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Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion

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Exotic species often face new environmental conditions that are different from those that they are adapted to. The tropical seagrass *Halophila stipulacea* is a Lessepsian migrant that colonized the Mediterranean Sea around 100 years ago, where at present the minimum seawater temperature is cooler than in its native range in the Red Sea. Here, we tested if the temperature range in which *H. stipulacea* can exist is conserved within the species or if the exotic populations have shifted their thermal breadth and optimum due to the cooler conditions in the Mediterranean. We did so by comparing the thermal niche (e.g. optimal temperatures, and upper and lower thermal limits) of native (Saudi Arabia in the Red Sea) and exotic (Greece and Cyprus in the Mediterranean Sea) populations of *H. stipulacea*. We exposed plants to 12 temperature treatments ranging from 8 to 40°C for 7 days. At the end of the incubation period, we measured survival, rhizome elongation, shoot recruitment, net population growth and metabolic rates. Upper and lower lethal thermal thresholds (indicated by 50% plant mortality) were conserved across populations, but minimum and optimal temperatures for growth and oxygen production were lower for Mediterranean populations than for the Red Sea one. The displacement of the thermal niche of exotic populations towards the colder Mediterranean Sea regime could have occurred within 175 clonal generations.

1. Introduction

Human activities accelerate introductions of marine exotic species beyond their native biogeographical boundaries [1], with widespread, but moderate impacts on native marine biodiversity [2]. Exotic species often experience different conditions at the recipient locations than those in their native range. For instance, several marine species translocations occur in locations with a cooler thermal regime than experienced at the native region [3]. These may affect the performance, both in terms of their competitive ability and expansion rates, of the exotic species at the receiving location.

Temperature is a major factor determinant of biological activity [4,5], constraining the niche of species. Specifically, species can operate within a certain temperature range that may be conserved within the species or vary depending on local conditions [6,7]. Biological performance is expected to vary within the species thermal niche. This is defined by a lower lethal thermal limit, an exponential increase, according to the Arrhenius equation, to reach a maximum at the optimum temperature and then steep decline to reach the upper thermal lethal limit [5]. The shapes and thermal limits of the performance curve can vary across functional levels, e.g. from physiology to individuals and

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populations [8]. The limits of the thermal breadth of biological activity at physiological and individual levels reflect sublethal thermal limits, whereas lethal thermal limits are defined by survival, thus, representing a population-level response [9]. Despite the importance of the thermal niche, this is typically assumed to be uniform for a species in the marine environment and represented from thermal performance curves obtained from a single population [10]. Little consideration (but see [11,12]) is given for the variability of the thermal niche along with the species biogeographical range, both among but also within populations [6].

Exotic species encountering new thermal conditions may (a) displace their thermal performance curve maintaining the width of the thermal range (modifying optimal and limit temperatures), (b) broaden the thermal range while maintaining their optimal temperatures or (c) perform within a narrow fraction of their thermal window matching the thermal range at the receiving site [13]. The capacity of exotic species to adjust to different thermal regimes at the receiving site is, therefore, fundamental to predict their impacts and expansion [14]. Unfortunately, this possibility has been largely overlooked in the marine environment until recently [15,16].

Species are able to modify their thermal limits by either phenotypic plasticity or adaptation [17]. Phenotypic plasticity is defined as the change in the phenotypic expression of a genotype in response to environmental factors [18]. Plasticity might become adaptive if the phenotypes produced in response to the new environment result in higher fitness [19]. Adaptation based on genetic change is expected to occur over a large timescale [20] although current evidence shows that species, including exotic ones [21] and those in their native range, can adapt over timescales of a few years to decades, depending on generation time, under climate change pressures [22]. Whereas rapid adaptation in native species is usually tested under laboratory experiments manipulating selective pressures [23,24], exotic species provide an opportunity to explore this in a natural setting [25–28]. The literature on rapid adaptation in marine organisms is increasing [29–31], with most tests for short generation time species, such as phytoplankton [23,24,32] amenable to experimental manipulation. None of these experiments have, however, addressed the capacity of exotic species relocated to a downshifted thermal range, which is the prevailing case in marine exotic species.

The Mediterranean Sea is a global hot spot for the introduction of exotic species due to the opening of the Suez Channel in 1869, which facilitated the transport of Indo-Pacific species through the Red Sea into the Mediterranean, the so-called Lessepsian migrants [33]. The transference from the Red Sea, the warmest of all seas [34] to the Mediterranean Sea involves, however, a significant downshift in thermal regimes affecting both the upper and lower temperatures [3]. The magnitude of this downshift increases from the Eastern Mediterranean towards the much cooler Western basin [3], thereby increasing the challenges to the colonization of Lessepsian migrants to extend westward along the Mediterranean Sea.

