Marine Environmental Research 197 (2024) 106443

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Patch age alters seagrass response mechanisms to herbivory damage

Rocío Jiménez-Ramos [a,*], Luis G. Egea [a], Claudia J. Pérez-Estrada [a,b], Eduardo F. Balart [b], Juan J. Vergara [a], Fernando G. Brun [a]

[a] Department of Biology, Faculty of Marine and Environmental Sciences, Institute of Marine Research INMAR, University of Cadiz, International Campus of Excellence of the Sea (CEIMAR), 11510, Puerto Real, Cádiz, Spain

[b] Centro de Investigaciones Biológicas Del Noroeste, S.C., Av. Instituto Politécnico Nacional 195, Col. Playa Palo de Santa Rita Sur, 23096, La Paz, B.C.S., Mexico

ARTICLE INFO

Keywords:
Seagrass
Halodule wrightii
Ecosystem disturbance
Habitat loss and fragmentation
Food webs
Grazing
Plant–herbivore interactions
Ontogeny
Phenolic compounds
Gulf of California

ABSTRACT

Natural disturbances can produce a mosaic of seagrass patches of different ages, which may affect the response to herbivory. These pressures can have consequences for plant performance. To assess how seagrass patch age affects the response to herbivory, we simulated the effect of herbivory by clipping leaves of *Halodule wrightii* in patches of 2, 4 and 6 years. All clipped plants showed ability to compensate herbivory by increasing leaf growth rate (on average 4.5-fold). The oldest patches showed resistance response by increasing phenolic compounds (1.2-fold). Contrastingly, the concentration of phenolics decreased in the youngest patches (0.26-fold), although they had a similar leaf carbon content to controls. These results suggest that younger plants facing herbivory pressure reallocate their phenolic compounds towards primary metabolism. Results confirm the *H. wrightii* tolerance to herbivory damage and provides evidence of age-dependent compensatory responses, which may have consequences for seagrass colonization and growth in perturbed habitats.

1. Introduction

Herbivores can negatively affect the performance and survival of individual plants. The removal of plant tissues by grazers causes structural damage to the plant, along with nutrient and carbohydrate losses (Prado et al., 2011). Together, these injuries can reduce plant ability to acquire resources, grow, and reproduce (Marquis, 1992; Strauss and Zangrel, 2002), and also have subsequent consequences for the structure of the community (Maron and Crone, 2006; Allan and Crawley, 2011). Seagrasses are marine foundation species that form one of the most important ecological coastal habitats (Short et al., 2011). These globally distributed species are well-recognized for the ecosystem services they provide, including primary production (Egea et al., 2023a), provision of shelter and food for marine animals (McHenry et al., 2021; Jiménez-Ramos et al., 2021), shoreline protection (Christianen et al., 2013), and organic carbon sequestration (Macreadie et al., 2014; de los Santos et al., 2023). Despite growing recognition of the crucial ecological role of this ecosystem, seagrass meadows have nevertheless declined by 19.1% globally since 1880 (Dunic et al., 2021), primarily due to human activities and climate change (McKenzie et al., 2020). Although seagrasses were traditionally considered unpalatable based on early studies that reported low consumption rates by marine herbivores, seagrass beds actually withstand intense rates of herbivore activity (Heck and Valentine, 2006). The diverse community of seagrass's primary consumers leads to complex interactions among seagrasses and herbivores that ultimately shape meadow characteristics such as biomass, productivity, and species diversity. Intense herbivory can even trigger trophic cascade effects in seagrass meadows, with far-reaching consequences for their ecological functioning and provision of ecosystem services (Scott et al., 2018).

Marine ecologists have studied herbivory in seagrasses since the early 1970s, but there is increased interest in this field of research among researchers as drivers of climate change are altering the established plant-herbivore interactions (Hernán et al., 2017; Valentine and Heck, 2021). Most seagrasses species are found in tropical regions (Green and Short, 2004), where they increasingly face extreme climatological events including storms, hurricanes, and typhoons (Correia et al., 2022). Because these extreme events act inconsistently over wide areas, they can heterogeneously influence meadow development, leading to a mosaic of seagrass with different patch ages (i.e., patches with different times after establishment following a disturbance event; Naiman et al., 2005; Egea et al., 2023b). In addition, poleward expansion of herbivore populations has led to the consumption of major habitat-forming plants in previously ungrazed areas, resulting in rapid

---

* Corresponding author.
E-mail address: rocio.jimenez@uca.es (R. Jiménez-Ramos).

https://doi.org/10.1016/j.marenvres.2024.106443
Received 25 November 2023; Received in revised form 7 March 2024; Accepted 8 March 2024

shifts in meadow structure (Vergés et al., 2014). In some areas, long-term conservation efforts to recover mega-herbivore populations like dugongs (*Dugong dugon*; Christianen et al., 2013; Cossa et al., 2023) or green turtles (*Chelonia mydas*; Lal et al., 2010; Mazaris et al., 2017) can increase herbivory pressure in seagrass meadows, resulting in a higher heterogeneity in seagrass bed, including a mixture of seagrass patches of different ages (Gulick et al., 2021). For example, green turtles repeatedly crop seagrass leaves in specific patches or zones that they periodically graze (probably because this stimulates the production of new and highly nutritious leaves; Christianen et al., 2012). Regarding dugongs, they graze by cropping on seagrass leaves (Preen, 1995) but also ripping up seagrass plants from the sediment generating feeding trails (Tol et al., 2016), which is periodically grazed by them when patches recover (Cossa et al., 2023).

Seagrasses exhibit a wide range of responses to herbivory. Induced responses are those that occur as a direct result of herbivore damage; these responses can involve both tolerance (i.e., the ability of plants to withstand and survive herbivory) and resistance (i.e., the development of traits to reduce leaf palatability) (Strauss and Agrawal, 1999), though tolerance responses are more common. Seagrasses tolerate grazing by intensifying recruitment of new shoots as a compensatory response (i.e., grazing stimulates the production of new photosynthetic tissue; Valentine et al., 1997; Vergés et al., 2008; Sanmartí et al., 2014). Other common mechanisms of herbivory tolerance in seagrasses include enhanced levels of N-metabolizing enzymes, reallocation of internal resources after herbivory damage, and changes in plant morphology (e.g., leaf biomass, shoot density, leaf area index; Zimmerman et al., 1996; Valentine et al., 1997; Vergés et al., 2008). On the other hand, resistance-based responses to herbivory encompass nutritional, chemical, and/or structural adaptations that lower plant palatability and thereby reduce herbivore preference or performance (Fritz and Simms, 1992). For example, seagrasses produce a variety of secondary metabolites, such as phenolic compounds, that act direct or indirectly as chemical defenses to resist herbivory (Martínez-Crego et al., 2015; Jiménez-Ramos et al., 2017).

Because both tolerance- and resistance-based responses to herbivory have a physiological cost, the availability of abiotic resources (e.g., light, nutrients) and patch age can shape the defense strategies adopted by a seagrass community. We accordingly hypothesized that seagrass responses to herbivory should be influenced by patch age. Recent studies showed mature patches may increase biodiversity (Pérez-Estrada et al., 2021) and enhance benthic metabolism through increased primary productivity and respiration (Egea et al., 2023b). Moreover, since seagrass biomass, particularly the belowground tissues, usually increases as the plant matures (Cebrián et al., 2000), we expect that old patches will exhibit higher compensatory responses (higher increase in aboveground biomass, shoot density, and leaf appearance and growth rates) and higher deterrent defenses (structural and phenolics) at the cost of nutritional quality (N content in leaves) (Agrawal and Fishbein, 2006). This hypothesis aligns with former studies in terrestrial plants, in which differing responses to herbivory along plant chronosequences (Cronin et al., 2001; Tiffin, 2002) have been attributed to shifts in defense patterns throughout succession (Rasmann et al., 2011; Hakes and Cronin, 2012). Although previous studies have described the various tolerance and resistance responses in seagrasses (e.g., Cebrián et al., 1998; Vergés et al., 2008; Sanmartí et al., 2014), researchers do not yet understand how these responses vary from young to mature seagrass meadows. However, a better understanding of how seagrass responses to herbivory differ with patch age is essential for developing sustainable conservation and restoration strategies for these vulnerable ecosystems (Bourque and Fourqurean, 2013).

Almost one-third of the seagrass species in North America can be found in the Gulf of California (McKenzie et al., 2020), a subtropical region of northwestern Mexico (Knutson et al., 2015). Among the seagrass species in this region, *Halodule wrightii* Ascherson (shoalgrass) is a fast-growing specie that inhabits shallow intertidal and subtidal areas (Rivera-Guzmán et al., 2017). Because the Gulf of California is regularly exposed to tropical storms and hurricanes that disrupt native plant communities (Jáuregui, 2003), many seagrass patches of different ages cohabit the same area (Pérez-Estrada et al., 2021; Egea et al., 2023b). As a foundation species, *H. wrightii* strongly influences the community composition of local fauna, including the abundance of benthic infauna (Booth and Heck, 2009; Pérez-Estrada et al., 2023). It also recolonizes after stressors more quickly than other seagrass species (Hall et al., 2006). These characteristics make *H. wrightii* a desirable candidate for assessing age-dependent responses to herbivory. In this study, we conducted a herbivory simulation experiment to evaluate the effects of herbivore damage on *H. wrightii* patches of different ages (i.e., different times since patch establishment). We measured tolerance responses by identifying compensatory growth (in terms of standing biomass, leaf appearance and growth rates, and shoot density) and assessing the associated cost to the plant (in the form of carbon, nitrogen, and non-structural carbohydrate content). We also measured resistance traits by quantifying the concentration of phenolic compounds in plant tissues.

