



Photo-physiology and morphology reveal divergent warming responses in northern and southern hemisphere seagrasses

Hung Manh Nguyen¹ · Fabio Bulleri² · Lázaro Marín-Guirao^{1,3} · Mathieu Pernice⁴  · Gabriele Procaccini¹

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Abstract

A better understanding of species and population responses to thermal stress is critical to predict changes in their distribution under warming scenarios. Seagrasses are a unique group of marine plants that play fundamental roles in marine environments and provide vital ecosystem services. Nevertheless, previous studies on seagrass thermal tolerance have focused exclusively on a handful of species, with the majority of these remaining virtually unexplored. Moreover, to date, no study has compared the response to thermal stress between northern and southern hemisphere seagrasses. Here, we conducted comparative mesocosm experiments using four seagrass species from the northern (i.e. Mediterranean: *Posidonia oceanica*, *Cymodocea nodosa*) and southern (i.e. Australia: *Posidonia australis* and *Zostera muelleri*) hemisphere as representative of two different life strategies, i.e. climax (*P. oceanica*, *P. australis*) and pioneer (*C. nodosa*, *Z. muelleri*). Plants acclimatized to the mesocosm conditions at ambient temperature (i.e. 26 °C) during a 5-week period, were exposed to a simulated marine heatwave (i.e. 32 °C) for 2 weeks. Measurements of plant responses, including photo-physiology, morphology, and pigment content, were performed at the end of the warming exposure. Results showed that warming had no significant effects on photosynthetic performances of northern hemisphere seagrasses while negatively impacted their southern hemisphere counterparts. Similarly, warming favored the growth of northern hemisphere plants, but strongly inhibited the development of southern hemisphere species. Furthermore, photo-physiological and pigment content results suggested pioneer seagrasses better dealt with warming than climax species. Our study provides more insights into the field of seagrass ecology and yields potential implication for future seagrass conservation and restoration activities.

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Lázaro Marín-Guirao, Mathieu Pernice and Gabriele Procaccini have contributed equally to this work.

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✉ Mathieu Pernice
mathieu.pernice@uts.edu.au

¹ Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

² Dipartimento di Biologia, University of Pisa, 56126 Pisa, Italy

³ Oceanographic Center of Murcia, Seagrass Ecology Group, Spanish Institute of Oceanography, C/Varadero, 30740 San Pedro del Pinatar, Murcia, Spain

⁴ Faculty of Science, Climate Change Cluster (C3), University of Technology Sydney, Sydney, NSW 2007, Australia

Introduction

Seagrasses are a unique group of marine angiosperms occupying thousands of square kilometers along the shorelines of all continents, except for Antarctica (Short et al. 2007). Being among the most important habitat-forming species in the marine environment, seagrasses provide high primary productivity and nursery habitats for fish and invertebrates (Unsworth et al. 2019). Seagrasses help to stabilize coastal sediments, thus preventing erosion (Orth et al. 2006), and reduce pathogenic threats for humans, fish, and invertebrates (Lamb et al. 2017). Given that a vast majority of the human population inhabits coastal areas, seagrasses directly or indirectly influence the livelihoods of billions of people worldwide (Bertelli and Unsworth 2014). Economically, seagrass meadows are ranked among the most valuable ecosystems on Earth, contributing nearly \$2 trillion annually in ecosystem services (Waycott et al. 2009; Costanza et al. 2014). Moreover, seagrasses represent the most significant natural carbon sink on our planet (Fourqurean et al. 2012; Macreadie and

Hardy 2018), which can potentially be a part of future ocean solutions helping us to mitigate the negative effect of greenhouse gas emissions (Gattuso et al. 2018).

Nonetheless, seagrasses have undergone a global decline mainly due to human-induced environmental changes (Orth et al. 2006; Waycott et al. 2009). Across the globe, human activities are wiping out over 100 km² of seagrass meadows per annum. As a result, nearly 29% of their areal extent has been lost since 1879 (Waycott et al. 2009). For instance, the Mediterranean endemic species, *Posidonia oceanica*, already lost approximately 13–50% of its total areal extent since the mid-nineteenth century (Telesca et al. 2015). The decline of *P. oceanica* meadows is likely to continue as this species' ecological functions have even been predicted to go extinct by the end of this century (Marbà and Duarte 2010; Chefaoui et al. 2018).

Among human-induced stressors to seagrasses, ocean warming appears to be a key player (Nguyen et al. 2021). While ocean warming is commonly known as the gradual increment in the mean of seawater temperature, it also affects species in the form of extreme climatic events (i.e. marine heatwaves). Marine heatwaves (MHWs) are defined as abnormal warming events that last for over 5 days, with temperatures exceeding the 90th percentile of a three-decade historical baseline database (Hobday et al. 2016). For seagrasses (but also for other benthic organisms), the impact of MHWs is generally more detrimental than increases in mean seawater temperatures because seagrasses are generally susceptible to sudden thermal changes (Smale et al. 2019). In fact, MHWs have produced devastating consequences for seagrasses and associated communities across the globe (Coma et al. 2009; Harley et al. 2012; Wernberg et al. 2016; Smale 2020). MHWs were the main cause of massive die-off events of seagrass species including *Zostera marina* (Jarvis et al. 2014) and *Amphibolis antarctica* (Arias-Ortiz et al. 2018; Strydom et al. 2020). MHWs not only caused the decline of seagrass meadows and all their ecological services but also fostered the release of greenhouse gases into the atmosphere, consequently contributing to the ongoing global warming (Arias-Ortiz et al. 2018; Macreadie and Hardy 2018; Salinas et al. 2020).

With insights from previous studies, we now know that the capacity to cope with warming (or especially MHWs) varies among different seagrass species (Marín-Guirao et al. 2016; Collier et al. 2017; Nguyen et al. 2020b), but also among populations of the same species from contrasting thermal environments (e.g. Bergmann et al. 2010; Winters et al. 2011; Marín-Guirao et al. 2018, 2019). However, to date, the tolerance to anomalous thermal events of the majority of seagrasses (especially in the region of southeast Asia and northern Australia, a hotspot of seagrass diversity) are yet to be investigated (see Nguyen et al. 2021 for a complete review). Indeed, to the best of our knowledge, no

study has compared the responses of northern versus southern hemisphere seagrasses to warming.

Along the ecological succession, plants can be divided in pioneer species (i.e. fast-growing, often with small body size and annual) and climax species (i.e. slow-growing, long-lived, often with large body size, and perennial), with different biological characteristics and ecological roles (Glenn-Lewin et al. 1992). Likewise, some seagrass species can be classified as climax (e.g. *Posidonia oceanica*, *P. australis*, *Zostera marina*, *Thalassia testudinum*) while others as pioneer (e.g. *Cymodocea nodosa*, *Z. muelleri*). The contrasting characteristics between the two groups underpin large variations in the number and type of ecosystem services they provide. In seagrasses, most of their ecological services (e.g. sediment stabilization, nursery habitat, and blue carbon burial, etc.) depend upon their physical structure and primary productivity and, hence, climax seagrasses are considered more ecologically valuable than pioneer ones.

