

Negative effects of warming on seagrass seedlings are not exacerbated by invasive algae

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ABSTRACT

The observed and projected rise in sea surface temperature challenges marine biodiversity worldwide, and particularly in temperate ecosystems dealing with the arrival of novel species of tropical provenance. When the impacted biota are early life stages of ecosystem engineers, the effects of those impacts are of major concern for ecologists and coastal managers. We experimentally examined the individual and potential additive effects of seawater warming and the presence of the invasive algae on the development of seedlings of the seagrass *Posidonia oceanica* in a three-month mesocosm experiment. Whereas the presence of the invasive algae (*Caulerpa cylindracea* and *Lophocladia lallemandii*) did not result in detrimental effects on seedlings, warming negatively affected seedling development. Interestingly, the presence of both invasive algae may ameliorate the negative effects of warming.

1. Introduction

The gradual increase of global sea surface temperature (SST) observed since the 1950s represents a threat to marine ecosystems worldwide (IPCC, 2014). Global responses of marine biota already indicate that climate change can have a strong impact on marine life (Díaz-Almela et al., 2009; Lejeune et al., 2010; Harley et al., 2012; Polozanska et al., 2013). The rise in SST is predicted to increase between 0.6 and 1.5 °C in the next 40 years (IPCC, 2014). This can be particularly relevant for temperate ecosystems dealing with novel species of tropical provenance, such as introduced species or species that have undergone a range expansion (Raitos et al., 2010; Vergés et al., 2014). Furthermore, when the species impacted by warming are ecosystem engineers, such as corals, kelps or seagrasses, the consequences of those impacts may cascade throughout the ecosystems they form, significantly magnifying overall impacts (e.g. Hoegh-Guldberg et al., 2007; Wernberg et al., 2012). Certainly, the introduction of invasive species and global warming are emerging as main threats to seagrass ecosystems (e.g. Orth et al., 2006b; Williams, 2007; Koch et al., 2013; Collier and Waycott, 2014).

Sexual reproduction is crucial for the persistence of seagrass beds, allowing for the consolidation of existing meadows and also for the formation of new ones via dispersal (Jarvis et al., 2014; Olesen et al., 2004; Orth et al., 1994, 2006a). Moreover, seedling production

represents a critical genetic diversity source that provides adaptive capacity to future environmental changes, essential for the long term persistence of the species (Jump et al., 2009; Kendrick et al., 2012, 2017). These early stages are considered the most critical phases in the life cycle of seagrasses, when plants experience the highest mortality rates (Orth et al., 2006a; Alagna et al., 2013). Indeed, the establishment rates of newly emerged seedlings are generally very low (Orth et al., 2006a), and are recognized as a major population bottleneck (Gómez-Aparicio, 2008). Given the ecological importance of seagrass seedlings, it is pressing to understand the environmental and biological factors which contribute to their successful establishment, survival and development.

The Mediterranean Sea is a hotspot of biodiversity (Myers et al., 2000). However, it exhibits rates of seawater warming that exceed threefold those of the global ocean (IPCC, 2007; Burrows et al., 2011). The increase in SST also stimulates the successful proliferation of alien species, particularly those from tropical and subtropical origin entering through the Suez Canal (Raitos et al., 2010; Vergés et al., 2014). In fact, the Mediterranean Sea is one of the areas with more introductions of non-native species (e.g. Galil et al., 2014; Streftaris and Zenetos, 2006), some of which are considered as invasive and are affecting the integrity of natural communities, including loss of seagrass meadows (Vitousek et al., 1997; Williams and Smith, 2007; Thomsen et al., 2010).

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The endemic seagrass *Posidonia oceanica* (L.) Delile is one of the most representative foundation species to inhabit littoral Mediterranean ecosystems due to its importance as a habitat former, and supporter of biodiversity (Duarte, 2000). It is a slow-growing species which forms extensive meadows, characterized by a very variable sexual reproduction in space and time (Green and Short, 2003; Díaz-Almela et al., 2009). These features contribute to a low capacity of resilience and adaptation to the rapid warming projected for the Mediterranean Sea (Raitos et al., 2010; Unsworth et al., 2015). Negative effects (e.g. increased mortality) in response to increasing temperatures have been observed in *P. oceanica* adult plants (e.g. Díaz-Almela et al., 2009; Marbà and Duarte, 2010) and there are indications that seedlings of this species are also sensitive to warming (Guerrero-Meseguer et al., 2017; Hernan et al., 2017). Presumably, the presence of invasive algae could further enhance the negative effects of warming on *P. oceanica* seedlings, as this species can suffer mortality, biomass loss, or deterioration of its sediment quality deterioration when colonized by some invasive algae (Marbà et al., 2014; Ballesteros et al., 2007; Holmer et al., 2009). On the other hand, invasive seaweed could also have facilitative interactions under elevated temperatures (Harley et al., 2012). For example, shading by the invaders could soften the effects of temperature rise by reducing photochemical stress (Wernberg et al., 2010).

In the Mediterranean Sea, *Caulerpa cylindracea* and *Lophocladia lallemandii* are two of the most invasive macroalgae threatening *P. oceanica* meadows (Verlaque, 1994; Streftaris and Zenetos, 2006; Ballesteros et al., 2007; Klein and Verlaque, 2008). Both species are able to colonize *P. oceanica* meadows, and can be particularly abundant at the edge of the meadows, over matte, as well as in low density seagrass patches (Ballesteros et al., 2007; Marín-Guirao et al., 2015), which are often the same habitats where *P. oceanica* seedlings successfully settle (Balestri et al., 1998; Piazzini et al., 1999; Balestri and Lardicci, 2008; Alagna et al., 2013). Therefore, these invasive and native macrophytes are likely to interact during the seedling establishment phase. In addition, the proliferation of both invasive species may be enhanced by increases in growth rates under warming (Samperio-Ramos et al., 2015), possibly accelerating the invasion of native habitats. Due to the important ecosystem services offered by seagrasses, including sediment and nutrient trapping, carbon fixation, nursery areas, and high primary production (Costanza et al., 1997; Orth et al., 2006b; Pergent et al., 2014), examination of the potential interactive impacts of warming and biological invasions on seagrasses is of main relevance for conservation and management strategies. The aim of the present work was to experimentally evaluate the potential independent and cumulative impacts of warming and the presence of invasive algae (*C. cylindracea* or *L. lallemandii*) on the biological status (development, physiology and survivorship) of seedlings of the Mediterranean seagrass *P. oceanica*. We conducted a 3-month laboratory experiment where *P. oceanica* seedlings were exposed to different seawater temperatures and the presence of the invasive algae, in order to test the following hypotheses: (1) the increase in temperature will negatively affect *P. oceanica* development, promoting seedling death (2) the presence of the invasive algae reduces the vegetative development of *P. oceanica*, and (3) the interaction of seedlings with the invasive algae alters the effects of warming on *P. oceanica* seedlings.

