

Warming intensifies the interaction between the temperate seagrass *Posidonia oceanica* and its dominant fish herbivore *Sarpa salpa*

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ABSTRACT

Apart from directly influencing individual life histories of species, climate change is altering key biotic interactions as well, causing community processes to unravel. With rising temperatures, disruptions to producer-consumer relationships can have major knock-on effects, particularly when the producer is a habitat-forming species. We studied how sea surface temperature (SST) modifies multiple pathways influencing the interaction between the foundational seagrass species, *Posidonia oceanica*, and its main consumer, the fish *Sarpa salpa* in the Mediterranean Sea. We used a combination of a field-based temperature gradient approaches and experimental manipulations to assess the effect of temperature on seagrass performance (growth) and fish early life history (larval development) as well as on the interaction itself (seagrass palatability and fish foraging activity). Within the range of temperatures assessed, *S. salpa* larvae grew slightly faster at warmer conditions but maintained their settlement size, resulting in a relatively small reduction in pelagic larval duration (PLD) and potentially reducing dispersion. Under warmer conditions (>24 °C), *P. oceanica* reduced its growth rate considerably and seemed to display fewer deterring mechanisms as indicated by a disproportionate consumption in choice experiments. However, our field-based observations along the temperature gradient showed no change in fish foraging time, or in other aspects of feeding behaviour. As oceans warm, our results indicate that, while *S. salpa* may show little change in early life history, its preference towards *P. oceanica* might increase, which, together with reduced seagrass growth, could considerably intensify the strength of herbivory. It is unclear if *P. oceanica* meadows can sustain such an intensification, but it will clearly add to the raft of pressures this threatened ecosystem already faces from global and local environmental change.

1. Introduction

The climate crisis continues apace, and the world's terrestrial and marine ecosystems are undergoing radical transformations as their constituent species struggle to adapt to rapidly changing environmental conditions. The difficulty in determining how ecosystems will respond stems from the complexity of species interactions. How species interactions are modified by climate change will have a disproportionate

impact on community assembly patterns and key ecosystem functions (Araújo and Luoto, 2007; Memmott et al., 2007; Tylianakis et al., 2008). As environmental conditions change, and species optima shift in space, time and predictability, each species can respond either by moving, acclimating, adapting, or dying. Every individual response, however, will necessitate all interacting species to respond in turn, resulting in assemblage-wide shifts in the strength and direction of species interactions (Gilman et al., 2010). In the case of habitat-forming species,

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the outcome of changing interactions can have ecosystem-wide consequences.

Predicting these changes is not straightforward. Even the simplest two-species interaction is the result of multiple individual life history traits and processes (determining species distribution, abundance, developmental stage, growth rate, behaviour, etc.), each of which can be independently affected by changing environmental conditions. Whether interactions reduce, intensify or remain unaffected will therefore depend on how these responses act together. Studies of consumer-resource relationships, both in terrestrial and aquatic systems, suggest that they may strengthen at higher temperatures as consumption rates increase to keep up with changing consumer metabolic requirements (Bale et al., 2002; Hernán et al., 2017; O'Connor, 2009; O'Connor et al., 2011; Pagès et al., 2018; Poore et al., 2013; Post and Pedersen, 2008). If resource species are unable to offset higher demand by ramping up production or by any other mechanism, they could decline under increased consumer pressure. The direction and strength of the interaction will also be influenced by shifting geographical patterns. Changes in temperature, seasonality, oceanic currents, etc. are affecting species' dispersal abilities and modifying distributional patterns (Cure et al., 2018). Unless both species show similar geographical shifts, the strength of the interaction is likely to vary considerably across space as the centres of species' ranges change in relation to each other, or as populations become more or less isolated. Interactions could even dissipate at range edges when one species moves away, or find novel configurations as new species take over as interactors.

Consumer-resource relationships are particularly strong in marine systems (Bakker et al., 2016; Cyr and Pace, 1993). Variations in water temperature has direct effects on survivorship, growth, reproduction and recruitment of both producers and consumers (Andrews et al., 2014; Bignami et al., 2017; Höffle et al., 2011; Howe and Marshall, 2002; Jordà et al., 2012; Poloczanska et al., 2013; Pörtner and Knust, 2007). In seagrasses, rising temperatures can affect plant performance by directly influencing photosynthesis/respiration ratios (Greve et al., 2003; Lee et al., 2007; Olsen et al., 2012; Ontoria et al., 2019b) or indirectly, by increasing sulphide concentration, which enhances plant mortality and reduces shoot growth (Höffle et al., 2011; Nejrup and Pedersen, 2008; Olsen et al., 2012). Early life-history stages are most affected by heating, resulting in very low recruitment and high rates of mortality (Andrews et al., 2014; Hernán et al., 2017; Marbà and Duarte, 2010; Olsen et al., 2012). Temperature can also influence grazers performance, such as echinoderms or fish (Deutsch et al., 2015; O'Connor, 2009; Pagès et al., 2018; Vergés et al., 2014; Yee and Murray, 2004). Additionally, temperature typically influences larval metabolism much more than juveniles or adults, given their narrower limits of thermal tolerance (Bignami et al., 2017; Pörtner and Farrell, 2008; Pörtner and Peck, 2010). Given the importance of larval developmental to the dispersal of marine species, this could have major consequences for distributions and connectivity (Leis et al., 2013). However, whether the effect of temperature on growth is positive or negative differs between studies depending on the species, life stage, region and temperature regime (Green and Fisher, 2004; McLeod et al., 2015; Poloczanska et al., 2013; Sponaugle et al., 2006).