Marine macrophytes rank among the exotic taxa causing most concern in the Mediterranean Sea [35]. Whereas there are several exotic macroalgae examples in the region [36], only one exotic seagrass species (*Halophila stipulacea*) has been established [37] (although the seagrass *H. decipiens* has also been reported recently [38]). This Lessepsian marine plant is native to the Indo-Pacific and entered the Eastern Mediterranean Sea [39] from the Red Sea across the Suez Canal and recently arrived to the Caribbean Sea through boat transport [40]. Even though it has been almost a century since the first meadow was reported in Rhodes Island, Greece [39], little evidence of negative impacts of *H. stipulacea* on native species in the Mediterranean Sea [41] has been published—contrary to situation in the Caribbean Sea where *H. stipulacea* can displace its native counterparts [42]. Since then, it has spread across the Eastern ([43] and references therein) and central Mediterranean [44]. The establishment and spread of this species in the Mediterranean Sea suggest a rapid adaption to its lower seawater temperatures, which are much cooler than those of the Red Sea. Indeed, it has been experimentally demonstrated that *H. stipulacea* is able to survive the winter Mediterranean water temperatures [10]. However, it is unknown if the tolerance to colder temperatures is conserved in native populations and if it is changing across the natural temperature gradient of the Mediterranean.

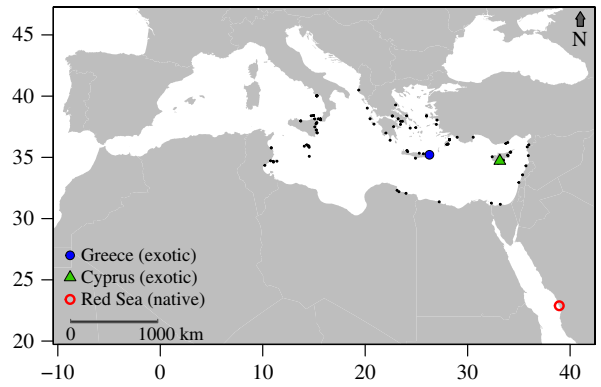

Figure 1. Current *H. stipulacea* distribution in the Mediterranean (black dots) and donor populations (Greece: filled circle in blue; Cyprus: triangle in green; the Red Sea: open circle in red) used for thermal tolerance experiments. (Online version in colour.)

Here, we experimentally test the null hypothesis that the temperature range of *H. stipulacea* is conserved in the Mediterranean Sea relative to that in the native Red Sea. We do so by comparing the thermal niche, as characterized by optimal temperatures, upper and lower thermal limits, of native populations of *H. stipulacea* sampled in the Red Sea (Saudi Arabian coast) and exotic populations in the Mediterranean Sea (Greece and Cyprus).

2. Methods

(a) Study site

We harvested the experimental *H. stipulacea* plants from two exotic populations in the Mediterranean Sea, in Greece (Crete, Maridati) and Cyprus (Limassol), and a native one in the Red Sea (Al Kahrar Lagoon; figure 1, electronic supplementary material, table S1). The specimens (rhizome fragments with shoots and roots attached) were randomly collected across the meadow by SCUBA divers, avoiding repetitive sampling from the same clone. The experiments with the native population were conducted at the Coastal and Marine Resource Core Lab (CMOR) at King Abdullah University of Science and Technology

(KAUST, Saudi Arabia), located 1 h away from the donor seagrass population. The experiments with exotic populations (Greece and Cyprus) were conducted at the Mediterranean Institute for Advanced Studies (IMEDEA, Spain) experimental facilities. Within 30 h after harvesting, the plants were carefully wrapped in seawater-soaked towels, placed inside an isothermal box containing cooling elements and transferred by plane. We were not able to conduct all experiments at the same laboratory facilities (IMEDEA) because of logistical complexity associated with the transportation of living plants from Saudi Arabia to Spain.

(b) Sea surface temperatures at donor population sites

The minimum and maximum sea surface temperature (SST) at the sites where the experimental *H. stipulacea* fragments were growing were obtained from temperature sensors (HOBO® Water Temp Pro v2 logger) deployed in Gouves (Greece; from July 2017 to May 2018) and Cape Greco (Cyprus; from September 2016 to July 2017) and from a CTD (Exosond 2, YSI inc., Yellow Springs, USA) deployed in Al Kahrar (Red Sea; from July to October 2015) (electronic supplementary material, table S1). Minimum and maximum temperatures were extracted as the 0.1th and 99.9th percentiles of SST, respectively, with the exception for the Red Sea where we used the minimum reported in Al-Dubai Talha *et al.* [45] for the lagoon of Al Kahrar (as the data obtained from CTD were limited to the summer period).

(c) Experimental set-up

We conducted two experiments per population, each to test the lower or upper range of the species thermal breadth, resulting in six experiments in total (electronic supplementary material, table S1). Upon the arrival of plants to the experimental facilities, we transferred them into 60 l seawater containers filled with air supply for acclimatizing at *in situ* temperature for 6 days. Acclimation temperature was set to the *in situ* temperatures at the time of collection of each population with the purpose to acclimate the plants to the conditions at the experimental facilities maintaining their *in situ* temperature to avoid exposing the plants to thermal stress before the onset experiment. Despite different acclimation temperatures between experiments, from the Red Sea experiments, we find that this did not affect plant performance (electronic supplementary material, table S2). The plants were exposed to a photoperiod of 12 L : 12 D, light intensity ranging from 250 to 400 µmol m^2 s^{-1} (measured with a photometric bulb sensor; LI-COR), similar inorganic nutrient concentrations (measured with a Bran Luebbe AA3 segmented nutrient analyser; electronic supplementary material, table S1) and a salinity range of 37–40 (refilling aquaria to compensate for evaporation) during both acclimation and experimental periods.