2. Materials and methods

2.1. Seed and algae collection and maintenance

Seeds of *Posidonia oceanica* were obtained from fruits collected on the shore during June 2015 in Mallorca (Balearic Islands, Western Mediterranean Sea). Fruits were opened and their seed removed and placed in 20 L aquariums with seawater (salinity, 37), at a temperature of 20 °C and illuminated by white fluorescent lamps that provided $76 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetically active radiation in a 14/

10 h light/dark photoperiod. Seeds successfully germinate under these conditions and first leaves and roots are produced within two weeks (Terrados et al., 2013). These seedlings were maintained on a gravel substrate for the following four months under the described conditions with monthly replacement of seawater and weekly manual elimination of epiphytic algae growing on seedling leaves. Seedlings were examined for proper vegetative development (presence of seed, leaves and roots) and 800 seedlings with homogeneous morphological characteristics (in terms of seed area, number and size of leaves and roots; Tables S1 and S2) were chosen for the experiment. *C. cylindracea* and *L. lallemandii* were collected by scuba-diving during October 2015 in Mallorca and were maintained during 15 days, in separate tanks, in the same conditions as the seagrass seedlings. *C. cylindracea* rhizoids were buried in the gravel, allowing for the usual establishment pattern of this species, attaching to the substrate. *L. lallemandii*, which typically grows as an epiphyte on other macrophytes, was maintained freely on the bottom. Both seaweeds remained in perfect condition during the acclimation period.

2.2. Experimental design

A factorial experiment was designed considering two factors: temperature (see details below) and presence of invasive algae (with three levels: absence, presence of *C. cylindracea*, and presence of *L. lallemandii*). We also analyzed the effects of both invasive species occurring concurrently, but we were only able to examine this effect at 27 °C due to logistical constraints.

According to Sureda et al. (2008), the ratio of *P. oceanica* biomass (including leaves, sheaths and rhizomes) versus *L. lallemandii* biomass in an invaded area was 10.7 (in dry weight). We used this ratio to estimate the biomass of *L. lallemandii* needed in each aquarium (which contained 20 seedlings; i.e. 5.62 g *P. oceanica* DW), which was equivalent to ca. 5 g of wet weight (WW). For *C. cylindracea*, we used the biomass quantified by Holmer et al. (2009), and Pereda-Briones et al. (2017) in the field (i.e. 8 g *C. cylindracea* DW/m²), which amounted to 7.5 g *C. cylindracea* WW in each aquarium. The biomass of both invasive algae was replaced by fresh material on a monthly basis to ensure the optimal condition of the invasive algae material.

We manipulated seawater temperature to reach three values: 25 °C as the control treatment [the summer mean SST in the Mediterranean Sea measured over the last 20 years (1982–2012); Shaltout and Omstedt, 2014]; 27 °C (temperature in the range of the mean summer SST forecasted for the end of this century; Shaltout and Omstedt, 2014); and 29 °C (temperature in the range of the mean summer SST forecasted for next century in the Mediterranean Sea; Shaltout and Omstedt, 2014).

Treatment temperatures were reached after an acclimation period in which temperature was increased at a rate of 1 °C per week, using the same methodology as Hernan et al. (2017). Seedlings and invasive algae were then exposed to the treatment temperatures for three months. Each treatment had 4 replicate 20 L aquaria each containing 20 randomly selected seedlings, commercial aquarium gravel as substratum, a heater, and a recirculating pump. Aquaria were filled with filtered seawater (10 µm plus UV filter), which was replaced monthly in concordance with the addition of new invasive algae biomass. Epiphytic algae different from *L. lallemandii* growing on seedling leaves were manually eliminated every week. Salinity and temperature were measured in alternating days, and maintained at 37 PSU and at 25, 27 and 29 °C respectively. Water samples of each aquarium were taken at the beginning, middle and end (coinciding with the water replacement events) of the experiment for nutrient content analysis.

2.3. Seedling survival and development

Survival of *P. oceanica* seedlings was calculated as the percentage of alive seedlings at the end of the experiment relative to the initial

number of seedlings contained in each replicate aquarium. Seedling development was assessed by measuring the following morphological characteristics on ten seedlings per aquarium at the beginning and at the end of the experiment: leaf width of the second youngest leaf, maximum leaf length, total leaf area, number of leaves, percentage of necrotic surface on each leaf, total root number, total root length and number of new roots (which are characterized by white coloration and being flexible).

2.4. Seedling biomass and physiology

Biomass of *P. oceanica* seedlings was measured by drying the samples in an oven at 60 °C to constant weight for approximately 48 h. Then, three seedlings of each aquarium were randomly selected to analyze carbon and nitrogen contents in leaves, seeds, rhizomes, and roots, using a Carlo-Erba CNH elemental analyzer. Additionally, carbohydrates in seeds (sucrose and starch) were measured using the methodology described by Invers et al. (2004). Sucrose and starch content were obtained after three sequential extractions with 95% (v/v) ethanol at 80 °C for 15 min. The remaining pellet of seeds was dissolved in 0.1 N NaOH for 24 h at room temperature for starch extraction. Sugars and starch content of extracts were determined by spectrophotometry using an anthrone assay with sucrose as standard.