Studies from terrestrial plants have documented dissimilarities in response to environmental stressors between climax versus pioneer plants. For instance, studies from the Brazilian Atlantic Forest showed that pioneer trees were more tolerant against oxidative stress than climax plants (Favaretto et al. 2011; Brandão et al. 2017; Esposito et al. 2018). In line with these studies from the southern hemisphere, a study from the Mediterranean region experimentally tested the responses of carbon assimilation under summer stress conditions (water deficits, high light, and temperature) in four Mediterranean trees, including climax and pioneer species (Faria et al. 1998). This study indicated that, although both groups of trees suffered a decline in their photosynthetic capacities, the climax plants exhibited the lowest photosynthetic rates and the highest proportion of carotenoids to chlorophyll (i.e. an indicator of photo-protective mechanism activated under stressful conditions) while pioneer species maintained higher photosynthetic rates (Faria et al. 1998). Hence, environmental stressors can impact more strongly climax species, favoring the persistence of less complex and stable ecosystems, and providing less valuable ecosystem services. This is true also for seagrasses (Johnson et al. 2003; Hyndes et al. 2016; Shields et al. 2019), where it appears essential to assess the response to stress of both climax and pioneer species, to support timely and effective conservation and/or restoration actions. Few studies have experimentally compared the response to warming of climax and pioneer seagrass species (e.g. see Masini and Manning 1997; Seddon and Cheshire 2001; Campbell et al. 2006; Collier and Waycott 2014; Marín-Guirao et al. 2016, 2018; Collier et al. 2017; Tutar et al. 2017). Most of them suggested that pioneer species are more thermal tolerant than climax ones. These studies demonstrated that the fast-growing pioneer seagrasses exhibited a better ability to maintain unaltered plant carbon balances through improved

photosynthetic thermal stability and performance as well as by inhibiting respiratory carbon consumption. Moreover, through a higher morphological plasticity, pioneer species can modify their plant architecture by increasing the above-ground (photosynthetic)/below-ground (non-photosynthetic) biomass ratio to deal with thermal stress (Collier et al. 2017; Marín-Guirao et al. 2018), and have also an increased ability to activate antioxidant defense mechanisms to protect themselves from heat-stress induced oxidative damage (Tutar et al. 2017). Notwithstanding these evidences, the number of studies on this topic, especially on species with overlapping geographical distribution, remains scarce and deserves more effort.

In the present study, four seagrass species including *P. oceanica* and *C. nodosa* from the Mediterranean (northern hemisphere) and *P. australis* and *Z. muelleri* from South East Australia (southern hemisphere) were selected for a comparative study of their responses to warming. Plants were collected in the same seasonal conditions (i.e. late summer-early autumn: Mar–May in the southern hemisphere and Sept–Nov in the northern hemisphere) from both geographic areas and two mesocosm experiments were conducted following the same design. Our study represents a unique opportunity to compare (1) two climax species of the genus *Posidonia* (*P. oceanica* and *P. australis*) with similar characteristics and ecological functions but distributed in the two hemispheres and (2) two couples of climax-pioneer species from both hemispheres (*P. oceanica* vs. *C. nodosa* and *P. australis* vs. *Z. muelleri*). On the first hand, we hypothesized that the responses to warming of the two *Posidonia* species (i.e. *P. oceanica* and *P. australis*) would be different because sampled populations live under different thermal regimes (i.e. 13–28 °C for *P. oceanica*; Fig. 1b and 17–26 °C for *P. australis*; Fig. 1c) and because the species thermal ranges are also different (i.e. 8–30 °C for *P. oceanica* and 12–28 °C for *P. australis*; Fig. S1). Additionally, we note that the collection sites of the Australian seagrasses in this study did not fall into any Mediterranean-climate regions (see Cowling et al. 1996 for a map of Mediterranean-climate regions and Fig. 1a for sample collection sites). On the other hand, in both hemispheres, the climax seagrasses are expected to suffer more from thermal stress than their pioneer counterparts.

Materials and methods

Experiment 1: northern hemisphere experiment

Targeted species and plant collection

Posidonia oceanica is endemic to the Mediterranean Sea (see Fig. S1 for the species distribution) and forms large and dense monospecific meadows on rocks and sandy seabeds

ranging from shallow water (less than 1 m) down to 45-m depth (Procaccini et al. 2003). It ranks as one of the slowest-growing plants and is among the longest-living plants on Earth with single clones extending over kilometers and living for hundreds to thousands of years (Arnaud-Haond et al. 2012). *Cymodocea nodosa* distributes throughout the Mediterranean Sea and extends also in nearby subtropical Atlantic areas (see Fig. S1 for *C. nodosa* distribution). *C. nodosa* is a relatively fast-growing species, commonly found in shallow waters in both sandy and mud substrates where it forms both monospecific and mixed meadows with other seagrass species (den Hartog 1970; Guidetti et al. 1998). Hereafter, we use Med-Climax for *P. oceanica* and Med-Pioneer for *C. nodosa*.

Plant fragments (i.e. ramets) of Med-Climax (40°44.020' N, 13°58.039' E at 5–6 m depth; Fig. 1a-1) and Med-Pioneer (40°47.021' N, 14°04.404' E at 8–10 m depth; Fig. 1a-2) were haphazardly collected by SCUBA diving in the Gulf of Naples (Italy) on the 18th September 2019. To reduce the likelihood of sampling the same genotype twice, plants were collected at a minimum distance of 10 m from each other. Both *P. oceanica* and *C. nodosa* experience a wide species thermal range from 8 °C in the winter (especially in the northern Adriatic Sea) to 30 °C in the summer (especially in the eastern Mediterranean Sea), see Fig. S1 for more details. The two populations used in this study came from a similar thermal condition (i.e. 13–28 °C, see Fig. 1b) which falls in the middle of the species thermal range, therefore excluding the existence of a potential range-edge effect for the selected populations. After collection, plants were kept in dark in a cooler filled with seawater at ambient temperature and transported to a benthic mesocosm facility at the Stazione Zoologica Anton Dohrn (SZN), Napoli, Italy (see Ruocco et al. 2019b for a detailed description of the experimental system). Light intensity, salinity, and seawater temperature were measured at the time of plant sampling for setting up the experimental system.

Experimental system

Once at the SZN experimental facility, twelve plant fragments (i.e. ramets) of each species composed by horizontal rhizomes of a similar size and a similar number of interconnected vertical shoots (~ 10 shoots) were selected to standardize the experiment. Med-Climax ramets were transplanted in six plastic pots (i.e. two ramets per pot) filled with coarse carbonate sediments as described in Ruocco et al. (2019b), while Med-Pioneer ramets were transplanted in twelve plastic pots (i.e. one ramet per pot) filled with natural sediments from the collection site as described in Marín-Guirao et al. (2018). After transplantation, pots of each species were randomly allocated into six 500-L aquaria with filtered and UV-treated natural