Each experiment was conducted over six 7-day periods (42 days in total) and consisted of an 'experimental temperature treatment' (electronic supplementary material, table S1) where temperature was manipulated every 7 days and a 'control temperature treatment' where temperature was maintained always at *in situ T*. Both treatments were set first to acclimation temperature (*in situ T*) for 6 days and then temperature in the experimental temperature treatment was increased/decreased at a rate of 2–3°C day^{-1} until reaching the target temperature. Then, the experiment started and the response of specimens was followed for 7 days, a sufficient time period to detect clonal growth responses for this species [10,46]. At the same time, plants in the control treatment were also assessed for 7 days. This procedure was repeated until reaching all the target temperatures (electronic supplementary material, table S1).

The experimental set-up of the exotic populations consisted of 60-l polypropylene freshwater tanks ($n = 8$ for the lower and $n = 6$ for the upper thermal range), with a pump recirculating the water, inside a temperature-controlled chamber. Each tank contained three replicated 4 l aquaria filled with seawater, air supply and four *H. stipulacea* fragments with five shoots. The temperature was maintained using a temperature control system (IKS Aquastar) connected to heaters and temperature sensors (recording every 15 min) deployed, respectively, in each tank and in one aquarium per treatment. For the native population (Red Sea), experiments were conducted in two experiments Percival Intellus environmental control incubators, one for the experimental target temperatures and the other for the control temperature. In each incubator, 24 replicated 2 l aquaria filled with seawater and two fragments of *H. stipulacea* with five shoots and constant aeration were placed.

(d) Response variables

Net population growth rate (in day^{-1}) was calculated using the following equation [47]:

$$\mu = \frac{(\ln(N_t/N_0))}{t}, \tag{2.1}$$

where N_0 and N_t are the number of shoots per fragment at the beginning and the end of the 7-day experiment, respectively, and t is the experiment time in days.

Shoot recruitment rate (in day^{-1}) was assessed according to [47]:

$$R = \frac{(\ln(N_0 + N_{new})/N_0)}{t}, \tag{2.2}$$

where N_{new} is the number of new shoots at the end of the experiment.

Survival was assessed at the end of the 7-day experiment by counting the fragments in the temperature treatment either presenting or not meristem mortality. Survivorship values were treated as binary data (0, for fragments with meristem mortality, and 1, for fragments with meristems alive). Binary data were plotted against temperature and a logistic dose–response model:

$$s = \frac{100}{1 + 10^{S(\mathrm{LT50} - T)}}, \tag{2.3}$$

where s is the fraction survival, T (in °C) is the temperature treatment, S is the hillslope of the regression, and LT50 is the lethal temperature at which 50% of the fragments presented meristem mortality was fitted using the GraphPad Prism.

Rhizome elongation rate (cm day^{-1}) was assessed using rhizome marking techniques: at the commencement of the 7-day experiment, a cable tie was placed near the apical meristem and rhizome elongation rate was calculated at the end of the experiment, by measuring the length of the rhizome between the apex and the cable tie divided by the duration of the experiment in days.

Metabolic rates (in mmol O_2 day^{-1} gDW^{-1}) were measured by incubating the plants in jars with seawater inside the experimental tanks at different experimental temperatures for 24 h (exposed 12 h to 'light' and 12 h to 'dark' conditions) the last day of the 7-day experiment. Mean O_2 concentration and temperature was continuously recorded every 15 min using optical dissolved oxygen sensors or miniDOTs (Precision Measurement Engineering) in four replicated 1 l jars with one *H. stipulacea* fragment and four replicated 2 l jars with two *H. stipulacea* for the exotic and native populations, respectively. Jars filled with seawater without plants (blank) were also incubated to register oxygen changes in the absence of plants.

The changes of oxygen concentration in jars were used to estimate respiration (R), net production (NP) and gross primary production (GPP). Data time series recorded for each jar was first separated into daytime (light) and night-time (dark), and the time steps were expressed in hours (h). R was calculated as follows:

$$\mathrm{R} = \sum \mathrm{O_2\ dark} \times [\mathrm{light(h)} + \mathrm{dark(h)}] \tag{2.4}$$

where O_2 dark was the slope of the linear regression fit between the oxygen concentration measurements at night-time and *light (h)* and *dark (h)* the duration (in h) of daytime and night-time periods, respectively.

NP was calculated from oxygen changes measured as follows:

$$\mathrm{NP} = \sum \mathrm{O_2\ light} \times \mathrm{light(h)} - \sum \mathrm{O_2\ dark} \times \mathrm{dark(h)} \quad (2.5)$$

where O_2 light representing the slope of the linear regression fit between the oxygen concentration measurements at daylight.

GPP was then estimated by the sum of NP and R:

$$\mathrm{GPP} = \mathrm{NP} + \mathrm{R} \quad (2.6)$$

Metabolic rates were multiplied by the corresponding volume (1 l jars for the exotic population and 2 l jars for native populations) and divided by the incubated fragments dry weight (DW, in g).