Beyond influencing each individual species, temperature can directly influence consumer-resource relationship (Pagès et al., 2018). Marine herbivores may increase foraging with temperature as respiratory and other metabolic costs mount (Gutow et al., 2016a; West and Post, 2016). If ingestion rates remain unchanged (Morelissen and Harley, 2007) or are unable to match increases in metabolism, herbivores can suffer significant energetic shortfalls due to intake inefficiency (Iles, 2014; Lemoine and Burkepile, 2012). Herbivore feeding preferences may also be modified directly if preferred resources become scarce under warmer conditions, or indirectly if the palatability of the resource changes. Herbivore preferences in seagrasses are strongly linked to leaf biomechanical and biochemical properties, including defences produced by the plant (De Los Santos et al., 2012; Jiménez-Ramos et al., 2018; LaTina

Steele and Valentine, 2015; Vergés et al., 2007). However, the effect of heating on the production of defences is poorly understood in seagrasses (Jiménez-Ramos et al., 2017). Although there is some evidence of temperature influencing C:N ratio and the content of fibre tissue in leaves (Hernán et al., 2017), it is unclear if seagrass produce phenolic compounds in response to temperature. Finally, marine plants may also have some ability to alleviate herbivore pressure with compensatory growth. Depending on the intensity and duration of herbivory, this strategy will be limited by the amount of carbon the plants have stored (Sanmartí et al., 2014; Vergés et al., 2008).

Because temperature can modify so many elements of the consumer-resource interaction, it is important to unpack each of them before determining how it is likely to change. We explore how warming seas are likely to influence plant-herbivore interactions in the Mediterranean. The Mediterranean is predicted to experience major temperature increases over the next century calling into question the integrity of its dominant benthic communities (Change, 2014). The seagrass *Posidonia oceanica* is one of its principal primary producers. The sea urchin *Paracentrotus lividus* and the fish *Sarpa salpa* are the main consumers of *P. oceanica* (Prado et al., 2007). Earlier studies suggest that herbivory by the sea urchin *P. lividus* on *P. oceanica* is unlikely to be affected by rising temperatures (Pagès et al., 2018). However, little is known about how the relationship between *P. oceanica* and its fish herbivore will change with warming. We explore how warming modifies the interaction between *P. oceanica* and the fish *Sarpa salpa*. We hypothesise that temperature could modify this plant-herbivore interaction by influencing (Fig. 1): (i) plant performance (higher temperatures will decrease plant growth), (ii) early life history of the fish (higher temperatures will enhance larval growth), (iii) herbivore foraging activity (higher temperatures will increase fish feeding rate) and (iv) herbivore preference (higher temperature will increase plant palatability). Most earlier studies used controlled ex-situ setups that may not translate into real world ecosystems and larger spatial scales (see Gutow et al., 2016a; Hernán et al., 2017; Pagès et al., 2018). We complemented our laboratory experiments with temperature-gradient based field studies for a

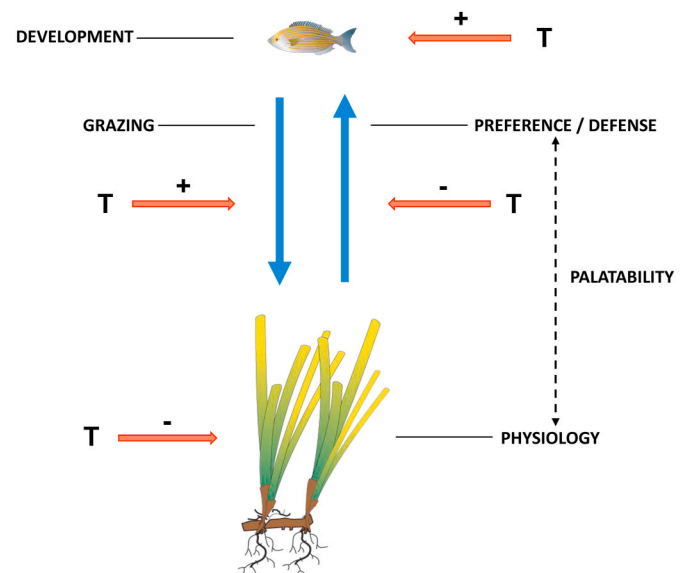


Fig. 1. The influence of increased temperature (T) on the interaction between *Posidonia oceanica* and *Sarpa salpa*. The figure shows the pathways that characterise this interaction and the hypothesised responses to temperature. The signs indicate a hypothesised strengthening (+) or weakening (-) of the pathway with increasing temperatures. From background literature, we assume that high temperature is likely to enhance both larval development and grazing activity of the herbivore. In contrast, plant performance is likely to decrease. These responses together should result in an overall intensification of grazing pressure on seagrass.

more realistic evaluation of how plant-herbivore interactions will likely evolve in a warming Mediterranean.

2. Methods

2.1. Study species and study design

The seagrass *Posidonia oceanica* is an iconic, long-lived foundational species in the Mediterranean Sea, forming vast meadows that provide a range of valuable ecosystem services (Francour, 1997; Lavery et al., 2013; Marbà et al., 1996). *P. oceanica* habitats are retreating at an alarming rate; over the last century, the Western Mediterranean has witnessed a loss of between 5 and 20% of its cover (Boudouresque et al., 2009; de los Santos et al., 2019). The seagrass has high thermal sensitivity (García et al., 2013; Jordà et al., 2012; Tomasello et al., 2007); it grows slower as temperature increases (Olsen et al., 2012; Ontoria et al., 2019a; Pagès et al., 2018) and its shoot mortality increases by 2% per year for each additional degree of maximum temperature (Marbà and Duarte, 2010).

