Warm and cool-edge populations reveal high resilience of seagrass (Posidonia oceanica) to warming

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Abstract

Disentangling spatial variation in climate change impacts is a pressing challenge. Here we compared the performance of Posidonia oceanica seagrass populations to temperature, throughout a year-long translocation experiment across 2800 km in the Mediterranean Sea. Transplants in central and warm-edge locations experienced temperatures >29 ºC during summer, representing thermal anomalies >5ºC above long-term maxima for cool-edge populations, 1.5ºC for central and <1ºC for warm-edge populations. At the onset of the experiment, a highly selective herbivory event removed 75% of cool-to-warm transplant biomass but left adjacent central and warm-edge treatments intact. Despite big differences in thermal stress and acute herbivory, cool-edge populations recovered and matched warm-edge populations across all performance metrics. Central populations displayed significantly lower growth and survivorship in response to thermal stress. Our findings reveal that intraspecific variation in thermal performance does not necessarily reflect thermal geography and suggest greater resilience to warming for Posidonia oceanica than previously recognised.

Introduction

Ocean warming is having profound effects on benthic marine ecosystems across the globe (Hoegh-Guldberg & Bruno 2010). Foundation species such as seaweeds, seagrasses and corals can be particularly susceptible to warming and have undergone extensive thermal stress (Hughes *et al.* 2018), mortality (Marba & Duarte 2010), and range contraction (Wernberg *et al.* 2016) over the past two decades. In conjunction with direct physiological impacts, ocean warming is leading to changes in species distributions resulting in new assemblages of species coming together and competing for space and resources within an ever-changing physical environment (Lenoir *et al.* 2020). This combination of factors creates a diverse and dynamic array of potential outcomes of climate change on the structure and function of marine ecosystems.

Temperature effects on populations across a species geographical range are not homogeneous and depend on the population's thermal range position and a species evolutionary history and adaptive capacity (Angilletta

2009; Somero 2010). For example, if individuals from different populations display similar optimal and upper thermal limits, then cool-edge populations will have lower sensitivity to warming than warm-edge populations where ambient temperatures are already close to upper thermal limits (Bennett *et al.* 2019). This is an inherent assumption of many species distribution models that rely on the realised distribution of species to predict the likelihood of extirpation or range shifts under climate change (Araújo & Peterson 2012). If, on the other hand, thermal performance differs between populations– through local adaptation or acclimatization – then the thermal safety margin between ambient conditions and an individual's upper thermal limit may remain relatively constant between populations across a species range (Bennett *et al.* 2015). This is an inherent assumption used to estimate the thermal sensitivity of reef building corals, for which the magnitude and the duration of local thermal anomalies are strong predictors of thermal bleaching (McClanahan *et al.* 2019). Determining whether thermal performance varies between populations and how this relates to local climatology or a species distribution is critically important for our capacity to anticipate and manage climate change impacts. For the vast majority of species, within-species patterns of thermal sensitivity remain unknown, highlighting a fundamental knowledge gap in climate change ecology.

An additional challenge for predicting the impacts of climate change on marine ecosystems is the influence of different ecological processes such as biotic interactions, on the outcomes of warming (Araújo & Luoto 2007). As species redistribute around the world, novel interactions are emerging that may either accelerate or buffer ecosystems from change under different environmental contexts (Gilman *et al.* 2010; Lurgi*et al.* 2012). Incorporating this complexity into models is challenging based on current approaches (Pagès *et al.* 2018). For example, thermal performance studies are routinely conducted in controlled laboratory environments which are invaluable for understanding complex physiological and evolutionary processes, but often miss key ecological processes and trade-offs that may determine an organism's success in the wild (Buñuel *et al.* 2020). While the ecological realism of experimental systems is improving (Ullah *et al.* 2018), calibrating laboratory-based experiments with real world multi-species ecological interactions remains a challenge. At the other end of the spectrum, direct impacts of thermal stress on natural ecosystems from marine heatwaves and long-term warming offer invaluable insights into the whole-of-ecosystem response to warming (Smale *et al.* 2019). However, such events are irregularly distributed and historically uncommon (Oliver *et al.* 2018). In addition, the ecological consequences of marine heatwaves can be catastrophic, undermining the health of the ecosystem for which the information is needed (Wernberg *et al.* 2016).

In this study we took an intermediate approach and conducted a 12-month translocation experiment of the seagrass *Posidonia oceanica*across its geographical range. Translocation experiments have the benefit of directly comparing the performance of geographically distant populations under common, relatively natural ecological settings. We conducted our study across a steep 5ºC gradient in average annual temperatures from the western to eastern Mediterranean Sea. The Mediterranean has experienced rapid warming between 0.25-0.65 ºC dec^{-1} over the past three decades, 2-3 times faster than the average global ocean (Marbà *et al.* 2015). Contemporary climate change has impacted central populations of *P. oceanica* in recent decades (Marba & Duarte 2010) and previous studies have predicted that the species could face functional extinction by 2050 (Jordà *et al.* 2012; Chefaoui *et al.* 2018). High thermal sensitivity in central populations raises questions about the sensitivity of warm-edge populations, which regularly face summer temperatures that are similar to heatwave conditions attributed to losses in the western basin. The aim of this experiment was to compare the eco-physiological performance of *P. oceanica* across its thermal distribution to quantify the thermal sensitivity of different populations to climate warming. We test the hypothesis that thermal performance reflects the thermal geography of a species either through local adaptation or thermal niche conservatism across its distribution.