(e) Thermal performance curve

The thermal tolerance of *H. stipulacea* populations was characterized by fitting growth and metabolic response variables to a Gaussian function [48]:

$$Y = a e^{[-0.5((T-b)/c)^2]}, \quad (2.7)$$

where Y is the response variable measured, T is the temperature (in °C), a (Amplitude) is the height of the centre of the distribution in Y units, b (Mean) is the temperature value at the centre of the distribution, and c (SD) is the temperature width of the distribution. From the fitted function, we calculated the minimum (lower thermal limit), maximum (upper thermal limit) and optimum temperature and their corresponding confidence intervals (CIs).

The thermal safety margin (TSM) is the difference between the lower or upper thermal limit and the minimum or maximum SST exposure in nature [49]. The lower TSM was calculated as the minimum local population SST minus the empirically observed minimum temperature for GPP, growth (rhizome elongation rate) or survival (LT50). The TSM for the upper thermal limit was similarly calculated, but using the maximum local SST at each site and the upper thermal limit of seagrass performance parameters.

(f) Generation time

The generation time (T_G, in days) of *H. stipulacea*, i.e. the time to duplicate the shoot population, was calculated as follows:

$$T_G = \frac{\ln 2}{B}, \quad (2.8)$$

where B was the birth rate (day^{-1}) estimated from the empirical equation reported for photosynthetic organisms in [50] as follows:

$$B = 0.0008\ (M^{-0.27}), \quad (2.9)$$

where M is shoot mass (in g DW).

(g) Statistical analysis

In order to determine whether different populations of *H. stipulacea* exhibit different thermal performance curves, we performed individual tests for each response variable as follows: a Gaussian curve function was fitted to temperature as a continuous predictor with population included as an independent categorical factor (with three levels: Greece, Cyprus and the Red Sea). A sum of squares *F*-test was used to compare the models with and without population (e.g. response variable ~ temperature + population versus response variable ~ temperature). If the effects of the population were significant, differences between populations were tested *a posteriori* with the additional sum of squares *F*-tests, applying a Bonferroni correction for multiple comparisons (p-value < 0.016). Finally, the temperature parameters (i.e. optimum, minimum and maximum temperatures) were compared across populations by 95% CIs: significant differences were determined when the 95% CIs did not overlap. All statistical analyses were performed using the GraphPad PRISM 8.0.1.

3. Results

SST at the sites where the experimental specimens were collected differed, with exotic populations being exposed at colder water than the native ones. SST at Greece, Cyprus and the Red Sea sites ranged between 14.96–27.67°C, 15.83–29.77°C and 21.82–35.30°C, respectively. The annual thermal amplitude across sites also differed, being the widest in Cyprus.

Exotic *H. stipulacea* populations maintained 100% survival at seawater temperatures ranging from 11 to 34°C, while native population fragments from 11 to 38°C (table 1, electronic supplementary material, figure S1). The logistic model fitted on plant survival indicated that the lower and upper lethal temperatures for 50% fragment survival were 8.00 ± 0.07 and 36.24 ± 0.64°C for Greece and 8.63 ± 0.68 and 36.82 ± 0.33°C for Cyprus, while they were 8.61 ± 0.70 and 38.25 ± 0.42°C in the native population (table 1).

Rhizome elongation and shoot recruitment rates of *H. stipulacea* showed clear bell-shaped curves across the temperature range examined, while thermal performance curves for net population growth rate were considerably flat (figure 2). *H. stipulacea* thermal performance curves for growth (rhizome elongation, shoot recruitment and net population growth rates) differed significantly between the population of Greece and the population of Cyprus and the Red Sea (electronic supplementary material, table S3). The width of the thermal niche for growth was narrower than that observed for survival for all seagrass populations tested. Rhizomes stopped growing at temperature treatments cooler than 14°C and exceeding 36°C in exotic populations, while they stopped growing below 17°C and above 38°C in the native population (figure 2*a*). Optimal temperatures for rhizome growth were 2-3°C lower in the exotic populations than in the native population (table 1). Differences were significant for minimum and optimum temperatures between exotic and native populations (table 1).

The lower thermal limit for shoot recruitment of *H. stipulacea* was at 17°C in native and exotic populations, whereas optimum temperature for shoot recruitment differed significantly between Greece and the Red Sea populations (figure 2*b*, table 1).

Net population growth increased (greater than 0) at seawater temperatures ranging between 20 and 34°C for Greece, 25 and 34°C for Cyprus and 25 and 38°C for the Red Sea. Population growth was in steady state (= 0) at temperatures from 8 to 17°C and from 31.5 to 34°C for Greece, from 8 to 11°C for Cyprus at temperature treatments of 8, 17, 20 and 40°C for the Red Sea. Net population growth declined (less than 0) when the temperature was warmer than 36°C for Greece and Cyprus, at treatments of 14 and 17°C for Cyprus and at treatments of 11 and 14°C for the

Table 1. Optimal temperature (T_{opt}) and lower (T_{min}) and upper (T_{max}) thermal limits estimated by fitting a logistic dose–response model to the thermal performance of survival and a Gaussian model to the thermal performance of growth and metabolic rates of *H. stipulacea* from the three studied populations. Confidence intervals at 95% of the temperature parameters are in brackets. N/A: not applicable due to unrealistic estimated values.

