Title: Heat wave intensity can vary the cumulative effects of multiple environmental stressors on *Posidonia oceanica* seedlings

Short title: Heat waves override other stressors

Author names and affiliations:

Laura Guerrero-Meseguer ^{1,2*¶}, Arnaldo Marín ¹, Carlos Sanz-Lázaro ^{3,4*¶}

¹ Departamento de Ecología e Hidrología, Facultad de Biología, Universidad de Murcia. Campus de Espinardo, 30100 Murcia, Spain

² Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Department of Biology, Faculty of Sciences, University of Porto, Campo Alegre s/n, 4150-181 Porto, Portugal (present address)

³ Departamento de Ecología, Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain

⁴ Multidisciplinary Institute for Environmental Studies (MIES), Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain

*Corresponding author

¶ Authors contributed equally to this work

Corresponding authors:

laura.guerrero@um.es (e-mail address)

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Department of Biology, Faculty of Sciences, University of Porto, Campo Alegre s/n, 4150-181 Porto, Portugal (present address)

carsanz@ua.es (e-mail address)

Multidisciplinary Institute for Environmental Studies (MIES), Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain

Abstract

Climate change is introducing new stressors into already stressed ecosystems. Among these, extreme events such as heat waves play a crucial role in determining the structure of ecosystems. We tested single and combined effects of overgrazing, burial and heat waves on the seedlings of the habitat-forming species *Posidonia oceanica*. At current heat wave temperatures, overgrazing in isolation had more deleterious effects than seed burial, and effects were synergistic and additive when both factors co-occurred. The combined effect of overgrazing and seed burial with current heat waves could hamper *P. oceanica* seedling development, with similar or even higher levels than the sole effect of heat waves in the near future (29°C). The effects of overgrazing and seed burial are expected to be overridden if heat waves temperatures exceed 29°C. These results suggest that co-occurring environmental stressors, in combination with current heat waves, could compromise the sexual recruitment of this seagrass.

Keywords: burial, climate change, extreme events, habitat-forming species, overgrazing, seagrass.

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Introduction

Nowadays, numerous anthropogenic activities have resulted in a complex scenario of multiple stressors that affect the environment (Halpern et al., 2008). Climate change is responsible

for new environmental stressors that, along with those already occurring, increase pressure on already impacted ecosystems. Under these conditions, stressors can act independently by producing additive cumulative effects, or can also interact by increasing (synergistic) or buffering (antagonistic) such effects (Vinebrooke et al. 2004; Thrush et al. 2009; Kelly et al. 2017).

Climate change is expected to increase the occurrence of extreme events (IPCC, 2012). Extreme events are caused by the abnormal high intensity of specific environmental variables (Diaz and Murnane, 2011; Easterling et al., 2000) and play a key role in structuring ecosystems because their effects can be more deleterious than several low-intensity disturbances (Sanz-Lázaro, 2016). For instance, marine heat waves can be caused by a rise in seawater temperature over a short period (Hegerl et al., 2011). The abrupt nature of this extreme event can have more lethal effects on marine organisms than a gradual temperature increase of seawater due to global warming (Garrahou et al., 2009; Smale and Wernberg, 2013). Marine heat waves especially affect sessile and photosynthetic organisms, including habitat-forming species such as seagrasses (i.e. Bergmann et al., 2010; Thomson et al., 2015; Winters et al., 2011).

Seagrasses provide key ecological functions in coastal systems (Hemminga and Duarte, 2000), promote habitat complexity and offer trophic resources by sustaining a large part of ecosystem biodiversity and production. They act as carbon sinks and nursery areas for many species, protect coasts from erosion and play a relevant role in the cycling of many elements (Duarte et al., 2010; Koch et al., 2009; Sanz-Lázaro et al., 2012). Temperatures above optimal (11.5-26° C, Lee et al., 2007) slow down growth rates and result in the mortality of temperate seagrass meadows (Lee et al., 2007; Marbà and Duarte, 2009). Excessive temperatures damage the light reception ability of photosystems and limit carbon assimilation, which makes respiration rates rise (Berry and Bjorkman, 1980). Consequently photosystems become less efficient (Campbell et al., 2006; Havaux, 1994), which alters nutrient uptake and protein synthesis

(Bulthuis, 1987; Hammer et al., 2018). The recession of seagrass beds due to heat waves can negatively affect the associated species of this ecosystem, which could lead to cascade effects (Hoegh-Guldberg et al., 2007; Moore and Duffy, 2016; Wernberg et al., 2012).

