (1): 650. https://doi.org/10.1038/s41467-018-03163-6.

Garrabou, J., R. Coma, N. Bensoussan, M. Bally, P. Chevaldonné, M. Cigliano, D. Diaz, et al. 2009. ‘Mass Mortality in Northwestern Mediterranean Rocky Benthic Communities: Effects of the 2003 Heat Wave’. *Global Change Biology* 15 (5): 1090–1103. https://doi.org/10.1111/j.1365-2486.2008.01823.x.

Gazeau, Frédéric, L Urbini, Te Cox, S Alliouane, and Jp Gattuso. 2015. ‘Comparison of the Alkalinity and Calcium Anomaly Techniques to Estimate Rates of Net Calcification’. *Marine Ecology Progress Series* 527 (May): 1–12. https://doi.org/10.3354/meps11287.

Ghedini, Giulia, Bayden D. Russell, and Sean D. Connell. 2015. ‘Trophic Compensation Reinforces Resistance: Herbivory Absorbs the Increasing Effects of Multiple Disturbances’. *Ecology Letters* 18 (2): 182–87. https://doi.org/10.1111/ele.12405.

Gilbert, Matthias, Christian Wilhelm, and Michael Richter. 2000. ‘Bio-Optical Modelling of Oxygen Evolution Using in Vivo Fluorescence: Comparison of Measured and Calculated Photosynthesis/Irradiance (P-I) Curves in Four Representative Phytoplankton Species’. *Journal of Plant Physiology* 157 (3): 307–14. https://doi.org/10.1016/S0176-1617(00)80052-8.

Gouvêa, Lidiane Pires, Nadine Schubert, Cintia Dalcuche Leal Martins, Marina Sissini, Fernanda Ramlov, Eva Regina de Oliveira Rodrigues, Eduardo Oliveira Bastos, et al. 2017. ‘Interactive Effects of Marine Heatwaves and Eutrophication on the Ecophysiology of a Widespread and Ecologically Important Macroalga’. *Limnology and Oceanography* 62 (5): 2056–75. https://doi.org/10.1002/lno.10551.

Grall, J., and J. M. Hall-Spencer. 2003. ‘Problems Facing Maerl Conservation in Brittany’, January. https://pearl.plymouth.ac.uk/handle/10026.1/1359.

Gunderson, Alex R., and Jonathon H. Stillman. 2015. ‘Plasticity in Thermal Tolerance Has Limited Potential to Buffer Ectotherms from Global Warming’. *Proceedings of the Royal Society B: Biological Sciences* 282 (1808): 20150401. https://doi.org/10.1098/rspb.2015.0401.

Hall-Spencer, J., N. White, E. Gillespie, K. Gillham, and A. Foggo. 2006. ‘Impact of Fish Farms on Maerl Beds in Strongly Tidal Areas’, January. https://pearl.plymouth.ac.uk/handle/10026.1/1351.

Hansson, Ingemar, and Daniel Jagner. 1973. ‘Evaluation of the Accuracy of Gran Plots by Means of Computer Calculations: Application to the Potentiometric Titration of the Total Alkalinity and Carbonate Content in Sea Water’. *Analytica Chimica Acta* 65 (2): 363–73. https://doi.org/10.1016/S0003-2670(01)82503-4.

Heijden, L. H. van der, and N. A. Kamenos. 2015. ‘Reviews and Syntheses: Calculating the Global Contribution of Coralline Algae to Total Carbon Burial’. *Biogeosciences* 12 (21): 6429–41. https://doi.org/10.5194/bg-12-6429-2015.

Hill, Ross, Alecia Bellgrove, Peter I. Macreadie, Katherina Petrou, John Beardall, Andy Steven, and Peter J. Ralph. 2015. 'Can Macroalgae Contribute to Blue Carbon? An Australian Perspective'. *Limnology and Oceanography* 60 (5): 1689–1706. https://doi.org/10.1002/lno.10128.

Hobday, Alistair J., Lisa V. Alexander, Sarah E. Perkins, Dan A. Smale, Sandra C. Straub, Eric C. J. Oliver, Jessica A. Benthuysen, et al. 2016. 'A Hierarchical Approach to Defining Marine Heatwaves'. *Progress in Oceanography* 141 (February): 227–38. https://doi.org/10.1016/j.pocean.2015.12.014.

Ichiki, S., H. Mizuta, H. Yasui, and H. Yamamoto. 2001. 'Effects of Irradiance and Water Temperature on the Photosynthesis and Growth of the Crustose Coralline Alga Lithophyllum Yessoense Foslie (Corallinales, Rhodophyceae)'. *Bulletin of Fisheries Sciences, Hokkaido University (Japan).* https://scholar.google.com/scholar_lookup?title=Effects+of+irradiance+and+water+temperature+on+the+photosynthesis+and+growth+of+the+crustose+coralline+alga+Lithophyllum+yessoense+Foslie+%28Corallinales%2C+Rhodophyceae%29&author=Ichiki%2C+S.+%28Hokkaido+Univ.%2C+Sapporo+%28Japan%29%29&publication_year=2001.

Ichiki, Shigeo, Hiroyuki Mizuta, and Hirotoshi Yamamoto. 2000. 'Effects of Irradiance, Water Temperature and Nutrients on the Growth of Sporelings of the Crustose Coralline Alga Lithophyllum Yessoense Foslie (Corallinales, Rhodophyceae)'. *Phycological Research* 48 (2): 115–20. https://doi.org/10.1046/j.1440-1835.2000.00193.x.

Jentsch, Anke, Juergen Kreyling, Michael Elmer, Ellen Gellesch, Bruno Glaser, Kerstin Grant, Roman Hein, et al. 2011. 'Climate Extremes Initiate Ecosystem-Regulating Functions While Maintaining Productivity'. *Journal of Ecology* 99 (3): 689–702. https://doi.org/10.1111/j.1365-2745.2011.01817.x.