2. Material and methods

2.1. Study area

The study was performed in Bahía Balandra, a shallow coastal wetland that covers approximately 52.5 ha in La Paz Bay in the southern Gulf of California, Mexico (Fig. 1). Bahía Balandra includes a coastal lagoon, mangroves, rocky reefs, and several populations of the seagrass *H. wrightii*. The area is in the subtropical arid zone, and the normal seawater temperature ranges from 12 to 39.5 °C (Sicard-González et al., 2012). Extreme climatological events such as tropical cyclones or hurricanes occur regularly in the area, and although their frequency and intensity can vary (Luja and Rodríguez-Estrella, 2010), these events are becoming increasingly severe and more common (Knutson et al., 2015). The area has belonged to the RAMSAR International Wetland Convention since 2008; it was declared as a Protected Area in 2012 by the Mexican Government and as a Natural Heritage Site by UNESCO in 2013 (Comisión Nacional de Área Naturales Protegidas, 2016). The seagrass populations in Bahía Balandra are composed of monospecific patches of the seagrass *H. wrightii* with a scattered distribution (Pérez-Estrada et al., 2021). This experiment was conducted at the subtidal site called la Poza (24° 19′ 0.54″ N, 110° 19′ 30.53″ W) in April 2016, during the period of maximum growth of *H. wrightii*.

The *H. wrightii* patches in the study area have been monitored since 2010 as part of research focused on seagrass establishment, community dynamics, and growth. As part of the monitoring program, three main zones were surveyed every six months, and every *H. wrightii* patch (including new ones) was measured and GPS-referenced (Pérez-Estrada et al., 2021). The data from this monitoring program allowed us to identify the location, size, and age of each *H. wrightii* patch in the area. At the time of our experiment, the study area included 59 noncontiguous patches covering a surface of approximately 4420 m^2. For our study, we selected subtidal patches at the same depth (0.5 m at low tide) and separated by at least 10 m with three different ages since establishment: i) patches established in 2014 (2 years old); ii) patches established in 2012 (4 years old); and iii) patches established in 2010 (6 years old). The average sizes of the 2-, 4-, and 6-year-old patches were 4.1 ± 1.2, 20.5 ± 4.6, and 36.2 ± 10.7 m^2, respectively. Average water temperature in the area during the study period was 23.3 ± 0.12 °C and mean underwater daily irradiance was 590 ± 120 μmol photons m^{-2} s^{-1} (Egea et al., 2023b).

2.2. Simulated herbivory experiment

Fishes in the study area, including members of the family Scaridae (e.g., parrotfish; Díaz-Uribe et al., 2007), preferentially feed on

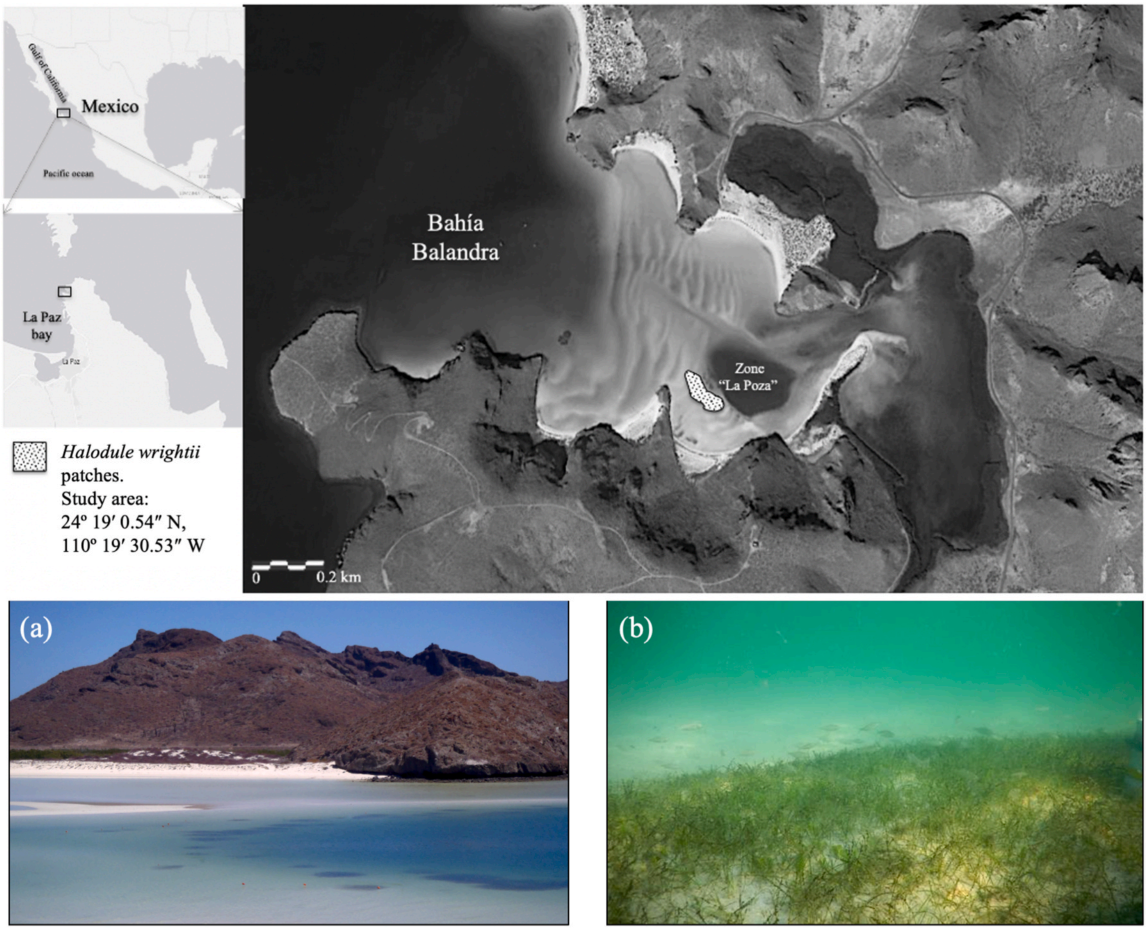

Fig. 1. Pictures of (a) the sampling area (black areas around deeper water represent *H. wrightii* patches) and (b) a representative 6-year-old seagrass patch. Map inset at the top shows the study area in Bahía Balandra, in the southern Gulf of California (Baja California Peninsula, Mexico).

fast-growing, early successional seagrass species, thus impacting the survival of the seagrass population (Burkholder et al., 2012). We simulated fish-induced herbivory damage and grazing pressure by manually clipping the leaves of *H. wrightii* individuals (e.g., Vergés et al., 2008; Sanmartí et al., 2014; Tomas et al., 2015). For each successional age (i.e., 2, 4, and 6 years), we randomly selected six similarly sized, equal-depth patches (2 m at high tide) spaced at least 7 m apart, for a total of 18 patches per age. Three patches in each age group were left unmodified (control), while leaves were manually clipped in the other three (clipped treatment). In each experimentally modified patch, we trimmed approximately 50% of leaf biomass within a 20 × 20 cm square at the center of the patch, aiming for a final blade length of 2 cm above sediment level. Rhizomes were severed around the edge of each clipped plot at the beginning of the experiment to prevent nutrient translocation into the plots. All clippings were removed from the plots to avoid any artefacts derived from detritus accumulation. The control treatment was left unmodified, and blades therefore remained at the normal height of ca. 4.5 ± 0.8 cm above sediment level. We examined the apex of control shoots at the end of the experimental period to confirm that no marks of recent grazing were observed. The experimental time span was one month and clipped treatments were kept at constant trimming one-week intervals throughout the experiment.

2.3. *Measurement of plant response variables*

To determine the tolerance responses of *H. wrightii* to simulated herbivory, we collected several physiological measurements from individual plants. These measurements included above- (leaf) and below-ground (rhizome, root) dry weight (after drying for 72 h at 60 °C; g DW m^{-2}), shoot density (shoot m^{-2}), leaf growth rate (LGR; mg DW $shoot^{-1}$ d^{-1}), and leaf appearance rate (LAR; number of new leaves $shoot^{-1}d^{-1}$). The LGR was measured in control and clipped treatments using a modified Zieman method (Peralta, 2000). In short, this method involves placing a recognizable element (e.g., staple, tied fishing line, etc) or making a hole on one leaf of the shoot (typically the younger or second younger one) at a convenient reference point (typically the junction between the leaf and the leaf sheath). Then, shoots are left for a period (e.g., 15 or 30 days) and the incremental growth between the leaf base and the recognizable element or hole is measured upon harvest. For this method, we selected five shoots with sufficient leaf width (1–2 mm) in each plot to employ a hole just above the ligule of the outermost leaf using a hypodermic needle. Marked shoots were collected at the end of the experimental period to estimate the leaf appearance rate (LAR, Eq. (1)) and leaf growth rate (LGR, Eq. (2)), as follows:

$$LAR = \sum NL \Big/ t_f - t_0 \tag{1}$$

$$LGR = \left(\frac{\sum_{i=1}^{n} \left[\left(LL_{i,j} - LLi, 0 \right)_{>0} \right]}{t_f - t_0} \right) * \frac{DW}{LL} \tag{2}$$

where *NL* represents the number of new leaves that appeared between the punching day (t_0) and collecting day (t_f), *LL* is the leaf length (cm); the subscript *i* refers to the *i*th leaf within the shoot; *n* is the total number of leaves at the end of the study period; the subscripts *f* and *0* refer to the final and initial conditions, respectively; and *DW/LL* is the dry weight/length ratio for leaves (g DW cm^{-1} leaf). (Peralta et al., 2021).

At the end of experimental period, samples were collected from the center of every plot using a 20 cm diameter corer. All rhizomes, roots, leaves, and sediment were removed and placed in plastic-sample bags. Samples were transported in an ice chest and then stored at −24°C in the laboratory until processing. In the laboratory, five shoots from each plot were randomly selected for analysis and carefully cleaned of epiphytes using a piece of soft paper. Physiological traits, including carbon (C), nitrogen (N), sucrose, starch in above- and below-ground tissues and the total non-structural carbohydrates (i.e., the sum of sucrose and starch in aboveground and belowground tissues; total NSC) contents were analyzed as compensation mechanisms of plants. For these analyses, dried leaves, rhizomes, and roots were ground to a fine powder. The total C and N contents in all tissues were measured in subsamples using a PerkinElmer 2400 elemental analyzer. Total NSC in above- and below-ground samples were measured in duplicate following the methods described by Alcoverro et al. (1999) and Brun et al. (2002). Sucrose was first solubilized by four sequential extractions in 96% (v/v) ethanol at 80 °C for 15 min. The ethanol extracts were then evaporated under a stream of 40 °C air, and the resulting residues were then redissolved in 10 ml of deionized water for analysis. Starch was extracted from the ethanol-insoluble residue by incubation for 24 h in 1 N NaOH. The sucrose and starch content of the extracts were determined spectrophotometrically using a resorcinol and anthrone assay at absorbances of 486 and 640 nm, respectively, with pure sucrose as the standard.