Rapid light curves (RLCs) were used to examine the effects of increased temperature and presence of invasive algae on seedling photophysiology. Fluorescence yield was measured in ten seedlings of each aquarium by pulse amplitude modulated (PAM) fluorometry, using an underwater device (Diving-PAM Walz GmbH, Germany). RLCs were generated automatically using an incremental sequence of actinic illumination periods, with light intensities increasing in 8 steps from 0 to 260 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ PAR at intervals of 15 s between measurements. The fraction of photosynthetic photon flux (PPF) absorbed by the leaf, the absorbance factor (AF), was derived from measuring the incident irradiance from a halogen lamp before and after the Diving-PAM's sensor was covered with 1 to 4 layers of the seedling leaves, then AF was calculated according to the method described by Beer et al. (1998).

Fluorescence measurements were done by placing the tip of the instrument's main optical fiber ca. 1 cm away from the base of the leaf. Then, a saturating-light with a pre-set PPF was irradiated. This allows to obtain the effective quantum yield (Y) of photosynthetic electron transport through PSII ($Y = (F_m' - F)/F_m'$), where F_m' is the maximal fluorescence of a light-adapted plant when all reaction centers are reduced, or closed, and F is the fluorescence in the light when part of the reaction centers are open. At that point, rates of electron transport (ETR) were calculated as: $ETR = Y \times \text{incident PAR} \times AF \times 0.5$ (assuming that half of the photons absorbed were absorbed by photosystem II; Schreiber et al., 1994). Photosynthetic quantum efficiency (α) was quantified as the initial slope of the linear portion of the RL curve. Saturating irradiance (E_k) was obtained by dividing the ETR_{max} by α .

2.5. Data analysis

Factorial ANOVAs (with two fixed and crossed factors; temperature and invasive algae) were used to test for differences in the initial morphological features of all the seedlings, and to compare final values of seedlings' morphological characteristics, carbon, nitrogen and carbohydrate content, as well as photophysiological variables. In addition, one-way ANOVAs were used to examine effects on seedling morphological traits between the four different levels of invasive algae presence (i.e. absence, *C. cylindracea*, *L. lallemandii*, and *C. cylindracea* + *L. lallemandii*) at 27 °C. Repeated measures ANOVAs were used to compare the concentration of dissolved inorganic nutrients present in the seawater of each aquarium at the beginning, in the middle and at the end of the experiment.

Prior to statistical analyses, data were examined for normality and

Table 1

Results of the Factorial ANOVAs analysing the morphological features of *Posidonia oceanica* seedlings in response to the presence of invasive algae (Inv), temperature (T) and their interaction. Significant effects highlighted in bold.

Variable	Source	df	MS	F	p
N° of leaves	Invasion	2	0,164	0,327	0,724
	Temperature	2	11,184	22,322	< 0,01
	Inv × T	4	1580	3154	0,03
	Error	27	0,501		
Width (cm)	Invasion	2	0,001	0,428	0,656
	Temperature	2	0,002	1257	0,301
	Inv × T	4	0,001	0,362	0,833
	Error	27	0,002		
Maximum leaf length (cm)	Invasion	2	6041	3848	0,03
	Temperature	2	4221	2688	0,086
	Inv × T	4	2483	1582	0,208
	Error	27	1570		
Total leaf area (cm ²)	Invasion	2	68,210	4788	0,017
	Temperature	2	468,830	32,912	< 0,01
	Inv × T	4	109,930	7717	< 0,01
	Error	27	14,250		
Necrotic leaf surface (cm ²)	Invasion	2	4145	1566	0,227
	Temperature	2	23,482	8873	< 0,01
	Inv × T	4	3452	1304	0,293
	Error	27	2646		
Seed area	Invasion	2	0,013	0,309	0,737
	Temperature	2	0,034	0,773	0,472
	Inv × T	4	0,022	0,494	0,740
	Error	27	0,044		
N° of roots	Invasion	2	31,001	0,971	0,391
	Temperature	2	29,659	0,929	0,407
	invasion × temperature	4	29,911	0,937	0,457
	Error	27	31,912		
Total root length (cm)	Invasion	2	35,940	2004	0,154
	Temperature	2	7990	0,446	0,645
	Inv × T	4	23,590	1315	0,289
	Error	27	17,930		
Maximum root length (cm)	Invasion	2	1192	0,946	0,401
	Temperature	2	1922	1526	0,236
	Inv × T	4	0,730	0,580	0,680
	Error	27	1259		
N° new roots	Invasion	2	74,104	1226	0,309
	Temperature	2	51,780	0,856	0,436
	Inv × T	4	55,269	0,914	0,470
	Error	27	60,460		
Total new roots length	Invasion	2	9723	3696	0,038
	Temperature	2	26,928	10,235	< 0,01
	Inv × T	4	0,435	0,165	0,954
	Error	27	2631		
Maximum new root length (cm)	Invasion	2	3864	3480	0,045
	Temperature	2	8761	7890	< 0,01
	Inv × T	4	0,150	0,135	0,968
	Error	27	1110		

The superscripted letter correspond to "squared" (square centimeters)

homogeneity of variances using Kolmogorov-Smirnov and Cochran's tests, respectively. Post-hoc Tukey test were conducted to examine differences between levels of each factor.

3. Results

All *Posidonia oceanica* seedlings survived for the entire duration of the experiment, but their development differed amongst treatments (Table 1). The total length of new roots decreased significantly with temperature, being significantly longer at 25 °C than the other temperatures (Fig. 1, Tukey). Similarly, the number of leaves and total leaf area decreased about 30% and 50% at 27 °C when seedlings were growing alone or with *C. cylindracea*, respectively. At 29 °C, the number