Posidonia oceanica (L.) Delile is an endemic seagrass species and the most abundant in the Mediterranean (Pasqualini et al., 2000). It forms meadows that are catalogued as a priority habitat for conservation by the European Union (Annexe I, Dir. 92/43/EEC). This species is in recession due to many anthropogenic pressures (Orth et al., 2006a; Waycott et al., 2009). *Posidonia oceanica* can be threatened by the current high temperatures caused by heat waves, especially in its early development stages (Guerrero-Meseguer et al., 2017; Pereda-Briones et al., 2019). Sexual reproduction is crucial for seagrass colonisation and maintaining the high genetic diversity needed to adapt to future environmental changes (Jump et al., 2009; Kendrick et al., 2012, 2017). The finite resources provided by seeds make seedlings more vulnerable to perturbations than adult seagrass shoots (Lambers et al., 2008; Schupp, 1995). In this critical development phase, the leaves and rhizome of *P. oceanica* seedlings are only slightly develop, which renders them very susceptible to disturbances from environmental stressors that can reduce their photosynthetic capacity, such as herbivory and burial (Balestri and Cinelli, 2003; Orth et al., 2006b).

Among other anthropogenic pressures, overgrazing in seagrasses has increased over in recent decades due to overfishing top predators, which has increased grazer abundance via cascading effects (Orth et al., 2006a; Short and Wyllie-Echeverria, 1996). Likewise, overgrazing is favoured by increases in leaf palatability due to eutrophication (Mazzella et al., 1992) or high seawater temperatures (Hernán et al., 2017). Seagrasses can suffer burial due to dredging and rising sedimentation rates owing to erosion in land enhanced by deforestation or extreme events like storms or floods (Cabaço et al., 2008; Short and Wyllie-Echeverria, 1996). Despite burial only

being partial, but mainly affects the seeds of *P. oceanica* seedlings, these seeds possess photosynthetic activity (Celdrán and Marín, 2011) and their good performance is crucial in early leaf and root development stages (Celdrán and Marín, 2013). Therefore, a reduction in seed photosynthesis by burial could have negative effects on seedling growth. The negative consequences of burial can be enhanced when other stressors that reduce seedling leaf surface co-occur, such as overgrazing. Thus if these environmental stressors simultaneously occur, the sexual recruitment of *P. oceanica* can be compromised by limiting its genetic diversity, which could pose a serious threat for the long-term survival of this seagrass.

As extreme events often occur simultaneously with other previous stressors in the environment, real scenarios that incorporate multiple stressors should be considered to fully understand their effects on organisms (Smith, 2011; Sanz-Lázaro 2019). Studies into multiple stressors with seagrasses that consider climate change scenarios primarily address the gradual increase in temperature and other stressors on adult seagrasses (Egea et al., 2018; Hendriks et al., 2017; Moreno-Marín et al., 2018; Ontoria et al., 2019; Pereda-Briones et al., 2019; Zimmerman et al., 2017). However, to the best of our knowledge, the effects of multiple stressors in extreme event scenarios, such as marine heat waves, have not been studied in seagrass seedlings. Given the expected rise in the frequency and intensity of extreme events, studying multiple stressors is crucial for environmental management, especially in areas of high anthropogenic pressure, such as coasts, on the early development stages of vulnerable species, as in seedlings of *P. oceanica*.

To this end, we ran a manipulative experiment in a microcosm system by simulating burial and overgrazing effects on *P. oceanica* seedlings in current and predicted heat waves scenarios. We hypothesised that: (i) the combined effect of all stressors would be additive or synergistic; (ii) heat waves would intensify the negative overgrazing and burial effects on *P. oceanica*.