To assess resistance-based responses to herbivore damage, we measured the total phenolic content (TPC) in five shoots from each plot using a modified Folin-Ciocalteau method (Bolser et al., 1998). Each sample was extracted in 1 ml 50% methanol at 4 °C for 24 h. We then mixed 0.1 ml of the supernatant into 7.9 ml distilled water and added 0.5 ml Folin-Ciocalteau reagent. After a 2 min incubation, 1.5 ml $NaCO_3$ solution was added to the sample. The sample was allowed to incubate for 2 h to allow for color development, and then absorbance was measured spectrophotometrically at 765 nm using chlorogenic acid as standard (Martínez-Crego et al., 2016).

2.4. Data and statistical analysis

Summary statistics are presented as mean and standard error (SE). We used generalized linear models (GLMs) to test how our different response variables varied with each of patch age (3 levels) and treatment (control and clipped). Response variables were modeled with a Gaussian distribution and identity link. The goodness of fit of each model was assessed by checking the normality and homoscedasticity of the residuals with the Shapiro-Wilk and Levene's tests, respectively. When the residuals of the Gaussian GLMs did not meet normality assumptions, as was the case for the sucrose content of aboveground biomass, we used GLMs with a Gamma error distribution and reciprocal link function. If a model was significant, we performed pairwise comparisons using estimated marginal means with a Bonferroni correction ("emmeans" R package; Lenth et al., 2019). The significance level (α) for all tests was 0.05, and all statistical analyses were performed using R 4.2.2 (R Development Core Team, 2022).

3. Results

Aboveground biomass and shoot density increased with patch age in control treatments, showing a trend to increase, on average, 2.5-fold from 2-year-old to 6-year-old patches. Likewise, belowground biomass increased 2.9-fold across patch age. Because belowground biomass increased more than aboveground biomass, the aboveground/belowground biomass ratio correspondingly decreased as patch age increased (1.15 ± 0.18, 1.08 ± 0.19, and 0.68 ± 0.06 for 2-, 4- and 6-year-old patches, respectively). The experimentally clipped patches had a higher aboveground biomass and shoot density than controls, regardless patch age. However, this difference was only statistically significant in the youngest patches, where aboveground biomass and shoot density were higher (2- and 2.4-fold, respectively) in clipped plot with respect to their controls counterparts (Fig. 2, Appendix A Table S1).

The leaf appearance rate (LAR) and leaf growth rate (LGR) were similar in control treatments across all patch ages. The experimental clipping triggered a sharp increase in both LAR and LGR relative to controls, independent of patch age. On average, LAR and LGR increased by 11.8- and 4.5-fold, respectively, in response to clipping. However, post-clipping LAR and LGR were significantly higher in the youngest patches compared to the oldest patches (Fig. 3, Appendix A Table S2).

With respect to nutrient composition, seagrass C content was not affected by patch age or experimental clipping, but the N content varied with both age and treatment. The N content of aboveground biomass

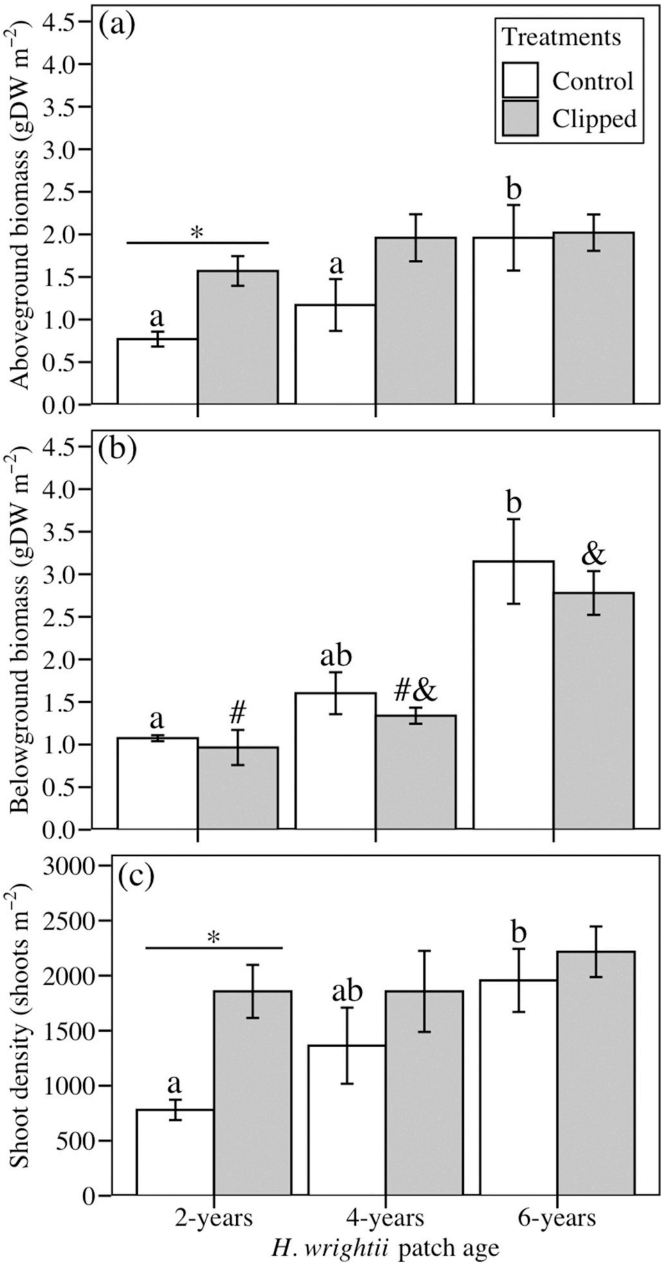

Fig. 2. Above- and belowground biomass (a, b) and shoot density (c) of *Halodule wrightii* plants in patches of different ages. Letters (for control patches) and symbols (for clipped patches) indicate significant differences among patch ages independent of the clipping treatment; asterisks above pairs of columns indicate significant differences between treatments (i.e., control and clipped) for each patch age. Data represent mean ± SE (n = 3), and significance was defined at $p < 0.05$.

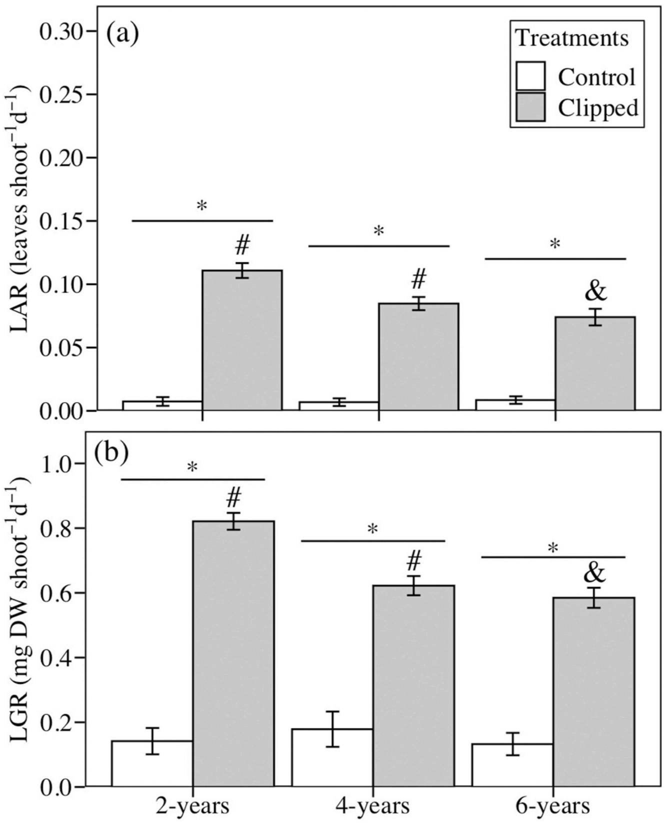

Fig. 3. Leaf appearance rate (a) and leaf growth rate (b) of *Halodule wrightii* plants in patches of different ages. Letters (for control patches) and symbols (for clipped patches) indicate significant differences among patch ages independent of the clipping treatment; asterisks above pairs of columns indicate significant differences between treatments (i.e., control and clipped) for each patch age. Data represent mean ± SE (n = 3), and significance was defined at $p < 0.05$.

was highest in 6-year-old control patches (2.99 ± 0.03% for this group compared to 1.96 ± 0.14% in 2-year-old control patches), which accounted this treatment having the lowest C/N ratio (Fig. 4a and c, Appendix A Table S3). In response to clipping, the N content of aboveground biomass decreased by 21% in the 6-year-old patches (from 2.99 ± 0.03% to 2.37 ± 0.29%) but increased by 25% in the 2-year-old patches (from 1.96 ± 0.14% to 2.44 ± 0.11%). A similar response was observed for the N content of belowground biomass, which decreased by 20% in 6-year-old patches after experimental clipping (from 2.65 ± 0.07% to 2.12 ± 0.25%) but increased by 11% in the 2-year-old patches (from 2.36 ± 0.16% to 2.61 ± 0.09%), though this difference was only significant for older patches. Clipping did not induce any significant differences in the N content of above- and belowground biomass in the 4-year-old patches (Fig. 4, Appendix A Table S3).

In control treatments, the sucrose content of the aboveground and belowground biomass increased with patch age. Aboveground starch content was also higher in the 6- and 4-year-old patches relative to the 2-year-old patches, but belowground starch did not vary significantly with patch age. Experimental clipping led to a reduced total NSC relative to same-age controls, especially in older patches (reductions of 73%, 36%, and 7% in 6-, 4-, and 2-year old patches, respectively). Although the clipping treatment did not cause significant changes in plant sucrose and starch content in the youngest patches; sucrose and starch content significantly decreased in the 4- and 6-year-old patches (Fig. 5; Appendix A Table S4).

The total phenolic concentration (TPC) in leaves also differed among patches with different ages, with the youngest control patches exhibiting the highest overall TPC content. At the end of the experimental period, the TPC of 6-year-old seagrass was 1.2-fold higher in the clipped patches relative to same-age controls (1.80 ± 0.07% vs. 1.50 ± 0.06%). By contrast, the TPC of 2-year-old seagrass was 0.26-fold lower after clipping. Both these differences were significant (Fig. 6, Appendix A Table S5).