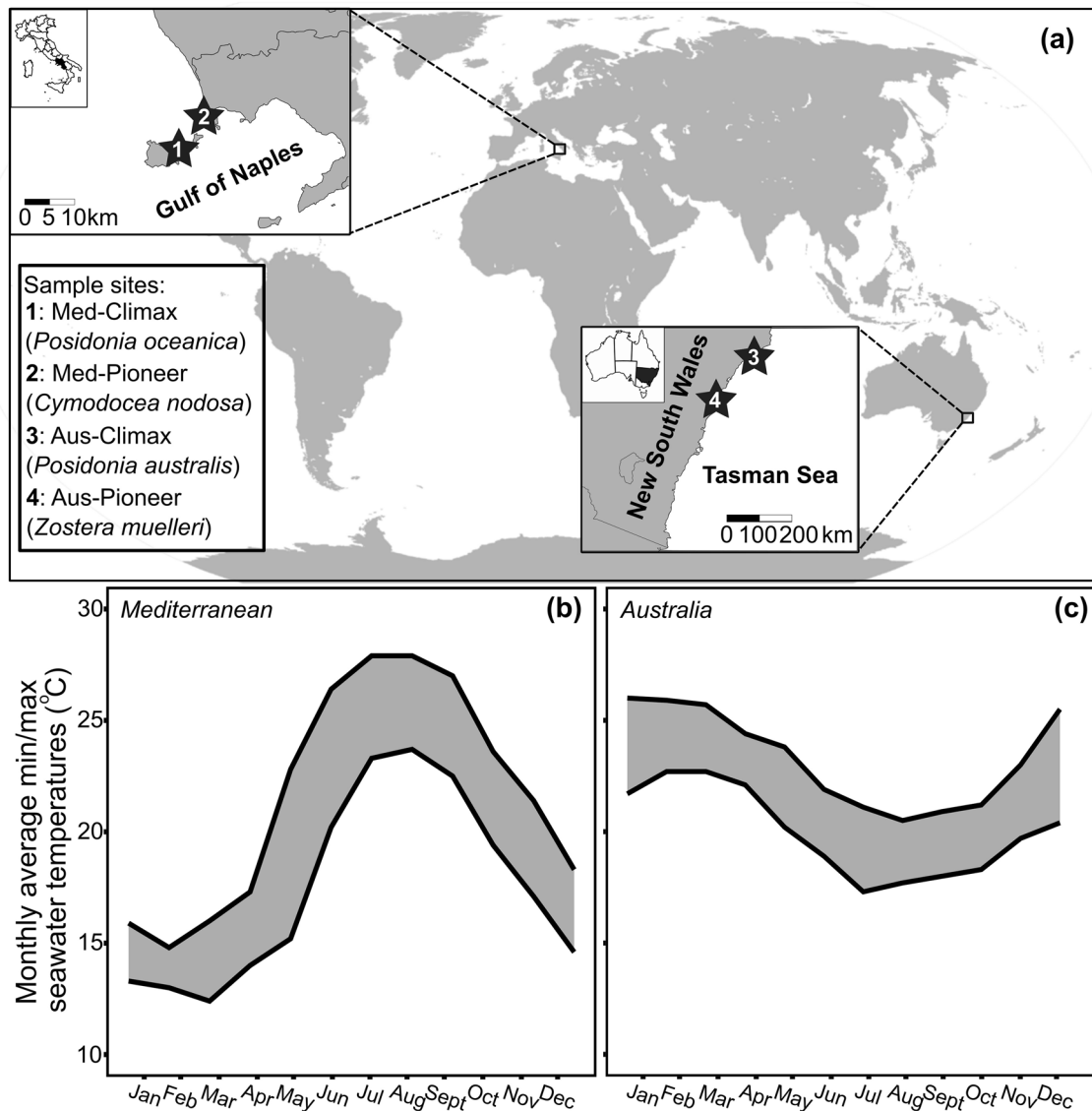


Fig. 1 Sample collection sites (a) and temperature conditions at collection sites (b, c). b Monthly average sea surface temperature in Ischia, Italy (Mediterranean sites: 1 & 2). c Monthly average sea tem-

perature in Port Stephens, NSW, Australia (Australian sites: 3 & 4) [Data were taken from World sea temperature of 2020 (<https://www.seatemperature.org/> data assessed on the 29th Nov 2020)]

seawater from a close area; each aquarium containing two ramets of the Med-Climax species and two ramets of the Med-Pioneer species. Transplant pots were distributed within aquaria to avoid crossed-species shading and their distance from the light source adjusted to reproduce similar light intensities to those measured at their collection sites (i.e. max noon irradiance: 300 and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ above the leaf canopy for Med-Climax and Med-Pioneer, respectively). A 12 h:12 h light:dark photoperiod was applied, starting from 7:00 a.m., and progressively increasing to the maximum irradiance at 13:00 before a gradual reduction until dark at 19:00. Water

temperature was measured automatically every 10 min using HOBO Pendant® Temperature/Light 64 K Data Logger (Onset, USA) and manually checked twice a day with WTW Cond 3310 Set 1 (Xylem Analytics, Germany). Seawater salinity of 37.5 ± 0.2 was kept constant throughout the experiment through regular additions of purified water. Seawater quality was controlled via continuous mechanical filtration, weekly-UV sterilizations, and partial renewals. An introductory video was made for this experiment and could be found on the website of Dr. Gabriele Procaccini's Laboratory (<https://gpgroupsxn.wixsite.com/webseite>; video: EpicSea2019).

Experiment 2: southern hemisphere experiment

Targeted species and plant collection

Posidonia australis is a slow-growing species found on sandy sediment between 1 and 15 m (Trautman and Borowitzka 1999). This species is distributed along the southern half of Australia, from Shark Bay in Western Australia to Port Macquarie in New South Wales, and along the northern coast of Tasmania (Fig. S1). With this distribution, *P. australis* exhibits the narrowest species thermal range (i.e. 12–28 °C) of the four seagrass species included in this study. *Zostera muelleri* is a fast-growing species, commonly found in shallow water (<4 m depth) on different sediments including fine sand, mud and others (Larkum et al. 2018). *Z. muelleri* is distributed along the eastern coast of Australia, Tasmania Kangaroo Island, Lord Howe Island, and New Zealand (Fig. S1; Waycott et al. 2004). The species' thermal range varies between 9 °C in the winter season (especially in the southern Tasmania Island and New Zealand) and 31 °C during summer (see Fig. S1). Hereafter, we use Aus-Climax for *P. australis* and Aus-Pioneer for *Z. muelleri*.

Ramets of Aus-Climax and Aus-Pioneer were collected, at distances > 25 m one from another to reduce the chance of sampling the same genotype twice. Plant fragments (i.e. ramets) were collected during low tides at ~ 70-cm depth at Port Stephens (PS), New South Wales (NSW), Australia (32°43'07.4" S 152°10'35.9" E; Fig. 1a) on the 19th of March 2019 and at Church Point (CP), NSW, Australia (33°38'46.8" S 151°17'11.9" E; Fig. 1a) on the 23rd of March 2019, respectively. Temperature ranges at both sampling locations between 17 °C in winter and 26 °C in summer. The maximum summer temperature is below the upper limits of species thermal ranges (i.e. 28 °C and 31 °C for *P. australis* and *Z. muelleri*, respectively; Fig. S1). Neither one of the two populations analyzed were at the edge of the species distribution range, but we acknowledged, in the interpretation of results, the difference in the upper limit of species thermal ranges. Light intensity and salinity were also measured at the time of sample collection. Plant materials were brought to the seagrass mesocosm facility at the University of Technology Sydney (UTS) soon after collection. A detailed description of the experimental system can be found in Nguyen et al. (2020b).