response variables		T_{opt} (°C)		T_{min} (°C)		T_{max} (°C)	
survival (LT50)	Greece	–		8.00	(7.87–8.13)	36.24	(34.96–37.51)
	Cyprus	–		8.63	(7.30–9.96)	36.82	(36.74–37.46)
	Red Sea	–		8.61	(7.24–9.98)	38.25	(37.42–39.07)
rhizome elongation rate (cm day^{-1})	Greece	27.24	(26.22–28.23)	14.94	(11.95–16.21)	39.53	(36.22–44.50)
	Cyprus	28.85	(28.22–29.42)	17.60	(16.56–18.34)	40.09	(38.09–42.27)
	Red Sea	30.47	(29.74–31.7)	20.24	(19.50–21.05)	40.69	(38.42–43.89)
shoot recruitment rate (day^{-1})	Greece	27.96	(27.13–28.51)	19.62	(17.77–20.57	36.29	(33.68–39.24)
	Cyprus	30.31	(29.74–30.83)	18.98	(17.78–19.74)	41.63	(39.73–43.87)
	Red Sea	30.06	(29.06–30.94)	17.84	(16.05–19.08)	42.27	(39.03–45.82)
net population growth rate (day^{-1})	Greece	19.64	(17.56–21.09)	N/A	N/A	N/A	N/A
	Cyprus	24.76	(22.61–27.84)	N/A	N/A	N/A	N/A
	Red Sea	30.01	(25.85–N/A)	N/A	N/A	N/A	N/A
GPP (mmol O_2 day^{-1} gDW^{-1})	Greece	24.89	(23.58–26.21)	11.93	(9.98–13.35)	37.84	(33.80–42.47)
	Cyprus	26.81	(25.70–28.14)	15.88	(14.42–19.96)	37.73	(31.43–41.85)
	Red Sea	29.92	(29.10–30.69)	16.28	(14.70–17.48)	43.55	(40.71–46.67)
R (mmol O_2 day^{-1} gDW^{-1})	Greece	26.90	(24.92–28.88)	9.00	(2.68–13.08)	44.79	(36.75–55.07)
	Cyprus	29.29	(28.41–30.18)	12.91	(10.72–14.48)	45.66	(42.33–49.63)
	Red Sea	31.06	(29.4–33.18)	14.44	(7.38–18.21)	47.67	(40.58–58.97)
NP (mmol O_2 day^{-1} gDW^{-1})	Greece	13.16	(N/A–20.95)	N/A	N/A	N/A	N/A
	Cyprus	18.36	(15.41–20.10)	N/A	N/A	N/A	N/A
	Red Sea	25.93	(23.91–28.38)	N/A	N/A	N/A	N/A

population of the Red Sea (figure 2*c*, table 1). The optimum temperature for net population growth of Greece populations differed significantly from those of the populations in Cyprus and the Red Sea (table 1). Statistical differences of the minimum and maximum temperature parameters between populations could not be tested due to high uncertainty of the Gaussian equation leading to undefined CIs, expressed as N/A (table 1).

H. stipulacea metabolic rates (GPP and R) varied along bell-shaped curves (figure 2*d*,*e*), while NP curves were rather flat along with temperature (figure 2*f*). Thermal performance curves for GPP and NP were different between all three populations, whereas those for R only differed between Greece population and the others (electronic supplementary material, table S3). *H. stipulacea* mostly fixed CO_2 (GPP) within a thermal range spanning from 11 to 34°C in Greece, from 11 to 38°C in Cyprus and from 17 to 38°C in the Red Sea (table 1). The minimum and optimum temperatures for GPP and R were significantly lower for the exotic population of Greece than for the Red Sea population, (table 1). The balance between GPP and R of *H. stipulacea* population in Greece was negative (i.e. NP < 0) across the tested thermal range with the exception for specimens at 25 and 26.5°C (figure 2*f*) and positive (i.e. autotrophic) from 14 to 29°C for Cyprus. On the contrary, the native *H. stipulacea* population was autotrophic within the thermal range from 20 to 38°C; otherwise, metabolism was heterotrophic (figure 2*f*). Significantly different optimum temperatures for NP were detected between the population of Greece and the Red Sea (table 1). The minimum and maximum temperature parameters among populations for NP could not be resolved due to the poor fit of the Gaussian curve, which led to undefined CIs, expressed as N/A (table 1).

In all studied populations the lower lethal temperature exceeded minimum annual SST, resulting in a lower TSM for minimum thermal limits for the survival of approximately 7°C in exotic populations and approximately 13°C in the native one. Lower sublethal thermal limits (growth and metabolism) in the native population were lower than the minimum SST observed, whereas in the exotic locations they were close, but above, the minimum SST (figure 3). Optimum temperatures for growth and metabolism of exotic populations were close to the maximum SST they were exposed to in the ecosystems sampled. Conversely, optimum temperatures for growth and metabolic rates of native populations were lower than the maximum SST they experience. The upper lethal and sublethal limits of all studied populations exceeded maximum SST at the locations where the plants grew. The current upper TSM *H. stipulacea* populations ranged from 2.95°C in the Red Sea to 7.05°C in Cyprus and 8.57°C in Greece.