Materials and Methods

115 ***Experimental setup***

116 Similar sized 4-month-old seedlings (experimental unit) were individually incubated under
117 controlled conditions in glass jars (500 ml) with aerated artificial seawater. Aeration was
118 maintained in each experimental unit using an air pump attached to a system of plastic capillary
119 tubes (1 mm diameter) inside the glass jars to ensure that the oxygen concentration was always
120 above 5.5 mg O₂/l⁻¹. Glass jars were covered with a diffusible top to allow air exchange, while
121 minimising changes in salinity and pH. Weekly variations were below the limit of detection for
122 salinity and pH, with 0.2 and 0.03, respectively. Artificial seawater was replaced weekly to
123 prevent the colonisation of epiphytes and changes in water quality. Seawater was prepared using
124 bidistilled water and marine salt (Ocean Fish, PRODAC International, Cittadella, Italy). No
125 nutrient supply was applied to seawater as *P. oceanica* uses the nutrient reserves of its seed during
126 the first 6-8 months of development (Balestri et al. 2009). Thus extra nutrient inputs should have
127 no relevant effect on plants.

128 The seedlings used in the experiment were germinated from seeds collected at the
129 Mazarrón Bay (Murcia, Spain) in May 2013. Before the experiment began, seedlings were grown
130 for 4 months in glass aquaria filled with seawater at 21±1°C and a salinity of 36. The light
131 intensity and the photoperiod in aquaria were 100 µmol m⁻² s⁻¹ and 14:10 (light:dark),
132 respectively

133 ***Simulation of environmental stressors***

134 The simulated environmental stressors were *heat wave*, *overgrazing* and *burial*. Each
135 environmental stressor constituted a factor that was fixed and orthogonal, and the experiment
136 design was fully factorial. Four temperatures were employed to simulate four heat wave scenarios:
137 27, 29, 31 and 33°C. Both factors *overgrazing* and *burial* had an effect [overgrazing (+G)/burial

(+B)], and their corresponding control [no overgrazing (-G)/no burial (-B)] treatments resulted in four experimental combinations: no grazing and no burial (-G-B); no grazing and burial (-G+B); grazing and no burial (+G-B); grazing and burial (+G+B). Each combination was replicated 6 times ($n=6$; i.e. 6 seedlings in 6 different glass jars). Thus 96 seedlings were used ($N=96$; Fig. 1).

The factor *heat wave* was simulated by placing the experimental units inside four environmental chambers for 1 month (CLIMACELL-404, MMM Medcenter Einrichtungen GmbH, München, Germany). Temperatures were selected based on the maximum sea surface temperatures (SST) reached at the end of summer near the area where seedlings were collected during the 2012-2015 period (Supplementary Table 1). The maximum SST in later summer months correspond to the period when the maximum SST is reached in the Mediterranean. The maximum SST temperatures in August increased by $3.3\pm0.2^{\circ}\text{C}$ above the mean temperature and in September, the maximum SST temperatures were $3.4\pm0.4^{\circ}\text{C}$ above the mean (Mean $\pm$ SD; Supplementary Table 1), which resulted in a maximum SST of 27°C . These heat waves temperatures occurred for up to 1 month and also reached values of 29°C on several days (Supplementary Table 1). Thus we incubated seedlings at 27°C for 1 month to simulate a current marine heat wave. By taking an expected increase of between $1\text{-}3.5^{\circ}\text{C}$ above the current SST for the rest of the century (IPCC 2007), we considered three other heat wave scenarios by incubating seedlings at 29 , 31 and 33°C . The 29°C temperature simulated a marine heat wave in the near future, while 31°C and 33°C simulated future heat wave scenarios, as predicted for the middle and end of the century in the Mediterranean. Before incubations, each seedling was firstly acclimatised from aquaria ($\sim 21^{\circ}\text{C}$) to ambient temperature ($\sim 25^{\circ}\text{C}$), and secondly from ambient temperature to each heat wave temperature in the corresponding environmental chamber for approximately 1 day. A temperature increase of 0.25°C per hour was never exceeded. The temperature of each environmental chamber was continuously recorded by data loggers (HOBO Onset Computer

Corporation, Bourne, MA, USA) to check that temperatures remained constant throughout the experiment. The light intensity and photoperiod in the environmental chambers were the same as during seedling maintenance (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 14:10 (light:dark), respectively).