4. Discussion

Our results showed that *H. wrightii* shoots of all ages compensate for herbivory pressure by increasing leaf growth rates. The growth rates measured in this study are similar to those from previous studies analyzing seagrass grazing response in the tropical seagrass *Thalassia testudinum* (Valentine et al., 1997; Moran and Bjorndal, 2005) and the temperate seagrasses *Posidonia oceanica* (Vergés et al., 2008) and *P. sinuosa* (Burnell et al., 2013). With respect to age-dependent compensatory responses, the youngest (2-year-old) patches exhibited the highest increase in aboveground biomass, shoot density, and leaf appearance and growth rates in response to experimental clipping. This finding contradicted our expectations: because older patches have more belowground reserve tissues and more interconnected ramets (i.e., modules composed by leaves, pieces of rhizome, roots, flowers, or inflorescences that often remain connected sharing resources; Kuo and Hartog, 2006), we had expected their growth rates to increase more rapidly (Rasheed, 2004; Grech et al., 2018). Instead, our results suggest that compensatory growth was more efficient in younger patches. We attribute this result to three separate processes. First, younger *H. wrightii* patches have been shown to have a higher rate of primary production per biomass unit. In our same study area, Egea et al. (2023b) reported that younger *H. wrightii* patches were more productive per biomass unit due to their higher aboveground/belowground biomass ratio, which necessarily decreases the proportion of non-photosynthetic tissues and thereby the respiration rate. The aboveground/belowground biomass ratio strongly influence the plants carbon budget because belowground respiratory tissues rely on photosynthates provided by shoots. This may constrain the growth of shoots in low-light environments or conditions (such as the loss of photosynthetic tissues by herbivory) (Peralta et al., 2002; Jiménez-Ramos et al., 2023). Second, we suspect that the higher total aboveground biomass and shoot density in the 4- and 6-year-old patches may increase self-shading of the canopy relative to 2-year-old patches, thus reducing their capacity to produce new leaf tissues (Enríquez and Pantoja-Reyes, 2005; Pérez-Estrada et al., 2021; Jiménez-Ramos et al., 2023). Third, the role of water flow within the patches might have influenced the differences among patch ages, although this process is probably of less relative importance than the previous two processes described. Despite all *H. wrightii* patches we studied were located in the same area and subjected to the same hydrodynamic conditions, it is possible that water flow within the canopy was higher in the youngest patches because they have significantly lower biomass and shoot density (Dahl et al., 2018; Egea et al., 2023c). Seagrass beds are well known for their ability to alter their local hydrodynamic environment, reducing current velocities and momentum within the canopy (Risandi et al., 2023). Increasing the residence time of dissolved nutrients within the meadow may promote the nutrient or resources uptake by the plants or by the associated fauna (González-Ortiz et al., 2014). However, the increase in current velocities have also been shown to enhance nutrient uptake by plants, which has been attributed to a decrease in the thickness of the diffusive boundary layer adjacent to plant cells and to the renovation of nutrients around the seagrass leaves (Morris et al., 2008; Pujol et al., 2019). The relatively low hydrodynamic condition in the area (as much as 14.1 cm s^{-1} during ebb tide; Gutiérrez-Sánchez, 1987) led us to suspect that the lower shoot density may benefit younger patches by enhancing volumetric flow rates within their canopy and, thereby, the nutrient uptake that may support the production of new leaf tissue (Morris et al., 2008, 2013).

Internal mobilization of nitrogen reserves is another important tolerance response that facilitates compensatory growth in seagrasses (Alcoverro et al., 2001). We correspondingly observed a significant

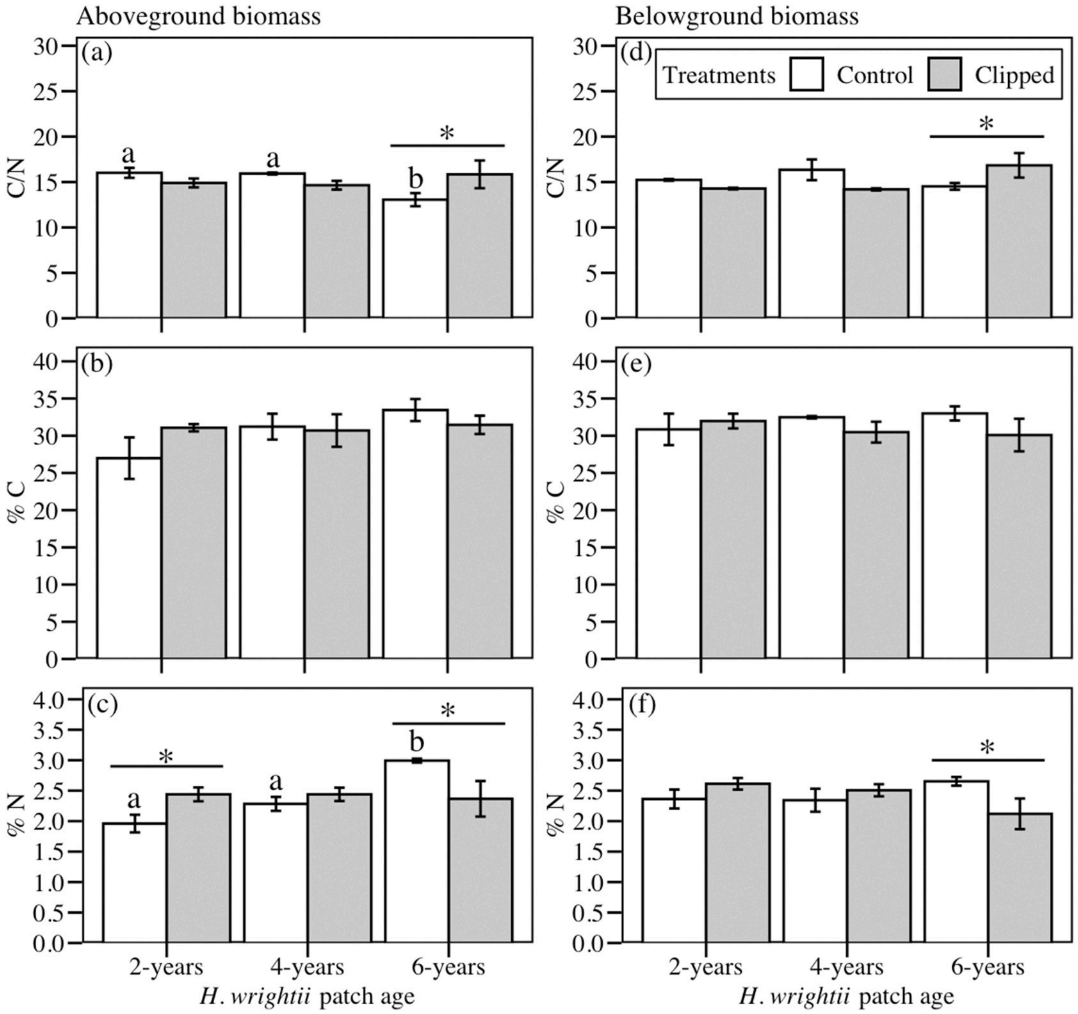

Fig. 4. The C/N ratio (a, d), carbon content (b, e) and nitrogen content (c, f) of the aboveground biomass (left panel) and belowground biomass (right panel) of *Halodule wrightii* plants in patches of different ages. Letters (for control patches) and symbols (for clipped patches) indicate significant differences among patch ages independent of the clipping treatment; asterisks above pairs of columns indicate significant difference between treatments (i.e., control and clipped) for each patch age. Data represent mean ± SE (n = 3), and significance was defined at $p < 0.05$.

increase in the leaf N content in clipped plants from the youngest patches, though we did not observe a parallel increase in rhizome N reserves. We therefore suspect that nutrient uptake from the environment was higher in the youngest patches, an assumption supported by previous studies (McNaughton, 1983; Jaramillo and Detling, 1988) and by the hypothesized increase in volumetric flow rate within younger patches (Morris et al., 2008, 2013). Greater N uptake and mobilization presumably allows younger plants to regrow swiftly after herbivory damage. However, younger patches also had the highest total N content per unit biomass, which may affect the palatability of the tissues; indeed, plant nutritional composition plays a central role in determining herbivore feeding patterns in marine habitats (Bjorndal, 1980; Goecker et al., 2005; Jiménez-Ramos et al., 2018). On the other hand, the reduced total N content in clipped plants from the oldest patches suggests that these plants lose some of their nutritional quality in response to herbivory. Some authors have suggested that a reduction in leaf N concentration could be a defense mechanism against future grazing (Augner, 1995; Valentine et al., 1997); however, this hypothesis needs to be more rigorously assessed in future research. Besides, the mobilization of carbohydrate reserves also play a major role in the ability of plants to withstand disturbances such as the loss of aboveground tissues (Eklöf et al., 2008; Egea et al., 2018) and it is considered as a tolerance response against herbivory (Vergés et al., 2008). We observed a sharp reduction in total NSC in 4- and 6-year-old patches in response to clipping, which may be linked to the relatively higher belowground biomass in these patches. Older patches have a lower aboveground/belowground biomass ratio, indicating that the proportion of non-photosynthetic tissues increases with patch age. With fewer photosynthetic tissues, older plants must metabolize their carbohydrate reserves to meet their higher respiratory demand (Brun et al., 2008; Jiménez-Ramos et al., 2023). Because *H. wrightii* is a fast-growing species, it has a relatively low rhizome storage capacity, meaning that the herbivory-induced reduction of internal carbohydrate reserves might make this species more vulnerable to additional stressors (Hauxwell et al., 2001; Villazán et al., 2015).

