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Superior growth traits of invaded (Caribbean) versus native (Red sea) populations of the seagrass *Halophila stipulacea*

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Abstract The seagrass *Halophila stipulacea* is native to the Red Sea. It invaded the Mediterranean over the past century and most of the Caribbean over the last two decades. Understanding the main drivers behind the successful invasiveness of *H. stipulacea* has become crucial. We performed a comprehensive study including field measurements, a mesocosm experiment, and a literature review to identify 'superior growth traits' that can potentially explain the

success story of *H. stipulacea*. We assessed meadow characteristics and plant traits of three invasive *H. stipulacea* populations growing off the Island of Sint Eustatius (eastern Caribbean). We compared similar parameters between native (Eilat, northern Red Sea) and invasive (Caribbean) *H. stipulacea* plants in a common-garden mesocosm. Lastly, we compared our field measurements with published data. The newly arrived *H. stipulacea* plants from St. Eustatius were characterized by higher percent cover, higher below- and above-ground biomasses, more apical shoots, and faster leaf turnover rates than those measured in

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both native and older invaded habitats. These results were further confirmed by the mesocosm experiment where the invasive *H. stipulacea* plants grew faster and developed more apical shoots than the native plants. Results suggest that increased growth vigour is one of the main invasive traits that characterize successful invasive *H. stipulacea* populations in the Caribbean and potentially in other invaded areas. We encourage long-term monitoring of *H. stipulacea* in both native and invaded habitats to better understand the future spread of this species and its impacts on communities and their ecosystem functions and services.

Introduction

Identifying typical traits that promote the success of invasive species has been one of the main topics of invasion biology in the last few decades (Moravcová et al. 2015). One approach for establishing these "invasive traits" is to compare, both in the field and/or in common garden setups, the differences in characteristics and performances between invasive and native species pairs (i.e., invasive species and similar species that are native to the invaded habitat) (Daehler 2003; Divíšek et al. 2018; Mathakutha et al. 2019). Another approach for establishing these "invasive traits" is to compare the traits of the same species in its native and invasive regions (i.e., comparing native vs. invasive populations). A fundamental question then arises: compared with their native regions, do invasive populations show similar, enhanced, or reduced "invasive trait" values (e.g., growth, reproduction, biomass, etc.) in their invaded region?

In the realm of plants, only a handful of studies have compared differences between native and invasive populations suggesting the answer to this question is that invasive populations have superior traits compared to the native population of the same species. For example, field surveys in North American wetlands showed that invasive populations of *Lythrum salicaria* (an invasive wetland plant) grew at higher densities than their counterparts growing in their native habitat (Edwards et al. 1998). Using both field and common garden comparisons, Zhu et al. (2020) demonstrated that invasive populations of the annual herb *Senecio vulgaris* exhibited greater plant sizes and more capitula than plants from native populations. Results from a common garden experiment (Caño et al. 2008) suggested that invasive populations of the shrub *Senecio pterophorus* (from north-east Spain) survived drought better and grew faster than plants from native populations (from South Africa). No such studies have been conducted on fully marine plants or macroalgae.

Halophila stipulacea is a tropical seagrass species, native to the Red Sea, the Persian Gulf, and the Indian Ocean (Winters et al. 2020). This species is characterized by high morphological and biochemical plasticity (Sharon et al. 2009; Rotini et al. 2017) which may partially explain its capacity to colonize new environments (Gambi et al. 2009; Winters et al. 2020). Following the opening of the Suez Canal in 1864, *H. stipulacea* became a Lessepsian migrant and has since become established, with a patchy distribution, in much of the eastern Mediterranean Sea (reviewed by Winters et al. 2020). With the recent doubling of the size of the Suez Canal (Galil et al. 2015) and the ongoing tropicalization of the Mediterranean (i.e., becoming warmer and saltier;Bianchi and Morri 2003; Borghini et al. 2014), a process that is happening even faster in the eastern Mediterranean (Pastor et al. 2019), *H. stipulacea* is predicted expected to become more prevalent in these waters in the coming years (Beca-Carretero et al. 2020b).