Experimental system

As soon as arrived at UTS, twelve ramets of each species with a similar number of shoots (i.e. 8–10 shoots) were selected and transplanted in individual plastic pots (i.e. one ramet per pot) filled with mini pebbles. Subsequently, pots were randomly distributed in tanks of the mesocosm facility: six 60-L aquaria for housing Aus-Climax pots and

six 40-L aquaria for Aus-Pioneer pots (i.e. two ramets per aquarium). For both species, the irradiance level was set with a max. noon irradiance of 350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at canopy height and a 12 h:12 h light:dark photo-period. Light cycle started from 7:30 a.m., with light levels progressively increasing to the maximum irradiance at 12:30 p.m. and kept for 2 h, before a progressive reduction until dark at 7:30 p.m. Water temperature was measured automatically every 30 min using iButton data logger (iButtonLink, USA) and manually checked twice a day using a digital thermometer (FLUKE 52II, USA). During the experiment, purified water was added periodically to maintain constant seawater salinity of 34 ± 0.2 similar to those in the fields. Approximately one third of seawater was renewed weekly in each aquarium to maintain water quality.

Experimental design

Both the northern and the southern hemisphere experiments shared the same experimental design. After transplantation and allocation within aquaria, plants of the four studied species were allowed to acclimate at 26 °C, which was similar to the seawater temperatures recorded during plant collection at the four studied populations (i.e. in every case the difference was lower than 1 °C). After a 5-week acclimation period, temperature in half of the aquaria containing each seagrass species (i.e. 3 aquaria) was progressively increased up to 32 °C at a heating rate of 1 °C day⁻¹ to simulate a marine heatwave (MHW); whereas the temperature in the rest of aquaria was maintained throughout the experiment (Fig. 2). Therefore, for each species, three tanks were randomly assigned to heat treatment (TM) and other three remained as controls (CT). Seagrass responses were analyzed at the end of the MHW exposure, which lasted 12 and 10 days in the northern and southern hemisphere experiments, respectively.

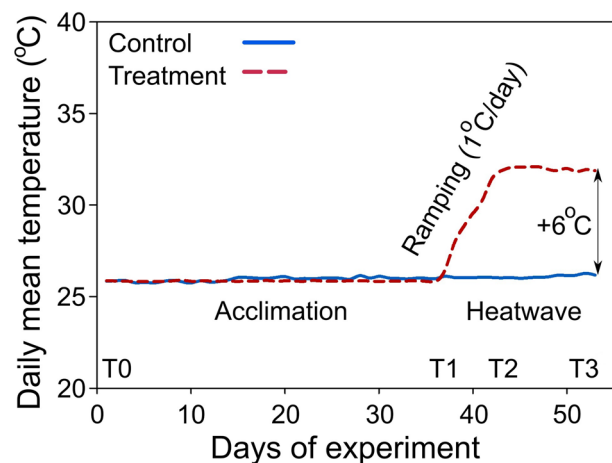


Fig. 2 Temperature profile during the two experiments

The aquarium is the true experimental unit for each seagrass species and variable, so that measurements performed on plants of the same aquarium (i.e. ‘pseudo replicates’) were averaged to obtain an independent replicated value. Therefore, the number of replicates used in statistical tests was $n = 3$.

Chlorophyll *a* fluorescence

Identical Diving-PAM fluorometers (WALZ, Germany) were used to determine the photo-physiological responses of the four studied seagrass species (Med-Climax, Med-Pioneer, Aus-Climax, and Aus-Pioneer) following the methodology described in Marín-Guirao et al. (2013). To standardize the procedure, two chlorophyll *a* fluorescence measurements were conducted on the same middle portion of the second youngest leaf of each plant (Ruocco et al. 2019a). Measurements included (a) maximum quantum yield (F_v/F_m) of photosystem II (PSII) measured on night dark-adapted plants (around 6:00–7:00 a.m. before the light cycle started), (b) effective quantum yield ($\Delta F/F_m'$) measured on light-adapted plants (around 12:30–13:30 while the irradiances were highest), and (c) non-photochemical quenching (NPQ) calculated using the method described elsewhere (Maxwell and Johnson 2000) to estimate the amount of photosynthetic energy lost as heat (i.e. the photo-protective mechanisms associated to the xanthophyll-cycle pigments; Marín-Guirao et al. 2013).

Plant growth

For both experiments, plant growth measurements were performed by adopting the leaf marking method (Zieman 1974). Two plants from each aquarium and species were marked at the same position above the ligule at the end of the acclimation period and subsequently collected at the end of the heatwave to measure leaf elongation (mm). Then, the newly developed leaf segments were cleaned of epiphytes and dehydrated at 70 °C for 24 h before being weighted to assess biomass production (mg dry weight).

Pigment content

At the end of the experiments, two plants of each species and from each aquarium were collected for the analysis of leaf pigment content. Approximately 50 mm of leaf tissue from the middle portion of the second youngest leaf of climax species (Med-Climax and Aus-Climax) and the whole second youngest leaf of pioneer species (Med-Pioneer and Aus-Pioneer) was used for the analysis. Epiphytes were immediately removed from the collected material, which was then kept on ice in darkness until further processing. Pigment extractions were done on the same day of sample collection.

After weight measurements, samples were homogenized in liquid nitrogen using pestles and mortars before being transferred into 1.5 mL tubes filled with 1 mL of 100% methanol. Thenceforward, samples were kept in complete darkness at 4 °C for 8 h before centrifugation. 200 μ L of the extracted solution was used to determine the absorbance at four different wavelengths (i.e. 470, 652, 665, and 750 nm) by the mean of microplate readers (TECAN Infinite[®] M1000PRO, Switzerland) to calculate chlorophyll *a*, chlorophyll *b*, chlorophyll *a + b*, chlorophyll *b/a* molar ratio and total carotenoids. Pigments were calculated using equations from Wellburn (1994) after converting microplate readings into 1-cm cuvette readings following Warren (2008). Finally, results were normalized to milligrams of fresh weight.

Statistical analyses

The response of seagrasses to experimental conditions was assessed using a three-way ANOVA ($n = 3$), including the following factors: hemisphere (2 levels: northern and southern, fixed), life-strategy (2 levels: climax and pioneer, fixed), and treatment (2 levels: control and treatment, fixed). Cochran’s *C* test was used to test homogeneity of variances and data were square-root transformed when necessary. Data were analyzed even when homogeneity of variances could not be achieved, as ANOVA is robust for this kind of assumption when the sizes of samples are equal (Underwood et al. 1997). However, in this case, the significance was judged more conservatively ($p < 0.01$) when interpreting results to reduce the likelihood of Type I error (which is inflated by heterogeneous variances). For each measurement, Student–Newman–Keuls (SNK) post hoc tests were used to identify significant differences between (1) control versus treatment plants of each hemisphere, each life-strategy, (2) northern versus southern plants of each life-strategy, each treatment, and (3) climax versus pioneer plants of each hemisphere, each treatment. All statistical analyses were conducted in R-studio v.1.2.5033 (R Core Team 2018) using package GAD (Sandrini-Neto and Camargo 2014).