Overgrazing was simulated by clipping the leaves of each seedling at the beginning of the experiment to approximately 2 cm above the rhizome to reduce the leaf area by 50-70%. Seed burial was done by completely burying the seeds of seedlings under coarse sediment that had been previously sterilised (grain size composition: 80.8% and 19.2% of pebble and gravel, respectively; Wentworth, 1922) and placing leaves above sediment. This sediment was chosen because it is not prone to suffer anaerobiosis. Thus no derived toxic by-products were expected to be generated, which could cause a possible bias in the experiment.

Measured parameters

Leaf growth, leaf senescence, leaf photosynthetic activity and seedling mortality were measured to evaluate the influence of multiple stressors on seedling development.

Leaf growth was calculated by the difference in leaf length at the beginning and end of the experiment. Leaf senescence was calculated as the percentage of the number of leaves that had fallen at the end of the experiment per seedling, divided by the initial number of leaves. Seedling mortality was calculated as the percentage of plants that had died at the end of the experiment. Both the seedlings without leaves and broken seeds were considered dead.

At the end of the heat wave simulations, minimum fluorescence after dark adaptation (F_0) and the C:N ratio of the leaves were measured to test any possible alterations in photosystem II (PSII) and in the nutrient translocation of seedlings, respectively. F_0 was measured in the leaves of the seedlings with a saturation light pulse by a pulse-amplitude-modulated fluorometer (MINI-PAM, Waltz, Effeltrich, Germany). This non-destructive method allowed us to take real-time

measurements of the physiological status of PSII. F_0 values are surrogates of photosynthetic efficiency under heat stress situations (Havaux, 1993; Yamane et al. 2000; Yamada et al. 1996) and, thus, indicate the basal state of PSII on long-term time spans (Guerrero-Meseguer et al. 2017a). In this way, the cumulative damages produced in PSII after 1 month of exposure to stressors were checked. F_0 measurements were taken after removing epiphytes by gently scraping leaves with a glass slide. Then dark-adaptation was applied by covering plants with aluminium foil for 15 minutes. Subsequently, double measurements were taken on the youngest leaves of seedlings. The average of these two measurements was used as a replicate.

The C:N ratio was assessed by quantifying the carbon (C) and nitrogen (N) contents in the leaves of seedlings. For this analysis, leaves were separated at the end of the experiment, and were stored frozen at -80°C after removing epiphytes. Afterwards, they were freeze-dried in an Alpha 1-2 LD plus freeze dryer (Martin Christ, Osterode, Germany) to constant weight before being finely ground. Total organic C and N were determined in a Carlo Erba Inst. EA 1108 Elemental Analyzer (Carlo Erba Strumentazione, Milan, Italy). Prior to the TOC analysis, samples were pre-treated by adding 1:1 HCl. Duplicate measurements were taken of two leaf subsamples. The average of these two measurements was used as a replicate.

Data analyses

The trends of the measured parameters with increasing heat wave temperatures (27, 29, 31 and 33°C) were analysed by adjusting data to the best fitted regression models using the Akaike information criterion (AICc; Supplementary Table 2). The normality and homoscedasticity of data variances were previously checked by Q - Q plots and the Bartlett test, respectively. Then an ANCOVA analysis was performed to compare the prototypical trajectories of the combinations of the treatments of the factors overgrazing and burial along temperatures using the factor heat wave as the co-variable. To test for significant differences among treatments specifically at 27°C , the

intercept of the regression model was set up at this temperature. Statistical analyses were performed with the R statistical software (v. 2.15.0) using packages “AICcmodavg” and “stringr” (R Core Team, 2019). Data were reported as mean±standard error (SE). Statistical tests were conducted at a significance level of $\alpha=0.05$.