The mean shoot mass of the experimental specimens was 0.004 ± 0.0004 g DW, yielding an estimated clonal generation time (using equation (2.8)) for *H. stipulacea* of about 197 days (approx. 0.54 year). Considering that the first *H. stipulacea* meadow observed in the Mediterranean dates back to the

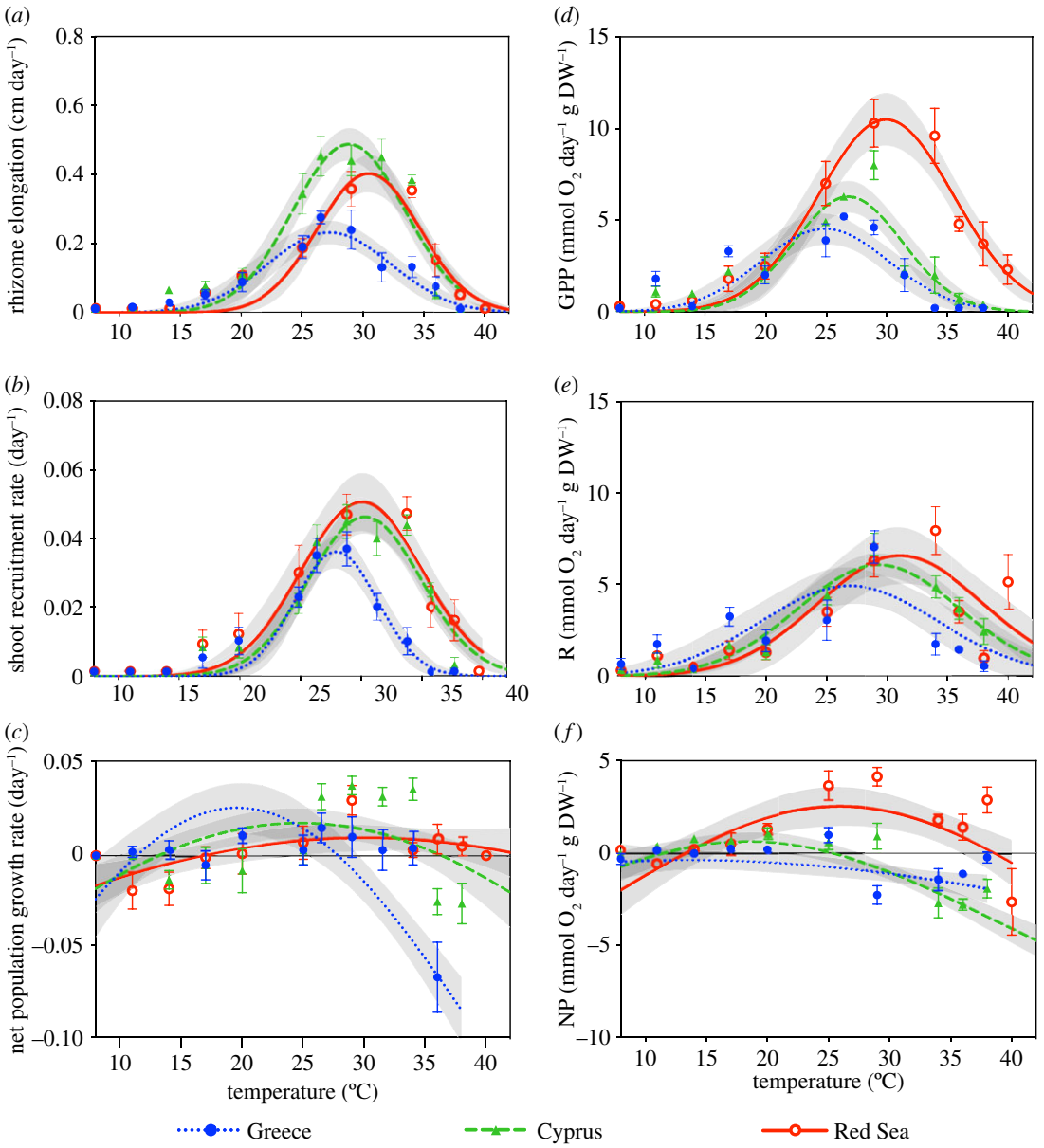

Figure 2. Thermal performance curves of (*a*) rhizome elongation rate, (*b*) shoot recruitment rate, (*c*) net populations growth rate, (*d*) gross primary production, (*e*) respiration and (*f*) net production of all three *H. stipulacea* populations (Greece: dotted lines and filled circles in blue; Cyprus: dashed lines and triangles in green; the Red Sea: solid lines and empty circles in red) exposed to a wide temperature range (8–40°C) fitted with a Gaussian equation. Circles represent the average treatment value and error bars denote standard errors (SEs). Shaded areas represent the 95% CI of each thermal performance curve. (Online version in colour.)

year 1923 [39], this species could have occurred in the Mediterranean for about 175 generations.