S. salpa is a demersal marine fish, commonly found in shallow Mediterranean waters and in the Eastern Atlantic (Braum, 1987; Hureau et al., 1984). It typically schools in groups of up to hundreds of individuals (Raventós et al., 2009). Seagrass consumption by *S. salpa* constitutes about 75% of the total herbivory on *P. oceanica* (Havelange et al., 1997; Prado et al., 2007); in some cases *S. salpa* can defoliate >40% of the seagrass' annual production (Prado et al., 2007). Although *P. oceanica* is capable of displaying a range of compensatory mechanisms and chemical defences when subjected to herbivory (Ruocco et al., 2018; Vergés et al., 2008), it is unclear how these mechanisms will play out as the oceans warm.

We evaluated the effects of warming on different components of the plant-animal interaction between *S. salpa* and *P. oceanica* (see Fig. 1) using a set of complementary field-based approaches including behavioural observations and controlled laboratory and field experiments. We chose these methods together with carefully controlled laboratory techniques to avoid the many problems of acclimation, scale and representativeness associated with studies that rely solely on laboratory techniques (Bailey, 2018; Tran et al., 2016; Wernberg et al., 2012). More realistic manipulative experiments are prohibitive and unfeasible particularly with species that have large home ranges (Pagès et al., 2013) and travel in large shoals (Buñuel et al., 2020). Carefully designed field studies can more closely reflect the likely trajectories of complex ecological interactions when exposed to change. We made use of a natural gradient in sea water temperature along the east coast of the Iberian Peninsula (~800 km), supplemented with a laboratory experiment. This gradient spans a maximum difference of up to 4 °C during the summer (average range of temperatures: from 18.5 ± 1.36 °C to 21 ± 1.8 °C in autumn 2017 and 22.5 ± 0.9 °C to 25 ± 0.9 °C in summer 2016), which represents the maximum amount of change the Mediterranean is likely to experience at the end of this century as a result of climate change (Change, 2014). This makes for an ideal space-for-time substitution, with the simplifying but useful assumption that spatial and temporal variation are equivalent. While less precise in determining the exact magnitude of change, it can be a powerful means of evaluating qualitative trends or to generate more accurate hypotheses (Elmendorf et al., 2015; Pickett, 1989). Because the sampled locations and seasons did not always coincide for all studies (Fig. 2a and b), we grouped locations in high temperature sites and low temperature sites to make them comparable.

2.2. Early life history of *S. salpa*

We used otolith analysis to assess how temperature influences the physiology and dispersal capability of *S. salpa* in the earliest development stages. We sampled 5 locations of the Mediterranean Spanish coast from October–November 2017 (Fig. 2a), grouping them in two

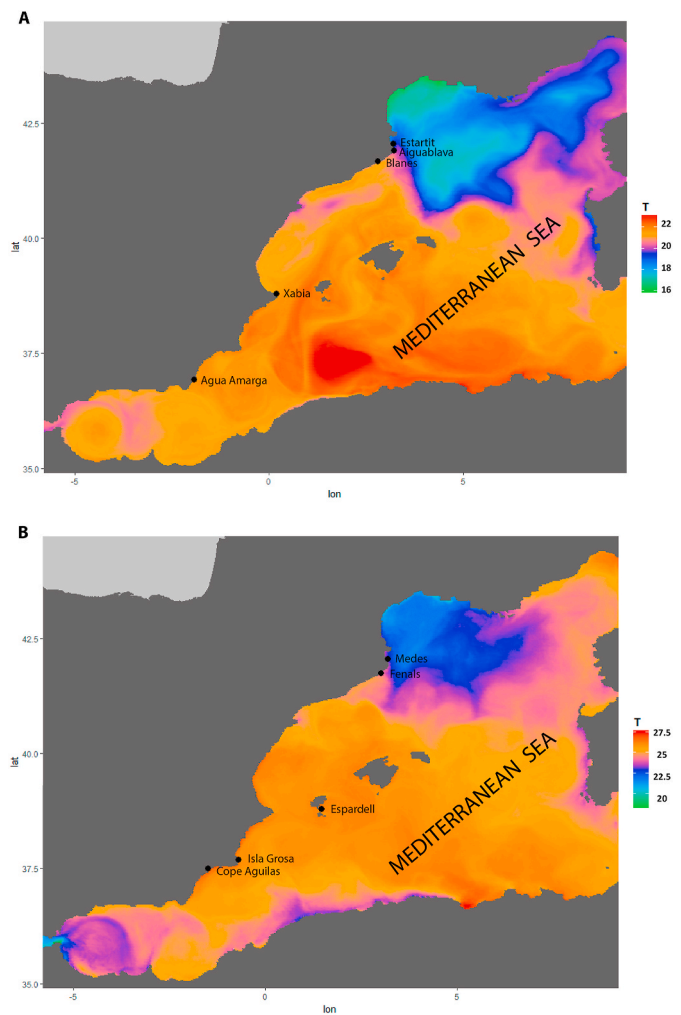


Fig. 2. Average sea surface temperatures at sampling sites during field surveys for a) larval development (across the Spanish coast in October–November 2017), and b) foraging activity recording and preference experiments (conducted in Medes and Isla Grosa in July–August 2016). Temperature data obtained from the Western Mediterranean OPERational forecasting system (WMOP) (Juza et al., 2016; Mourre et al., 2018), developed at the Balearic Islands Coastal Observing and Forecasting System (www.socib.es).

temperature regimes: low temperature locations (18.5 °C average temperature during sampling period) - Estartit and Aiguablava - and high temperature locations (21 °C average temperature during sampling period) - Blanes, Xàbia and Agua Amarga. Note that temperature groups do not follow a strict latitudinal pattern. Blanes is grouped with southern locations due to a warm current that influenced this location during the sampling period. We collected 112 individuals along the gradient on SCUBA using hand nets at depths from 0 to 5 m. We then removed the otoliths and preserved them in 96% ethanol.