In addition to tolerance-based responses to herbivory, seagrasses may also produce phenolic compounds as a chemical defense against herbivory (Arnold and Targett, 2002). These C-based organic compounds modify seagrass palatability, though the literature on their effect is not consistent: while some studies have found that phenolic production increases palatability (Martínez-Crego et al., 2015), others have found the opposite effect (Vergés et al., 2008). In this study, we observed two contrasting responses in chemical defense production depending on patch age. First, clipped leaves from the oldest patches had higher total phenolic content (TPC) than same-age controls, in addition to reduced nutritional quality due to their reduced leaf N content. This combined response supports the *defense syndrome hypothesis* against herbivory (Agrawal and Fishbein, 2006; also called the *syndrome of low*

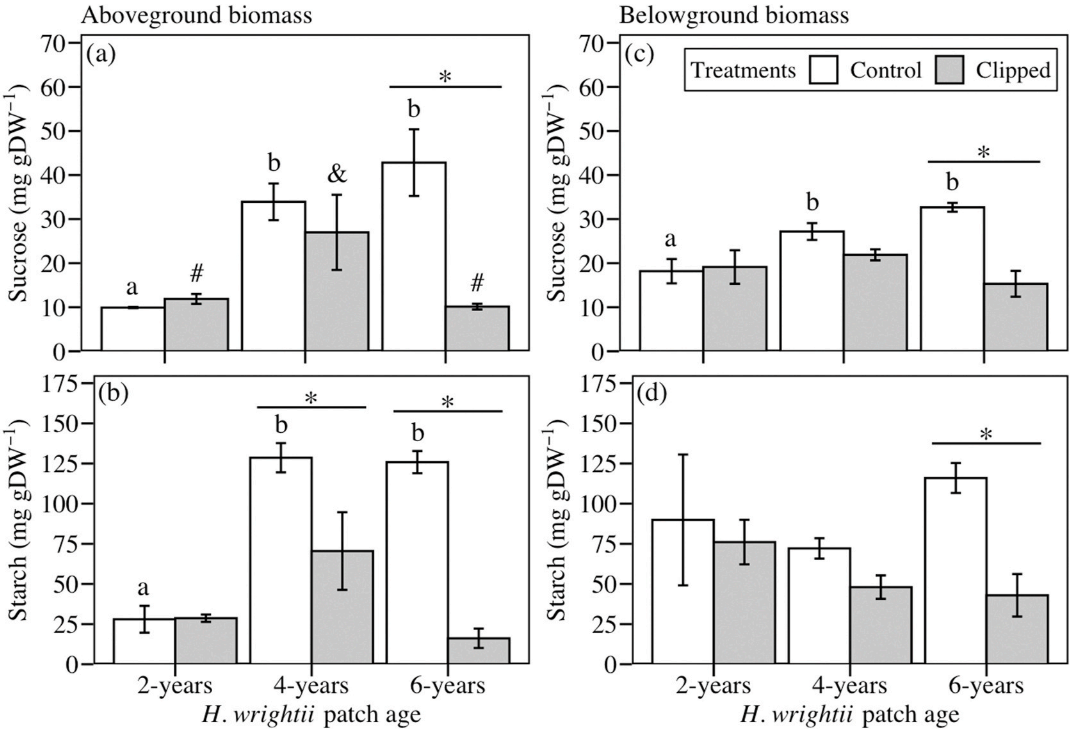

Fig. 5. Sucrose (a, b) and starch (c, d) content in aboveground biomass (left panel) and belowground biomass (right panel) of *Halodule wrightii* plants in patches of different ages. Letters (for control patches) and symbols (for clipped patches) indicate significant differences among patch ages independent of the clipping treatment; asterisks above pairs of columns indicate significant difference between treatments (i.e., control and clipped) for each patch age. Note difference in y-axis scale. Data represent mean ± SE (n = 3), and significance was defined at $p < 0.05$.

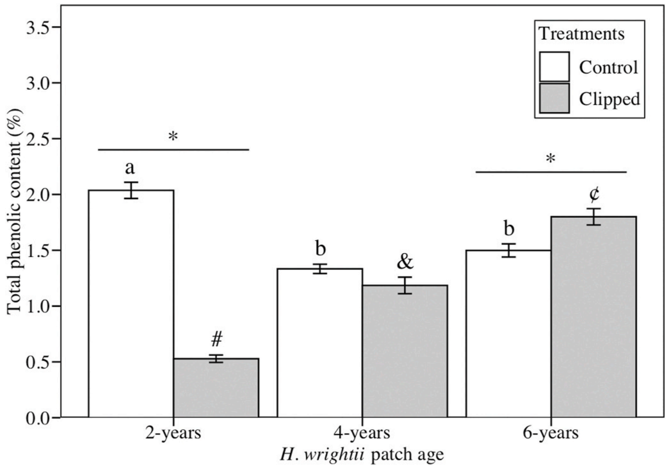

Fig. 6. Total phenolic content (%) in leaves of *H. wrightii* plants in patches of different ages. Letters (for control patches) and symbols (for clipped patches) indicate significant differences among patch ages independent of the clipping treatment; asterisks above pairs of columns indicate significant difference between treatments (i.e., control and clipped) for each patch age. Data represent mean ± SE (n = 3), and significance was defined at $p < 0.05$.

vulnerability plants), which suggests that plants suffering from herbivory pressure invest more in defenses (structural and phenolics) at the cost of nutritional quality (N content in leaves). On the other hand, clipped leaves from the youngest patches had lower TPC than same-age controls. These younger plants also maintained higher growth rates and carbohydrate reserves than other patches despite having a similar total carbon content. Importantly, phenolic compounds not only function as defensive secondary metabolites against herbivory but can also participate in a wide number of plant functions (e.g., antioxidants: Hodzic et al., 2009; antimicrobials: Vergeer and Develi, 1997; anti-fungal: Jensen et al., 1998). Previous studies in terrestrial plants have reported that some of the carbon used for cell wall construction during plant growth comes from phenolic compounds (Abdulrazzak et al., 2006; Merali et al., 2012); for example, phlorotannin, a tannin produced by brown seaweeds, is predicted to accumulate not only in response to the need for chemical defense but also in response to the demand for it during cell wall construction (Arnold and Targett, 2002). The role of phenolics as primary metabolites has also been suggested in simulated herbivory experiments in temperate seagrasses such as *Posidonia oceanica* (Vergés et al., 2008) and *Cymodocea nodosa* (Sanmartí et al., 2014). Our study accordingly supports the importance of phenolics in primary metabolic processes.

Our results evidenced that herbivores are a key force affecting seagrass survival during meadow colonization and growth. Plant defense responses are the result of a complex interplay among habitat selection pressures (Agrawal, 2011), nutrient availability (Tomas et al., 2011), genetic variability (Hernán et al., 2022), and patch ages (this study). Here we reported significant differences in the strategy followed by *H. wrightii* to face herbivory damage depending on the patch age, indicative of age-dependent plasticity. This has important ecological consequences. Our result indicated that younger patches used compensatory responses (i.e., higher regrowth) instead of using of chemical (phenolic content) or physiological (reduction in total NSC) defenses, which were observed in older patches along with a lower compensatory response. Consequently, our results indicate that herbivores, by preferentially feeding on younger seagrass patches, make meadows more susceptible to additional stressors (Stockbridge et al., 2020). The Gulf of California is changing, as exotic invaders and climate change modify the functioning and environmental status of this sea (Páez-Osuna et al., 2016). As temperatures rise, existing trophic interactions are also likely to be modified by climate change (Valentine and Heck, 2021). How *Halodule wrightii* will respond to these new pressures is still an open question, but its resilience to herbivory is most likely going to be dependent on the continued effectiveness of the mechanisms we described. Our results confirm the importance of incorporating the patch age to develop accurate decisions for seagrass ecosystem management like restoration projects, illustrating some of the yet undocumented links between patch chronosequence and the success of restoration (Kindeberg et al., 2023). For example, our results showed that young *H. wrightii* patches evidence higher regrowth rates after shoot damage. We, therefore, suspect that the horizontal rhizomatic growth, needed to rooting, extend and stablish the meadow, could be relatively higher at the beginning of the restoration process using shoots from younger donor meadows. Likewise, the recovery of the donor meadow might be relatively faster. However, if restoration places are subjected to high herbivory rates, shoots from older meadows probably will be more adequate to face this natural stress in the area at the beginning and critical period of restoration process. These hypotheses should be rigorously assessed in future research.

5. Conclusions

H. wrightii populations exhibit an age-dependent plasticity in response to simulated herbivory. Specifically, simulated herbivory damage (via experimental clipping) can induce substantial compensatory responses, the most notable of which was an increase in leaf growth rate in patches of all ages. Other tolerance responses included the mobilization of internal resources, which was more prominent in older patches. The resistance response, which we defined as increased leaf phenolic content following clipping, was only observed in plants from the oldest patches; in younger patches, leaf phenolic content decreased while compensatory growth increased and total carbon content remained unchanged, suggesting that phenolic compounds were being reallocated towards growth and metabolism. Overall, our results suggest that herbivores are a key force affecting seagrass survival during meadow colonization and growth. As natural disturbances become increasingly common in an era of rapid global change, an understanding of how seagrass establishment and colonization dynamics change in response to grazing damage will be increasingly relevant to the conservation and restoration strategies for these vulnerable ecosystems.

CRediT authorship contribution statement

Rocío Jiménez-Ramos: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Luis G. Egea:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Claudia J. Pérez-Estrada:** Writing – review & editing, Resources, Investigation. **Eduardo F. Balart:** Writing – review & editing, Resources. **Juan J. Vergara:** Writing – review & editing, Supervision. **Fernando G. Brun:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study was funded by the Spanish national project CTM 2011-24482 (SEA-LIVE); the Excellence Project from *Junta de Andalucía* RNM-P12-3020 (PRODESCA); and was supported by the Spanish National Project RECOUNT (PID 2020-120237RJ-I00), financed by MCIN/AEI/10.1339/501100011033. The permits for working in the protected site were issued by the province of Baja California Sur (local government) under the numbers F00-CONANP/AFPBALANDRA/236/2016. We thank to the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR) and their kindly staff for the use of their facilities and warmly attention. We also thank to Dr. Ricardo Rodríguez Estrella (Proy-PC027-CIBNOR) and Dr. Enrique Morales Bojórquez (PEP-CIBNOR) for their support. We also thank to the three anonymous referees for their constructive comments on an early version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106443.