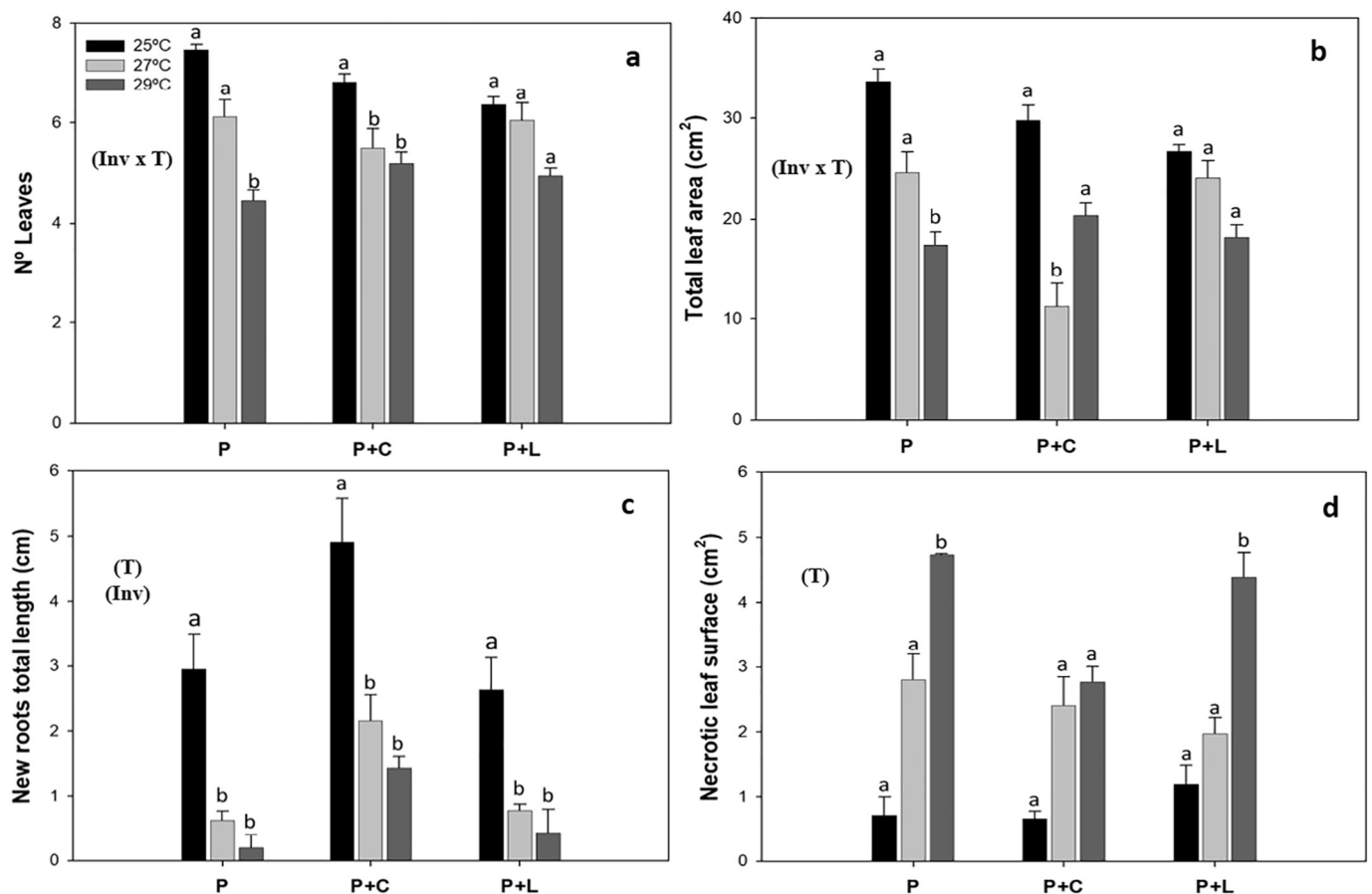


Fig. 1. *P. oceanica* seedling's number of leaves (a), total leaf surface (b), total length of new roots (c) and necrotic leaf surface (d) in the different experimental treatments: P (only *P. oceanica* seedlings), P + C (*P. oceanica* seedlings and *C. cylindracea*), P + L (*P. oceanica* seedlings + *L. lallemandii*). Error bars represent +1 SE. Significant results (T = temperature; Inv = invasive species; n.s. = non-significant) are shown in brackets and Tukey results are indicated with lowercase letters.

of leaves continued to decrease. In addition, total leaf area decreased at 29 °C but only when *P. oceanica* seedlings were growing alone, whereas seedlings co-occurring with *L. lallemandii* did not exhibit such decreases in either number of leaves or leaf area at 29 °C (Fig. 1, Tukey). On the other hand, the percentage of necrotic leaf surface tended to increase with temperature, being significantly higher at 29 °C for seedlings growing alone or with *L. lallemandii* (Fig. 1, Tukey).

Carbon content in seedling tissues was generally not affected by temperature nor the presence of invasive algae (Table 2), except for in the presence of *L. lallemandii*, whereby carbon in new roots was significantly lower than for seedlings growing alone or in the presence of *C. cylindracea*. Nitrogen content of leaves and new roots did not differ amongst treatments, whereas nitrogen content of old roots, rhizomes and seeds was generally higher at 29 °C than the other temperatures (Table 2; Fig. 2; Tukey). Regarding soluble carbohydrates in seeds, sucrose content was higher in the presence of *L. lallemandii* than in the other invasive treatments for all temperatures, and starch content was not affected by either treatment or their interaction (Table 3; Fig. 3, Tukey).

Whereas saturating irradiance did not differ amongst treatments (Table 4), seedling maximum electron transport rate (ETR_{max}) and photosynthetic efficiency (α) increased significantly at 27 °C, and decreased again at 29 °C ($29 = 25 < 27$; Table 4; Fig. 4, Tukey).

The presence of the two invasive species (tested only at 27 °C) had very few effects on the development of *P. oceanica* seedlings; with only total leaf area changing, which was lower in seedlings interacting with invasive species, due to the presence of *C. cylindracea*, in comparison to seedlings growing alone (Table S3; Tukey).

Water nutrient content changed through time (i.e. with each water

change) but did not differ between treatments (Table S4). Ammonium concentrations decreased through time from 7.1 ± 0.41 to $2.9 \pm 0.32 \mu\text{M}$, whereas nitrate increased from 0.5 ± 0.02 to $3.5 \pm 0.29 \mu\text{M}$.

4. Discussion

Our results indicate that warming can negatively affect the seedlings of *Posidonia oceanica*, and that the presence of invasive seaweed can potentially alter some of these responses to warming. Increasing temperatures have affected not only the photophysiology of the plants, but also leaf development and the production of new roots. Interestingly, the presence of invasive algae does not necessarily result in negative effects on seedlings.