4. Discussion

Our results indicate a remarkable thermal range for *H. stipulacea*, spanning from 8 to 38°C for survival, well above the thermal range experienced *in situ* (14.96–27.67°C in Greece, 15.83–29.77°C in Cyprus and 21.82–35.30°C in the Red Sea). This led to large TSM between the *in situ* lower temperature and the thermal limits of both exotic and native *H. stipulacea* populations, although TSM were narrower for the lower thermal limit in exotic populations, and only approximately 3°C for the upper thermal limit for the Red Sea population. Hence, *H. stipulacea* is able to perform over a wide range of temperatures. The very low thermal limit reported for the Red Sea population (8.61°C for survival compared to a minimum *in situ* temperature of 21.82°C) is remarkable and certainly key to enable this species to grow and spread in the Mediterranean, where minimum seawater temperatures are much lower than those in the Red Sea. The broad thermal breath of *H. stipulacea* probably contributes to the capacity of the species to expand outside its native biogeographical range [37], as this species has been reported in the Mediterranean [43,44] and Caribbean Sea [40].

H. stipulacea showed lower thermal limits and optimal temperatures in its exotic range than in its native range, providing evidence for a thermal niche shift to the lower seawater temperatures in the Mediterranean Sea. These differences were not observed at a lethal level (i.e. survival at LT50), but at sublethal level, when comparing the thermal performance curve obtained for growth and metabolic rates between exotic and native populations. Similarly, lionfish from Indonesia showed a lethal minimum at 10.0°C and a feeding cessation (sublethal) at 16.1°C, as thermal limits can vary across functional levels due to different effects of temperature on vital functions [51].

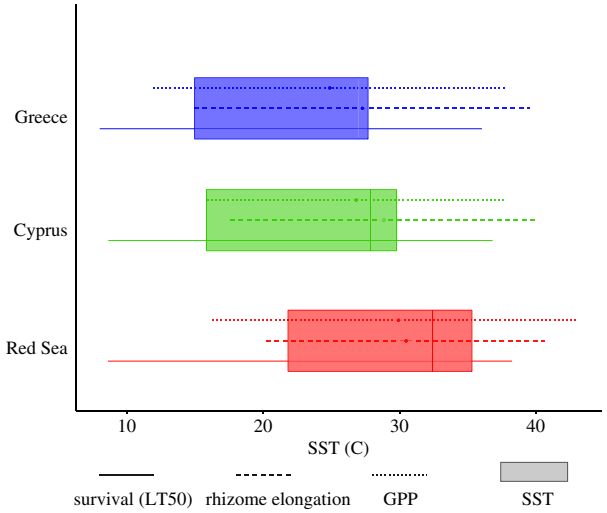

Figure 3. SST, minimum and maximum temperatures for survival (solid line), rhizome elongation growth rate (dashed line) and GPP (dotted line) of all three populations of *H. stipulacea*. Dots represent optimum temperatures and vertical lines mean summer SST. (Online version in colour.)

The lower lethal temperatures of *H. stipulacea* were similar in the native and exotic populations, demonstrating that this plant exhibits cold tolerance in its exotic range and is capable to resist thermal conditions that are far beyond those occurring at the ancestral range (minimum seawater temperature in the central Red Sea of 21.82°C). Hence, the survivorship of this tropical seagrass species is not limited by the low winter temperatures it experiences in the Mediterranean Sea, in agreement with previous observations [10]. However, the minimum temperature required for rhizome elongation and shoot recruitment of *H. stipulacea* in exotic populations is 14°C and 17°C, respectively, a winter temperature that in the Mediterranean Sea is currently restricted to the Eastern basin. The fact that minimum seawater temperatures in the Western Mediterranean (from 10.1 to 14.1°C; [52]) are below the lower thermal limit for *H. stipulacea* shoot recruitment can explain why this species has not been reported yet in the Western Mediterranean. While the species could survive at such temperatures, the fact that it cannot elongate the rhizome and produce new shoots implies that it is unable to grow and disperse [46]. In addition, any fragment that currently might reach the Western Mediterranean would die after weeks or months (i.e. based on *H. stipulacea* shoot life span) given its small size [50].

The optimum temperatures measured at the cellular (metabolic rates) and organismal level (growth rates) of the exotic populations met the maximum SST at Cyprus and Greece. In contrast, the optimum temperatures of the native population were much lower than the maximum SST of the Red Sea. This indicates that current Mediterranean temperatures allow *H. stipulacea* to display the full performance of the seagrass. As the Eastern Mediterranean has been warming at a fast rate (0.65°C decade^{-1}; [53]), our results suggest that the performance of exotic *H. stipulacea* must have increased since its arrival at the Mediterranean about one century ago, and that it will continue to do so with projected future warming [54].

The upper thermal tolerance thresholds of *H. stipulacea* varied between exotic and native populations, although the differences were not significant. Moreover, the experimental upper thermal limits observed here exceeded those required to perform in their habitats, which is a common trait among exotic species and might influence their capacity to establish in a warming ocean [55].