Jentsch, Anke, Jürgen Kreyling, and Carl Beierkuhnlein. 2007. 'A New Generation of Climate-Change Experiments: Events, Not Trends'. *Frontiers in Ecology and the Environment* 5 (7): 365–74. https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2.

Johnson, Maggie D., and Robert C. Carpenter. 2018. 'Nitrogen Enrichment Offsets Direct Negative Effects of Ocean Acidification on a Reef-Building Crustose Coralline Alga'. *Biology Letters* 14 (7): 20180371. https://doi.org/10.1098/rsbl.2018.0371.

Kamenos, Nicholas A., Heidi L. Burdett, Elena Aloisio, Helen S. Findlay, Sophie Martin, Charlotte Longbone, Jonathan Dunn, Stephen Widdicombe, and Piero Calosi. 2013. 'Coralline Algal Structure Is More Sensitive to Rate, Rather than the Magnitude, of Ocean Acidification'. *Global Change Biology* 19 (12): 3621–28. https://doi.org/10.1111/gcb.12351.

Kamenos, Nicholas A., P. Geoffrey Moore, and Jason M. Hall-Spencer. 2004. 'Nursery-Area Function of Maerl Grounds for Juvenile Queen Scallops Aequipecten Opercularis and Other Invertebrates'. *Marine Ecology Progress Series* 274 (June): 183–89. https://doi.org/10.3354/meps274183.

Kolzenburg, Regina, Katy R. Nicastro, Sophie J. McCoy, Alex T. Ford, Gerardo I. Zardi, and Federica Ragazzola. 2019. 'Understanding the Margin Squeeze: Differentiation in Fitness-Related Traits between Central and Trailing Edge Populations of Corallina Officinalis'. *Ecology and Evolution* 9 (10): 5787–5801. https://doi.org/10.1002/ece3.5162.

Koop, K., D. Booth, A. Broadbent, J. Brodie, D. Bucher, D. Capone, J. Coll, et al. 2001. 'ENCORE: The Effect of Nutrient Enrichment on Coral Reefs. Synthesis of Results and Conclusions'. *Marine Pollution Bulletin* 42 (2): 91–120. https://doi.org/10.1016/S0025-326X(00)00181-8.

Levring, Tore. 1974. 'The Marine Algae of the Archipelago of Madeira'. http://localhost:8080/xmlui/handle/100/1231.

Lewis, Bonnie Emma. 2016. 'Growth and Calcification of Reef-Building Coralline Algae and Their Response to Ocean Acidification'. Griffith thesis, Griffith University. https://doi.org/10.25904/1912/1902.

Littler, M., and D. S. Littler. 2013. 'The Nature of Crustose Coralline Algae and Their Interactions on Reefs'. *Undefined*. https://www.semanticscholar.org/paper/The-Nature-of-Crustose-Coralline-Algae-and-Their-on-Littler-Littler/0398f38ded57b81184369e8d93597ffda5604ee5.

Lüning, K. 1991. 'Seaweeds: Their Environment, Biogeography, and Ecophysiology.' *Journal of the Marine Biological Association of the United Kingdom* 71 (1): 246–246. https://doi.org/10.1017/S0025315400037632.

Marbà, Núria, and Carlos M. Duarte. 2010. 'Mediterranean Warming Triggers Seagrass (Posidonia Oceanica) Shoot Mortality'. *Global Change Biology* 16 (8): 2366–75. https://doi.org/10.1111/j.1365-2486.2009.02130.x.

Martin, Sophie, Marie-Dorothée Castets, and Jacques Clavier. 2006. 'Primary Production, Respiration and Calcification of the Temperate Free-Living Coralline Alga Lithothamnion Corallioides'. *Aquatic Botany* 85 (2): 121–28. https://doi.org/10.1016/j.aquabot.2006.02.005.

Martin, Sophie, Stéphanie Cohu, Céline Vignot, Guillaume Zimmerman, and Jean-Pierre Gattuso. 2013. 'One-Year Experiment on the Physiological Response of the Mediterranean Crustose Coralline Alga, Lithophyllum Cabiochae, to Elevated PCO2 and Temperature'. *Ecology and Evolution* 3 (3): 676–93. https://doi.org/10.1002/ece3.475.

Martin, Sophie, and Jason M. Hall-Spencer. 2017. 'Effects of Ocean Warming and Acidification on Rhodolith/Maërl Beds'. In *Rhodolith/Maërl Beds: A Global Perspective*, edited by Rafael Riosmena-Rodríguez, Wendy Nelson, and Julio Aguirre, 55–85. Coastal Research Library. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-29315-8_3.

McCoy, Sophie J., and Nicholas A. Kamenos. 2015. 'Coralline Algae (Rhodophyta) in a Changing World: Integrating Ecological, Physiological, and Geochemical Responses to Global Change'. *Journal of Phycology* 51 (1): 6–24. https://doi.org/10.1111/jpy.12262.

Nelson, W. A.,. 2009. 'Calcified Macroalgae – Critical to Coastal Ecosystems and Vulnerable to Change: A Review'. *Marine and Freshwater Research* 60 (8): 787–801. https://doi.org/10.1071/MF08335.

Neves, Pedro, João Silva, Viviana Peña, and Cláudia Ribeiro. 2021. '"Pink Round Stones"—Rhodolith Beds: An Overlooked Habitat in Madeira Archipelago'. *Biodiversity and Conservation* 30 (12): 3359–83. https://doi.org/10.1007/s10531-021-02251-2.

Nixon, Scott W. 1995. 'Coastal Marine Eutrophication: A Definition, Social Causes, and Future Concerns'. *Ophelia* 41 (1): 199–219. https://doi.org/10.1080/00785236.1995.10422044.

Oliver, Eric C. J., Michael T. Burrows, Markus G. Donat, Alex Sen Gupta, Lisa V. Alexander, Sarah E. Perkins-Kirkpatrick, Jessica A. Benthuysen, et al. 2019. 'Projected Marine Heatwaves in the 21st Century and the Potential for Ecological Impact'. *Frontiers in Marine Science* 6. https://www.frontiersin.org/articles/10.3389/fmars.2019.00734.