Results

Impact of overgrazing and burial with current heat waves

At current heat waves temperatures, seedling mortality was $12.5\pm2.7\%$. Of the seedlings that died, one seedling was from treatment -G-B, another from +G-B and one from +G+B. Thus 5-6 seedlings per treatment were alive at the end of the experiment. At 27°C, leaf growth significantly lowered due to overgrazing (+G-B), but not to burial (-G+B; Fig. 2A). When both stressors were combined, they had a synergistic effect that reduced leaf growth in seedlings by 3-fold (+G+B; Fig. 2A, Table 1). Leaf senescence showed negative responses at 27°C in the overgrazed seedlings (+G-B; Table 1), but not in the burial treatment (-G+B). However, no synergistic effects were found when overgrazing and seed burial co-occurred (+G+B; Fig. 2B, Table 1).

No significant differences were found for any treatment for F_0 at 27°C (Fig. 2C, Table 1). However at 27°C, the C:N ratio in leaves was significantly higher in the burial than in the other treatments (-G+B; Figure 2D, Table 1).

Interactions of overgrazing and burial with the forecasted marine heat waves

At temperatures above 27°C, many plants presented deterioration symptoms: leaves paled and seedlings showed fragile rhizomes, hollow seeds and roots (Supplementary Figure 1). No seedling died in the heat wave predicted for the near future (29°C), while the plants grown at 31°C

and 33°C obtained a mortality rate of 21 ± 2.7 %, which resulted in 4-6 alive seedlings per treatment at the end of the experiment.

Leaf growth along the temperature gradient followed a second-degree polynomial trend along temperatures with considerable differences among treatments, especially when overgrazing and burial co-occurred (+G-B; Table 1, Supplementary Table 2). Differences among treatments gradually diminished with temperature, with very homogeneous values close to 0 at 31 and 33°C (Fig. 2A, Table 1). As regards the leaf senescence percentage, seedlings were similarly affected in all the treatments along the temperature gradient (Table 1, Supplementary Table 2), except in the buried seedlings, which had a less marked effect than on the other treatments (-G+B; Fig. 2B, Table 1). A maximum 50% leaf senescence was noted at 33°C for treatments -G-B and -G+B. However at 33°C, the mean values went above 60% in treatments +G-B and +G+B (Fig. 2B).

F_0 underwent a linear decrease with temperature irrespectively of treatment (Fig. 2C, Table 1 and Supplementary Table 2). Temperature generally incremented the C:N of leaves by a significantly linear trend (Figure 2D, Table 1, Supplementary Table 2). However, the C:N of the leaves of the buried seedlings did not change with temperature (-G+B; Fig. 2D).

Discussion

Our results revealed that at current heat wave temperatures *P. oceanica* seedlings were more negatively affected when overgrazing and seed burial occurred together than when both stressors occurred in isolation. The effects for leaf growth were synergistic and additive for the other measured parameters, which could significantly hamper seedling development. Above this temperature, heat waves became the predominant stressors, and the negative effects of overgrazing and seed burial were overridden by the effect of heat wave above 29°C.

Effects of overgrazing at current heat waves

At 27°C, the seedlings affected by overgrazing showed lower leaf growth and higher leaf senescence than the non-grazed seedlings, while the C:N content and photosynthetic activity of the grazed leaves were similar to those in the other treatments. Diminished leaf growth could be due to the reduction in leaf surface and consequently, in their photosynthetic efficiency.

Leaf surface loss was more marked in the seedlings that were clipped than in the other treatments, and consequently, their photosynthetic area also reduced. This fact is especially important in 4-month-old *P. oceanica* seedlings because, at this age, the photosynthetic surface of leaves is larger than in seeds. Thus leaves are the main contributor to the photosynthesis of the whole plant (Guerrero-Meseguer et al., 2017a).

In our study, at 27°C the F_0 values were *ca.* 600, which suggests that the photosynthetic machinery of leaves was not affected at this temperature. Under short-term exposures to high temperature, the F_0 value is used as an indicator of PSII functioning, which usually increases up to a critical threshold (Yamada et al., 1996; Yamane et al., 2000). Conversely during long periods at high temperature, the F_0 values in the leaves of *P. oceanica* seedlings can lower as temperature rises (Guerrero-Meseguer et al. 2017a). Adult *P. oceanica* shoots have a large photosynthetic area that allows them to compensate overgrazing by increasing their leaf production (Vergés et al., 2008). Notwithstanding, this compensatory capacity is limited in high herbivory situations (Hernán et al., 2019). This compensatory capacity is much more limited in *P. oceanica* seedlings, which have a poorly developed leaf system, and the marked loss of their leaf surface is expected to diminish the photosynthetic capacity of seedlings, which could explain the reduction in leaf growth.