Methods

Experiment locations and climate

Trans-Mediterranean translocation of *Posidonia oceanica* fragments took place between Catalunya (Spain), Mallorca (Spain) and Cyprus in July 2018 and were monitored until July 2019 (Fig. 1). Sea surface temperature data for each transplant site were based on daily SST maps with a spatial resolution of 1/4°, obtained from the National Center for Environmental Information (NCEI,*https://www.ncdc.noaa.gov/oisst* (Reynolds *et al.*2007). These maps have been generated through the optimal interpolation of Advanced Very High Resolution Radiometer (AVHRR) data for the period 1981-2019. Underwater temperature loggers (ONSET Hobo pro v2 Data logger) were deployed at the transplant sites in Catalunya, Mallorca and Cyprus and recorded hourly temperatures throughout the duration of the experiment (one year). In order to obtain an extended time series of temperature at transplant sites, a calibration procedure was performed comparing logger data with sea surface temperature from the nearest point on SST maps. In particular, SST data were linearly fitted to logger data for the common period. Then, the calibration coefficients were applied to the whole SST time series to obtain corrected-SST data and reconstruct daily habitat temperatures from 1981-2019. Local climate data was also compared to the global thermal distribution of *P. oceanica* to assess how representative experimental sites were of the thermal distribution of the species (Supplementary materials). Collectively, seawater temperatures from the three locations span the 16^{th} - 99^{th} percentile of temperatures observed across the global thermal distribution of *P. oceanica.* As such Catalunya, Mallorca and Cyprus are herein considered to represent the cool-edge, centre and warm-edge of *P. oceanica*distribution, respectively.

Transplantation took place toward warmer climates and procedural controls were conducted within each source location, resulting in six source-to-recipient treatments (Fig. 1). Initial collection of *P. oceanica* , handling and transplantation was carried out simultaneously by coordinated teams in July 2018 (Table S1). Each recipient location was subsequently resampled four times throughout the experiment, in August/September 2018 (T1), October 2018 (T2), April 2019 (T3) and May/June 2019 (T4, Table S1).

Between 60-100 fragments were collected for each treatment. A fragment was defined as a section of *P. oceanica* containing one apical shoot connected with approximately five vertical shoots and 10-15 cm of rhizome with intact roots. Collection occurred at two sites within each location, separated by approximately 1 km. Within sites, collections were conducted between 4 – 5 m depth and were spaced across the meadow to minimise the dominance of a single clone and damage to the meadow. Upon collection, fragments were transported for up to one hour back to the nearest laboratory in shaded seawater.

Handling methods

In the laboratory, fragments were placed into holding tanks with aerated seawater, at ambient temperature and a 14:10 light-dark cycle. All shoots were clipped to 25 cm length (from meristem to the tip of the longest leaves), to standardise initial conditions and reduce biomass for transportation. For transport by plane or ferry between locations, fragments were packed in layers within cool-boxes. Each layer was separated by frozen cool-packs wrapped in wet tea towels (rinsed in sea water). All fragments spent 12 hrs inside a cool-box irrespective of their recipient destination, including procedural controls (i.e. cool-cool, centre-centre and warm-warm) to simulate the transit times of the plants travelling furthest from their source location (Fig. 1a). On arrival at the destination, fragments were placed in holding tanks with aerated seawater at ambient temperature as described above in their recipient location for 48 hrs, prior to field transplantation.

Measurement methods

One day prior to transplantation, fragments were tagged with a unique number and attached to a U-shaped peg. Morphological traits for each fragment were measured and included: 1) length of the longest apical leaf, width and number of leaves 2) total number of bite marks on leaves of three vertical shoots per fragment, 3) number of vertical shoots, 4) leaf count of three vertical shoots per fragment and 5) overall horizontal rhizome length. A subset (n=10) of fragments per treatment were marked prior transplantation to measure shoot growth. To do this, all shoots within a single fragment were pierced using a hypodermic needle. Two holes were pierced side-by-side at the base of the leaf/top of the meristem.

Transplant methods

All transplant sites were located in 4 – 5 m depth in area of open dead-matte, surrounded by *P. oceanica* meadow. In Mallorca and Cyprus, fragments were distributed between two sites, separated by approximately 1 km. In Catalunya, lack of suitable dead matte habitat meant that all fragments were placed in one site.

Fragments were planted along parallel transects at 50 cm intervals and with a 50 cm gap between parallel transects (Fig. S1). Different treatments were mixed and deployed haphazardly along each transect.

Resampling methods and herbivory

On day 10 of the experiment, a severe herbivory event was recorded at both warm-edge translocation sites. Scaled photos of all fragments were taken at this time to record the effects of herbivory on transplants. At the end of each main sampling period (T0 – T1, T1-T2 and T3 – T4), all pierced fragments were collected and taken back to the laboratory to measure shoot growth. At T1, T2 and T3, additional sets of fragments (n = 10 per treatment) were marked using the piercing method to record growth in the subsequent time period. In addition, at T1 and T3, n = 20 shoots within the natural meadow at each site were marked to compare growth rates between the native meadow and transplants. Underwater shoot counts and a scaled photo was taken to record fragment survivorship, shoot mortality, bite marks, and shoot length among all remaining fragments within each site and sampling time.