Oliver, Eric C. J., Markus G. Donat, Michael T. Burrows, Pippa J. Moore, Dan A. Smale, Lisa V. Alexander, Jessica A. Benthuysen, et al. 2018. 'Longer and More Frequent Marine Heatwaves over the Past Century'. *Nature Communications* 9 (1): 1324. https://doi.org/10.1038/s41467-018-03732-9.

Page, Tessa M., Ellie Bergstrom, and Guillermo Diaz-Pulido. 2021. 'Acclimation History of Elevated Temperature Reduces the Tolerance of Coralline Algae to Additional Acute Thermal Stress'. *Frontiers in Marine Science* 8. https://www.frontiersin.org/articles/10.3389/fmars.2021.660196.

Pardo, Cristina, Lua Lopez, Viviana Peña, Jazmin Hernández-Kantún, Line Le Gall, Ignacio Bárbara, and Rodolfo Barreiro. 2014. 'A Multilocus Species Delimitation Reveals a Striking Number of Species of Coralline Algae Forming Maerl in the OSPAR Maritime Area'. *PLOS ONE* 9 (8): e104073. https://doi.org/10.1371/journal.pone.0104073.

Qui-Minet, Zujaila Nohemy, Jérôme Coudret, Dominique Davoult, Jacques Grall, Miguel Mendez-Sandin, Thierry Cariou, and Sophie Martin. 2019. 'Combined Effects of Global Climate Change and Nutrient Enrichment on the Physiology of Three Temperate Maerl Species'. *Ecology and Evolution* 9 (24): 13787–807. https://doi.org/10.1002/ece3.5802.

Qui-Minet, Zujaila Nohemy, Dominique Davoult, Jacques Grall, Coralie Delaunay, Christophe Six, Thierry Cariou, and Sophie Martin. 2021. 'Physiology of Maerl Algae: Comparison of Inter- and Intraspecies Variations'. *Journal of Phycology* 57 (3): 831–48. https://doi.org/10.1111/jpy.13119.

Ragazzola, Federica, Agnese Marchini, Mario Adani, Andrea Bordone, Alberto Castelli, Gabriella Cerrati, Regina Kolzenburg, et al. 2021. 'An Intertidal Life: Combined Effects of Acidification and Winter Heatwaves on a Coralline Alga (Ellisolandia Elongata) and Its Associated Invertebrate Community'. *Marine Environmental Research* 169 (July): 105342. https://doi.org/10.1016/j.marenvres.2021.105342.

Rendina, Francesco, Phil J. Bouchet, Luca Appolloni, Giovanni F. Russo, Roberto Sandulli, Regina Kolzenburg, Aditya Putra, and Federica Ragazzola. 2019. 'Physiological Response of the Coralline Alga Corallina Officinalis L. to Both Predicted Long-Term Increases in Temperature and Short-Term Heatwave Events'. *Marine Environmental Research* 150 (September): 104764. https://doi.org/10.1016/j.marenvres.2019.104764.

Rendina, Francesco, Sara Kaleb, Annalisa Caragnano, Federica Ferrigno, Luca Appolloni, Luigia Donnarumma, Giovanni Fulvio Russo, Roberto Sandulli, Valentina Roviello, and Annalisa Falace. 2020. 'Distribution and Characterization of Deep Rhodolith Beds off the Campania Coast (SW Italy, Mediterranean Sea)'. *Plants* 9 (8): 985. https://doi.org/10.3390/plants9080985.

Rhodolith/Maërl Beds: A Global Perspective. n.d. Accessed 16 December 2022. https://link.springer.com/book/10.1007/978-3-319-29315-8.

Ribeiro, C. 2008. 'Comparison of Rocky Reef Fish Communities among Protected, Unprotected and Artificial Habitats in Madeira Island Costal Waters Using Underwater Visual Techniques.' *PhD, Universidade de Lisboa, Faculdade de Ciências*, 2008.

Riosmena-Rodríguez, Rafael. 2017. 'Natural History of Rhodolith/Maërl Beds: Their Role in Near-Shore Biodiversity and Management'. In *Rhodolith/Maërl Beds: A Global Perspective*, edited by Rafael Riosmena-Rodríguez, Wendy Nelson, and Julio Aguirre, 3–26. Coastal Research Library. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-29315-8_1.

Rothäusler, Eva, and Veijo Jormalainen. 2016. 'Eutrophication and the Challenge of Changing Biotic Interactions'. In , 179–94. https://doi.org/10.4324/9781315370781-9.

Russell, Bayden D., Jo-Anne I. Thompson, Laura J. Falkenberg, and Sean D. Connell. 2009. 'Synergistic Effects of Climate Change and Local Stressors: CO2 and Nutrient-Driven Change in Subtidal Rocky Habitats'. *Global Change Biology* 15 (9): 2153–62. https://doi.org/10.1111/j.1365-2486.2009.01886.x.

Schlegel, R. W., and Smit, A. J. 2018. 'heatwaveR: a central algorithm for the detection of heatwaves and cold-spells.' J. Open Sour. Softw. 3:821. Doi: 10.21105/joss.00821

Schubert, N., V. W. Salazar, W. A. Rich, M. Vivanco Bercovich, A. C. Almeida Saá, S. D. Fadigas, J. Silva, and P. A. Horta. 2019. 'Rhodolith Primary and Carbonate Production in a Changing Ocean: The Interplay of Warming and Nutrients'. *Science of The Total Environment* 676: 455.

Schubert, N., V. W. Salazar, W. A. Rich, M. Vivanco Bercovich, A. C. Almeida Saá, S. D. Fadigas, J. Silva, and P. A. Horta. 2019. 'Rhodolith Primary and Carbonate Production in a Changing Ocean: The Interplay of Warming and Nutrients'. *Science of The Total Environment* 676 (August): 455–68. https://doi.org/10.1016/j.scitotenv.2019.04.280.