References

Abdulrazzak, N., Pollet, B., Ehlting, J., Larsen, K., Asnaghi, C., Ronseau, S., Proux, C., Erhardt, M., Seltzer, V., Renou, J.-P., Ullmann, P., Pauly, M., Lapierre, C., Werck-Reichhart, D., 2006. A coumaroyl-ester-3-hydroxylase insertion mutant reveals the existence of nonredundant meta -hydroxylation pathways and essential roles for phenolic precursors in cell expansion and plant growth. Plant Physiol. 140, 30–48. https://doi.org/10.1104/pp.105.069690.

Agrawal, A.A., Fishbein, M., 2006. Plant defense syndromes. Ecology 87 (7), 132–149. https://doi.org/10.1890/0012-9658(2006)87[132:pds]2.0.co;2.

Agrawal, A.A., 2011. Current trends in the evolutionary ecology of plant defence. Funct. Ecol. 25, 420–432. https://doi.org/10.1111/j.1365-2435.2010.01796.x.

Alcoverro, T., Zimmerman, R., Kohrs, D., Alberte, R., 1999. Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 187, 121–131. https://doi.org/10.3354/meps187121.

Alcoverro, T., Cerbiān, E., Ballesteros, E., 2001. The photosynthetic capacity of the seagrass *Posidonia oceanica*: influence of nitrogen and light. J. Exp. Mar. Biol. Ecol. 261, 107–120. https://doi.org/10.1016/S0022-0981(01)00267-2.

Allan, E., Crawley, M.J., 2011. Contrasting effects of insect and molluscan herbivores on plant diversity in a long-term field experiment. Ecol. Lett. 14, 1246–1253. https://doi.org/10.1111/j.1461-0248.2011.01694.x.

Arnold, T.M., Targett, N.M., 2002. Marine tannins: the importance of a mechanistic framework for predicting ecological roles. J. Chem. Ecol. 28, 1919–1934. https://doi.org/10.1023/A:1020737609151.

Augner, M., 1995. Low nutritive quality as a plant defence: effects of herbivore-mediated interactions. Evol. Ecol. 9, 605–616. https://doi.org/10.1007/BF01237658.

Bjorndal, K.A., 1980. Nutrition and grazing behavior of the green turtle *Chelonia mydas*. Mar. Biol. 56, 147–154. https://doi.org/10.1007/BF00397131.

Bolser, R.C., Hay, M.E., Lindquist, N., Fenical, W., Wilson, D., 1998. Chemical defenses of freshwater macrophytes against crayfish herbivory. J. Chem. Ecol. 24, 1639–1658. https://doi.org/10.1023/A:1020816511924.

Booth, D., Heck, K., 2009. Effects of the American oyster *Crassostrea virginica* on growth rates of the seagrass *Halodule wrightii*. Mar. Ecol. Prog. Ser. 389, 117–126. https://doi.org/10.3354/meps08163.

Bourque, A.S., Fourqurean, J.W., 2013. Variability in herbivory in subtropical seagrass ecosystems and implications for seagrass transplanting. J. Exp. Mar. Biol. Ecol. 445, 29–37. https://doi.org/10.1016/j.jembe.2013.03.014.

Brun, F., Hernández, I., Vergara, J., Peralta, G., Pérez-Lloréns, J., 2002. Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. Mar. Ecol. Prog. Ser. 225, 177–187. https://doi.org/10.3354/meps225177.

Brun, F.G., Olivé, I., Malta, E., Vergara, J.J., Hernández, I., Pérez-Lloréns, J.L., 2008. Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Mar. Ecol. Prog. Ser. 365, 67–75. https://doi.org/10.3354/meps07512.

Burkholder, D.A., Heithaus, M.R., Fourqurean, J.W., 2012. Feeding preferences of herbivores in a relatively pristine subtropical seagrass ecosystem. Mar. Freshw. Res. 63, 1051–1058. https://doi.org/10.1071/MF12029.

Burnell, O.W., Connell, S.D., Irving, A.D., Russell, B.D., 2013. Asymmetric patterns of recovery in two habitat forming seagrass species following simulated overgrazing by urchins. J. Exp. Mar. Biol. Ecol. 448, 114–120. https://doi.org/10.1016/j.jembe.2013.07.003.

Cebrián, J., Duarte, C.M., Agawin, R., N, S., Merino, M., 1998. Leaf growth response to simulated herbivory: a comparison among seagrass species. J. Exp. Mar. Biol. Ecol. 220, 67–81. https://doi.org/10.1016/S0022-0981(97)00084-1.

Cebrián, J., Pedersen, M., Kroeger, K., Valiela, I., 2000. Fate of production of the seagrass *Cymodocea nodosa* in different stages of meadow formation. Mar. Ecol. Prog. Ser. 204, 119–130. https://doi.org/10.3354/meps204119.

Christianen, M.J.A., Govers, L.L., Bouma, T.J., Kiswara, W., Roelofs, J.G.M., Lamers, L.P.M., van Katwijk, M.M., 2012. Marine megaherbivore grazing may increase seagrass tolerance to high nutrient loads. J. Ecol. 100, 546–560. https://doi.org/10.1111/j.1365-2745.2011.01900.x.

Christianen, M.J.A., van Belzen, J., Herman, P.M.J., van Katwijk, M.M., Lamers, L.P.M., van Leent, P.J.M., Bouma, T.J., 2013. Low-canopy seagrass beds still provide important coastal protection services. PLoS One 8, e62413. https://doi.org/10.1371/journal.pone.0062413.

Comisión Nacional de Área Naturales Protegidas, 2016. Programa de Manejo: Area de Protección de Flora y Fauna Balandra. CONANP, SEMARNAT, Colectivo Balandra. México.

Correia, K.M., Smee, D.L., 2022. A meta-analysis of tropical cyclone effects on seagrass meadows. Wetlands 42, 108. https://doi.org/10.1007/s13157-022-01611-0.

Cossa, D., Cossa, M., Timba, I., Nhaca, J., Macia, A., Infantes, E., 2023. Drones and machine-learning for monitoring dugong feeding grounds and gillnet fishing. Mar. Ecol. Prog. Ser. 716, 123–136. https://doi.org/10.3354/meps14361.

Cronin, J.T., Abrahamson, W.G., Craig, T.P., 2001. Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. Oikos 93, 312–320. https://doi.org/10.1034/j.1600-0706.2001.930214.x.

Dahl, M., Infantes, E., Clevesjö, R., Linderholm, H.W., Björk, M., Gullström, M., 2018. Increased current flow enhances the risk of organic carbon loss from *Zostera marina* sediments: insights from a flume experiment. Limnol. Oceanogr. 63, 2793–2805. https://doi.org/10.1002/lno.11009.

de los Santos, C.B., Egea, L.G., Martins, M., Santos, R., Masqué, P., Peralta, G., Brun, F.G., Jiménez-Ramos, R., 2023. Sedimentary organic carbon and nitrogen sequestration across a vertical gradient on a temperate wetland seascape including salt marshes, seagrass meadows and rhizophytic macroalgae beds. Ecosystems 26, 826–842. https://doi.org/10.1007/s10021-022-00801-5.

Díaz-Uribe, J.G., Arreguín-Sánchez, F., Cisneros-Mata, M.A., 2007. Multispecies perspective for small-scale fisheries management: a trophic analysis of La Paz Bay in the Gulf of California, Mexico. Ecol. Model. 201, 205–222. https://doi.org/10.1016/j.ecolmodel.2006.09.015.

Dunic, J.C., Brown, C.J., Connolly, R.M., Turschwell, M.P., Côté, I.M., 2021. Long-term declines and recovery of meadow area across the world's seagrass bioregions. Global Change Biol. 27, 4096–4109. https://doi.org/10.1111/gcb.15684.

Egea, L.G., Infantes, E., Jiménez-Ramos, R., 2023c. Loss of POC and DOC on seagrass sediments by hydrodynamics. Sci. Total Environ. 901, 165976 https://doi.org/10.1016/j.scitotenv.2023.165976.

Egea, L.G., Jiménez-Ramos, R., Vergara, J.J., Hernández, I., Brun, F.G., 2018. Interactive effect of temperature, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa*. Mar. Pollut. Bull. 134, 14–26. https://doi.org/10.1016/j.marpolbul.2018.02.029.

Egea, L.G., Jiménez-Ramos, R., Romera-Castillo, C., Casal-Porras, I., Bonet-Melià, P., Yamuza-Magdaleno, A., Cerezo-Sepúlveda, L., Pérez-Lloréns, J.L., Brun, F.G., 2023a. Effect of marine heat waves on carbon metabolism, optical characterization, and bioavailability of dissolved organic carbon in coastal vegetated communities. Limnol. Oceanogr. 68, 467–482. https://doi.org/10.1002/lno.12286.

Egea, L.G., Pérez-Estrada, C.J., Jiménez-Ramos, R., Hernández, I., López-López, S., Brun, F.G., 2023b. Changes in carbon metabolism and dissolved organic carbon fluxes on seagrass patches (*Halodule wrightii*) with different ages in Southern Gulf of California. Mar. Environ. Res. 191, 106136 https://doi.org/10.1016/j.marenvres.2023.106136.

Eklöf, J.S., Gullström, M., Björk, M., Asplund, M.E., Hammar, L., Dahlgren, A., Öhman, M.C., 2008. The importance of grazing intensity and frequency for physiological responses of the tropical seagrass *Thalassia hemprichii*. Aquat. Bot. 89, 337–340. https://doi.org/10.1016/j.aquabot.2008.03.009.

Enríquez, S., Pantoja-Reyes, N.I., 2005. Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*. Oecologia 145, 234–242. https://doi.org/10.1007/s00442-005-0111-7.

Fritz, R.S., Simms, E.L., 1992. Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics. University of Chicago Press, Chicago, pp. 1–600.

Goecker, M., Heck, K., Valentine, J., 2005. Effects of nitrogen concentrations in turtlegrass *Thalassia testudinum* on consumption by the bucktooth parrotfish *Sparisoma radians*. Mar. Ecol. Prog. Ser. 286, 239–248. https://doi.org/10.3354/meps286239.

González-Ortiz, V., Egea, L.G., Jiménez-Ramos, R., Moreno-Marín, F., Pérez-Lloréns, J.L., Bouma, T.J., Brun, F.G., 2014. Interactions between seagrass complexity, hydrodynamic flow and biomixing alter food availability for associated filter-feeding organisms. PLoS One 9, e104949. https://doi.org/10.1371/journal.pone.0104949.