Our observations supported our first hypothesis (i.e. the increase in temperature will negatively affect *P. oceanica* development, promoting seedling death), as we detected seedling deterioration with increasing temperatures. Optimal growth temperatures for temperate seagrasses are reported to be between 11.5 and 26 °C (Lee et al., 2007), and once the threshold temperature is exceeded, seagrass performance typically declines (Nejrup and Pedersen, 2008). Critical temperature threshold for adult *P. oceanica* plants are considered to be between 29 °C and 30 °C (Marbà and Duarte, 2010; Olsen et al., 2012), and similar to those of seedlings (Guerrero-Meseguer et al., 2017; Hernan et al., 2017). Our four-month-old seedlings survived along the 90 days of the experiment in all the treatments, including those maintained at 29 °C, whereas previous studies with six-month old seedlings observed an increase of ca. 13% in mortality rates at this temperature (Hernan et al., 2017). Such contrasting responses may be related to carbohydrate reserves (i.e.

Table 2

Factorial ANOVAs analysing the differences in percentage of nitrogen in the *P. oceanica* seedlings tissue components: seeds, old roots, new roots, rhizome and leaves, according to the presence of invasive algae (Inv), temperature (T) and their interaction. Significant effects are highlighted in bold.

Nutrient	Variable	Source	df	MS	F	p
Carbon	Seeds	Invasion	2	17,300	2908	0,071
		Temperature	2	0,790	0,134	0,875
		Inv × T	4	5380	0,904	0,475
		Error	27	5950		
	Old roots	Invasion	2	12,070	1647	0,211
		Temperature	2	18,880	2578	0,094
		Inv × T	4	12,880	1758	0,166
		Error	27	7330		
	New roots	Invasion	2	43,950	4557	0,020
		Temperature	2	1170	0,122	0,886
		Inv × T	4	3670	0,381	0,820
		Error	27	9640		
	Leaves	Invasion	2	1,29	2,44	0,106
		Temperature	2	0,54	1,02	0,373
		Inv × T	4	0,23	0,43	0,784
		Error	27	0,53		
	Rhizomes	Invasion	2	0,790	0,570	0,570
		Temperature	2	3620	2640	0,091
		Inv × T	4	1430	1051	0,404
		Error	27	1,37		
Nitrogen	Seeds	Invasion	2	0,200	3123	0,060
		Temperature	2	1089	17,002	< 0,01
		Inv × T	4	0,135	2121	0,105
		Error	27	0,064		
	Old roots	Invasion	2	0,006	0,475	0,626
		Temperature	2	0,069	5515	< 0,01
		Inv × T	4	0,005	0,421	0,792
		Error	27	0,012		
	New roots	Invasion	2	0,032	3035	0,066
		Temperature	2	0,006	0,600	0,556
		Inv × T	4	0,012	1162	0,350
		Error	27	0,010		
	Leaves	Invasion	2	0,003	0,151	0,860
		Temperature	2	0,012	0,663	0,523
		Inv × T	4	0,015	0,813	0,527
		Error	27	0,018		
	Rhizomes	Invasion	2	0,102	0,650	0,531
		Temperature	2	1086	6894	< 0,01
		Inv × T	4	0,141	0,898	0,480
		Error	27	0,157		

starch and sucrose) of seeds, which were not affected by temperature in our experiment. Carbohydrates are crucial storage compounds of plants (Tetlow et al., 2004), which reflect the recent energetic balance of the plant, and their reduction can strongly influence plant survival (Genot et al., 1994) and make seagrass meadows more vulnerable to additional stress (Eklof et al., 2009). Presumably, our younger seedlings should have higher carbon reserves than older ones, which may have increased their resistance to warming. Consequently, we suggest that the lethal temperature threshold of young seedlings of *P. oceanica* is age-dependent based on carbon reserves available in the seed and would be above 29 °C for younger seedlings. The results of Guerrero-Meseguer et al. (2017), who worked with five-month old *P. oceanica* seedlings, support this notion since they did not observe mortality at 27 nor at 29 °C, whereas mortality peaked to 33% when temperatures went over 29 °C.

Even though our seedlings did not suffer mortality under warming, we did observe sublethal responses which may affect seedling survival success in the longer term. For example, during seedling development, *P. oceanica* seedlings typically undergo a decrease in the number of leaves formed, but an increase in total leaf area as these new leaves are larger (Terrados et al., 2013). In our experiment, seedlings experiencing warming underwent a stronger reduction in leaf formation and an overall decrease in total leaf surface. This response indicates that increasing temperatures reduce leaf formation rates, which has also been reported for adults (Mayot et al., 2005) and seedlings (Olsen et al.,

2012; Guerrero-Meseguer et al., 2017; Hernan et al., 2017) of *P. oceanica* as well as for adult plants of other temperate (Nejrup and Pedersen, 2008) and tropical (Edwards, 1995) seagrasses. In addition, the proportion of necrotic leaf surface increased, indicating that warming creates stressful conditions for temperate seagrass seedlings (Niu et al., 2012; Guerrero-Meseguer et al., 2017; Hernan et al., 2017). Increased necrotic surface may be stimulated by a deterioration of the photosynthetic apparatus when temperature overpasses certain values. In fact, warming can lead to improved fitness and an increase in photosynthesis rate (with an estimated optimum temperature range for net photosynthesis of 16° to 35 °C in temperate seagrasses; Lee et al., 2007) until the thermal threshold of a species is exceeded and damage occurs (Pörtner and Farrel, 2008; Pedersen et al., 2016). We observed that maximum electron transport rate (ETR_{max}) and photosynthetic efficiency (α) of seedlings increased significantly at 27 °C, but decreased again at 29 °C, suggesting that the photosynthesis process suffers when temperatures are above 27 °C (see also Guerrero-Meseguer et al., 2017).

Regarding seedlings' nutrient content, we detected higher nitrogen at higher temperatures in seeds, old roots and rhizomes, but no differences in leaves or new roots. Presumably, as the number of leaves and leaf surface decrease with temperature, seedlings don't need to mobilize a larger fraction of their nitrogen reserves (stored in seeds, old roots and rhizomes) to produce leaf biomass, resulting in higher final nitrogen concentrations in these tissues. In contrast, new roots may have higher nitrogen requirements since they are an active growth tissue where the available resources are mobilized and used, and their development may be prioritized regardless of the environmental conditions.