Schubert, Nadine, Laurie C. Hofmann, Antonella C. Almeida Saá, Anderson Camargo Moreira, Rafael Güntzel Arenhart, Celso Peres Fernandes, Dirk de Beer, Paulo A. Horta, and João Silva. 2021. 'Calcification in Free-Living Coralline Algae Is Strongly Influenced by Morphology: Implications for Susceptibility to Ocean Acidification'. *Scientific Reports* 11 (1): 11232. https://doi.org/10.1038/s41598-021-90632-6.

Schubert, Nadine, Viviana Peña, Vinícius W. Salazar, Paulo A. Horta, Pedro Neves, Cláudia Ribeiro, Francisco Otero-Ferrer, et al. 2022. 'Rhodolith Physiology Across the Atlantic: Towards a Better Mechanistic Understanding of Intra- and Interspecific Differences'. *Frontiers in Marine Science* 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.921639.

Schubert, Nadine, Rui Santos, and João Silva. 2021. 'Living in a Fluctuating Environment Increases Tolerance to Marine Heatwaves in the Free-Living Coralline Alga Phymatolithon Lusitanicum'. *Frontiers in Marine Science* 8. https://www.frontiersin.org/articles/10.3389/fmars.2021.791422.

Schubert, Nadine, Kathryn M. Schoenrock, Julio Aguirre, Nicholas A. Kamenos, João Silva, Paulo A. Horta, and Laurie C. Hofmann. 2020. 'Editorial: Coralline Algae: Globally Distributed Ecosystem Engineers'. *Frontiers in Marine Science* 7. https://www.frontiersin.org/article/10.3389/fmars.2020.00352.

Shayka, Bridget Francine. 2018. 'Effects of Nutrient Addition and Ocean Acidification on Tropical Crustose Coralline Algae. California State University, Northridge.', 2018.

Smale, Dan A., Thomas Wernberg, Eric C. J. Oliver, Mads Thomsen, Ben P. Harvey, Sandra C. Straub, Michael T. Burrows, et al. 2019. 'Marine Heatwaves Threaten Global Biodiversity and the Provision of Ecosystem Services'. *Nature Climate Change* 9 (4): 306–12. https://doi.org/10.1038/s41558-019-0412-1.

Smale, Dan A., Anna L. E. Yunnie, Thomas Vance, and Stephen Widdicombe. 2015. 'Disentangling the Impacts of Heat Wave Magnitude, Duration and Timing on the Structure and Diversity of Sessile Marine Assemblages'. *PeerJ* 3 (March): e863. https://doi.org/10.7717/peerj.863.

Steller, Dl, Jm Hernandez-Ayón, R Riosmena-Rodríguez, and A Cabello-Pasini. 2007. 'Effect of Temperature on Photosynthesis, Growth and Calcification Rates of the Free-Living Coralline Alga Lithophyllum Margaritae'. *Ciencias Marinas* 33 (4): 441–56. https://doi.org/10.7773/cm.v33i4.1255.

Steller, D.l., R. Riosmena-Rodríguez, M.s. Foster, and C.a. Roberts. 2003. 'Rhodolith Bed Diversity in the Gulf of California: The Importance of Rhodolith Structure and Consequences of Disturbance'. *Aquatic Conservation: Marine and Freshwater Ecosystems* 13 (S1): S5–20. https://doi.org/10.1002/aqc.564.

Stenseth, Nils Chr., and Atle Mysterud. 2002. 'Climate, Changing Phenology, and Other Life History Traits: Nonlinearity and Match–Mismatch to the Environment'. *Proceedings of the National Academy of Sciences* 99 (21): 13379–81. https://doi.org/10.1073/pnas.212519399.

Steven, A.D.L. 2000. 'Effects of Inorganic Nitrogen and Phosphorus Enrichment on the Calcification and Growth of Hermatypic Corals and Coral-Reef Communities.' *PhD. James Cook University, Australia*, 2000.

Straub, Sandra C., Thomas Wernberg, Mads S. Thomsen, Pippa J. Moore, Michael T. Burrows, Ben P. Harvey, and Dan A. Smale. 2019. 'Resistance, Extinction, and Everything in Between – The Diverse Responses of Seaweeds to Marine Heatwaves'. *Frontiers in Marine Science* 6. https://www.frontiersin.org/article/10.3389/fmars.2019.00763.

Tanaka, Yasuaki, Atsushi Suzuki, and Kazuhiko Sakai. 2017. 'Effects of Elevated Seawater Temperature and Phosphate Enrichment on the Crustose Coralline Alga Porolithon Onkodes (Rhodophyta)'. *Phycological Research* 65 (1): 51–57. https://doi.org/10.1111/pre.12152.

Turpin, David H. 1991. 'Effects of Inorganic N Availability on Algal Photosynthesis and Carbon Metabolism'. *Journal of Phycology* 27 (1): 14–20. https://doi.org/10.1111/j.0022-3646.1991.00014.x.

Turpin, David H., Ivor R. Elrifi, Douglas G. Birch, Harold G. Weger, and Jody J. Holmes. 1988. 'Interactions between Photosynthesis, Respiration, and Nitrogen Assimilation in Microalgae'. *Canadian Journal of Botany* 66 (10): 2083–97. https://doi.org/10.1139/b88-286.

Vásquez-Elizondo, Román Manuel, and Susana Enríquez. 2016. 'Coralline Algal Physiology Is More Adversely Affected by Elevated Temperature than Reduced PH'. *Scientific Reports* 6 (1): 19030. https://doi.org/10.1038/srep19030.

Villas-Bôas, Alexandre Bigio, Frederico Tapajós De Souza Tâmega, Mauricio Andrade, Ricardo Coutinho, and Marcia Abreu De Oliveira Figueiredo. 2014. 'Experimental Effects of Sediment Burial and Light Attenuation on Two Coralline Algae of a Deep Water Rhodolith Bed in Rio de Janeiro, Brazil'. *Cryptogamie, Algologie* 35 (1): 67–76. https://doi.org/10.7872/crya.v35.iss1.2014.67.