In 2002, *H. stipulacea* was reported for the first time from the Caribbean Sea (Ruiz and Ballantine 2004). While the spread of *H. stipulacea* in the Mediterranean Sea has been slow and limited (Gambi et al. 2009), in the Caribbean its spread has been rapid, reaching most eastern Caribbean islands, as well as the coast of South America, within less than a decade after its first sighting (Scheibling et al. 2018; Fig. 1A). In the Caribbean, *H. stipulacea* has physically displaced local seagrass species (e.g., *Syringodium filiforme, Halodule wrightii,* and *Halophila decipiens*) by monopolizing the spaces of these local seagrasses (Willette and Ambrose 2012), leading to

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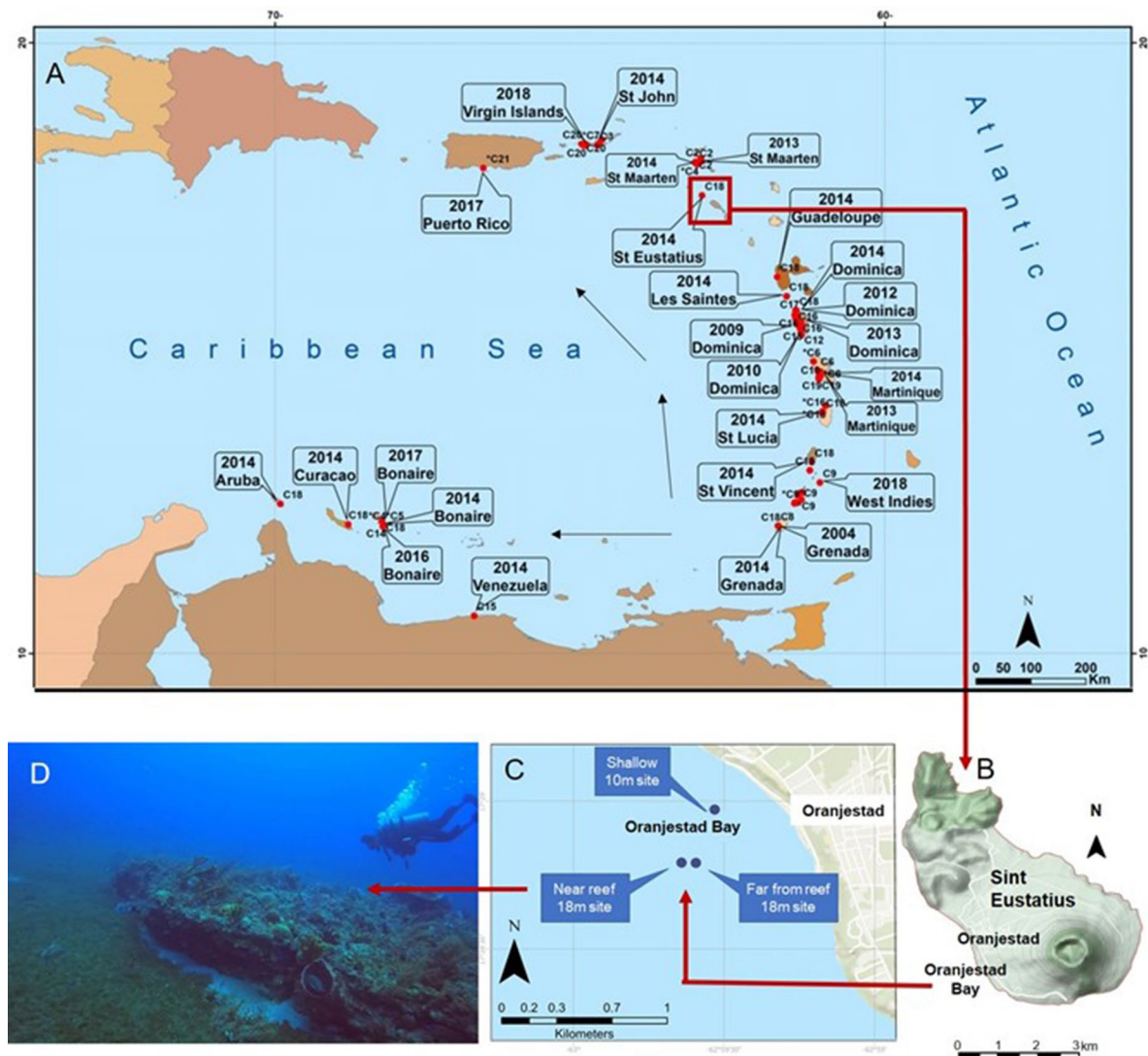
Fig. 1 Study sites. **A** Distribution of studies on *Halophila stipulacea* in the Caribbean. Numbers and labels refer to the appropriate source and year of the first report of *H. stipulacea* (see Table S1 in Winters et al. 2020). Black arrows represent the spreading of *H. stipulacea* (adapted from Winters et al.