Interestingly, concerning our second hypothesis (i.e. the presence of the invasive algae reduces seedlings' vegetative development), our results suggest the opposite. While it has been demonstrated that *C. cylindracea* and *L. lallemandii* can negatively affect adult plants of *P. oceanica*, decreasing shoot size and increasing leaf necrosis and shoot mortality (Holmer et al., 2009; Ballesteros et al., 2007; Marbà et al., 2014), effects on seedlings remain practically unknown. In fact, *C. cylindracea* can favor the production of new roots of *P. oceanica* seedlings, facilitating their early establishment (Pereda-Briones et al., 2017), and effects of *L. lallemandii* on *P. oceanica* seedlings have not been examined thus far. The lack of negative effects of invasive algae on the seedlings observed in our experiment and detected in the field (Pereda-Briones et al., 2017), in addition to the enhancement of root development in the presence of *C. cylindracea*, and the tendency of the presence of *L. lallemandii* to improve the negative effects of warming on seedling leaf development, all suggest that there may be positive rather than negative influences of invasive algae on seedlings during their recruitment phase under the tested conditions. Yet, and considering the cautionary principle, it is worth highlighting that, albeit not statistically significant, we did observe a trend towards lower leaf area in the presence of *L. lallemandii*. Perhaps other experimental conditions (e.g. duration of the experiment or biomass of *L. lallemandii*), would have yield more obvious negative effects.

Warming also led to a decrease of total new root length, which is likely to negatively affect the establishment capacity of seedlings if they do not reach the adequate root length for successful anchoring (Infantes et al., 2011). In addition to the length of the root system, the ability of the seedlings to remain anchored to the substratum is strongly dependent on the hydrodynamic conditions (Frederiksen et al., 2004; Infantes et al., 2009; Rivers et al., 2011, Pereda-Briones et al., 2018) and the substratum type (Balestri and Lardicci, 2008; Alagna et al., 2013; Pereda-Briones et al., 2018). In this sense, the presence of *C. cylindracea*, by favoring the production of new roots, could facilitate the establishment of seedlings, and thus, maximize the possibilities of remaining in the substratum facing waves and currents. Indeed, the survival and growth of one-year old *P. oceanica* seedlings transplanted to dead matte substrate are enhanced by the presence of *C. cylindracea* (Pereda-Briones et al., 2017). Furthermore, this facilitative interaction

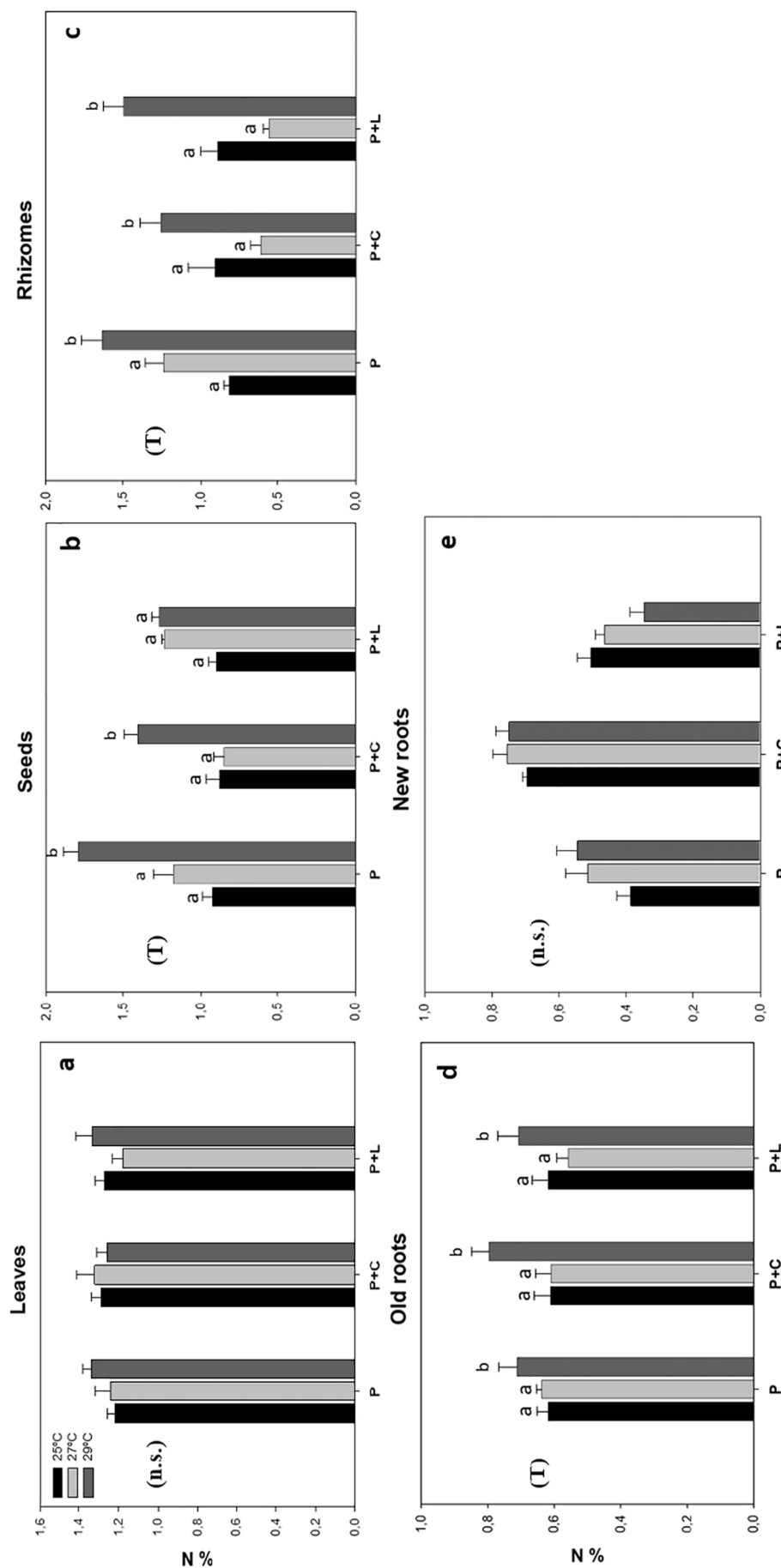


Fig. 2. *Posidonia oceanica* mean nitrogen concentration (% of DW) of seedling tissues: leaves (a), seeds (b), rhizomes (c), old roots (d) and new roots (e), across experimental treatments: P (only *P. oceanica* seedlings), P + C (*P. oceanica* seedlings and *C. cylindracea*), P + L (*P. oceanica* seedlings + *L. lallemandii*). Error bars represent + 1 SE. Significant results (T = temperature; Inv = invasive species; n.s. = non-significant) are shown in brackets and Tukey results are indicated with lowercase letters.