In the laboratory, morphological measurements (described above) were repeated on the collected fragments and growth of transplant and natural meadow shoots was measured. Growth (shoot elongation, cm d^{-1}) of the marked shoots was obtained by measuring the length from the base of meristem to marked holes of each leaf (new growth) of the shoot and dividing the leaf elongation per shoot by the marking period (in days). For each shoot, total leaf length (cm shoot^{-1}) and the number of new leaves was recorded. The rate of new leaf production (new leaves shoot^{-1}d^{-1}) was estimated dividing the number of new leaves produced per shoot and the marking period. New growth was dried at 60 ºC for 48 hrs to determine carbon and nitrogen content of the leaves, and carbon to nitrogen (C:N) ratios. Carbon and nitrogen concentrations in the new growth leaf tissue was measured at the beginning of the experiment and each subsequent time point for each treatment. Nutrient analyses were conducted at Unidade de Técnicas Instrumentais de Análise (University of Coruña, Spain) with an elemental analyser FlashEA112 (ThermoFinnigan).

Underwater photos of shoots were analysed using ImageJ software (https://imagej.net). Maximum leaf length on each shoot in warm-edge transplant sites (cool-warm, centre-warm and warm-warm) were recorded for the initial (day 10) herbivore impact, T1, T2 and T3 and related to transplant nutrient concentrations. Herbivore impact was estimated as the proportional change in length of the longest leaf relative to initial length at T0.

Thermal stress

Long term maximum temperatures were recorded as the average of annual maximum daily temperatures in each transplant site, averaged between years from 1981-2019. Maximum thermal anomalies were calculated as the difference between daily temperatures in a recipient site over the course of the experiment and the long-term maximum temperature in the source site for each corresponding population. 'Heat stress' and 'recovery' growth periods of the experiment were defined as T0 -T2 (July-October) and T2-T4 (November-June), respectively, corresponding to periods of positive and negative maximum thermal anomalies. Thermal anomalies experienced by the different transplant treatments were plotted using the 'geom_flame', function in the 'HeatwavesR' package (Schlegel & Smit 2018) of R (version 3.6.1, 2019) .

Statistical analyses

Differences in fragment survivorship between source-recipient treatments were analysed with a chi-squared test at the end of the heat stress period (T2) and end of the recovery period (T4). Differences in 1) Shoot mortality per fragment, 2) herbivory impact, 3) C:N of leaves, 4) growth, 5) new leaf production and 6) total leaf length were each compared between treatments (n = 6) within discrete time periods using Analysis of variance (ANOVA). Normality was tested using Shapiro-Wilks test and Q-Q plots, and homogeneity of variance was tested with Levene's test and residuals plot. No transformations were applied. Tukey-HSD analysis was used to examine differences between groups for significant main effects. To assess the relationship between thermal performance, source location and 1) maximum absolute realised temperature and 2) maximum thermal stress anomalies, linear mixed effects models were used using the lme4 package in R (Bates *et al.* 2015). Source location and temperature metric were treated as fixed effects and recipient location' and 'time point' were treated as random effects, to account for differences in performance across locations and seasonality. Separate models were conducted for each temperature metric (i.e. max temperature or heat stress anomaly) and performance metric (i.e. growth, new leaf production and total leaf length) combination. We started with a full model based on *a priori* hypotheses about the inclusion of terms and higher order interactions. We identified the minimum adequate model by a stepwise removal of non-significant terms using likelihood ratio tests of the model with the effect in question against the model without the effect in question. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality and therefore no transformations were conducted. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. All analyses were conducted in R (version 4.0.3, 10/10/2020).

Results

Realised thermal anomalies

The western Mediterranean Sea experienced marine heatwave conditions during 2018 summer, coinciding with the translocation experiment. Maximum daily cool-edge temperatures were 24.9 ºC, 1.3 ºC above long term summer maximum (averaged 1981-2019). Maximum daily temperatures in central sites were 29.1ºC, also 1.3 ºC above long term summer maximum and the second highest temperature on record. Maximum daily temperatures at *P. oceanica's* warm-edge in the eastern Mediterranean were more typical at 29.3 ºC and 0.18 ºC above long term maximum.

As a result of the marine heatwave in the western Mediterranean, thermal anomalies experienced by cool-edge and central transplants were similar in central and warm-edge locations. The highest anomalies were experienced by cool-centre and cool-warm fragments, which experienced maximum anomalies of 5.4 ºC and 5.7 ºC above long-term maximum temperatures in the cool-edge, respectively. Positive thermal stress anomalies lasted for 130 days for cool-warm fragments and 102 days for cool-centre fragments over the course of the experiment (Fig. 2, Table S1). Centre-warm and centre-centre fragments experienced maximum anomalies of 1.53 ºC and 1.3 ºC, respectively for a duration of 37 and 33 days above long-term summer maxima in *P. oceanica's* range centre. Warm-warm transplants experienced equally high temperatures to cool-warm and centre-warm transplants, but the smallest relative anomaly (0.18 ºC for 2 days) above long-term summer maxima. The coolest daily temperatures recorded throughout the course of the experiment ranged from 10.5 ºC in the cool-edge, 13.9 ºC in the centre and 16.2 ºC in the warm-edge.