2020). **B** Location of Oranjestad Bay (St. Eustatius, eastern Caribbean; Source: Google Maps) and (**C**) the three sampling sites (blue circles) within the bay (Source: ArcGIS Pro). **D** Invasive *H. stipulacea* growing right up to the coral reef at Near-reef 18 m site (Photograph: Winters G.)

major changes in the Caribbean's seagrass seascape (Steiner and Willette 2015b). Due to its invasive character, there is growing interest in better understanding the mechanisms that allowed the invasion success of *H. stipulacea* in the Caribbean. However, information about differences or similarities in traits between invasive and native populations (that may explain its success) is still missing.

In St. Eustatius, a relatively small (21 km^2) Dutch island in the eastern Caribbean (Fig. 1A-C), *H. stipulacea* was first sighted in 2012 (Fig. 1A–D). Since then, it has vastly displaced the native seagrasses *Syringodium filiforme* and *Thalassia testudinum* that previously dominated local meadows (Maitz et al. 2020). Around the island, *H. stipulacea* grows both far from and in close contact with Caribbean coral reef patches (Fig. 1D).

Our main objective was to assess whether biological traits associated with *H. stipulacea* are similar or different between its invaded vs. native habitats. Due to its very rapid invasion of the Caribbean, we hypothesized that the invasive population might have superior fitness-related (growth, reproduction, etc.) traits over the native populations from the northern Red Sea.

To this end, we (*i*) described plant and population traits of invasive *H. stipulacea* growing in three sites at St. Eustatius (eastern Caribbean; Fig. 1) to test for possible small-scale habitat-related differences concerning depth and proximity to the coral reef that may affect seagrass by the movement of grazers between habitats (potential negative effect) or fertilization from excretion or urination produced by reef dwellers such as fish (potential positive effect), (*ii*) measured several traits in invasive (St. Eustatius, Caribbean) and native (Gulf of Aqaba, GoA) *H. stipulacea* plants growing side by side, under the same controlled conditions in a mesocosm setup, and (iii) compared some traits and population descriptors from the population at St. Eustatius with published data (using the same methods) from the Caribbean, Mediterranean (150-year-old invasion) and northern Red Sea (where it is native) (e.g., Mejia et al. 2016; Rotini et al. 2017).