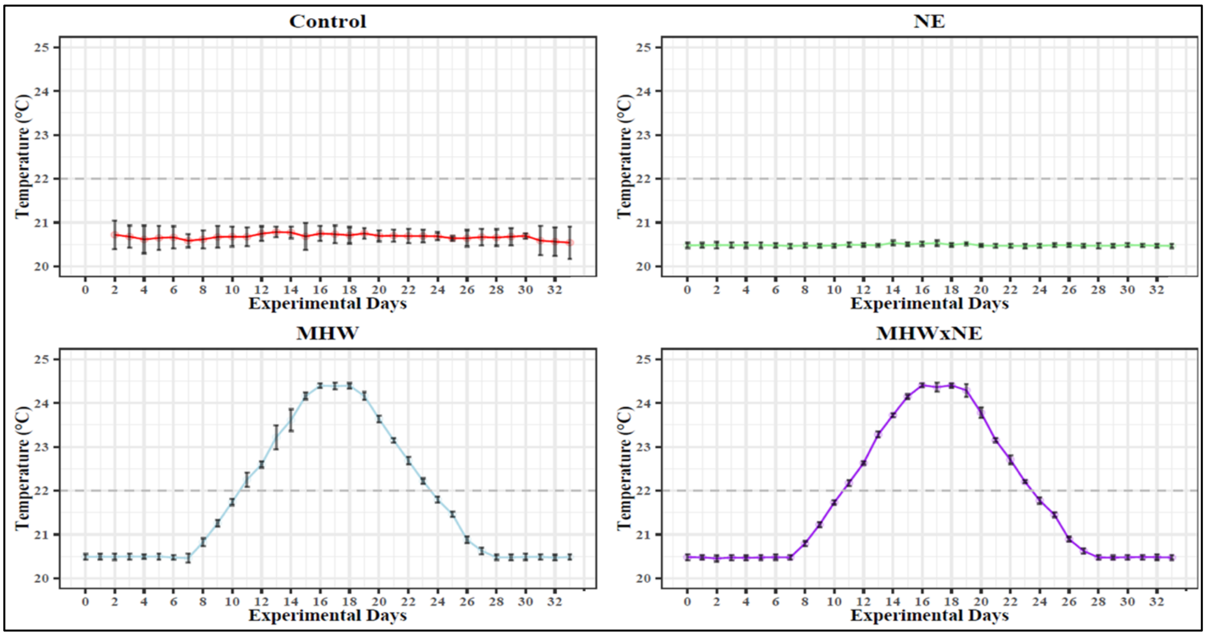
Weisscher, F. C. M. 1983. 'Marine Algae from Selvagem Pequena (Salvage Islands) [CANCAP-Project]'. http://localhost:8080/xmlui/handle/100/1199.

Wernberg, Thomas, Scott Bennett, Russell C. Babcock, Thibaut de Bettignies, Katherine Cure, Martial Depczynski, Francois Dufois, et al. 2016. 'Climate-Driven Regime Shift of a Temperate Marine Ecosystem'. *Science* 353 (6295): 169–72. https://doi.org/10.1126/science.aad8745.

Wernberg, Thomas, Dan A. Smale, Fernando Tuya, Mads S. Thomsen, Timothy J. Langlois, Thibaut de Bettignies, Scott Bennett, and Cecile S. Rousseaux. 2013. 'An Extreme Climatic Event Alters Marine Ecosystem Structure in a Global Biodiversity Hotspot'. *Nature Climate Change* 3 (1): 78–82. https://doi.org/10.1038/nclimate1627.

Wilson, Sian, Charmaine Blake, John A Berges, and Christine A Maggs. 2004. 'Environmental Tolerances of Free-Living Coralline Algae (Maerl): Implications for European Marine Conservation'. *Biological Conservation* 120 (2): 279–89. https://doi.org/10.1016/j.biocon.2004.03.001.

7. Annex



Annex Figure 1. Mean Temperature data of experimental tanks (n=5) per day (33 days), for each treatment.

Annex Table 1. Mean Nutrient concentrations (in µM) over the full experimental period. Incoming represents the nutrient values from the general water input of the experimental set-up, feeding all tanks

Date		Incoming	MHW	MHW x NE	NE	Control
27/05/2022	[NH4]				16,22 ± 1,9	
	[PO4]				11,74 ± 0,8	
27/05/2022	[NO3]				56,13 ± 5,0	
29/05/2022	[NH4]	0	0,00	4,82 ± 2,50	7,96 ± 2,1	
	[PO4]	1,17 ± 0,001	0,93 ± 0,06	7,47 ± 0,76	8,13 ± 1,0	
29/05/2022	[NO3]	4,225 ± 0,37	1,24 ± 0,41	40,38 ± 4,23	43,83 ± 5,5	
03/06/2022	[NH4]	0	0,00	8,20 ± 2,99	8,90 ± 8,2	0,00
	[PO4]	0,615 ± 0,01	0,48 ± 0,17	7,60 ± 1,11	8,83 ± 1,6	0,38 ± 0,3
03/06/2022	[NO3]	3,75 ± 0,03	0,49 ± 0,08	33,88 ± 4,84	37,92 ± 5,5	0,54 ± 0,2
10/06/2022	[NH4]	0	0,00	0,00	4,11 ± 3,0	0,00
	[PO4]	0,65 ± 0,07	0,43 ± 0,11	8,87 ± 4,62	8,14 ± 1,0	4,00 ± 5,0
10/06/2022	[NO3]	3,365 ± 1,27	1,75 ± 1,30	38,61 ± 8,13	40,73 ± 6,8	4,02
17/06/2022	[NH4]	0		0,00	0,00	
	[PO4]	0,53		4,24 ± 0,34	5,49 ± 1,1	
17/06/2022	[NO3]	2,05		23,35 ± 4,85	24,67 ± 6,4	
	[NH4]		0,00	0,00	1,19 ± 3,3	0,00
24/06/2022	[PO4]		0,25 ± 0,07	6,12 ± 0,81	6,66 ± 0,7	0,53 ± 0,1
24/06/2022	[NO3]		0,70 ± 0,31	31,26 ± 4,87	30,77 ± 3,0	1,63 ± 0,4

Annex Figure 2. (Left and Right) Open Acrylic incubation chamber, set on magnetic stirrer, with rhodolith being mounted. (Bottom) Closed Incubation Chamber with set Light Filter cover.