Table 3

Factorial ANOVAs analysing the percentage of sucrose and starch in the seeds of *P. oceanica* seedlings according to the presence of invasive algae (Inv), temperature (T) and their interaction. Significant effects highlighted in bold.

Variable	Source	df	MS	F	p
Sucrose	Invasion	2	72,920	6852	< 0,01
	Temperature	2	2437	0,229	0,797
	Inv × T	4	23,380	2197	0,112
	Error	17	10,640		
Starch	Invasion	2	2,37E+09	1829	0,191
	Temperature	2	5,61E+08	0,431	0,656
	Inv × T	4	5,94E+08	0,457	0,765
	Error	17	1,29E+09		

was not diminished under warming conditions, thus potentially counteracting negative warming effects by enabling the anchoring of the seedlings and the consequent nutrient uptake in adverse thermal scenarios.

Elevated temperatures can increase or decrease competition, and

Table 4

Factorial ANOVAs analysing the photophysiology parameters: ETR_{max}, alpha and E_k of *Posidonia oceanica* seedlings according to the presence of invasive algae (Inv), temperature (T) and their interaction. Significant effects highlighted in bold.

Variable	Source	df	MS	F	p
ETR max	Invasion	2	5197	0,268	0,765
	Temperature	2	169,055	8717	< 0,01
	Inv × T	4	9032	0,466	0,761
	Error	99	19,395		
Alpha	Invasion	2	0,003	0,469	0,627
	Temperature	2	0,076	13,928	< 0,01
	Inv × T	4	0,000	0,061	0,993
	Error	99	0,005		
Ek	Invasion	2	767,100	0,677	0,511
	Temperature	2	1009,900	0,891	0,414
	Inv × T	4	1002,900	0,885	0,476

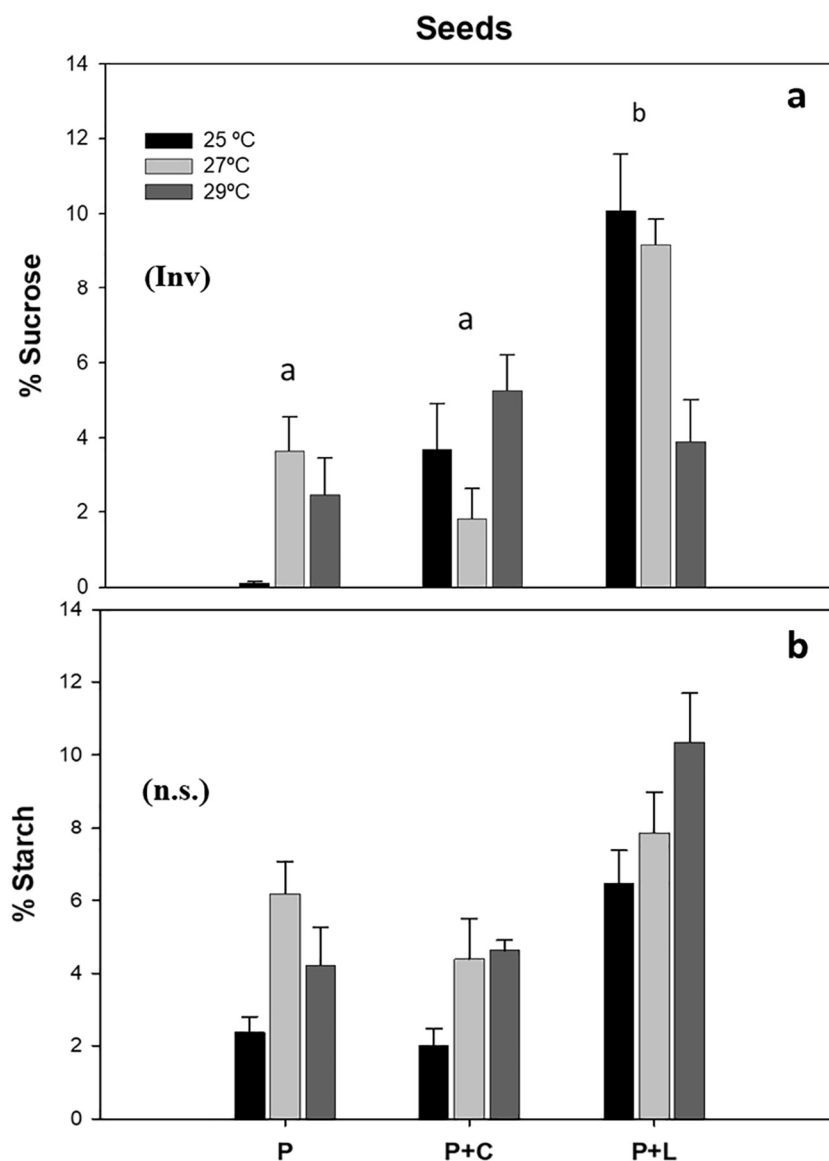


Fig. 3. *Posidonia oceanica* mean carbohydrate concentration (% of DW) of seedling seeds: sucrose (a) and starch (b), across experimental treatments: P (only *P. oceanica* seedlings), P + C (*P. oceanica* seedlings and *C. cylindracea*), P + L (*P. oceanica* seedlings + *L. lallemandii*). Error bars represent +1 SE. Significant results (T = temperature; Inv = invasive species; n.s. = non-significant) are shown in brackets and Tukey results are indicated with lowercase letters.