Graphs were made with R-studio using package ggplot2 (Wickham 2009).

Results

Photo-physiological responses

Northern versus southern hemisphere seagrasses

Warming had strong impacts on the southern hemisphere seagrasses while did not result in any significant changes for the northern hemisphere plants (ANOVA: $H \times T$, $F(1,16) = 12.030$, $p < 0.01$) (Fig. 3). Warming significantly

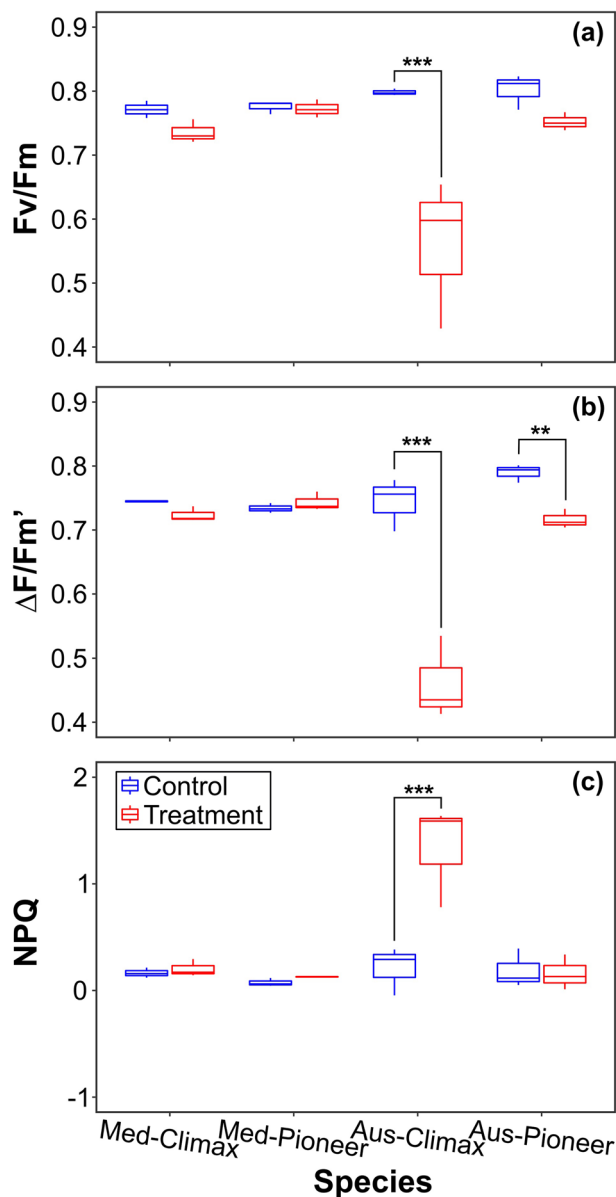


Fig. 3 Boxplot graphs present photo-physiological results at the end of the experiment ($n=3$). **a** Maximum quantum yield (F_v/F_m), **b** Effective quantum yield ($\Delta F/F_m'$), and **c** non-photochemical quenching (NPQ). Asterisks indicate statistical differences between control and treatment within each species (Student–Newman–Keuls post hoc test, ** $p < 0.01$, *** $p < 0.001$, more details can be found in Supplementary data, Table S1)

reduced F_v/F_m of the Aus-Climax plants (SNK test for 'H:L:T' among 'T' within 'H:L': $p < 0.001$; Fig. 3, Table S1), while slightly impacted the Med-Climax plants. Similarly, the Aus-Climax plants enhanced their NPQ with warming (SNK test for 'H:L:T' among 'T' within 'H:L': $p < 0.001$; Fig. 3, Table S1) while Med-Climax's NPQ remained relatively unchanged (Fig. 3). Both Aus-species dramatically lowered their $\Delta F/F_m'$ as a result of thermal

stress (Fig. 3; Table 1), while the $\Delta F/F_m'$ values of Med-plants were not negatively affected but, rather, slightly increased under warming in the case of the Med-Pioneer species (Fig. 3; Table 1). As a consequence, we detected a significant interaction in $H \times L \times T$ for $\Delta F/F_m'$ measurements (ANOVA: $F(1,16) = 14.267$, $p < 0.01$). It is important to highlight that while the control plants exhibited a similar level of performance, heated Climax plants from the two hemispheres responded differently and significant differences were detected from all photo-physiological measurements (SNK test for 'H:L:T' among 'H' within 'L:T': $p < 0.001$; Table S1).

Climax versus pioneer seagrasses

The simulated MHW strongly impacted the photosynthetic performances (both F_v/F_m and $\Delta F/F_m'$) of Aus-Climax plants, however, the level of warming impacts were much lower in the Aus-Pioneer plants (Fig. 3). Climax-pioneer dissimilarities in response to warming were also found in the activation of NPQ machinery. While Aus-Climax plants significantly activated their NPQ machinery (Fig. 3) as mentioned above, on the other hand, Aus-Pioneer plants did not alter their NPQ even at the same warming condition (Fig. 3). This is also evidenced from the SNK results for 'H:L:T' among 'L' within 'H:T' when no significant difference detected for Aus-control plants but Aus-treatment plants (SNK test: $p < 0.001$; Table S1).

Plant growth responses

Northern versus southern hemisphere seagrasses

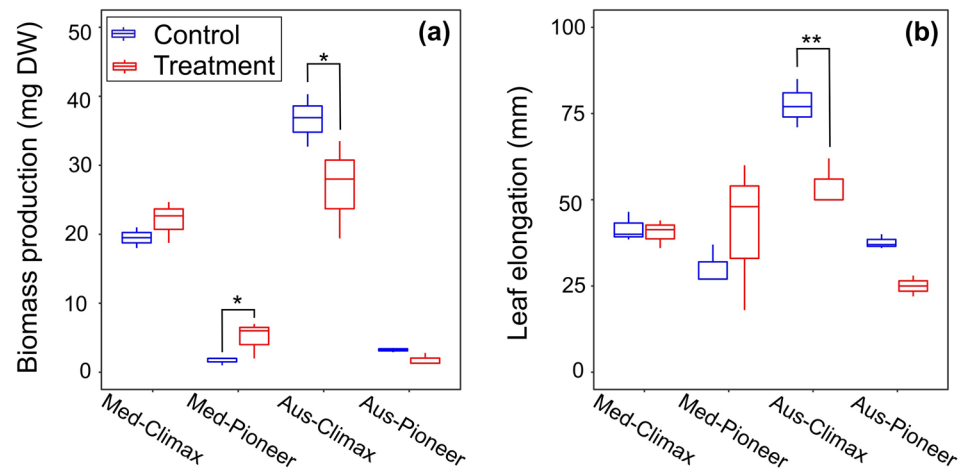
There were differences in response to warming between northern versus southern hemisphere seagrasses in both biomass production and leaf elongation measurements (Fig. 4), as shown by the significant $H \times T$ interactions (ANOVA: $F(1,16) = 14.532$, $p < 0.01$ and $F(1,16) = 10.151$, $p < 0.01$, respectively). Among climax plants, warming significantly reduced biomass production (SNK test: $p < 0.05$; Fig. 4a) as well as leaf elongation (SNK test: $p < 0.01$; Fig. 4b) of the southern plants. On the other hand, warming favored the developments of the northern ones in terms of productivity (Fig. 4a, b). Differently, warming increased the growth of northern pioneer plants (e.g. a significant difference between control versus treatment detected for biomass production, SNK test: $p < 0.05$; Fig. 4a). In contrast, the southern pioneer plants suffered a reduction in growth as a result of their exposure to a simulated MHW (Fig. 4a, b).