Materials and methods

Field sampling

Study sites

Field sampling was performed in Oranjestad Bay (St. Eustatius, eastern Caribbean; Fig. 1A- B) using SCUBA-diving between February 10th–24th 2020 representing the local dry season that runs between February-May, whereas the rainy season runs between October-January (https://www.meteo.cw/climate.php, accessed November 22nd, 2021). We compared *H. stipulacea* meadows from three different sites (Fig. 1C) including "Shallow 10 m" (17°28.934'N, 62°59.509'W; where *H. stipulacea* was growing in a dense meadow at 10 m), "Near-reef 18 m" (17°28.792'N, 62°59.641'W; where *H. stipulacea* was growing in dense meadows at 18 m neighboring the local coral reef, Fig. 1D) and "Far-reef

18 m" (17°28.792'N, 62°59.640'W; where *H. stipulacea* was growing in dense meadows at 18 m and 50 m away from the reef). The use of these three sites allowed us to compare (i) the effects of depth (two similar-looking meadows growing at different depths, 10 and 18 m—"Shallow 10 m" vs. "Far-reef 18 m") and (ii) the potential effect of growing near the coral reef with potential nutrient fertilization effects, or vice versa, grazing effects by reef fish ("Near-reef 18 m" vs. "Far-reef 18 m").

Environmental variables

Salinity at the three sites during our field campaign was 35.6 PSU (partial salinity units; measured using a WTW 340i). Annual averages of monthly sea-surface temperatures (SSTs) around St. Eustatius for 2020 (sampling year) were 28.1 ± 0.3 °C, with the coldest SSTs measured around February–March (26.8 °C; the time of sampling campaign) and the warmest SSTs around September–October (29.3 ± 0.1 °C; https://seatemperature.info, accessed November 22nd, 2021). In situ water temperatures during the sampling period in these three sites ranged between 25 and 26 °C, with daily means between 25.66 ± 0.05 °C to 26.85 ± 0.02 °C SE in Near-reef 18 m and Far-reef 18 m sites, respectively, while 26.44 ± 0.07 °C SE in Shallow 10 m (measured in situ using the miniDOT Logger, PME, California).

Daily data of SST (Jan 2019–March 2020) from both native (GoA) and invasive (Mediterranean and Caribbean Seas) habitats of *H. stipulacea* were acquired from NOAA's ERDDAP global oceanographic data server (https://coastwatch.pfeg.noaa.gov/erddap/griddap/jplMURSST41.html. accessed April 12th, 2022). Daily data of global irradiation on the horizontal plane at ground level (global horizontal irradiance, GHI; W m^{-2} day^{-1}) from both native (GoA) and invasive (Mediterranean and Caribbean Seas) habitats of *H. stipulacea* were acquired from Copernicus Atmosphere Monitoring Service (CAMS; http://www.soda-pro.com/web-services/radiation/cams-radiation-service; accessed September 22nd, 2021).

Plant traits

In all three sites, we used photo quadrats to measure the percentage of cover, cores to measure meadow

characteristics (e.g. shoot density, frequency of apical shoots, root density, leaf surface area and biomass), samples collected for biochemical analysis (pigment concentration and fatty acid composition) and in situ marking of plants for investigating growth rates (daily above- and below-ground biomass production rates, and rhizome elongation rate).

Seagrass cover Photo quadrats (50×50 cm) were taken along 30-m transects parallel to the shore ($n=15$ photographs with 2-m intervals) at each of the three sites using a custom-made tripod (Sony rx100 with 2 YS-D2 Sea & Sea strobe, Nauticam WWL Fisheye lens) enabling all images to be captured from a 90° angle and 30 cm distance from the meadows (Supplementary data, Fig. S2A). CoralNet (https://coralnet.ucsd.edu/; Beijbom et al. 2015) was used to calculate *H. stipulacea*'s percent cover with 25 randomly placed spots on each image. Results of seagrass cover were subsequently averaged for each site.