Annex Table 2. Control group mean physiological response and standard error to temperature and nutrient enrichment per time point (t1 to t4). Tukey Post-Hoc results of significant difference between time points is marked by difference in letters: Control differences are marked with lowercase letters (e.g., a, b, c), NE treatment with uppercase letters (e.g., A, B, C).

	Control				NE			
Incubation Day	t1	t2	t3	t4	t1	t2	t3	t4
Temperature (°C)	20,5	20,5	20,5	20,5	20,5	20,5	20,5	20,5
GPP (µmol O_2 g^{-1} h^{-1})	0,693 ± 0,094 ^{a}	0,798 ± 0,073 ^{b}	0,807 ± 0,082 ^{b}	0,833 ± 0,079 ^{b}	0,948 ± 0,137 ^{A}	1,034 ± 0,157 ^{AB}	1,119 ± 0,173 ^{B}	1,027 ± 0,162 ^{AB}
NPP (µmol O_2 g^{-1} h^{-1})	0,417 ± 0,080 ^{a}	0,472 ± 0,053 ^{ab}	0,485 ± 0,065 ^{ab}	0,535 ± 0,064 ^{b}	0,658 ± 0,092 ^{A}	0,678 ± 0,111 ^{A}	0,719 ± 0,126 ^{A}	0,694 ± 0,116 ^{A}
Respiration (µmol O_2 g^{-1} h^{-1})	-0,276 ± 0,019 ^{a}	-0,326 ± 0,020 ^{b}	-0,321 ± 0,023 ^{b}	-0,298 ± 0,020 ^{ab}	-0,291 ± 0,046 ^{A}	-0,356 ± 0,048 ^{B}	-0,400 ± 0,052 ^{C}	-0,333 ± 0,051 ^{B}
DNP (µmol O_2 g^{-1} day^{-1})	8,595 ± 1,258 ^{a}	9,867 ± 0,941^{ab}	10,008 ± 1,082^{ab}	10,469 ± 1,038 ^{b}	12,113 ± 1,744 ^{A}	13,054 ± 2,013^{AB}	14,065 ± 2,225 ^{B}	13,050 ± 2,077 ^{AB}
Light Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	0,140 ± 0,036 ^{a}	0,171 ± 0,060 ^{a}	0,177 ± 0,043 ^{a}	0,230 ± 0,016 ^{a}	0,059 ± 0,016 ^{A}	0,226 ± 0,013 ^{B}	0,367 ± 0,023 ^{C}	0,290 ± 0,046 ^{BC}
Dark Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	0,005 ± 0,026 ^{a}	0,019 ± 0,039 ^{a}	0,028 ± 0,029 ^{a}	0,038 ± 0,023 ^{a}	-0,055 ± 0,030 ^{A}	0,059 ± 0,018 ^{B}	0,116 ± 0,018 ^{C}	0,018 ± 0,010 ^{B}
DNC (µmol $CaCO_3$ g^{-1} day^{-1})	1,902 ± 0,329 ^{a}	2,200 ± 0,510 ^{a}	2,204 ± 0,340 ^{a}	2,840 ± 0,153 ^{a}	1,380 ± 0,361 ^{A}	2,574 ± 0,219 ^{AC}	4,088 ± 0,208 ^{B}	3,880 ± 0,603 ^{BC}

Annex Table 3. Control group mean physiological response and standard error to temperature and nutrient enrichment per time point (t1 to t4). Data is displayed in percentage of the initial value (t1) and Standard error as percentage of its timepoints' data..