Effects of burial at current heat waves

The growth, senescence and photosynthetic performance of the leaves of buried seedlings were comparable to those of the non-buried ones. The C:N ratio showed slightly high values, which indicates lack of stress in seedlings. Thus, current heat waves in the Mediterranean are not expected to affect seedlings that only suffer from seed burial.

Similarly to our results, *P. oceanica* seedlings transplanted in sand do not seem to be affected by different burial levels (Guerrero-Meseguer et al., 2017b). The leaf development of adult *P. oceanica* shoots has also displayed resistance to low burial levels (Ceccherelli et al., 2018). The clonal development of seagrass meadows allows neighbouring and unburied shoots to transfer metabolites to sustain the survival of stressed shoots. Contrarily to adult shoots, seagrass seedlings lack clonality, but can grow independently by using nutrients of seeds during the first 6-8 months of development (Balestri et al. 2009). Low burial levels enhance seedling colonisation in other seagrass species, such as *Cymodocea nodosa* and *Zostera marina* (Marba and Duarte, 1994; Marion and Orth, 2009, 2012). However, this study demonstrates that burial in *P. oceanica* does not influence seedling recruitment. The differences could be related to the colonisation strategy of each seagrass species. *C. nodosa* and *Z. marina* form seed banks in sediment and their seeds remain dormant until adequate environmental conditions appear to germinate (Buia and Mazzella, 1991; Hootsmans et al., 1987; Terrados, 1993). In contrast, *P. oceanica* seeds germinate once fruit are released from the parent plant, and are transported by winds and currents until the fruit opens and the seed sinks (Inglis, 2000; Kuo and Den Hartog, 2006, Guerrero-Meseguer et al. 2018). Thus in seagrasses that produce dormant seeds, burial can develop positive responses to enhance germination in seed banks, while these benefits seem to be lacking in non-dormant seagrass seeds with floating fruits, such as *P. oceanica*.

Effects of overgrazing and burial at current heat waves

Our results suggest that, at current heat waves, the negative effects of overgrazing on *P. oceanica* seedlings could notably intensify when seeds are buried. The negative effect on the leaf growth of *P. oceanica* seedlings was more marked than the sum of the effects of overgrazing and seed burial in isolation, which evidences a synergistic effect. Likewise, leaf senescence had more negative effects when overgrazing and burial co-occurred at current heat waves but, in this case cumulative effects were additive. Therefore in an overgrazing situation at current heat wave temperatures, the development of *P. oceanica* seedlings could be limited and this effect could be amplified when seeds are buried. The co-occurrence of these three stressors can, therefore, endanger the survival and colonisation capacity of this seagrass species in the Mediterranean. However, the fact that knowledge on these issues is scarce means that it is necessary to conduct more research into this topic to completely understand the consequences of overgrazing on *P. oceanica* seedlings, and to verify the possible interactions with heat waves.

Effects of future heat waves

The heat waves predicted in the near future (29°C) are expected to notably affect seedling physiology and development. In the near future, heat waves are expected to increase in intensity (Easterling et al. 2000; IPCC, 2007; Giorgi and Lionello, 2008) and their negative effects would be comparable to current scenarios of multiple co-occurring stressors. However, the heat waves expected for the mid- and end of the century could have an even stronger impact on *P. oceanica* seedlings. This means that as heat waves temperatures intensify, the effects of other stressors could be masked by heat waves.