Meadow characteristics Cores (10 cm diameter, area = 78.54 cm^2, $n=7$–9 cores per site) were collected in random spots at each site, placed in Ziplock bags filled with seawater and brought back to the laboratory. In the laboratory, core content was sieved from the sediment and plants within each core were counted for their number of shoots (apical and non-apical) and roots, traits that provide a measure of seagrass abundance (Beca-Carretero et al. 2020a, b). Data from these cores were then normalized per m^{-2}. Subsequently, core material was separated into above-ground (leaves and sheaths) and below-ground (rhizomes and roots) compartments. Leaves were scanned (Canon Lide 120 digital scanner) for the measurement of leaf surface area (an important indicator of photosynthetic capacity in plants; Rotini et al 2017; Beca-Carretero et al. 2020a, b), using ImageJ (version 1.47; Abramoff et al. 2003). Colour threshold settings were kept constant between all images (brightness = 0.210) as was the scale (i.e., the ratio between the number of pixels per 1 cm). Measurements of leaves from the same core (12–15 leaves, from the 3rd–5th shoots only) were averaged and then data from all replicated cores from the same site were averaged. Lastly, dry weight (DW) was determined for above- and below-ground compartments after drying at 70 °C for 48 h. Results were normalized to one square meter and above-ground to below-ground dry-weight biomass

ratios (AG/BG) were calculated (Beca-Carretero et al. 2020a, b). Average shoot and root densities and biomass parameters represent means (± SE) of 7–9 biomass cores from each site.

Biochemical analysis The concentrations of total chlorophyll content (Chlorophyll a + Chlorophyll b) and total carotenoid content ($x+c$) were analysed in plants from the three sites. In the lab, samples for pigment evaluation ($n=5$ plants from each sampling site) were packed into aluminum foils and immediately frozen at −20 °C. Once frozen, samples were moved into a freeze drier and lyophilized. Then, 50 mg of freeze-dried plant material was crushed with mortar and pestle and moved to Eppendorf tubes where Chlorophyll extraction took place by adding 1 ml of 96% EtOH v/v. Extractions were kept overnight in the dark at 4 °C. After centrifugation, extracted pigments were quantified by reading spectrophotometer absorptions at 664.1, 648.6, 470, and 750 nm. The following equations (Lichtenthaler and Buschmann 2001) were used to evaluate the pigment contents:

$$\text{Chlorophyll } a(\text{mg/ml}) = 13.36\, A_{664.1} - 5.19\, A_{648.6} \tag{1}$$

$$\text{Chlorophyll } b(\text{mg/ml}) = 27.43\, A_{648.6} - 8.12\, A_{664.1} \tag{2}$$

$$\begin{aligned}&\text{Carotenoids } (x+c)(\text{ug/ml}) \\ &= \left(1000\, A_{470} - 2.13_{\text{Chl}a} - 97.64_{\text{Chl}b}\right)/209\end{aligned} \tag{3}$$

Pigment content was expressed as mg g^{-1} of leaves dry weight (DW) (Rotini et al. 2017).

The content and composition of fatty acids in *H. stipulacea* leaves were determined by modifying a protocol previously applied in leaves of other seagrass species (Beca-Carretero et al. 2018) including *H. stipulacea* (Beca-Carretero et al. 2019). Analyses were performed only on healthy leaf tissue (avoiding damaged areas or epiphytes) on the second youngest shoot (Beca-Carretero et al. 2019). Selected leaves were cleaned with filtered seawater before analysis. Leaf samples (1.5–2 g FW) were dried in 15–20 g of silica gel in small plastic bags for 12–24 h in darkness until the biomass was fully dehydrated (see protocol in Beca-Carretero et al. 2018). The samples were then kept at −20 °C until analyzed. 24-h before conducting the measurements samples were again freeze-dried to remove any residual humidity and ground up to a fine

powder using a bead mill homogenizer (Beadmill 4 machine, Fisher Scientific, USA) at 5 m s^{-1} for 3 min.