Grech, A., Hanert, E., McKenzie, L., Rasheed, M., Thomas, C., Tol, S., Wang, M., Waycott, M., Wolter, J., Coles, R., 2018. Predicting the cumulative effect of multiple disturbances on seagrass connectivity. Global Change Biol. 24, 3093–3104. https://doi.org/10.1111/gcb.14127.

Green, E.P., Short, F., 2004. World atlas of seagrasses. Bot. Mar. 47, 259–260. https://doi.org/10.1515/BOT.2004.029.

Gulick, A.G., Johnson, R.A., Pollock, C.G., Hillis-Starr, Z., Bolten, A.B., Bjorndal, K.A., 2021. Recovery of a cultivation grazer: a mechanism for compensatory growth of *Thalassia testudinum* in a Caribbean seagrass meadow grazed by green turtles. J. Ecol. 109, 3031–3045. https://doi.org/10.1111/1365-2745.13718.

Gutiérrez Sánchez, S.S., 1987. Geomorfología, agua y sedimentos de la caleta-laguna de Balandra, Baja California Sur. Tesis Licenciatura. Universidad Autónoma de Baja California Sur, La Paz, Baja California Sur, México.

Hakes, A.S., Cronin, J.T., 2012. Successional changes in plant resistance and tolerance to herbivory. Ecology 93, 1059–1070. https://doi.org/10.1890/10-1637.1.

Hall, L., Hanisak, M., Virnstein, R., 2006. Fragments of the seagrasses *Halodule wrightii* and *Halophila johnsonii* as potential recruits in Indian River Lagoon, Florida. Mar. Ecol. Prog. Ser. 310, 109–117. https://doi.org/10.3354/meps310109.

Hauxwell, J., Cebrián, J., Herrera-Silveira, J.A., Ramírez, R.J., Zaldivar J, A., Gomez, N., Aranda-Cirerol, N., 2001. Measuring production of *Halodule wrightii*: additional evidence suggests clipping underestimates growth rate. Aquat. Bot. 69, 41–54. https://doi.org/10.1016/S0304-3770(00)00137-6.

Heck, K.L., Valentine, J.F., 2006. Plant–herbivore interactions in seagrass meadows. J. Exp. Mar. Biol. Ecol. 330, 420–436. https://doi.org/10.1016/j.jembe.2005.12.044.

Hernán, G., Ortega, M.J., Gándara, A.M., Castejón, I., Terrados, J., Tomas, F., 2017. Future warmer seas: increased stress and susceptibility to grazing in seedlings of a marine habitat-forming species. Global Change Biol. 23, 4530–4543. https://doi.org/10.1111/gcb.13768.

Hernán, G., Ortega, M.J., Tomas, F., 2022. Specialized compounds across ontogeny in the seagrass *Posidonia oceanica*. Phytochemistry 196, 113070. https://doi.org/10.1016/j.phytochem.2021.113070.

Hodzic, Z., Pasalic, H., Memisevic, A., Srabovic, M., Saletovic, M., Poljakovic, M., 2009. The influence of total phenols content on antioxidant capacity in the whole grain extracts 472. Eur. J. Sci. Res. 28, 471–477.

Jaramillo, V.J., Detling, J.K., 1988. Grazing history, defoliation, and competition: effects on shortgrass production and nitrogen accumulation. Ecology 69, 1599–1608. https://doi.org/10.2307/1941657.

Jáuregui, E., 2003. Climatology of landfalling hurricanes and tropical storms in Mexico. Atmósfera 16 (4), 193–204. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-62362003000400001&lng=es&tlng=en.

Jensen, P.R., Jenkins, K.M., Porter, D., Fenical, W., 1998. Evidence that a new antibiotic flavone glycoside chemically defends the sea grass *Thalassia testudinum* against zoosporic fungi. Appl. Environ. Microbiol. 64, 1490–1496. https://doi.org/10.1128/AEM.64.4.1490-1496.1998.

Jiménez-Ramos, R., Egea, L.G., Vergara, J.J., Brun, F.G., 2018. Nutrient load and epiphytes are drivers of increased herbivory in seagrass communities. Mar. Ecol. Prog. Ser. 599, 49–64. https://doi.org/10.3354/meps12622.

Jiménez-Ramos, R., Brun, F.G., Pérez-Lloréns, J.L., Vergara, J.J., Delgado-Cabezas, F., Sena-Soria, N., Egea, L.G., 2023. Resistance and recovery of benthic marine macrophyte communities to light reduction: insights from carbon metabolism and dissolved organic carbon (DOC) fluxes, and implications for resilience. Mar. Pollut. Bull. 188, 114630 https://doi.org/10.1016/j.marpolbul.2023.114630.

Jiménez-Ramos, R., Egea, L.G., Ortega, M.J., Hernández, I., Vergara, J.J., Brun, F.G., 2017. Global and local disturbances interact to modify seagrass palatability. PLoS One 12, e0183256. https://doi.org/10.1371/journal.pone.0183256.

Jiménez-Ramos, R., Egea, L.G., Vergara, J.J., Brun, F.G., 2021. Factors modulating herbivory patterns in *Cymodocea nodosa* meadows. Limnol. Oceanogr. 66, 2218–2233. https://doi.org/10.1002/lno.11749.

Kindeberg, T., Attard, K.M., Hüller, J., Müller, J., Quintana, C.O., Infantes, E., 2023. Structural complexity and benthic metabolism: resolving the links between carbon cycling and biodiversity in restored seagrass meadows. Biogeosci. Discuss. https://doi.org/10.5194/bg-2023-173.

Knutson, T.R., Sirutis, J.J., Zhao, M., Tuleya, R.E., Bender, M., Vecchi, G.A., Villarini, G., Chavas, D., 2015. Global projections of intense tropical cyclone activity for the late twenty-first century from dynamical downscaling of CMIP5/rcp4.5 scenarios. J. Clim. 28, 7203–7224. https://doi.org/10.1175/JCLI-D-15-0129.1.

Kuo, J., Hartog, C. den, 2006. Seagrass morphology, anatomy, and ultrastructure. In: Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, Dordrecht, pp. 51–87. https://doi.org/10.1007/978-1-4020-2983-7_3.

Lal, A., Arthur, R., Marbà, N., Lill, A.W.T., Alcoverro, T., 2010. Implications of conserving an ecosystem modifier: increasing green turtle (*Chelonia mydas*) densities substantially alters seagrass meadows. Biol. Conserv. 143, 2730–2738. https://doi.org/10.1016/j.biocon.2010.07.020.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2019. Emmeans: estimated marginal means, aka least-squares means. In: R Package (2019). Version 1.3.3.

Luja, V.H., Rodríguez-Estrella, R., 2010. Are tropical cyclones sources of natural selection? Observations on the abundance and behavior of frogs affected by extreme climatic events in the Baja California Peninsula, Mexico. J. Arid Environ. 74, 1345–1347. https://doi.org/10.1016/j.jaridenv.2010.04.005.

Macreadie, P.I., Baird, M.E., Trevathan-Tackett, S.M., Larkum, A.W.D., Ralph, P.J., 2014. Quantifying and modelling the carbon sequestration capacity of seagrass meadows – a critical assessment. Mar. Pollut. Bull. 83, 430–439. https://doi.org/10.1016/j.marpolbul.2013.07.038.

Maron, J.L., Crone, E., 2006. Herbivory: effects on plant abundance, distribution and population growth. Proc. R. Soc. B Biol. Sci. 273, 2575–2584. https://doi.org/10.1098/rspb.2006.3587.

Marquis, R.J., 1992. The selective impact of herbivores. In: Fritz, R.S., Simms, E.L. (Eds.), Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago, IL, pp. 301–325.

Martínez-Crego, B., Arteaga, P., Tomas, F., Santos, R., 2016. The role of seagrass traits in mediating *Zostera noltei* vulnerability to mesograzers. PLoS One 11, e0156848. https://doi.org/10.1371/journal.pone.0156848.

Martínez-Crego, B., Arteaga, P., Ueber, A., Engelen, A.H., Santos, R., Molis, M., 2015. Specificity in mesograzer-induced defences in seagrasses. PLoS One 10, e0141219. https://doi.org/10.1371/journal.pone.0141219.

Mazaris, A.D., Schofield, G., Gkazinou, C., Almpanidou, V., Hays, G.C., 2017. Global sea turtle conservation successes. Sci. Adv. 3 (9), e1600730 https://doi.org/10.1126/sciadv.1600730.

McHenry, J., Rassweiler, A., Hernan, G., Uejio, C.K., Pau, S., Dubel, A.K., Lester, S.E., 2021. Modelling the biodiversity enhancement value of seagrass beds. Divers. Distrib. 27, 2036–2049. https://doi.org/10.1111/ddi.13379.

McKenzie, L.J., Nordlund, L.M., Jones, B.L., Cullen-Unsworth, L.C., Roelfsema, C., Unsworth, R.K.F., 2020. The global distribution of seagrass meadows. Environ. Res. Lett. 15, 074041 https://doi.org/10.1088/1748-9326/ab7d06.

McNaughton, S.J., 1983. Compensatory plant growth as a response to herbivory. Oikos 40, 329–336. https://doi.org/10.2307/3544305.

Merali, Z., Mayer, M.J., Parker, M.L., Michael, A.J., Smith, A.C., Waldron, K.W., 2012. Expression of a bacterial, phenylpropanoid-metabolizing enzyme in tobacco reveals essential roles of phenolic precursors in normal leaf development and growth.

Physiol. Plantarum 145, 260–274. https://doi.org/10.1111/j.1399-3054.2012.01583.x.

Moran, K., Bjorndal, K., 2005. Simulated green turtle grazing affects structure and productivity of seagrass pastures. Mar. Ecol. Prog. Ser. 305, 235–247. https://doi.org/10.3354/meps305235.

Morris, E.P., Peralta, G., Brun, F.G., van Duren, L., Bouma, T.J., Perez-Llorens, J.L., 2008. Interaction between hydrodynamics and seagrass canopy structure: spatially explicit effects on ammonium uptake rates. Limnol. Oceanogr. 53, 1531–1539. https://doi.org/10.4319/lo.2008.53.4.1531.