Selective herbivory in transplants with higher nutritional quality

Ten days after initial deployment, transplants were surveyed revealing selective overgrazing on cool-warm *P. oceanica* (Fig. 2). Shoot lengths of cool-warm transplants were reduced by 74.5 ± 4% across both transplant sites in Cyprus, from 22.3 ± 3 cm to 5.7 ± 9 cm. In contrast, centre-warm and warm-warm plants that were interspersed with cool-warm plants, lost significantly less biomass, from 25 ± 3% and 27.9 ± 5 % of initial length, respectively (ANOVA, $F_{(2,194)} = 37.64$, $p<0.001$, Fig. 2A). Selective grazing patterns were consistent between two warm-edge sites separated by 1.6 km. No other transplants in cool-edge or central locations were over-grazed by herbivores. Selectivity patterns in grazing rates reflected differences in nutritional quality between seagrass populations and regional differences in herbivore assemblages. C:N of cool-edge *P. oceanica* leaves were 18.1 ± 1.4 (mean ± SE), significantly lower (i.e. higher nutritional quality) at the start of the experiment than C:N ratios for central 34.7 ± 1.4 or warm-edge 52.3 ± 1.8 populations (ANOVA, $F_{(2,8)} = 97.76$, $p < 0.001$, Fig. 2B).

By week six, significant differences in shoot length were still observed between treatments (ANOVA, $F_{(2,245)} = 87.69$, $p<0.001$), but difference between treatments was reduced (Fig. 2A). Similarly, C:N in leaf tissue of the transplants started to equilibrate with local conditions. By the recovery period, no significant differences in C:N ($p = 0.83$) and no differences in herbivory impacts (i.e. shoot length, $p = 0.79$) were detectable between source populations in warm-edge sites (Fig. 2). The morphology of the bite scars observed on the consumed seagrass in Cyprus suggested that herbivorous fishes (*Siganus rivulatus, S. luridus, Sparisoma* *cretense*) and potentially sea turtles (*Chelonia mydas*) were responsible for the intense grazing rates, although relative contributions of each species could not be quantified (Fig. S2).

Survivorship

Despite experiencing extreme thermal anomalies and grazing rates at the start of the experiment, cool-warm transplants displayed high survivorship (number of surviving rhizome fragments) with no significant differences observed between treatments at the end of the thermal stress period (χ^2 = 10.65, df = 5, p = 0.06, Fig. 3). Survivorship then declined in all treatments over winter months (between October and April), potentially due to dislodgement from storms. Notably however, both centre-centre and centre-warm treatments displayed significantly lower survivorship than all cool-edge and warm-edge populations by the end of the experiment (χ^2= 58.07, df = 5, p<0.001, Fig. 3A).

The number of living shoots per fragment of rhizome was relatively stable over time, with slight declines over the period of thermal stress (Fig. 3B). Warm-warm and cool-centre treatments displayed significantly lower rates of shoot mortality than central and other cool-edge treatments at the end of the thermal stress period (ANOVA, $F_{(5,388)}$ = 8.118, p <0.001). By the end of the recovery period, changes in shoot numbers on the surviving fragments had stabilised and cool-centre fragments had recovered to display no net change from the beginning of the experiment.

Growth patterns

The number of leaves per shoot remained relatively stable through time and retained an average of 4.8 ± 0.13 leaves in cool-cool treatments, 4.3 ± 0.75 in centre-centre treatments and 4.3 ± 0.16 leaves per shoot in warm-warm treatments by the end of the experiment. Centre-warm treatments were the exception and declined from 4.0 ± 0.29 leaves shoot^{-1} at the beginning, to 2.5 ± 0.79 leaves shoot^{-1} by the end of the experiment. Underlying this pattern of stability, however, were marked differences in leaf production and turnover between treatments. New leaf production displayed a positive relationship with maximum temperature and significant differences between source populations, characterised by a higher rate of new leaf production in treatments sourced from cool-edge population, than centre or warm-edge populations (Table S2, Fig. 4A). In relation to heat stress anomalies, new leaf production displayed a strong positive relationship and no difference between source populations (Table S2, Fig. 4B), suggesting local thermal adaptation or acclimatization within source populations. *P. oceanica* growth rates (shoot elongation) and total leaf length per shoot displayed non-significant relationships with maximum temperatures and heat stress anomalies (p> 0.05, Fig. 4D, E, G, H). Rather, source populations were the best predictor of growth and total leaf length over the course of the experiment (Table S2). At the end of the thermal stress period, growth rates were significantly different among transplant treatments (ANOVA, $F_{(5,112)}$ = 3.8, p = 0.003, Fig. 4F). Cool-cool and warm-warm treatments displayed the highest growth rates despite experiencing up to 6ºC difference in temperature, and centre-centre and centre-warm treatments displayed significantly lower growth rates (Fig. 4F). By the end of the recovery period, growth rates had increased in all treatments except centre-warm shoots which remained significantly lower than cool-edge (Tukeys-HSD, p = 0.005) and warm-edge controls (Tukeys-HSD, p = 0.018). For total leaf length, shoots recorded their shortest length at the end of the heat stress period and recovered to be significantly longer by end of the recovery period (Fig. 4I). Moreover, at the end of the thermal stress period, cool-edge procedural controls were significantly longer than all other treatments and warm-edge procedural controls were significantly longer than cool-warm or central-warm treatments (Table S2). These relative differences held to the end of the recovery period (Fig. 4I).