For the extraction of fatty acids, we used ~15 mg of freeze-dried powdered leaf material. To quantify total and individual fatty acid contents a known quantity of 0.1 mL of the saturated fatty acids (SFAs; 19:0 in 0.4 mg mL^{-1}) in Toluene (99%, Sigma Aldrich, catalog no. 89680) as an internal standard before starting the direct transmethylation. Fatty acid methyl esters (FAMEs) were obtained by direct transmethylation with 2 mL dry methanol containing 2% (v/v) H_2SO_4. Extraction of FAMEs was conducted at 78 °C for 2 h under continuous stirring. To prevent oxidation, vials were sealed with nitrogen gas before heating. After transmethylation, 1 mL of Milli-Q water was added and FAMEs later extracted using 0.5 mL of n-hexane. Identification of FAMEs was achieved by co-chromatography with authentic commercially available FAME standards of fish oil (Menhaden Oilcatalogueog no. 47116; Supelco) using an Agilent 7890A/5975C gas chromatography/mass selective detector (GC/MSD) Series (Agilent Technologies, USA) equipped with a flame ionization detector and a fused silica capillary column (DB-WAXETR, 0.25 mm×30 m×0.25 μm, Agilent Technologies, Catalogue No.: 122–7332). Results represent the mean of 4 replicates ($n=4$) ± SE.

In situ growth rates To directly measure growth rates, in each of the three sampling sites, we followed Azcárate-García et al. (2020). Briefly, eight "spots" were haphazardly chosen within the meadow (5 m away from each other) and in each spot, 3–5 plants were marked with a cable tie just behind the apical shoot (Supplementary data, Fig. S3). Selected plants within each spot were located approximately 50–100 cm apart from each other. Only plants with an apical shoot bearing four full-grown leaves were selected. These four leaves were excluded from analyses. After 7–8 days, marked plants were carefully harvested, and plants were enclosed in zip-lock bags full of seawater, and placed in cooler boxes for transport to the laboratory. In the laboratory, plant fragments were carefully cleaned and analysed. Daily above-ground biomass production rate (gDW plant^{-1} day^{-1}), belowground biomass production rate (gDW plant^{-1} day^{-1}) and rhizome elongation rate (cm plant^{-1} day^{-1}) were determined by measuring the new above- and belowground biomasses, rhizome elongation, respectively,

produced over the time interval (7–8 days) (Azcárate-García et al. 2020).

Comparing the performance of native and invasive populations using a mesocosm experiment

To compare growth rate and physiological functioning traits between the invasive and native populations, plants from the Caribbean (St. Eustatius) shallow site ("Shallow 10 m") were collected (February 20th, 2020; $n=150$ plants, each with 4–6 shoots, at a distance of 5 m away from each other), inserted in zip lock bags filled with seawater, brought back to the lab and prepared for transport the following morning. Twelve zip lock bags containing the seawater and seagrass individuals were inserted into a cooler box and flown to Israel (it took some 48 h from the seagrass collection in the Caribbean until planting in the Israeli mesocosm). Plants from the northern shores of the GoA ("North Beach", Eilat, Israel) were similarly collected (5th March 2020). Both invasive and native populations were planted in the seagrass-dedicated mesocosm (details on this mesocosm and conditions within, can be found inOscar et al. 2018; Nguyen et al. 2020b; Szitenberg et al. 2022) that included 20 aquaria (60L each, layered with 7 cm of Eilat's sediment), 10 with invasive Caribbean plants, and another 10 with native Eilat plants (12–14 shoots in each aquarium; (Supplementary data, Fig. S4). Plants were kept under the following conditions: 40 PSU (partial salinity units; salinity levels that fit the year-round average of the Eilat population) 25 °C and 250 μmol photons m^{-2} s^{-1} at the aquarium's surface (photoperiod L:D 12:12 h). Previous experiments in our group (Oscar et al. 2018) tested the effects of different salinities on *H. stipulacea* from Eilat and demonstrated a very wide salinity tolerance (ranging between 20 and 60 PSU), with smaller ± 10 PSU changes from the natural salinity levels (40 PSU) having no effects on seagrass photophysiology, it was assumed that for the Caribbean population, the change from 35.6 to 40.0 PSU was negligible. Irradiance levels were chosen to fit the values at the collection depth (10 m, both sites). In all aquaria, water temperatures were kept at 25 °C to mimic spring conditions and to prevent flowering (Malm 2006; Nguyen et al. 2018), which would have added further variability to the measurements.