Table 1 Results of three-way ANOVA analyses

Source of variation	Fv/Fm			$\Delta F/Fm'$			NPQ			Biomass production			Leaf elongation								
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p					
Hemisphere (H)	1	0.008	3.902	ns	1	0.021	24.429	***	1	0.340	15.795	**	1	1.183	7.162	*	1	596.670	7.381	ϕ	***
Life-strategy (L)	1	0.021	10.778	**	1	0.036	42.631	***	1	0.497	23.113	***	1	70.854	429.089	***	1	2327.230	28.790	***	***
Treatment (T)	1	0.040	20.650	***	1	0.051	60.076	***	1	0.180	8.377	*	1	0.015	0.091	ns	1	251.340	3.109	ns	ns
H×L	1	0.009	4.629	ϕ	1	0.032	37.824	***	1	0.185	8.620	**	1	2.236	13.540	**	1	1315.230	16.271	***	***
H×T	1	0.023	12.030	**	1	0.045	52.699	***	1	0.068	3.174	ns	1	2.398	14.523	**	1	820.560	10.151	**	**
L×T	1	0.018	9.423	**	1	0.022	25.552	***	1	0.139	6.442	*	1	0.395	2.394	ns	1	214.000	2.647	ns	ns
H×L×T	1	0.009	4.669	ϕ	1	0.012	14.267	**	1	0.186	8.642	**	1	0.017	0.103	ns	1	1.340	0.017	ns	ns
Residual	16	0.002			16	0.001			16	0.022			16	0.165			16	80.830			
Transformation		None				None				Sqrt				Sqrt				None			
Cochran's C test		$p < 0.001$				$p < 0.01$				$p > 0.05$				$p > 0.05$				$p < 0.001$			
Source of variation	Chlorophyll a			Chlorophyll b			Chlorophyll a+b			Total carotenoids			Chlorophyll b/a molar ratio								
Hemisphere (H)	1	6.422	80.732	***	1	2.983	176.301	***	1	18.158	109.855	***	1	0.308	37.524	***	1	0.097	131.972	***	***
Life-strategy (L)	1	2.086	26.225	***	1	0.648	38.290	***	1	5.059	30.606	***	1	0.116	14.151	**	1	0.007	9.783	**	**
Treatment (T)	1	0.585	7.360	*	1	0.061	3.633	ns	1	1.026	6.209	*	1	0.041	5.054	*	1	0.016	21.394	***	***
H×L	1	3.395	42.674	***	1	1.680	99.275	***	1	9.850	59.591	***	1	0.138	16.805	***	1	0.072	97.368	***	***
H×T	1	0.432	5.424	*	1	0.008	0.464	ns	1	0.556	3.362	ns	1	0.021	2.595	ns	1	0.046	62.871	***	***
L×T	1	0.596	7.491	*	1	0.230	13.619	**	1	1.567	9.483	**	1	0.024	2.907	ns	1	0.004	5.737	*	*
H×L×T	1	0.945	11.884	**	1	0.157	9.305	**	1	1.874	11.339	**	1	0.095	11.544	**	1	0.003	4.561	*	*
Residual	16	0.080			16	0.017			16	0.165			16	0.008			16	0.001			
Transformation		None				None				None				None				None			
Cochran's C test		$p > 0.05$				$p > 0.05$				$p > 0.05$				$p > 0.05$				$p > 0.05$			

Significant codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns $p > 0.05$; ϕ means $0.01 < p < 0.05$ but not interpreted as significant because of variance heterogeneity in Cochran's C test
Sqrt square root

Fig. 4 Boxplot graphs present plant growth response results at the end of the experiments ($n=3$). Asterisks indicate statistical differences between control and treatment within each species (Student–Newman–Keuls post hoc test, $*p<0.05$, $**p<0.01$, more details can be found in Supplementary data, Table S1)



Climax versus pioneer seagrasses

Even if we did not detect any significant difference between climax versus pioneer species within each hemisphere (ANOVA: $L \times T$, $p > 0.05$ for both plant growth response measurements), it is interesting to note that there were significant interactions of $H \times L$ for both biomass production and leaf elongation (ANOVA: $F(1,16) = 13.540$, $p < 0.01$ and $F(1,16) = 16.271$, $p < 0.001$, respectively). For Med-seagrasses, even if warming generally enhanced the developments of both Med-Climax plants and Med-Pioneer plants, the levels of increments were significantly higher in Med-Pioneer plants in comparison with its climax counterpart (Fig. 4a, b). Differently, Aus-Climax plants exhibited greater impact of warming when compared with their pioneer counterparts (Fig. 4a, b) with significant differences between control versus treatment detected for both plant growth response measurements only for Aus-Climax plants (SNK test: $p < 0.05$ and $p < 0.01$; Fig. 4a, b).

Pigment content responses

Results from pigment content measurements showed complex interactions between northern versus southern as well as climax versus pioneer seagrasses in response to warming. Significant interactions were detected in $H \times L \times T$ for all pigment measuring parameters (ANOVA, $p < 0.05$, Table 1). Details are presented below.

Northern versus southern hemisphere seagrasses

Warming significantly reduced all pigments content of Med-Climax plants such as Chl *a* (SNK test: $p < 0.01$, Fig. 5a), Chl *b* (SNK test: $p < 0.01$, Fig. 5b), Chl *a + b* (SNK test: $p < 0.01$, Fig. 5c), and total carotenoids (SNK test: $p < 0.01$, Fig. 5d) but did not result in any significant reduction in pigment content for Aus-Climax plants (except for the case

of Chl *a*, although the level of reduction was greater in Med-Climax plants; Fig. 5a–d). Interestingly, while Med-Climax plants maintained their Chl *b/a* molar ratio during HW, Aus-Climax plants significantly increased the ratio (SNK test: $p < 0.01$, Fig. 5e). It is worth mentioning that while Med-Pioneer plants accumulated more pigment content under the increased temperature, Aus-Pioneer plants reduced the accumulation of these pigments (see Fig. 5a–d). Likewise, Aus-Pioneer plants increased their Chl *b/a* molar ratio as a result of warming, while Med-Pioneer plants exposed to warming showed values similar to control plants (Fig. 5e).

Climax versus pioneer seagrasses

Warming greatly impacted the Med-Climax plants in terms of pigment contents including Chl *a*, Chl *b*, Chl *a + b* as well as total carotenoids with significant differences detected between control versus heated plants across all these measurements (Fig. 5a–d). On the contrary, warmed Med-Pioneer plants significantly improved pigment contents as a result of warming (Fig. 5a–d). Furthermore, we assessed a statistical difference between heated Med-Climax plants versus heated Med-Pioneer plants in terms of total carotenoids' response (SNK test for 'H:L:T' among 'L' within 'H:T': $p < 0.001$; Table S1). For the southern hemisphere plants, warming negatively affected both climax and pioneer plants in terms of pigments (Fig. 5).