Following Raventós and Macpherson (2001), we extracted and mounted otoliths (lapilli) on microscope slides using thermoplastic glue (Crystalbond 509). To expose daily increments, we polished mounted otolith using two different grained sandpapers (3 µm and 1 µm Imperial lapping film, 3M) to obtain a section containing the nucleus and the first growth ring. We read otolith growth increments using a high-powered microscope with transmitted light (AxioPlan, Zeiss) connected to a ProgRes C10 camera (Jenoptic) and an image analysis system (Raventós and Macpherson, 2005). Otolith extraction and processing was performed at CEAB's Otolith Research Lab (www.ceab.csic.es/en/otolith-research-lab-2).

Otolith settlement marks in this species were always very clear

(Raventós and Macpherson, 2001). We counted growth increments of the otoliths from the centre to the edge along the longest radius when possible. If not, we used other axes for the counts, where increments were clearly visible. We determined the length of the pelagic larval duration (PLD) for each individual by counting the number of daily growth increments between the core and the settlement mark, and determined the age by counting the total number from core to margin, obtaining the day of birth. We also recorded size-at-settlement (along the longest axis of the otolith), and growth rate during pelagic phase (larval growth, GRPLD) before settlement. We calculated pre-settlement growth by dividing size-at-settlement by PLD. To minimize errors, we repeated all measurements three times.

2.3. Plant performance (growth)

We collected *P. oceanica* fragments (rhizomes) each with at least four shoots, in dense and healthy meadows at 5m depth in Isla Grosa (South Eastern coast of Spain, 37° 43.7' N, 0° 42.75' W) in April 2016. Fragments were transported to the laboratory, planted in pots and transferred to tanks in the mesocosm facilities of the Spanish Institute of Oceanography (IEO, Oceanographic Centre of Murcia, Spain). The experimental system consisted of 12 tanks (500L) with independent seawater circulation systems and control of temperature and light (see details in Marín-Guirao et al., 2013), with ca. 50 shoots per tank. Plants were then acclimated in the mesocosms for 3 weeks at conditions similar to the field at the time of sampling (temperature: 20 °C, salinity: 37.5 psu, and light regime of 250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, with a 12:12 h photoperiod). After the acclimation period, three tanks were maintained at 20 °C, while temperatures in the other nine tanks were progressively increased (2 °C day⁻¹) to reach three target temperatures (24 °C, 28 °C, and 32 °C, three replicates per temperature). These four temperatures were chosen to represent the (i) temperature of the study site at the time of collection (20 °C); (ii) the present day monthly averaged summer maximum (24 °C); (iii) an anomalously high temperature, occasionally reached in this region during heat waves (28 °C, pers. obs.); and (iv) an extremely high temperature, to reflect extreme heat wave conditions predicted for the coming decades (32 °C(Change, 2014)). The exposure period lasted for five weeks, after which plant growth was measured using a punching technique (Zieman 1974, adapted to this species (Alcoverro et al., 1995). At the start of the warming period, we pierced the leaves of 12 shoots per tank (chosen at random) with a needle at the height of the ligule. At the end of the experiment, we measured leaf growth (expressed in $\text{cm}^2 \cdot \text{shoot}^{-1} \cdot \text{day}^{-1}$) by tracking the displacement of the piercing scar. The experimental unit was the individual tank (n = 3 per experimental condition).

2.4. Herbivore foraging activity

To assess the effect of temperature on individual and shoal feeding behaviour, we filmed a total of 93 shoals feeding in *P. oceanica* seagrass

Table 1

Results of larval development, feeding activity and preference of *S. salpa* in relation to temperature. p-values correspond to those provided by each test. D.f = degrees of freedom, significant effects are in bold.

Response variable	Descriptive variable	Test	d. f	Statistic	p-Value
PLD	Temperature	Kruskal-Wallis	1	F = 4.1096	0.042
GRPLD (resid)	Temperature	Anova	1	F = 7.44	0.002
Settlement size	Temperature	Kruskal-Wallis	1	F = 2.151	0.142
Plant shoot growth	Temperature	Anova	3	F = 47.043	< 0.001
Shoal feeding time	Temperature	Kruskal-Wallis	1	$\chi^2 = 0.003$	0.952
Bite rate	Temperature	Kruskal-Wallis	1	$\chi^2 = 0.707$	0.401
Preference	Temperature	T-test	34	T = 2.155	0.038