The results presented here provide evidence for a shift of the thermal niche of exotic *H. stipulacea* populations, suggesting a rapid adaptation towards the lower thermal regimes in the Eastern Mediterranean. Although no molecular evidence was assessed, as this would have been out of the scope of this study, rapid adaptation could have occurred either through epigenetic alteration of the seagrass genome, variation in the associated microbiome, or due to transposable elements [56]. Changes in thermal tolerance from the native to the exotic range occurred (a) in the lower sublethal thermal limit of the species in response to the local minimum SST and (b) in the optimum temperature for plant performance in response to the local maximum SST. Hence, these results reveal that the conservatism often assumed in the temperature niche within species [6,7] does not apply to *H. stipulacea* due to its capacity to cope with colder thermal conditions by a downward displacement of their thermal niche, rather than broadening its amplitude, which is already exceptionally broad. There is previous evidence of marine species supporting higher cold tolerance in the exotic than in the native range: exotic populations of the marine macroalgae *Gracilaria vermiculophylla* [57] and the green crab *Carcinus maenas* [55] show higher survivorship compared to native populations after being exposed to extreme cold shock experiments (the seaweed was exposed to 20°C and the crab to 6°C). Those approaches, however, could not identify how many degrees the thermal range shifted. On the contrary, our results demonstrate for the first time a downward shift of 2°C of the minimum and optimum temperature towards lower ranges to match the thermal conditions found in their exotic range.

We estimated that *H. stipulacea* is present in the Mediterranean since approximately 175 clonal generations, which indicates that it had enough time to shift its thermal performance curve. Within much less generations, populations from the exotic plant *Hypericum canariense* (approx. 25 generations) in Hawaii, San Diego and California differed in growth rates, size and flowering from the native population in the Canary Islands [58]. Also, the invasive cane toad (*Rhinella marina*) in Australia showed morphological changes after 50 generations [27,28] (electronic supplementary material, table S4).

The experiments conducted here tested the thermal niche of two exotic and one native population of *H. stipulacea*. We cannot, therefore, rule out that other exotic and native populations may present a broader thermal range [6]. In particular, the native population was collected at 22°N, whereas populations further north in the Red Sea may be adapted to cooler temperatures. However, the lower thermal limit of the native population tested extended to temperatures 13°C below the minimum temperatures experienced in the environment and anywhere in the Red Sea, so that Red Sea populations are not under selective pressure to adapt to lower temperatures. A recent study [59] indicated that the levels of polyunsaturated fatty acid (PUFA) of *H. stipulacea* are more similar to seagrasses found in cooler regions, which could be an indication of tolerance of the species to cope with the colder Mediterranean waters.

Our results suggest that under global warming scenarios [60], minimum annual seawater temperatures in the

Mediterranean would enable *H. stipulacea* expansion to the Western Mediterranean basin by the year 2100 when minimum temperatures may have increased by 3°C and exceeded the minimum temperature required for shoot recruitment. Moreover, the increase of annual maximum seawater temperature projected along the current century for the Mediterranean Sea (0.25°C decade^{-1} in the Western and 0.65°C decade^{-1} in the Eastern Mediterranean; [53]) would lead to improve *H. stipulacea* performance (growth, recruitment and metabolic rates) in this region. Global atmospheric ocean circulation models projected for the Western Mediterranean (Balearic Islands) indicate that, under a moderate greenhouse gas emissions scenario, SST will increase by 3.4 ± 1.2°C by the end of this century [60]. Conversely, *H. stipulacea* has changed its thermal tolerance by decreasing its minimum sublethal and optimum temperatures approximately 2°C since its establishment in the Mediterranean in about 95 years (175 clonal generations).

At present, there is little evidence that *H. stipulacea* is outcompeting the native Mediterranean seagrasses [41,61]. A recent study based on ecological niche modelling showed that, under the most severe climate change scenario, the distribution of *Posidonia oceanica* could be completely lost by 2100 and the meadows of *C. nodosa* could be reduced by between 20.8 and 46.5% [62]. As *H. stipulacea* would be able to perform under these climate change scenarios in the future Mediterranean Sea, a shift in the dominant flora could be implied, with the native warm tolerant species *C. nodosa* [63] and the exotic *H. stipulacea* occupying the empty space left by *P. oceanica* or even outcompeting the *Cymodocea* native species.

In summary, our results show evidence for a thermal niche shift of *H. stipulacea* to the colder thermal regime of the Mediterranean Sea this species colonized about one century ago. Differences were found in the lower sublethal limit and in the optimum temperature due to the exposure to the colder Mediterranean seawaters. These results together with the trajectories of increase of annual maximum seawater temperature projected along the current century for the Mediterranean Sea reveal that the plant will expand and improve its performance. Considering this, *H. stipulacea* is likely to increase its ecological role in the future, warmer Mediterranean Sea, where the endemic dominant *P. oceanica* may become ecologically extinct [62]. Whereas most experimental research on the thermal tolerance of marine species focuses on the capacities of species to cope with warmer waters [30] the capacity of exotic species, which tend to colonize environments colder than their native ranges [6], to cope with cooler regimes, such as demonstrated here for *H. stipulacea*, would determine their success and capacity to impact on competing native species and ecosystems.

Data accessibility. Data are available at the public data repository digital CSIC: http://hdl.handle.net/10261/200469 [64].

Authors' contributions. C.M.D., N.M., I.E.H., and M.W. conceived the experimental design. I.E.H., I.S., E.T.A., A.A. and M.W. collected plant material for experiments. M.W. conducted the experiments. A.A. and M.W. analysed the data. C.M.D., N.M., I.E.H., S.A. and M.W. interpreted the results. All authors contributed to the writing of the manuscript and gave final approval for publication.

Competing interests. We declare we have no competing interests.

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