Discussion

Difference between northern versus southern hemisphere seagrasses in response to warming

When two sister species of the genus *Posidonia* were exposed to a similar simulated MHW (i.e. 32 °C), their photo-physiological and plant growth responses clearly

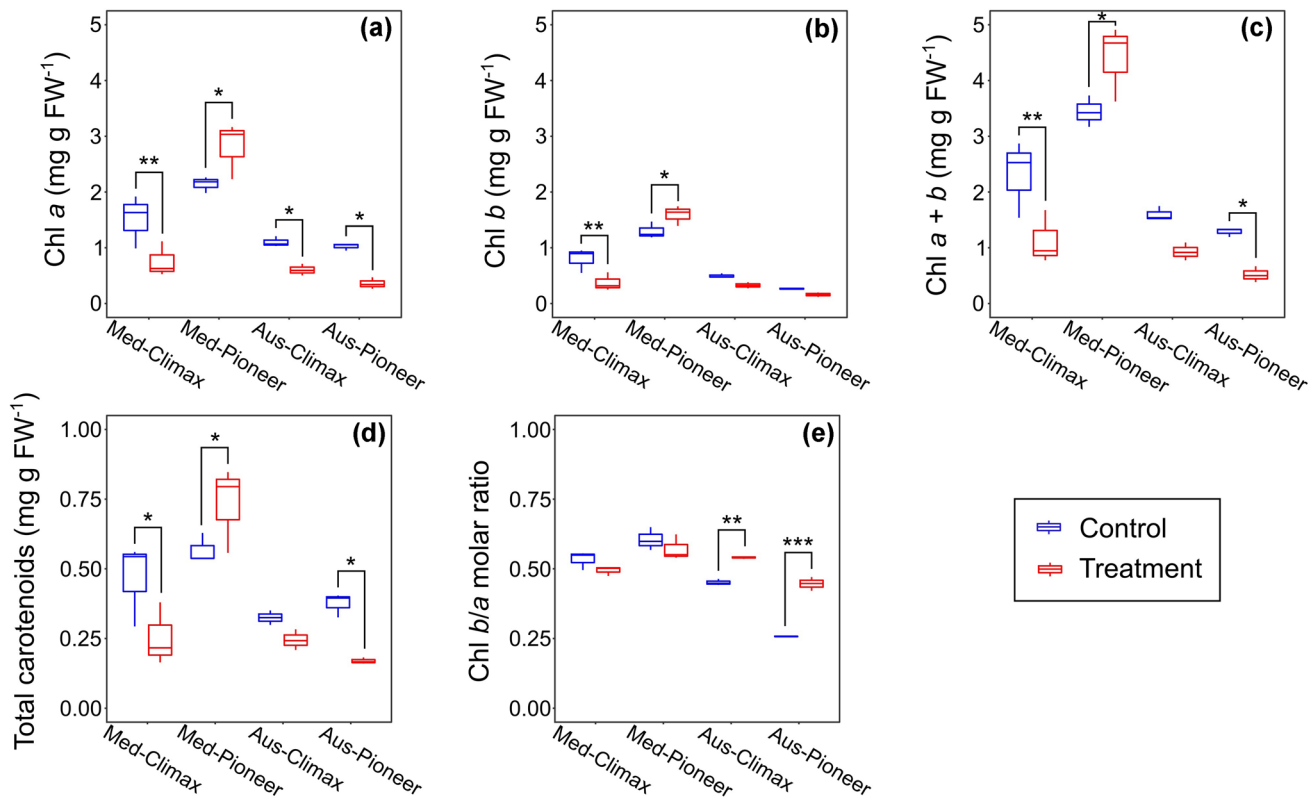


Fig. 5 Boxplot graphs of pigment results at the end HW exposure ($n=3$). Asterisks indicate statistical differences between control and treatment within each species (Student–Newman–Keuls post hoc

test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, more details can be found in Supplementary data, Table S1)

demonstrated that the southern hemisphere species *P. australis* (i.e. Aus-Climax) is more sensitive to anomalous thermal events than the northern hemisphere species *P. oceanica* (i.e. Med-Climax). Warming dramatically affected the photosynthetic performance of *P. australis*, while the photosynthetic functioning of *P. oceanica* was unaffected. The impairment of the photosynthetic apparatus, reflected as a reduction in the maximum (i.e. Fv/Fm) and effective photochemical efficiency (i.e. $\Delta F/Fm'$), is a common response in seagrasses subjected to thermal stress (e.g. see Winters et al. 2011; Marín-Guirao et al. 2016; Ruocco et al. 2019a; Nguyen et al. 2020a for some recent studies) and evidenced a higher photosynthetic thermal sensitivity in *P. australis* with regard to *P. oceanica*. This was further supported by the fact that only *P. australis* activated the NPQ machinery, a well-known photo-protective mechanism in plants (including seagrasses) that mitigates the damaging effects of a heat-induced photosynthetic malfunction by dissipating excess energy as heat (e.g. see Ashraf and Harris 2013 for a review in plants and Marín-Guirao et al. 2016; Ontoria et al. 2019 for some recent studies in seagrasses). Moreover, only *P. australis* experienced a significant growth inhibition during the warming exposure. Reduction in plant growth is a major consequence of growing under stress conditions and is

commonly associated with photosynthetic constrains under high temperatures and with the diversion of resources from growth to sustain a heat-stress response and to repair heat-induced damage (e.g. Wahid et al. 2007; Bitá and Gerats 2013; York et al. 2013; Collier et al. 2017; Marín-Guirao et al. 2018). Interestingly, while warming reduced the overall pigment content (i.e. Chl *a*, Chl *b* and carotenoids content) of *P. oceanica* plants, the same level of warming only reduced Chl *a* content in the southern hemisphere plants. This resulted in a significant Chl *b/a* molar ratio increment (i.e. a proxy of PSII antenna size), suggesting that *P. australis* attempted to counterbalance their heat-impaired photosynthetic performance by enhancing their light harvesting efficiency.

Both *P. oceanica* and *P. australis* together with seven other species including *P. sinuosa*, *P. angustifolia*, *P. coriacea*, *P. denhartogii*, *P. kirkmanii*, *P. ostenfeldii*, and *P. robertsoniae* belong to the genus *Posidonia* which is among the most primitive marine angiosperm genus (den Hartog 1970; Kuo and Cambridge 1984). Interestingly, while *P. oceanica* is endemic to the Mediterranean, the other eight species (including *P. australis*) occur exclusively in the subtropical and temperate Australian seas (Kuo and Cambridge 1984). It is still unclear when the single Mediterranean species

and the Australian congeneric counterparts diverged. Phillips and Menez (1988) suggested it could have happened during the late Eocene, about 40 million years ago (Mya), while Les et al. (2003) estimated a more recent separation of 16.7 ± 12.3 Mya. A more recent study from Aires et al. (2011) predicted this divergence would have taken place much earlier in the ancient Tethys Sea (i.e. over 60 Mya). In any case, the disconnection of Mediterranean *Posidonia* with the Australian ones has allowed the two groups to evolve in two contrasting environmental and evolutionary conditions (i.e. Mediterranean Sea versus Australian Seas). Compared to the Australian Seas, the Mediterranean has undergone massive changes during its history (Bianchi and Morri 2017). Especially, due to anthropogenic climate change, the Mediterranean Sea waters have warmed up at a faster pace (Bianchi and Morri 2017; Ozer et al. 2017; Nguyen et al. 2020a), become saltier (Borghini et al. 2014), and exhibited more frequent and intense extreme oceanic events (e.g. MHWs, see Darmaraki et al. 2019). In addition, not only the species but also the studied *P. oceanica* population thrives in a broader thermal regime (i.e. 13–28 °C, Fig. 1b; see Fig. S1 for more details about species distribution and species thermal range) than *P. australis* (i.e. 17–26 °C, Fig. 1c; see Fig. S1 for more details about species distribution and species thermal regime); and this, together with the evolutionary differences among both *Posidonia* species stated above, may explain why the northern hemisphere *Posidonia* was less affected by warming than its southern hemisphere counterpart.