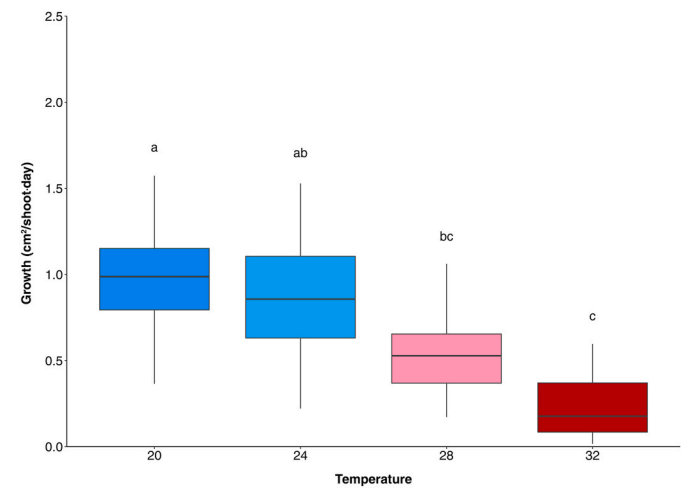


Fig. 4. *Posidonia oceanica* shoot growth at four different incubating temperatures. Letters indicate significant differences ($p < 0.05$) according to post-hoc tests.

meadows between 5 and 8 m in 5 locations of NW and W Spanish coast with different temperature regimes. All videos were shot during the summer of 2016, when *S. salpa* feeding activity is typically at its peak (Prado et al., 2007; Tomas et al., 2005). As before, we grouped locations into low temperature locations (Medes and Fenals: 22.5 °C average temperature during sampling period) and high temperature locations (Espardell, Isla Grosa and Cabo Cope: 25° average temperature during sampling period, Fig. 2b). We allowed 3–4 min for the fish to acclimatise to the diver's presence and then recorded the shoal with a hand-held underwater video camera, with an average of 7 min of footage per

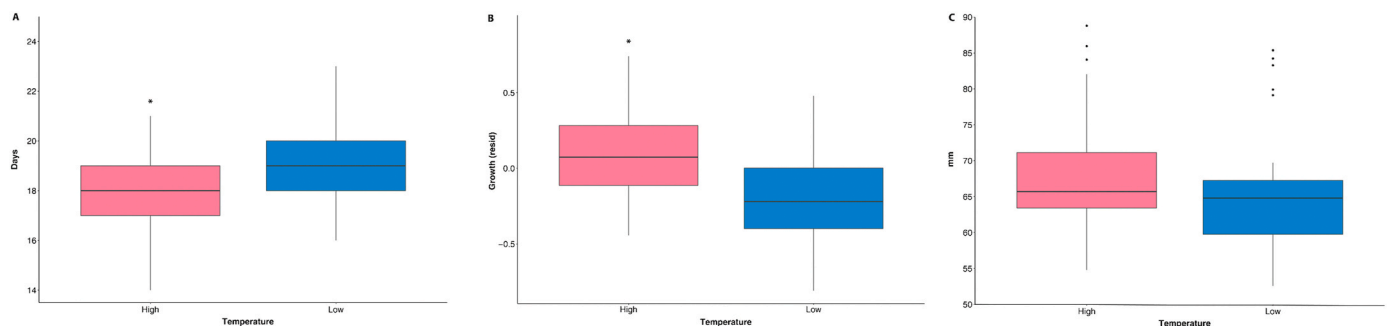


Fig. 3. a) Pelagic larval duration (PLD), b) growth rate during PLD (GRPLD) and c) settlement size of *Sarpa salpa* larvae at high and low temperature sites. Growth values were extracted from the residuals of the model $\text{GRPLD} \sim \text{PLD}$ due to their being collinear. Significant differences ($p < 0.05$) are marked with asterisks.

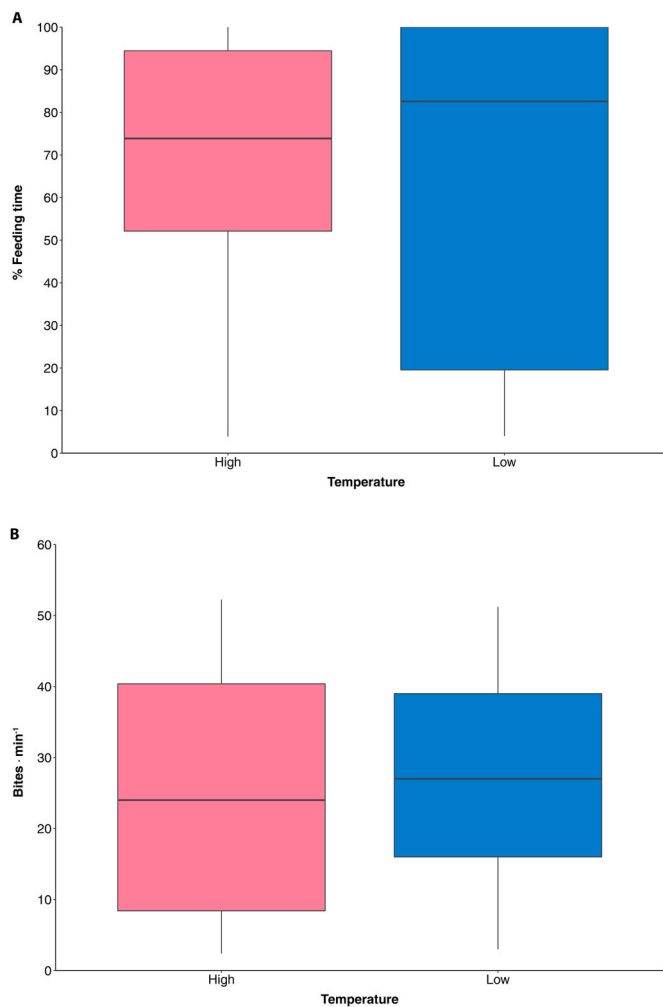


Fig. 5. Foraging behaviour of *Sarpa salpa* at high and low temperature sites. a) Percentage time the shoal spent feeding (shoal feeding time) and b) bite rate (number of bites per minute).