Growth rates in natural meadows displayed similar patterns to transplants, albeit with higher rates over the same heat stress and recovery periods (Fig. S2). During the thermal stress period, growth rates were significantly higher in cool-edge meadows than warm-edge meadows and both cool-edge and warm-edge meadows displayed significantly higher growth rates than central meadows (ANOVA, $F_{(2,66)}$= 17.12, p<0.001). New leaf production was highest in central meadows and significantly lower in cool-edge and warm-edge meadows during the thermal stress period (ANOVA, $F_{(2,66)}$ = 13.56, p<0.001).

Discussion

Improving predictions for how species and communities will be affected by climate change and extreme events is an urgent challenge. Here we examined how populations of the functionally important seagrass *Posidonia oceanica* could be affected by heat stress, by conducting a translocation experiment across its range. Cool-edge, central and warm-edge populations displayed clear differences in thermal performance when grown under common conditions, but patterns contrasted with *a priori* expectations based on their thermal geography. Cool-edge populations performed equally well to warm-edge populations under common conditions and significantly better than central populations in terms of growth rates and survivorship. The strong performance of cool-edge populations was remarkable given they experienced thermal anomalies of >5ºC above long term summer maxima, compared to 1.5ºC for central populations and <1ºC for warm-edge populations. Moreover, cool-edge transplants suffered and subsequently recovered from severe overgrazing at the warmest site that removed >75% of aboveground biomass, highlighting remarkable resilience of cool-edge populations to combined effects of thermal stress and herbivory – two pervasive impacts of climate change in marine ecosystems.

Effects of thermal stress on seagrass populations

Patterns of thermal performance in *P. oceanica* varied among populations and deviated from expectations with respect to range position, highlighting the challenges of extrapolating climate change vulnerability predictions from local observations. Among cool, central and warm-edge populations, only measures of new leaf production supported models of local-adaptation/acclimatization and no metrics consistently supported expectations of thermal-niche conservatism between populations. The positive relationship between new-leaf production and thermal anomalies indicates higher rates of leaf turnover (i.e., shorter lifespan) under warmer conditions, potentially reflecting a coping strategy for thermal stress within fragments. Cool-edge and warm-edge populations did display similar rates of growth and survival under warm-edge (29ºC) conditions, suggestive of niche conservatism, however central populations exhibited comparatively poor performance, in contrast to expectations. Moreover, patterns of poor thermal performance by central populations at the warm edge were consistent with results among centre-centre procedural controls, which experienced similar absolute temperatures to warm-edge treatments. Growth patterns from the natural meadows over the same period further support these findings, showing lower growth rates and higher new-leaf production in centre-populations relative to cool and warm-edge meadows following the thermal stress period, reinforcing that experimental findings were not an experimental artefact.

Unlike centre-populations, the high resilience of cool-edge *P. oceanica* to thermal stress was consistent with documented responses of congeneric *Posidonia* species to a natural heatwave of similar magnitude. In 2011, Western Australia experienced the most intense marine heatwave on record (Hobday *et al.* 2016) and *Posidonia australis* displayed relatively minor impacts despite experiencing thermal anomalies in excess of 5ºC and absolute temperatures reaching 30ºC (Strydom *et al.* 2020). In contrast to *P. australis,* other seagrasses (i.e. *Amphibolis antarctica*) experienced severe losses (Fraser *et al.* 2014) and kelp forests (*Ecklonia radiata*) suffered a 100 km range contraction (Wernberg *et al.* 2016) in response to the 2011 heatwave. While the response by *P. oceanica* to large anomalies in the current study was relative to cool-edge (c.f. warm-edge in *P. australis*) conditions, the similarity between species to survive temperatures up to 30ºC highlights a remarkable latent capacity to withstand thermal stress in these edge populations. Latent tolerance to extreme events potentially reflects the warm evolutionary origins of the genus and the high resilience *Posidonia* has displayed to climatic upheaval over the past 60 mya (Por 2009; Bianchi *et al.* 2012).