Regarding the pioneer seagrass species, this study also pinpoints some dissimilarities in the response to warming between *C. nodosa* (i.e. Med-Pioneer) and *Z. muelleri* (i.e. Aus-Pioneer). For example, warming significantly impacted the photosynthetic functioning of *Z. muelleri* (i.e. reduced $\Delta F/F_m'$ values) while no significant changes were detected for *C. nodosa*. Likewise, warming favored the growth and biomass production of *C. nodosa* but not for *Z. muelleri*, and similar divergences were also found in their responses at the level photosynthetic pigments. These divergent responses to seawater warming manifested that the *C. nodosa* species, which is indeed benefited by increased temperatures, is more tolerant to anomalous heat events than the *Z. muelleri*. This finding suggests that the differences in response to warming among northern and southern hemisphere seagrasses may not be limited to the genus *Posidonia*, but extended to other seagrass species across hemispheres. However, since both pioneer species belong to different families with contrasting origins and estimated ages (*Cymodoceaceae*: 67 Mya vs. *Zosteraceae*: 47 Mya; Janssen and Bremer 2004; Waycott et al. 2007), the comparison is not as direct as in the two studied *Posidonia* species. Hence, further studies to compare the responses to warming of other seagrass species across hemisphere are warranted.

Difference between climax versus pioneer seagrasses in response to warming

The northern hemisphere climax and pioneer species reacted almost in the same way to warming in terms of photo-physiology (i.e. no significant changes along with warming) and growth (i.e. greatly enhancements along with warming), whereas their responses differed in regard to pigment content modifications. The climax plants reduced all pigments (i.e. Chl *a*, Chl *b* and total carotenoids) during the warming exposure, while on the contrary, the pioneer plants increased the overall pigment content as a result of warming. These results indicated that, although both species came from the same thermal regime (both population and species), the climax seagrass was slightly impacted by the simulated MHW while the pioneer species even benefited from the warming exposure. The differences between the southern hemisphere species in response to warming was stronger for the photo-physiological parameters. *P. australis* plants experienced greater reductions in both Fv/Fm and $\Delta F/F_m'$ values compared with *Z. muelleri* plants (Fig. 3a, b). In addition, only the climax plants significantly increased their NPQ as a result of thermal stress. Additionally, the Australian species did not show differences between climax and pioneer species in regard to pigment content response. These results are in line with findings from the northern hemisphere experiment on demonstrating that climax seagrasses are more prone to be adversely affected by warming than pioneer species. The fact that we observed the ‘climax-pioneer’ pattern in different traits for the two hemispheres suggests that species also differed in their mechanisms of response to warming.

To correctly interpret this result, we should take into consideration that the difference in the species thermal range can affect the species response to the temperature imposed during the experiment. *P. australis* was exposed to a warming treatment that goes above its theoretical thermal tolerance, possibly masking the effect due to its life-strategy. Nevertheless, species temperature range was here assessed looking at species distribution, but maximum temperature tolerance limits can be higher. This is the case of *P. oceanica*, where the species occurs in a coastal enclosed lagoon (i.e. the Stagnone di Marsala, Italy) exhibiting temperature and salinity tolerant levels above its theoretical tolerance limit (Tomasello et al. 2009).

Differences between the response of climax versus pioneer species to environmental stressors have been previously documented in other seagrasses. For instance, Masini and Manning (1997) showed the pioneer seagrasses (i.e. *Amphibolis griffithii* and *A. antarctica*) were more resilient to changes in light and temperature when compared to two other climax seagrasses (i.e. *P. sinuosa* and *P. australis*) inhabiting in the same region of Western Australia. Similarly, the Mediterranean pioneer *C. nodosa* was also shown

to be more thermal tolerant than the Mediterranean climax *P. oceanica* (Marín-Guirao et al. 2018), but also to other abiotic stress factors including light (Olesen et al. 2002) and salinity (Sandoval-Gil et al. 2014), which seems to be related to their different levels of phenotypic plasticity (Pazzaglia et al. 2021). Seddon and Cheshire (2001) also suggested that the climax *P. australis* is more vulnerable to desiccation in high-temperature conditions than the pioneer *A. antarctica*. All these evidences imply that warming can reshape the seagrass landscape by reducing the presence of climax species while enhancing the distribution of pioneer seagrasses. For instance, in Mission Bay, San Diego Bay, and Chesapeake Bay (USA), the climax seagrass *Z. marina* was replaced by the pioneer *Ruppia maritima* following extreme climatic events (Johnson et al. 2003; Shields et al. 2019). The same phenomenon is predicted to occur also in the Mediterranean, where ocean warming is expected to cause a decline of *P. oceanica* (Marbà and Duarte 2010; Chefaoui et al. 2018) while favoring the expansion of some pioneer species (e.g. *C. nodosa*, *Halophila stipulacea*) (Savva et al. 2018; Winters et al. 2020). Changes in seagrass meadow composition at the landscape scale would ultimately reduce their ecological value (Orth et al. 2006; Lamb et al. 2017; Unsworth et al. 2019) and, hence, affect the livelihoods of billions of people living in coastal areas (Bertelli and Unsworth 2014). The replacement of climax seagrass species, generally characterized by high biomass and productivity, by pioneer species will also decrease the capacity of seagrass meadows to mitigate the effects of carbon emissions (Gattuso et al. 2018). Under some warming scenarios, seagrass ecosystems may even switch their metabolism from autotrophic to heterotrophic (Burkholz et al. 2019), and enhance CO₂ and methane fluxes from the meadows into the atmosphere (Burkholz et al. 2020).

Future perspectives

Our study brings evidence of differences in the response to warming between climax and pioneer seagrasses and suggests that these differences are consistent between the northern and southern hemispheres. Ocean warming is happening fast and accelerating the tropicalization of temperate seagrass meadows (see review by Hyndes et al. 2016). Our results emphasize the need to protect climax seagrasses because they are more ecologically valuable but also more susceptible to warming than pioneer species. Optimistically, we can enhance the resilience of climax seagrasses to warming through genotype selection, synthetic biology, assisted evolution, and the use of microbiome (Bulleri et al. 2018). Such approaches require, however, a deeper understanding of the relationship between seagrasses and the surrounding environments.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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