shoal (see Buñuel et al., 2020 for details). To assess shoal feeding behaviour, we noted the percentage of time each shoal spent grazing inside the meadow compared to swimming and hovering. For individual activity, we used three metrics: (i) bite rates per individual (number of bites per unit of time); (ii) bout duration per individual (i.e. time each fish spent between its first descent into the seagrass leaves to feed and its exit towards the water column); and (iii) number of bouts during the feeding time. We used different clips to assess bouts and bites, since the quality of the video needed to accurately estimate bite rate was considerably higher than that needed for determining if a fish was feeding within the meadow or not (bout duration and number of bouts). Therefore, for bite rate analysis, we selected clips of any duration, but where the mouthparts of individual fish could be clearly seen. Wherever possible, we followed a maximum of 5 individuals from each shoal to measure individual activity. We were less restrictive with the quality of the video for estimates of bout duration and number, but sought a minimum video length (i.e. 30 s) in order to standardise our assessment. We extracted an average value of bite rate for each location; this value was multiplied by number of bouts and bout duration for each replicate (i.e. total bite rate = average bite rate (location) × bout duration × number of bouts). This was done to get past the opportunistic nature of bite rate data, and to obtain an integrative estimate of bite rate in a given feeding time.

2.5. Herbivore preference

Finally, we conducted a field choice experiment to indirectly test if temperature modified the palatability of seagrasses. Fish could choose between plants previously incubated in the laboratory at warmer or cooler conditions to evaluate their preference, with the assumption that a preference would either signal lower plant defences (mechanical or chemical) or more nutritious plant tissues. This experiment was repeated twice in two different locations (each differing in their mean summer temperature): Medes (42°02'N, 3°21'E, NW Mediterranean, mean summer temperature = 22.5 °C) and Isla Grosa (37°43'N, 0°42'W, W Mediterranean, mean summer temperature = 25 °C). We collected shoots of *P. oceanica* at both sites, and half of the shoots from each location were incubated in cold water aquaria (22 °C) and the other half in warm water aquaria (30 °C) for one week. At the end of the week, plants were transplanted to each field site in pairs (plants incubated in warm and cool water placed next to each other) to evaluate fish preference. We measured the area of all treated shoots at the start of the experiment, ensuring that there were no herbivory marks on any of the selected shoots. We transplanted the shoots at the same locations they had been picked from, using 23 replicates (pairs) in Medes and 13 in the Isla Grosa. We collected the shoots after 5 days, checked them for signs of herbivory and measured the total area once again to evaluate consumption by herbivores.

2.6. Statistical analysis

The effect of heating on pelagic larval duration (PLD) was tested using a Kruskal-Wallis test, due to the non-normal distribution of data, assessing the difference in PLD between the high and low temperature sites. Growth during PLD (GRPLD) was tested using a linear mixed effects model, where temperature was included as fixed factor (2 levels: low and high) and location as a random factor (5 levels). Given the strong collinearity we found between GRPLD and PLD (the longer the PLD, the lower the GRPLD, $p < 0.05$), we used the residuals of the linear model of GRPLD vs. PLD as the response variable instead of GRPLD. In this way, we could test the effect of warming on larval growth regardless of the amount of time the larvae spent in the water column. Finally, we tested for differences in settlement size between temperature regimes using a Kruskal-Wallis test. Normality and homogeneity of variance were checked and fulfilled for linear models. We set the significance level at $p = 0.05$.

To assess the influence of warming on seagrass performance, we analysed the effect of the predictor variable 'temperature' coded as a fixed factor (4 levels: 20, 24, 28 and 32 °C) on the response variable 'plant shoot growth' with linear models, using the average value for each aquarium. Assumptions of normality and homoscedasticity were visually checked and fulfilled.

To evaluate if water temperature (two levels: high and low) had any effect on the response variable 'time spent feeding by the shoal', we used a Kruskal-Wallis test since data were strongly non-normal. Similarly, Kruskal-Wallis tests were used to test the effect of water temperature on the response variable 'bite rate'.

Finally, a paired *t*-test was used in choice experiments to check for fish preference towards plants incubated in cold or warm water. Assumptions of normality and homoscedasticity were visually checked and fulfilled.

3. Results

3.1. Early life history of *S. salpa*

There was a small but significant negative relationship between pelagic larval duration (PLD) and temperature (Fig. 3a, Table 1). While larvae spent around 18 days in the water column at high temperature locations, individuals from colder regions spent one day more on

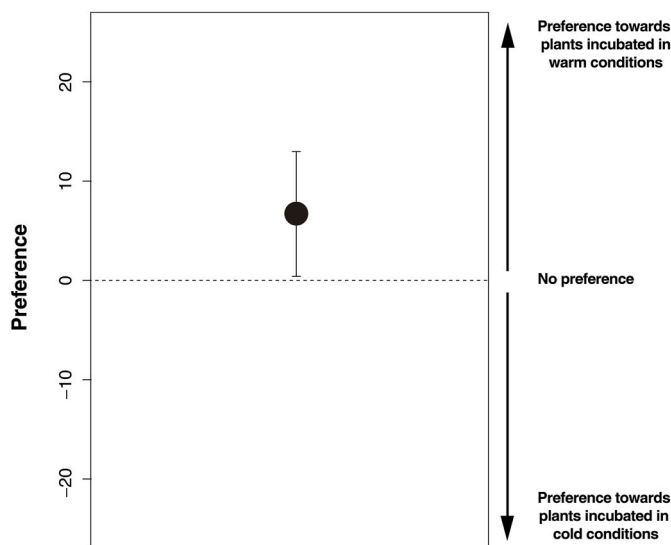


Fig. 6. Preference experiments: palatability of plants incubated at warm and cold conditions. Positive values indicate a preference for plants incubated at warm temperatures and the effect is significant when the confidence interval does not intercept 0.