The idea of *P . oceanica* as resilient to heat stress may appear at odds with the prevailing literature, which has demonstrated (Diaz-Almela *et al.* 2009; Marba & Duarte 2010) and projected (Jordà *et al.* 2012; Chefaoui *et al.* 2018) high sensitivity of *P. oceanica* to warming. However, the results of the current study are consistent with these previous findings and help place them in a broader geographical context. Central populations of *P. oceanica* were severely affected by thermal anomalies up to 1.5ºC above long term summer maxima in the current study. Anomalies of this magnitude are slightly cooler than those experienced during the 2003 Mediterranean heatwave, which caused a steep increase in shoot mortality in central populations (Marba

& Duarte 2010). Our findings therefore support previous evidence that *P. oceanica* in central populations are living close to their thermal limits but suggest that populations elsewhere may indeed have greater tolerance of high temperatures. The reason why central populations of *P. oceanica* are more severely affected by high temperatures than cool and warm-edge populations is unclear. The genetic structure of *P. oceanica* is characterised by strong separation between the eastern and western basins, consistent with a vicariance event during the last glacial maxima (Arnaud-Haond*et al.* 2007). Within the western basin, genetic connectivity is relatively low for both the cool-edge and central experimental collection locations, but both these locations are directly connected to the same central node within the regional metapopulation (Rozenfeld*et al.* 2008). Central and cool-edge populations, therefore, share more genetic and climatological similarities over geological timescales than cool-edge and warm-edge populations (Chefaoui *et al.* 2017), whereas central and warm-edge populations share more contemporary similarities in terms of selection pressure on upper thermal limits. Despite this, central populations exhibit the greatest sensitivity to temperature, across the distribution of *P. oceanica* forcing a re-evaluation of the vulnerability and management of this species in response to climate change.

While field-based evidence of performance could not separate cool-edge and warm-edge populations, the maximum realised temperature in our experiment was 29.3oC. Therefore, we cannot rule out the possibility that differences in performance between populations occur at higher temperatures. Indeed, previous laboratory-based experiments have suggested that warm-edge populations can survive temperature up to 36oC, whereas cool-edge populations and central populations of *P. oceanica* display upper thermal limits around 30oC (Bennett *et al.* 2021). Such experimental evidence would suggest that cool-edge populations were close to their upper threshold during the current experiment whereas warm-edge populations retained a larger thermal buffer. Previous laboratory experiments, however, did not document the subsequent recovery of *P. oceanica* following thermal stress. While cool-warm and cool-centre treatments clearly showed signs of thermal stress in the current study, our results suggest that they have greater capacity to endure and recover from heat stress than central-populations.

Selectivity and recovery of seagrass from herbivory

In addition to direct impacts of temperature on *P. oceanica,* high rates of grazing by herbivores on cool-warm transplants highlights the importance of indirect impacts of warming on ecosystem function. The selective patterns of grazing at warm-edge sites were consistent with the contrasting patterns of nutritional quality of transplants from different locations. These results are consistent with previous studies that have demonstrated higher rates of herbivory on nutrient enriched plants (Prado & Heck Jr 2011; Campbell *et al.* 2018). Interestingly, increased grazing on nutrient-rich seagrass was only observed in warm-edge, not central sites, where low nutrient meadows were also present. This pattern, in conjunction with feeding scars suggests that warm-affiliated fishes and sea turtles were likely responsible for the observed over-grazing. Tropical range extending fishes such as the rabbitfish *Siganus rivulatus* and *S. luridus* are the dominant herbivores in Cyprus (Santana-Garcon *et al.* personal communication) and while currently absent from Spain, are expected to establish in the coming decades (Daniel *et al.* 2009). Herbivore pressure by native cool-affiliated species is already high in the western Mediterranean (Pages *et al.* 2012) and therefore the cumulative effects of cool and warm water herbivores on nutrient rich seagrass in the western Mediterranean needs to be assessed and monitored carefully.

Conclusions

The combination of warming temperatures, extreme events and species redistributions has the potential to fundamentally change marine ecosystems in the Anthropocene. Identifying general principles, developing models and testing predictions about how these impacts will unfold across species distributions is critical to anticipate and adaptively manage ecosystems over coming decades. Here we demonstrate that cool, central and warm-edge populations of seagrass differ in their response to temperature, however these responses are not necessarily linear or conform to *a-priori* expectations. Moreover, the highly selective effects of warm-affiliated herbivores on cool-edge populations, highlight how species redistributions and species interactions can have unexpected and consequential effects on ecological performance under climate change. Our findings make a strong case for ongoing empirical comparisons of thermal performance for functionally important species and highlight the complexity of inferring thermal sensitivity from a single location. In doing so these findings provide hope for greater resilience to warming in Mediterranean seagrasses than previously recognised.

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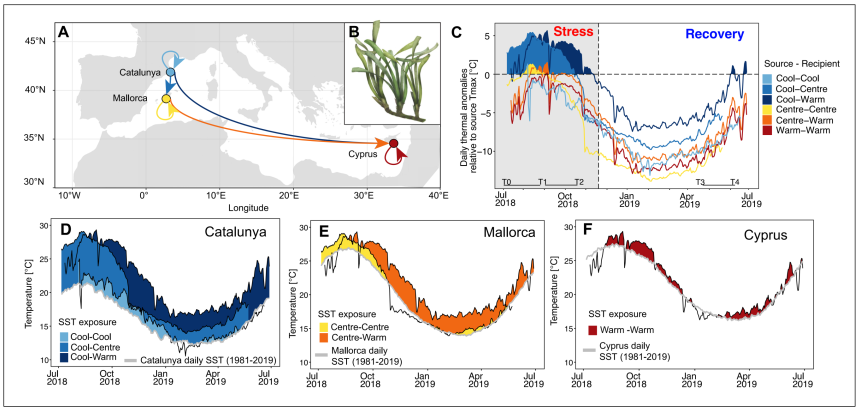
Figures