Morris, E.P., Peralta, G., Van Engeland, T., Bouma, T.J., Brun, F.G., Lara, M., Hendriks, I. E., Benavente, J., Soetaert, K., Middelburg, J.J., Lucas Perez-Llorens, J., 2013. The role of hydrodynamics in structuring in situ ammonium uptake within a submerged macrophyte community. Limnol. Oceanogr. Fluid. Environ. 3, 210–224. https://doi.org/10.1215/21573689-2397024.

Naiman, R.J., Décamps, H., McClain, M.E., Likens, G.E., 2005. Structural patterns. In: Ecology, Conservation, and Management of Streamside Communities. Riparia. Academic Press.

Páez-Osuna, F., Sanches-Cabeza, J.A., Ruiz Fernández, A.C., Alonso-Rodríguez, R., Piñón-Gimate, A., Cardoso-Mohedano, J.G., Flores-Verdugo, F.J., Carballo, J.L., Cisneros-Mata, M.M., Álvarez-Borrego, S., 2016. Environmental status of the Gulf of California: a review of responses to climate change and climate variability. Earth-Sci. Rev. 162, 253–268. https://doi.org/10.1016/j.earscirev.2016.09.015.

Peralta, G., 2000. Estudios sobre el crecimiento en *Zostera noltii* Hornem. Dinámica estacional y aspectos ecofisiológicos. PhD. Cádiz University.

Peralta, G., Godoy, O., Egea, L.G., de los Santos, C.B., Jiménez-Ramos, R., Lara, M., Brun, F.G., Hernández, I., Olivé, I., Vergara, J.J., González-Ortiz, V., Moreno-Marín, F., Morris, E.P., Villazán, B., Pérez-Lloréns, J.L., 2021. The morphometric acclimation to depth explains the long-term resilience of the seagrass *Cymodocea nodosa* in a shallow tidal lagoon. J. Environ. Manag. 299, 113452 https://doi.org/10.1016/j.jenvman.2021.113452.

Peralta, G., Pérez-Lloréns, J., Hernández, I., Vergara, J., 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. J. Exp. Mar. Biol. Ecol. 269, 9–26. https://doi.org/10.1016/S0022-0981(01)00393-8.

Pérez-Estrada, C.J., Rodríguez-Estrella, R., Brun-Murillo, F.G., Gurgo-Salice, P., Valles-Jiménez, R., Morales-Bojórquez, E., Medina-López, M.A., 2023. Diversity and seasonal variation of the molluscan community associated with the seagrass *Halodule wrightii* in a marine protected area in the southern Gulf of California. Aquat. Ecol. 57, 299–319. https://doi.org/10.1007/s10452-023-10011-3.

Pérez-Estrada, C.J., Falcón-Brindis, A., Rodríguez-Estrella, R., Morales-Bojórquez, E., Crespo-Domínguez, J.M., Brun-Murillo, F.G., 2021. Seasonal shifts in morphology, physiology and population traits in the seagrass *Halodule wrightii* (Cymodoceaceae) in a subtropical arid area. Aquat. Bot. 172, 103381 https://doi.org/10.1016/j.aquabot.2021.103381.

Preen, A., 1995. Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. Mar. Ecol. Prog. Ser. 124, 201–213. https://doi.org/10.3354/meps124201.

Prado, P., Collier, C.J., Romero, J., Alcoverro, T., 2011. Distinctive types of leaf tissue damage influence nutrient supply to growing tissues within seagrass shoots. Mar. Biol. 158, 1473–1482. https://doi.org/10.1007/s00227-011-1664-0.

Pujol, D., Abdolahpour, M., Lavery, P., McMahon, K., Oldham, C., 2019. Flow velocity and nutrient uptake in marine canopies. Mar. Ecol. Prog. Ser. 622, 17–30. https://doi.org/10.3354/meps12987.

Rasheed, M.A., 2004. Recovery and succession in a multi-species tropical seagrass meadow following experimental disturbance: the role of sexual and asexual reproduction. J. Exp. Mar. Biol. Ecol. 310, 13–45. https://doi.org/10.1016/j.jembe.2004.03.022.

Rasmann, S., Bauerle, T.L., Poveda, K., Vannette, R., 2011. Predicting root defence against herbivores during succession. Funct. Ecol. 25, 368–379. https://doi.org/10.1111/j.1365-2435.2010.01811.x.

Risandi, J., Rifai, H., Lukman, K.M., Sondak, C.F.A., Hernawan, U.E., Quevedo, J.M.D., Hidayat, R., Ambo-Rappe, R., Lanuru, M., McKenzie, L., Kohsaka, R., Nadaoka, K., 2023. Hydrodynamics across seagrass meadows and its impacts on Indonesian coastal ecosystems: a review. Front. Earth Sci. 11 https://doi.org/10.3389/feart.2023.1034827.

Rivera-Guzmán, N.E., Moreno-Casasola, P., Espinosa, E.C., Ruiz, A.E.L., Vega, C.M., Peralta-Peláez, L.A., Higueredo, L.E.S., Medina, K.P.A.R., Aguayo, K.V.S., 2017. The biological flora of coastal dunes and wetlands: *Halodule wrightii* Ascherson. J. Coast Res. 33, 938–948. https://doi.org/10.2112/JCOASTRES-D-14-00162.1.

Sanmartí, N., Saiz, L., Llagostera, I., Pérez, M., Romero, J., 2014. Tolerance responses to simulated herbivory in the seagrass *Cymodocea nodosa*. Mar. Ecol. Prog. Ser. 517, 159–169. https://doi.org/10.3354/meps11084.

Scott, A.L., York, P.H., Duncan, C., Macreadie, P.I., Connolly, R.M., Ellis, M.T., Jarvis, J. C., Jinks, K.I., Marsh, H., Rasheed, M.A., 2018. The role of herbivory in structuring tropical seagrass ecosystem service delivery. Front. Plant Sci. 9, 127. https://doi.org/10.3389/fpls.2018.00127.

Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., Erftemeijer, P.L.A., Fortes, M.D., Freeman, A.S., Jagtap, T.G., Kamal, A.H.M., Kendrick, G.A., Judson Kenworthy, W., La Nafie, Y.A., Nasution, I.M., Orth, R.J., Prathep, A., Sanciangco, J. C., Tussenbroek, B. van, Vergara, S.G., Waycott, M., Zieman, J.C., 2011. Extinction risk assessment of the world's seagrass species. Biol. Conserv. 144, 1961–1971. https://doi.org/10.1016/j.biocon.2011.04.010.

Sicard-González, M.T., Tripp-Valdéz, M.A., Ocampo, L., Maeda-Martínez, A.N., Lluch-Cota, S.E., 2012. Coastal sea surface temperature records along the Baja California Peninsula. CICIMAR Oceánides 27, 65–69. https://doi.org/10.37543/oceanides.v27i2.118.

Stockbridge, J., Jones, A.R., Gillanders, B.M., 2020. A meta-analysis of multiple stressors on seagrasses in the context of marine spatial cumulative impacts assessment. Sci. Rep. 10, 11934 https://doi.org/10.1038/s41598-020-68801-w.

Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185. https://doi.org/10.1016/S0169-5347(98)01576-6.

Strauss, S.Y., Zangrel, A.R., 2002. Plant–insect interactions in terrestrial ecosystems. In: Herrera, C.M., Pellmyr, O. (Eds.), Plant–animal Interactions. An Evolutionary Approach. Blackwell Science, Oxford, UK, pp. 77–106.

Tiffin, P., 2002. Competition and time of damage affect the pattern of selection acting on plant defense against Herbivores. Ecology 83, 1981–1990. https://doi.org/10.2307/3071780.

Tol, S.J., Coles, R.G., Congdon, B.C., 2016. Dugong dugon feeding in tropical Australian seagrass meadows: implications for conservation planning. PeerJ 4, e2194. https://doi.org/10.7717/peerj.2194.

Tomas, F., Abbott, J.M., Steinberg, C., Balk, M., Williams, S.L., Stachowicz, J.J., 2011. Plant genotype and nitrogen loading influence seagrass productivity, biochemistry, and plant–herbivore interactions. Ecology 92, 1807–1817. https://doi.org/10.1890/10-2095.1.

Tomas, F., Martínez-Crego, B., Hernán, G., Santos, R., 2015. Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. Global Change Biol. 21, 4021–4030. https://doi.org/10.1111/gcb.13024.

Valentine, J.F., Heck, K.L., 2021. Herbivory in seagrass meadows: an evolving paradigm. Estuar. Coast 44, 491–505. https://doi.org/10.1007/s12237-020-00849-3.

Valentine, J.F., Heck, K.L., Busby Jr., J., Webb, D., 1997. Experimental evidence that herbivory increases shoot density and productivity in a subtropical turtlegrass (*Thalassia testudinum*) meadow. Oecologia 112, 193–200. https://doi.org/10.1007/s004420050300.

Vergeer, L.H.T., Develi, A., 1997. Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. Aquat. Bot. 58, 65–72. https://doi.org/10.1016/S0304-3770(96)01115-1.

Vergés, A., Pérez, M., Alcoverro, T., Romero, J., 2008. Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish. Oecologia 155, 751–760. https://doi.org/10.1007/s00442-007-0943-4.

Vergés, A., Steinberg, P.D., Hay, M.E., Poore, A.G.B., Campbell, A.H., Ballesteros, E., Heck, K.L., Booth, D.J., Coleman, M.A., Feary, D.A., Figueira, W., Langlois, T., Marzinelli, E.M., Mizerek, T., Mumby, P.J., Nakamura, Y., Roughan, M., van Sebille, E., Gupta, A. Sen, Smale, D.A., Tomas, F., Wernberg, T., Wilson, S.K., 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. Proc. R. Soc. B Biol. Sci. 281, 20140846 https://doi.org/10.1098/rspb.2014.0846.

Villazán, B., Salo, T., Brun, F.G., Vergara, J.J., Pedersen, M.F., 2015. High ammonium availability amplifies the adverse effect of low salinity on eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 536, 149–162. https://doi.org/10.3354/meps11435.

Zimmerman, R.C., Kohrs, D.G., Alberte, R.S., 1996. Top-down impact through a bottom-up mechanism: the effect of limpet grazing on growth, productivity and carbon allocation of *Zostera marina* L. (eelgrass). Oecologia 107, 560–567. https://doi.org/10.1007/BF00333949.