average. Additionally, growth during PLD (GRPLD) was significantly higher at higher temperatures (using the residuals of GRPLD ~ PLD model to avoid collinearity, see Methods) (Fig. 3b, Table 1). Individuals in the high temperature regime showed 10% higher larval growth than individuals in colder locations. As a result, the size of settlers was similar in cold and warm regions (Fig. 3c).

3.2. Plant performance

P. oceanica growth rates were negatively affected by increasing temperatures (Fig. 4). Thus, while growth at control (20 °C) and 24 °C did not differ significantly, shoots subjected to 28 °C and 32 °C grew significantly less than controls (30% and 50% decrease relative to the controls, respectively, see Fig. 4 and Table 1).

3.3. Herbivore foraging activity

Sarpa salpa shoals observed both in warm and cold locations spent a similar amount of time feeding (68.5% and 63.3%, respectively (Fig. 5a, Table 1). At the individual level, fish bite rates were around 25 bites/min in both regions and were apparently unaffected by temperature (Fig. 5b).

3.4. Herbivore preference

Feeding choice experiments showed that fish had a significant preference for plants incubated at warmer water temperatures (30 °C) than those incubated at colder ones (22 °C) (Fig. 6, Table 1).

4. Discussion

The strength of species interactions can be modified by temperature even when one of the actors remains relatively unaffected. Our results indicate that under warmer conditions, the herbivorous fish *Sarpa salpa* experienced only a small reduction in its potential dispersal ability, growing faster to a largely invariant settlement size. In addition, *S. salpa* foraging activity was similar across temperature regimes. In contrast, its primary resource, the long-lived seagrass *Posidonia oceanica* showed distinct responses to warming, growing slower and becoming more palatable under high temperature conditions. As a result, the impact of

S. salpa herbivory on *P. oceanica* may intensify in the coming decades even if temperature does not influence its grazing activity.

The impacts of climate change on species distribution in both marine and terrestrial systems is likely to be large. As global temperatures rise, species ranges shift, expand or contract, and populations disappear particularly from the “warm” edges of their distributions. In species with a dual life history, where most dispersal takes place during the larval phase, temperature can disproportionately impact how far they can travel, by modifying larval growth and influencing settlement processes. Numerous studies on benthic fish are equivocal on the impacts of temperature on larval dispersal. Thus, while some species may increase their pelagic larval duration (PLD, a good indicator of how much time the larvae has to disperse in the plankton), others may show reduced PLDs at higher temperatures (Bignami et al., 2017; Green and Fisher, 2004; McLeod et al., 2015; Moyano et al., 2017). The herbivorous fish *S. salpa* shows a clear decrease in PLD, as it grows considerably faster (GRPLD) to a largely invariant settlement size at higher temperatures. However, all in all, a larva from the fish species *S. salpa* growing at warm temperatures sees its plankton stage reduced by only 5.5%. While several other factors including the current regime and post-settlement movement could certainly influence dispersal patterns; the average distance larvae can travel in the study area, in a day is around 12 km (Schunter et al., 2011), which is unlikely to be ecologically significant at the scale of the Mediterranean. Of course, should this pattern be maintained at even higher temperatures it could affect population connectivity at regional scales (Álvarez-Romero et al., 2018; Kleypas et al., 2016). Under these circumstances *S. salpa* ranges may contract and find themselves in more disconnected pockets across the coast (Pascual et al., 2017), which could then result in a lumpy distribution of herbivory on *P. oceanica* meadows. While the influence of temperature on PLD was small, faster growing larvae could potentially influence post-recruitment processes like growth and settlement survival. *A priori*, an increase of larval growth rate and a faster recruitment could increase fish survivorship, since the pelagic larval phase is the most vulnerable life stage (Moyano et al., 2017; Pörtner and Farrell, 2008), but there is no clear evidence that temperature influenced post-settlement growth and mortality for *S. salpa*. As it stands, *S. salpa* distributions and dispersal patterns are likely to be more affected by current regimes, post-settlement movement, or incidental bycatch than by temperature alone. In fact, the largest shoals of *S. salpa* currently occur in fishing-free locations like MPAs (Raventos et al., 2009), where they can have a disproportionate impact on *P. oceanica* meadows (Pagès et al., 2012; Planes et al., 2011; Prado et al., 2008).

As environments change, behavioural modifications are among the first to manifest as a phenotypic response (Sih et al., 2011). However, the foraging behaviour of *S. salpa* was quite unaffected by temperature regimes. Temperature is known to modify essential metabolic rates since it accelerates consumer physiology (Gutow et al., 2016a; Pörtner, 2012). The resulting energy costs require herbivores to compensate either by increasing feeding rates or by modifying their feeding choices. However, neither the time spent foraging nor the rate at which *S. salpa* foraged changed between temperature regimes. It is possible that the temperature regimes present within the Mediterranean are well within the wide optima of *S. salpa*. As a species, it occurs in geographies as far apart as Mozambique and the Atlantic coast of France (Braum, 1987; Hureau et al., 1984), and is likely to be metabolically adapted to a range of environmental conditions. Although it feeds largely on seagrass as an adult (Havelange et al., 1997), it can supplement its diet with macroalgae and detritus, showing a dietary plasticity that may allow it to easily adapt to changing metabolic conditions.