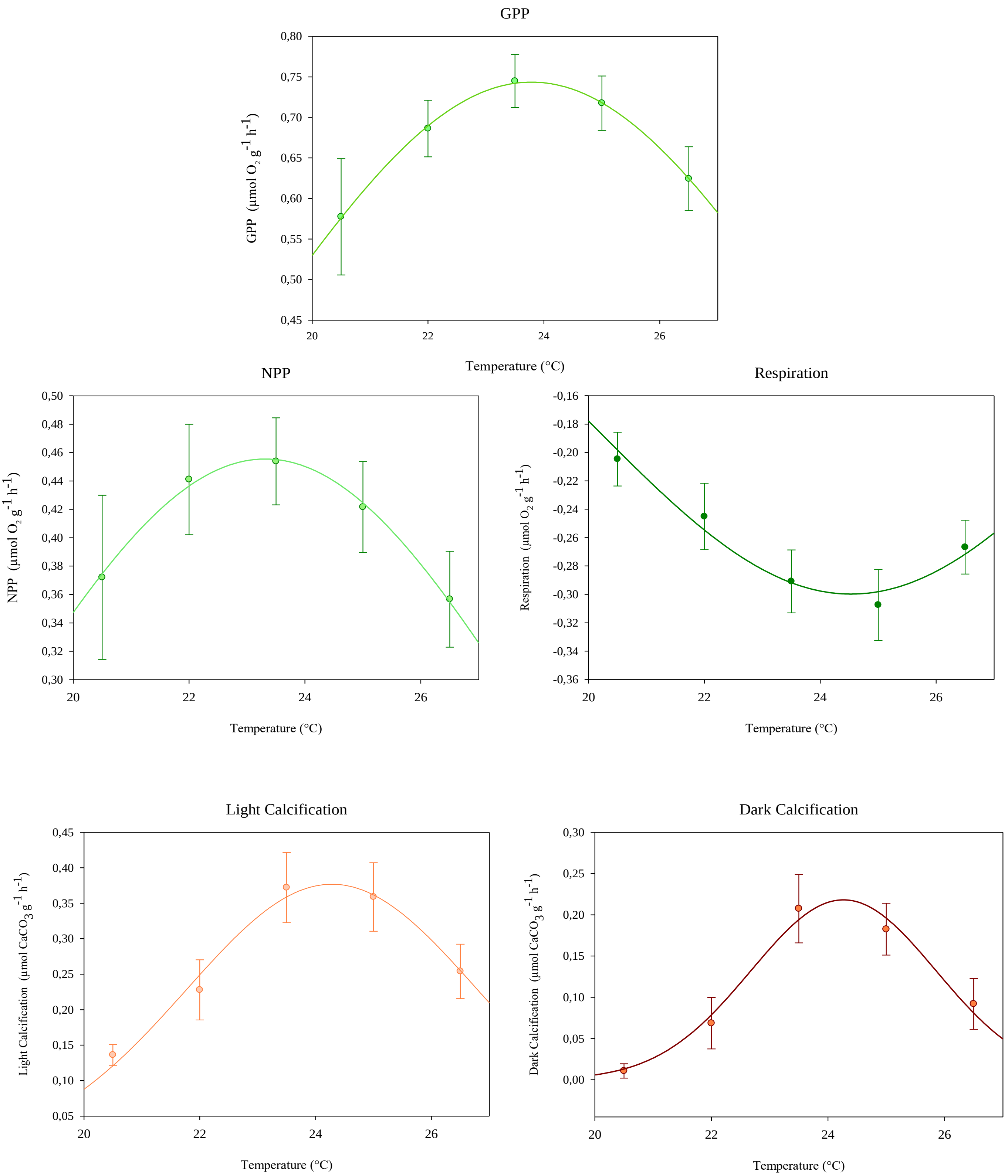
	Control				NE			
Incubation Day	t1	t2	t3	t4	t1	t2	t3	t4
Temperature (°C)	20,5	20,5	20,5	20,5	20,5	20,5	20,5	20,5
GPP **(µmol O_2 g^{-1} h^{-1})**	100% ± 14%	115% ± 11%	116% ± 12%	120% ± 11%	100% ± 15%	109% ± 17%	118% ± 18%	108% ± 17%
NPP **(µmol O_2 g^{-1} h^{-1})**	100% ± 19%	113% ± 13%	117% ± 16%	128% ± 15%	100% ± 14%	103% ± 17%	109% ± 19%	106% ± 18%
Respiration **(µmol O_2 g^{-1} h^{-1})**	100% ± 5%	118% ± 5%	116% ± 6%	108% ± 5%	100% ± 7%	123% ± 7%	138% ± 8%	115% ± 8%
DNP **(µmol O_2 g^{-1} day^{-1})**	100% ± 15%	115% ± 11%	116% ± 13%	122% ± 12%	100% ± 14%	108% ± 17%	116% ± 18%	108% ± 17%
Light Calcification **(µmol $CaCO_3$ g^{-1} h^{-1})**	100% ± 26%	122% ± 35%	127% ± 24%	165% ± 7%	100% ± 26%	380% ± 6%	618% ± 6%	488% ± 16%
Dark Calcification **(µmol $CaCO_3$ g^{-1} h^{-1})**	100% ± 494%	373% ± 201%	538% ± 102%	731% ± 60%	100% ± 54%	208% ± 31%	312% ± 16%	133% ± 54%
DNC **(µmol $CaCO_3$ g^{-1} day^{-1})**	100% ± 17%	116% ± 27%	116% ± 18%	149% ± 8%	100% ± 26%	187% ± 16%	296% ± 15%	281% ± 44%

Annex Table 4. MHW group mean physiological response and standard error to temperature and nutrient enrichment per time point (t1 to t5). Tukey Post-Hoc results of significant difference between time points is marked by difference in letters: Control differences are marked with lowercase letters (e.g., a, b, c), NE treatment with uppercase letters (e.g., A, B, C).

	MHW					**MHW x NE**				
Incubation Day	t1	t2	t3	t4	t5	t1	t2	t3	t4	t5
Temperature (°C)	20,5	24,5	24,5	20,5	20,5	20,5	24,5	24,5	20,5	20,5
GPP (µmol O_2 g^{-1} h^{-1})	0,692 ± 0,080 ^{a}	0,610 ± 0,090 ^{ab}	0,702 ± 0,061 ^{ab}	0,727 ± 0,062 ^{ab}	0,728 ± 0,068 ^{b}	0,751 ± 0,082 ^{A}	0,794 ± 0,077 ^{AB}	0,728 ± 0,065 ^{AB}	0,877 ± 0,097 ^{B}	0,894 ± 0,085 ^{B}
NPP (µmol O_2 g^{-1} h^{-1})	0,477 ± 0,073 ^{a}	0,403 ± 0,059 ^{a}	0,457 ± 0,044 ^{a}	0,478 ± 0,050 ^{a}	0,475 ± 0,053 ^{a}	0,539 ± 0,050 ^{AB}	0,523 ± 0,064 ^{AB}	0,462 ± 0,043 ^{A}	0,545 ± 0,066 ^{AB}	0,562 ± 0,060 ^{B}
Respiration (µmol O_2 g^{-1} h^{-1})	-0,215 ± 0,009 ^{a}	-0,207 ± 0,044 ^{a}	-0,245 ± 0,028 ^{a}	-0,249 ± 0,015 ^{a}	-0,253 ± 0,017 ^{a}	-0,211 ± 0,045 ^{A}	-0,271 ± 0,020 ^{AB}	-0,265 ± 0,024 ^{AB}	-0,332 ± 0,037 ^{B}	-0,332 ± 0,031 ^{B}
DNP (µmol O_2 g^{-1} day^{-1})	8,831 ± 1,089 ^{a}	7,714 ± 1,115 ^{a}	8,850 ± 0,775 ^{a}	9,182 ± 0,814 ^{a}	9,185 ± 0,893 ^{a}	9,663 ± 0,998 ^{A}	10,029 ± 1,021 ^{AB}	9,125 ± 0,825 ^{A}	10,952 ± 1,226 ^{B}	11,184 ± 1,088 ^{B}
Light Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	0,113 ± 0,018 ^{a}	0,207 ± 0,040 ^{a}	0,197 ± 0,042 ^{a}	0,172 ± 0,018 ^{a}	0,184 ± 0,022 ^{a}	0,064 ± 0,010 ^{A}	0,255 ± 0,066 ^{B}	0,272 ± 0,065 ^{B}	0,281 ± 0,064 ^{B}	0,288 ± 0,059 ^{B}
Dark Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	-0,022 ± 0,009 ^{a}	0,059 ± 0,029 ^{b}	0,055 ± 0,040 ^{b}	0,042 ± 0,010 ^{b}	0,038 ± 0,016 ^{b}	-0,058 ± 0,013 ^{A}	0,065 ± 0,038 ^{B}	0,040 ± 0,031 ^{B}	0,042 ± 0,022 ^{B}	0,053 ± 0,010 ^{B}
DNC (µmol $CaCO_3$ g^{-1} day^{-1})	1,798 ± 0,201 ^{a}	2,311 ± 0,303 ^{a}	2,208 ± 0,223 ^{a}	1,993 ± 0,166 ^{a}	2,196 ± 0,179 ^{a}	1,464 ± 0,177 ^{A}	2,918 ± 0,563 ^{B}	3,411 ± 0,605 ^{B}	2,808 ± 0,879 ^{B}	3,500 ± 0,722 ^{B}