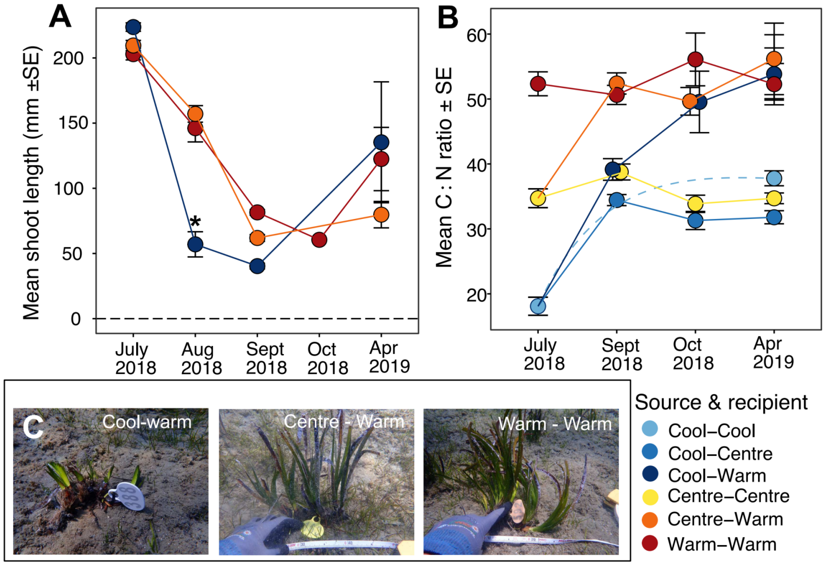
Fig. 1 A) Map of source and recipient transplant sites across the Mediterranean Sea. Colours indicate the source and direction of transplants in Catalunya (blue), Mallorca (yellow and orange) and Cyprus (red). B) Illustrated example of a transplant fragment, characterised by an apical shoot and multiple vertical shoots. C) Daily temperature anomalies experienced by *P. oceanica* transplants, with respect to the maximum annual temperature recorded in the site of origin (averaged between 1981-2019). Dashed vertical line, highlights switch between positive (stress) and negative (recovery) thermal anomalies. Maximum annual temperatures recorded in Catalunya = 25oC, Mallorca = 29.1oC and Cyprus = 29.3oC. Daily temperatures experienced by transplants relative to their thermal regimes of origin in D) Catalunya, E) Mallorca and F) Cyprus. Shaded areas represent daily temperatures above thermal regime of origin. Different shades of blue, yellow, orange and red within D, E and F, illustrate temperature profiles in the different recipient sites. Grey line represents the long-term daily temperature, averaged between 1981-2019.

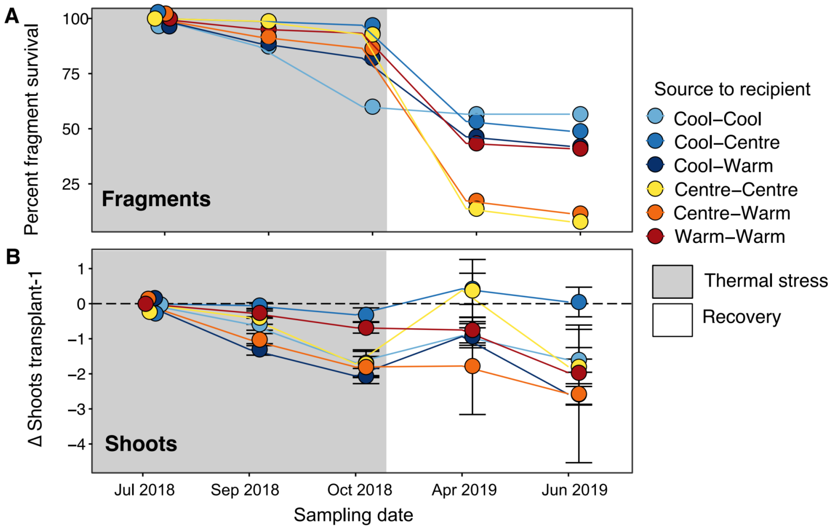
Fig. 2 Patterns of shoot length and nutritional quality of seagrasses over time in response to herbivory. A) Effects of a selective feeding event in August 2018 in Cyprus where herbivores selectively overgrazed cool-edge transplants (marked by asterisk). B) Carbon:Nitrogen ratios of *P. oceanica* leaves in the different treatments throughout the course of the experiment. C:N ratios in cool-cool plants (blue dashed line) were only measured in July 2018 and April 2019. C) Differences in herbivory on cool-warm transplants, compared to adjacent centre-warm and warm-warm transplants.