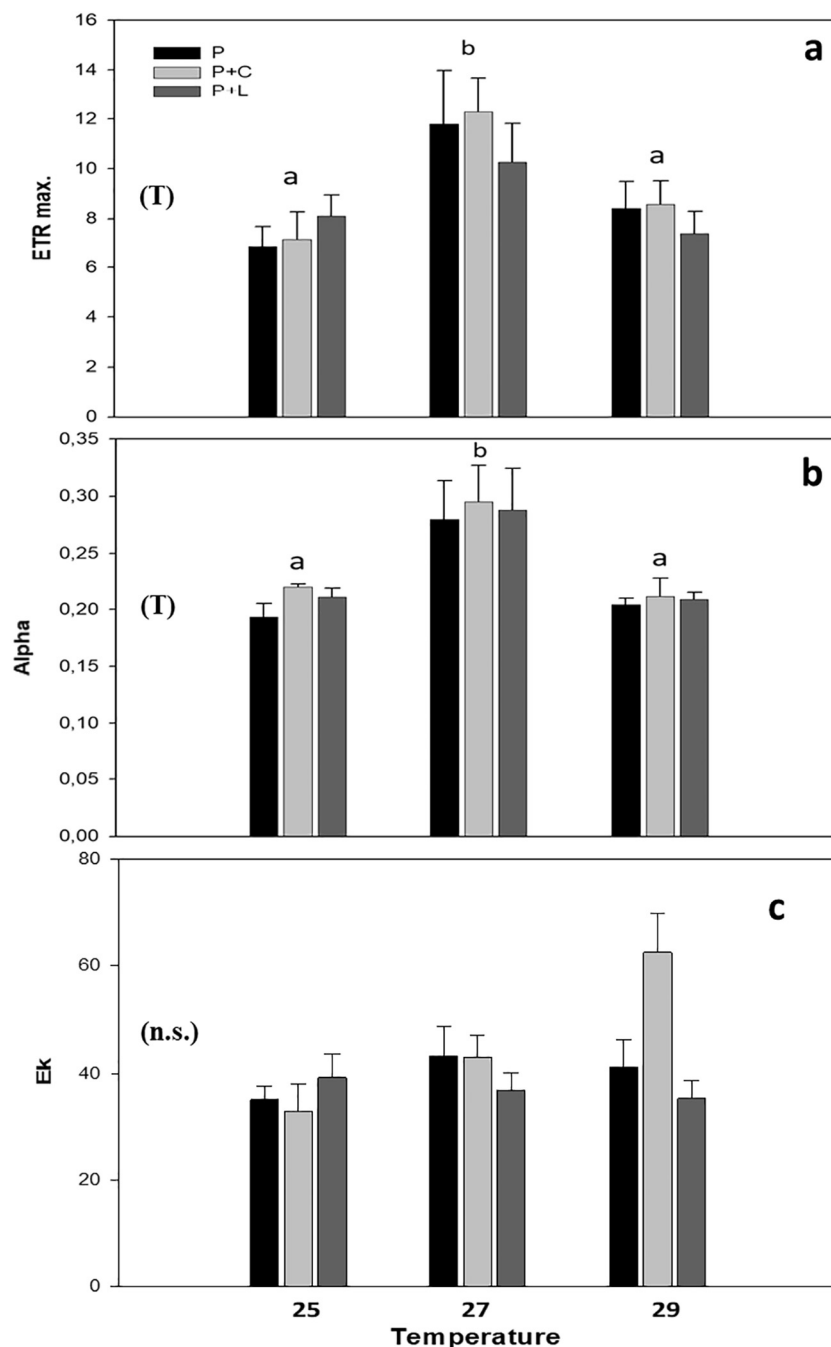


Fig. 4. *Posidonia oceanica* photophysiology parameters: ETR_{max} (a), alpha (b) and E_k (c) across experimental treatments: P (only *P. oceanica* seedlings), P + C (*P. oceanica* seedlings and *C. cylindracea*), P + L (*P. oceanica* seedlings + *L. lallemandii*). Error bars represent +1 SE. Significant results (T = temperature; Inv = invasive species; n.s. = non-significant) are shown in brackets and Tukey results are indicated with lowercase letters.

even transform competitive interactions into facilitative ones (Harley et al., 2012). In our case study, the effects of rising temperatures on *P. oceanica* seedlings have not been modified in the presence of invasive algae, rejecting our third hypothesis (i.e. the interaction of seedlings with the invasive algae alters the effects of the temperature on *P. oceanica* seedlings). Although the presence of *C. cylindracea* attenuated the decrease in the number of leaves and total leaf area of *P. oceanica* seedlings at 29 °C, and in the presence of *L. lallemandii* there were no temperature effects in these two variables, the general trend of decreasing foliar development with higher temperatures is maintained independently of the presence of invasive algae.

Warming is expected to continue in the coming years (IPPC, 2014), and this may also modify the response of both invasive algae, with

further consequences for their interactions with *P. oceanica* seedlings. For instance, *L. lallemandii* may be more vulnerable to warming as it does not tolerate well temperatures above 29 °C, while *C. cylindracea* maintains high growth rates from 23 to 31 °C (Samperio-Ramos et al., 2015). On the other hand, global warming on marine ecosystems is accelerating the settlement of new alien species (Raitos et al., 2010), and there are numerous examples of invasive species being more tolerant to higher temperatures than native ones (e.g. Smith et al., 2004; Sorte et al., 2010). This is particularly relevant for the Mediterranean Sea, where the predicted warming exceeds threefold that of the global ocean (Burrows et al., 2011), further encouraging the proliferation of invasive species from tropical and subtropical origin entering through the Suez Canal (Bianchi, 2007). Therefore, even though *P. oceanica*

seedlings appear to be resistant in their interaction with *C. cylindracea* and *L. lallemandii* (at least in the conditions of our study), since they are sensitive to warming (Guerrero-Meseguer et al., 2017; Hernan et al., 2017; this study), they may not cope well with these or other invasive species if global warming trends continue.

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Contributors

L.P., F.T. and J.T. conceived and conducted the experiment. L.P. conducted plant analyses. F.T., and J.T. contributed with reagents/material/laboratory equipment. L.P., F.T. and J.T. prepared and reviewed the manuscript and have approved the final article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2019.01.049>.

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