Heat waves above 29°C are expected to affect *P. oceanica* seedlings by not only minimising leaf growth and photosynthetic activity, but by also increasing leaf senescence and C:N ratios. The C:N ratio in leaves generally rises due to N mobilisation to other plant parts as a strategy to minimise nutrient loss when leaves are senescent or damaged (Avice and Etienne,

2014). In line with a previous study (Pereda-Brions et al. 2019), our results demonstrate that *P. oceanica* seedlings can translocate the N of leaves to seeds and roots to limit loss of nutrients, which could be promoted by the senescence of the leaves at temperatures above 29°C. Therefore, the heat waves predicted for the mid- and end of the century are expected to profoundly affect *P. oceanica* seedlings by compromising their development and threatening their survival. This agreement with previous studies suggests a critical threshold for the negative effects of heat above 29°C in early *P. oceanica* stages (Guerrero-Meseguer et al., 2017; Hernán et al., 2017; Pereda-Briones et al., 2019).

Heat waves increasing occur due to climate change (Easterling et al., 2000) and, unfortunately, other environmental stressors are already present in ecosystems. This study is a clear example of how the maximum temperatures forecasted for the end of the century can act as a dominant stressor compared to others. According to our results, the growth and photosynthetic activity of *P. oceanica* seedlings can be compromised with current heat waves when overgrazing and seedling burial co-occur. These negative effects observed during the first months of seedling life could also be important in the second summer of development as seedlings have no nutrient reserves from their seeds and their rhizome is still moderately developed (Balestri et al. 2009).

The negative consequences of heat waves on *P. oceanica* are expected to depend very much on depth. The simulated heat wave values were taken from estimations based on temperature values from the coastal sea surface in late summer. At this time of the year in temperate areas, thermal stratification is observed in the upper water column layer, the seasonal thermocline, which keeps water above this thermocline at a relatively constant temperature. This thermocline can go down to a depth of 20 m (Houpert et al. 2015). As the bathymetric distribution of *P. oceanica* goes from the surface to a depth of 40-45 m (Telesca et al. 2015), the impact of heat waves is expected to affect mainly shallow *P. oceanica* meadows.

Asexual reproduction is widely used by *P. oceanica* and could buffer the negative effect on seedlings in heat stress situations to some extent (Marin-Guirao et al. 2017). Nevertheless, sexual reproduction is a key mechanism for maintaining the genetic diversity of seagrasses and favours adaptation to environmental changes (Reusch et al., 2005). Additionally, the buoyant fruits of *P. oceanica* produced through sexual reproduction boost its dispersal capacity (Inglis, 2000; Kendrick et al., 2012). Consequently, the highly detrimental cumulative effects of current heat waves with overgrazing and seed burial can pose a serious threat for this species' long-term survival. Nevertheless, longer-term experiments that monitor the status of seedlings after a heat wave are needed to test if the effects caused by heat waves are transient or can fully compromise seedling development.

Conclusion

This study suggests that the combination of several environmental stressors with current heat waves in the Mediterranean can threaten the survival of *P. oceanica* seedlings and, thus, this species' sexual recruitment. The cumulative effect of overgrazing and seed burial with current heat waves can have similar deleterious consequences for *P. oceanica* than the sole effect of heat waves in the near future (29°C). Future temperatures brought about by heat waves that exceed 29°C can have an overwhelming effect on *P. oceanica* seedlings by overriding the effects of other environmental stressors. Specific experiments should be performed to test these effects on other types of extreme events, species and communities, as well as the effect of the other environmental stressors after extreme events have occurred. These topics will provide key information for environmental agencies to improve their forecasting ability and management strategies for future scenarios with multiple stressors, including extreme events.

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624

625 **Table 1.** Coefficients (mean±SE) of the regression models by testing the effects of environmental stressors overgrazing (+G) and seed
626 burial (+B) with a temperature gradient for each measured parameter. Regression models can be first-degree (^a) or second-degree
627 polynomial (^b) according to the AICc (corrected Akaike information criterion; Supplementary Table 1). Significant effects (p < 0.05)
628 are indicated in bold.