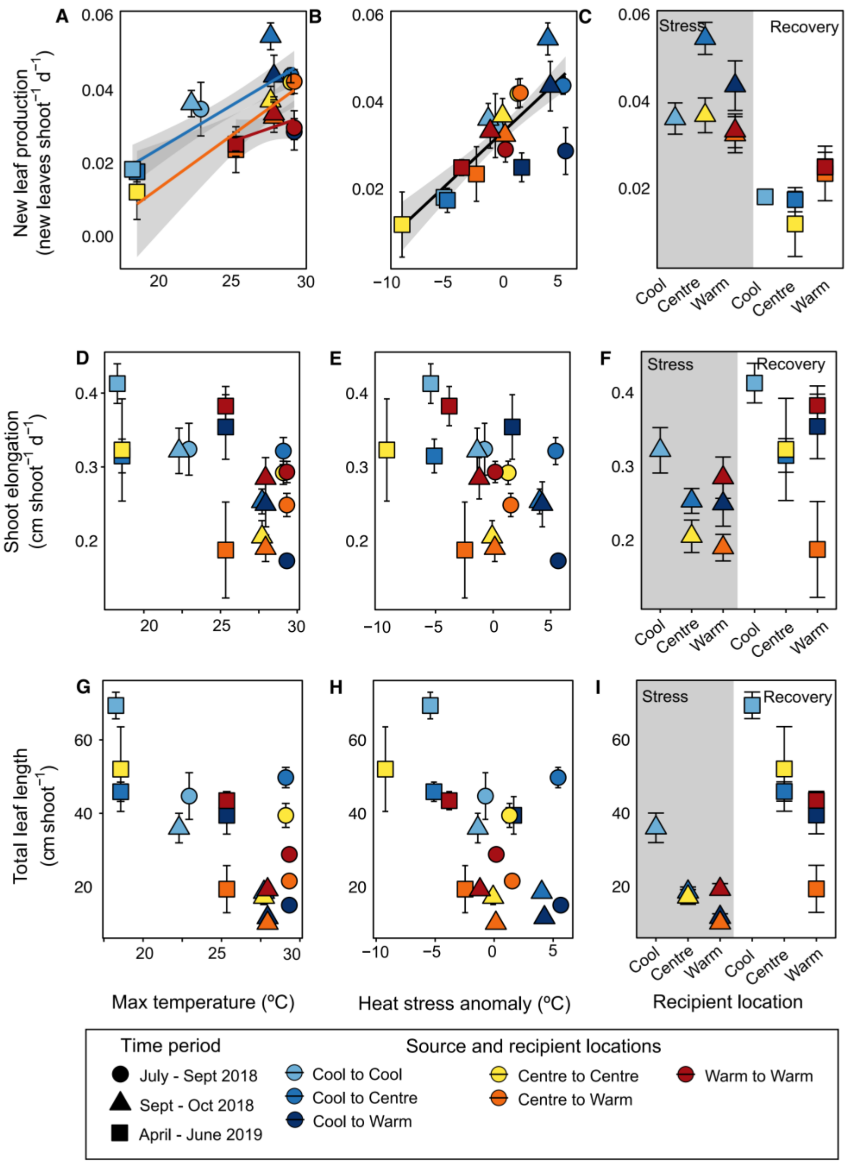
Fig. 3 Patterns of transplant survivorship (A) and transplant shoot survival (B) over the course of the experiment. Transplant fragment survival is represented as the proportion of transplants remaining at the end of each growth period, relative to the expected number of transplants (i.e. initial conditions minus fragments removed for growth measurements). Shoot survivorship is the change in the number of shoots per transplant, relative to initial conditions. Blue points and lines represent the fragments from Catalunya (cool edge), orange lines and points represent fragments from Mallorca (central) and red represents fragments from Cyprus (warm edge).

Fig. 4 Thermal performance of *Posidonia oceanica*transplants across the Mediterranean Sea. Rows represent performance metrics of new leaf production (top), shoot elongation (middle) and total leaf length (bottom). Columns represent temperature metrics of absolute maximum temperature (left), maximum thermal stress anomaly (middle) and a comparison between heat stress period (July-Oct 2018, left, grey) and recovery periods (Nov – June 2019, white, right). Points represent mean +- SE rates per treatment. Shape of point illustrates the time-period measurements were recorded in. Colour of point represents the different transplant treatments and procedural controls as per previous Figures.






A
Percent fragment survival
100
75
50
25
Fragments
B
Δ Shoots transplant-1
1
0
−1
−2
−3
−4
Shoots
Jul 2018
Sep 2018
Oct 2018
Apr 2019
Jun 2019
Sampling date
Source to recipient
Cool–Cool
Cool–Centre
Cool–Warm
Centre–Centre
Centre–Warm
Warm–Warm
Thermal stress
Recovery


A
B
C
New leaf production
(new leaves shoot^{-1} d^{-1})
0.06
0.04
0.02
0.00
Stress
Recovery
D
E
F
Shoot elongation
(cm shoot^{-1} d^{-1})
0.4
0.3
0.2
G
H
I
Total leaf length
(cm shoot^{-1})
60
40
20
20
25
30
−10
−5
0
5
Cool
Centre
Warm
Max temperature (ºC)
Heat stress anomaly (ºC)
Recipient location
Time period
July - Sept 2018
Sept - Oct 2018
April - June 2019
Source and recipient locations
Cool to Cool
Cool to Centre
Cool to Warm
Centre to Centre
Centre to Warm
Warm to Warm