Annex Table 5. MHW group mean physiological response and standard error to temperature and nutrient enrichment per time point (t1 to t4). Data is displayed in percentage of the initial value (t1) and Standard error as percentage of its timepoints' data.

	MHW					MHW x NE				
Incubation Day	t1	t2	t3	t4	t5	t1	t2	t3	t4	t5
Temperature (°C)	20,5	24,5	24,5	20,5	20,5	20,5	24,5	24,5	20,5	20,5
GPP (µmol O_2 g^{-1} h^{-1})	100% ± 12%	88% ± 13%	101% ± 9%	105% ± 9%	105% ± 10%	100% ± 11%	106% ± 10%	97% ± 9%	117% ± 13%	119% ± 11%
NPP (µmol O_2 g^{-1} h^{-1})	100% ± 15%	84% ± 15%	96% ± 10%	100% ± 10%	100% ± 11%	100% ± 9%	97% ± 12%	86% ± 8%	101% ± 12%	104% ± 11%
Respiration (µmol O_2 g^{-1} h^{-1})	100% ± 4%	96% ± 21%	114% ± 11%	116% ± 6%	118% ± 7%	100% ± 8%	128% ± 4%	125% ± 4%	157% ± 7%	157% ± 6%
DNP (µmol O_2 g^{-1} day^{-1})	100% ± 12%	87% ± 13%	100% ± 9%	104% ± 9%	104% ± 10%	100% ± 10%	104% ± 11%	94% ± 9%	113% ± 13%	116% ± 11%
Light Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	100% ± 16%	184% ± 19%	175% ± 22%	153% ± 11%	163% ± 12%	100% ± 16%	402% ± 26%	428% ± 24%	442% ± 23%	453% ± 20%
Dark Calcification (µmol $CaCO_3$ g^{-1} h^{-1})	100% ± 39%	366% ± 49%	348% ± 72%	288% ± 24%	271% ± 42%	100% ± 23%	213% ± 58%	169% ± 77%	173% ± 52%	191% ± 20%
DNC (µmol $CaCO_3$ g^{-1} day^{-1})	100% ± 11%	128% ± 17%	123% ± 12%	111% ± 9%	122% ± 10%	100% ± 12%	199% ± 38%	233% ± 41%	192% ± 60%	239% ± 49%



Annex Figure 3. Thermal Performance curves of *Phymatolithon* sp.. Data represents mean physiological response (n=5) and standard error.

Annex Table 6. Primary Production Physiological Parameters Formulas, using the Oxygen concentration measured during incubation time, under Light and Dark Conditions

Primary Production (PP) Physiological Parameters	**Formula**
Net PP (NPP) ***(μmol O_2/ (g * h))***	$[(O_{2(final)}-O_{2(initial)})_{Light} * V_{chamber}] / (Dry\ Weight * Time_{Incubation})$
Respiration (Resp) ***(μmol O_2/ (g * h))***	$[(O_{2(final)}-O_{2(initial)})_{Dark} * V_{chamber}] / (Dry\ Weight * Time_{Incubation})$
Gross PP (GPP) ***(μmol O_2/ (g * h))***	NPP + Resp
Daily Net PP (DNP) ***(μmol O_2/ (g * day))***	$(NPP * Time_{Diurnal}) + (Resp * Time_{Nocturnal})$

Annex Table 7. Calcification Physiological Parameters Formulas, using the Calcium Carbonate concentration measured during incubation time, under Light and Dark Conditions (TA- Total alkalinity)

Calcification Physiological Parameters	**Formula**
Light Calcification (LC) ***(μmol $CaCO_3$/ (g * h))***	$[(TA_{initial} - TA_{final})_{Light} * 0.5 * V_{chamber}] / (Dry\ Weight * Time_{Incubation})$
Dark Calcification (DC) ***(μmol $CaCO_3$/ (g * h))***	$[(TA_{initial} - TA_{final})_{Dark} * 0.5 * V_{chamber}] / (Dry\ Weight * Time_{Incubation})$
Daily Net Calcification (DNC) ***(μmol $CaCO_3$/ (g * day))***	$(LC * Time_{Diurnal}) + (DC * Time_{Nocturnal})$