	Treatments	At 27°C	Temperature linear trend	Temperature quadratic trend
Leaf growth^b	-G-B	1.315 ± 0.134	-0.393 ± 0.107	0.034 ± 0.017
	+G-B	-0.478 ± 0.189	0.118 ± 0.151	-0.008 ± 0.024
	-G+B	0.177 ± 0.189	-0.166 ± 0.151	0.022 ± 0.024
	+G+B	-0.571 ± 0.268	0.446 ± 0.214	-0.058 ± 0.034
Leaf senescence^a	-G-B	27.64 ± 10.01	3.341 ± 2.671	-
	+G-B	45.15 ± 14.16	-1.618 ± 3.777	-
	-G+B	15.69 ± 14.16	-1.680 ± 3.777	-
	+G+B	-9.434 ± 20.03	5.341 ± 0.462	-
F₀^a	-G-B	640.4 ± 62.4	-93.1 ± 16.6	-
	+G-B	-43.7 ± 88.2	8.02 ± 23.5	-
	-G+B	-20.3 ± 88.2	23.8 ± 23.5	-
	+G+B	-135.6 ± 124.8	15.4 ± 33.3	-
Leaf C:N^a	-G-B	11.3 ± 2.14	2.14 ± 0.57	-
	+G-B	-1.23 ± 3.03	0.42 ± 0.81	-

-G+B	7.66 ± 3.03	-2.05 ± 0.81	-
+G+B	-7.86 ± 4.28	2.55 ± 1.14	-

629 Figures:

Factor:

Heat wave

Overgrazing (G)

Burial (B)

630

631 **Figure 1.** Scheme of the experimental setup herein used. Different colours represent the four temperatures used to simulate each heat wave
632 scenario: 27, 29, 31, 33°C. Brown rectangles and dashed lines represent the burial and overgrazing of seedlings, respectively.

Figure 2. The effects of overgrazing (+G) and seed burial (+B) with a temperature gradient on *Posidonia oceanica* seedling development (mean±SE). Net leaf growth (A, $n=6$, $R^2=0.14$, $p<0.001$), leaf senescence (B, $n=6$, $R^2=0.30$, $p<0.001$), minimum fluorescence after dark adaptation in leaves (F_0 ; C, $n=6$, $R^2=0.50$, $p<0.001$) and C:N ratio in leaves (D, $n=4$, $R^2=0.47$, $p<0.001$). R^2 and p -values refer to the whole regression model. Solid lines indicate significant prototypical trajectories of the response variables along the temperature gradient ($p < 0.05$). Background colours denote the current (white), near future (grey) and

from mid-century (dark grey) heat wave scenarios. All the treatments were tested at 27, 29, 31 and 33°C, but treatments moved slightly from the exact temperature on the graph to avoid overlapping and to facilitate visualisation.

Supplementary Material

Supplementary Table 1. Mean and maximum sea surface temperatures reached in the Mediterranean in the warmest summer months (August and September) between 2012 and 2015 (data from an oceanographic buoy belonging to the Port Authority, Ministry of Development, Spain; Cabo de Palos).

Year	August		September	
	Mean $\pm$ SD	Maximum	Mean $\pm$ SD	Maximum
2012	24.7 $\pm$ 0.2	27.3	23.4 $\pm$ 1.4	25.3
2013	24.4 $\pm$ 1.0	26.9	23.9 $\pm$ 0.8	26.3
2014	24.5 $\pm$ 0.4	28.4	23.6 $\pm$ 1.2	27.6
2015	24.8 $\pm$ 0.3	29.1	22.3 $\pm$ 1.1	27.6
<i>Mean $\pm$ SD</i>	24.6 $\pm$ 0.2	27.9 $\pm$ 1.0	23.3 $\pm$ 0.7	26.7 $\pm$ 1.1

Supplementary Table 2. Summary of the Akaike information criterion (AICc) results.

Lower values (in bold) determine the best fitted regression model.

	First-degree polynomial	Second-degree polynomial	Exponential
	$y_i = \beta_0 + \beta_1 x_i$	$y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2$	$y_i = \beta_0 + \beta_1 e^{x_i}$
Net leaf growth	87.88	79.78	134.7
Leaf senescence	932.3	939.9	932.5
F_0	1283.7	1284.0	1317.4
Leaf C:N	403.2	408.2	410.5

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658 **Supplementary Figure 1.** A *P. oceanica* seedling grown at 33°C. The picture shows
659 deterioration symptoms on leaves due to high temperature: not as green, fragile rhizome,
660 hollow seeds and roots.