In sharp contrast to its main herbivore, the seagrass *P. oceanica* shows clear responses to increased temperature, which are likely to translate into functionally relevant and ecosystem-wide impacts over the coming century. *P. oceanica* is a temperate-adapted seagrass which does best in cooler waters. Our results show that in warmer waters, its growth rate reduced substantially, confirming trends that have been documented in

other field and mesocosm studies (Jordà et al., 2012; Marbà and Duarte, 2010; Ontoria et al., 2019a; Pagès et al., 2018). Similarly, its capacity to deter *S. salpa* appears to diminish in warmer environments. Our results show that under warmer conditions, *P. oceanica* defences are likely to reduce considerably, making the plant more palatable to fish herbivores. While observations on other macrophyte species suggest that incubations at high temperatures modify plant palatability to consumers (Mannino et al., 2016; O'Connor, 2009; Sudatti et al., 2011), the exact pathway by which temperature influences the acquisition of defences is poorly understood in seagrasses (Jiménez-Ramos et al., 2017). This is crucial, since the principal strategy *P. oceanica* employs to deal with herbivory is to resist offtake as much possible with a suite of structural and chemical defences (de los Santos et al., 2019; Vergés et al., 2008). Whether this strategy continues to remain effective in a hotter world is an open question, but we cannot discard other physiological factors involved in plant palatability. In fact, Hernán et al. (2017) also found higher herbivore preference for heated *P. oceanica* seedlings using the sea urchin *Paracentrotus lividus*. Although these results were not related to any alteration in leaf chemical content, higher temperatures did affect physical factors (e.g. leaf thickness) which could interfere with herbivore choice. It is important to clarify that our field experiment was set up specifically to test for fish preferences, with the assumption that fish would choose leaves with less defences or more nutritive conditions. We cannot directly infer that higher palatability would translate to higher consumption in the field, since several other factors (including the availability of other preferred resources and fish metabolic needs, among others) will influence absolute consumption. Whatever the mechanism, given the increased palatability and lower growth observed in this study, *P. oceanica* will certainly be more vulnerable to herbivory pressure in a warmer Mediterranean Sea.

The agents in our simple two-species interaction respond quite differently to temperature. However, the consequence of these separate responses still implies a significant intensification of the interaction as both species adjust to changes both in their environment as well as to each other. Even if fish distribution, survival and foraging behaviour remain unaffected at higher temperatures, the reduced ability of *P. oceanica* to offset herbivory and regrow after it is grazed, may lead to increased herbivory pressure. The consequences of such an increase for the ecosystem are unclear. For one, even this simple two-species interaction is governed by more processes than we have examined here. For instance, fish physiological responses and population dynamics could be strongly influenced as climate change modifies temperatures, seasonality, oceanographic currents etc. How these changes modify herbivory pressures by *S. salpa* is still uncertain. For another, in its evolutionary history, *P. oceanica* has likely had to deal with considerably greater herbivory in the past from other herbivorous fish and green turtles. It may well be able to resist herbivory with novel or old evolutionary strategies. That this species displays phenotypic plasticity in response to warming is evident: at higher temperatures, field and laboratory studies show that it is capable of shifting from primarily vegetative growth to the production of sexual propagules, sometimes using pseudoviviparous strategies for dispersal (Ballesteros et al., 2007; Ruiz et al., 2018).

That said, climate change is introducing several new interactors to this system. Herbivore preference can also change as a result of shifting distributions or because their alternate food sources are better able to produce defences. Under temperature stress, secondary metabolite production can be crucial in determining how strongly different macrophytes will be defended (Staehr and Wernberg, 2009; Sudatti et al., 2011). In addition, as the oceans heat, herbivorous fish from the Indo-Pacific are increasingly finding their way into the Mediterranean through the Suez Canal (Bianchi and Morri, 2003; Vergés et al., 2014). In the complex real world of ongoing climate change, *P. oceanica* will have to contend not merely with an intensification of its interaction with *S. salpa*, but with this growing assemblage of tropical herbivores as well. In many parts of the eastern Mediterranean, this novel guild of herbivores has already converted the simple two-species system into a

multi-herbivore assemblage, all grazing on the same seagrass resource. Between habitat loss due to local anthropogenic stressors and intensifying herbivory pressure, the iconic meadows of *P. oceanica* are undergoing major transformations. Whether they survive these combined impacts will depend on our ability to understand and manage these growing pressures.

Credit authorship contribution statement

Xavier Buñuel: Conceptualization, Formal analysis, Writing - original draft, Visualization. Teresa Alcoverro: Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Funding acquisition. Javier Romero: Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. Juan M. Ruiz: Methodology, Investigation, Writing - review & editing. Rohan Arthur: Conceptualization, Writing - review & editing, Visualization. Marta Perez: Conceptualization, Methodology, Investigation, Writing - review & editing. Yaiza Ontoria: Methodology, Formal analysis, Investigation, Writing - review & editing. Nuria Raventós: Methodology, Investigation, Writing - review & editing. Enrique Macpherson: Methodology, Investigation, Writing - review & editing. Hector Torrado: Methodology, Investigation, Writing - review & editing. Jordi F. Pagès: Conceptualization, Formal analysis, Writing - review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no competing financial or personal interests that could influence this article.

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