After 5 months of acclimation to the mesocosm conditions (March–August 2020; we were unable to

access the lab for experimental work due to COVID-19 lockdowns), two plants in each aquarium were marked with a cable tie just behind the apical shoot (Fig. S3; Azcárate-García et al. 2020). Measurements took place after another two weeks and included maximum quantum yield of photosystem II (F_v/F_m; Diving-PAM, Walz, Germany), overall shoot formation rates (number of shoots day^{-1}), apical shoot formation rates (number of apical shoots day^{-1}), apical shoot frequency (%), rhizome elongation rates (cm plant^{-1} day^{-1}) and leaf formation rates (number of new leaves plant^{-1} day^{-1}). For each measurement, we averaged results from two plants per aquarium ($n=9$–10 different aquaria for each population).

Comparisons with previous studies

To assess how Oranjestad Bay's *H. stipulacea* meadows and plant characteristics/traits might be similar or different from other *H. stipulacea* meadows in the Caribbean and elsewhere, we updated (the recent "*Halophila stipulacea* database" (based on 164 "*Halophila stipulacea*" publications. see Table S1 in Winters et al. 2020 for details). We updated this database using the same methodology (currently containing a total of 171 studies; Table 4). Plant-related parameters from this database, including *H. stipulacea's* percent cover, shoot and root densities, frequency of apical shoots, and above- and below-ground biomasses, were collected and compared across all regions with our data from Oranjestad Bay.

Statistical analysis

For the field sampling, the effects of the site (3 sites in St. Eustatius) on the different plant traits measured were evaluated using a one-way Analysis of Variance (one-way ANOVA). Before the analysis, the assumptions of normality were evaluated using the Shapiro–Wilk normality test (Sokal and Rohlf 2012) and homogeneity of variances using Levene's test (Levene 1960; Sokal and Rohlf 2012). When the parametric assumptions were not met, data were analyzed using Kruskal–Wallis test (Daniel 1990). A Tukey HSD post hoc test was applied whenever significant differences were determined. For the mesocosm

experiment, the effect of the population (i.e., Eilat versus the Caribbean) was assessed using a two-sample student *t*-test (Cressie and Whitford 1986). Before the analysis, the homogeneity of variance assumption was validated using the *F* test (Lix et al. 1996). In case the homogeneity of variance assumption was not met, data were analyzed using Welch Two Sample Student *t*-test (Keselman et al. 2004). All statistical analyses were conducted in R-studio v. 2021.09.0 (R Core Team 2020). Graphs were made with R-studio using the *ggplot2* package (Wickham 2009). Boxplots represent the medians and quartiles (0.25, 0.75).

Results

Field sampling and comparisons with previous studies

Meadow characteristics in Oranjestad Bay and comparisons with previous studies

In all three sites sampled within Oranjestad Bay, the percent cover of *H. stipulacea* was very high (90–97%; Fig. 2A, D Fig. S2B). The highest cover (97%) was found at the shallowest site (Shallow 10 m), followed by the deeper (18 m) Near- (95.6%) and Far-reef sites (90.6%, significantly lower than the other two sites; One-Way ANOVA, $p < 0.01$, Fig. 2A). Shoot density (numbers of shoots m^{-2}; Fig. 2B) was highest in the shallow site (8348 ± 649 SE shoots m^{-2}), followed by the deeper sites (18 m) near- (7543 ± 1066 SE shoots m^{-2}) and Far-reef (6351 ± 399 SE shoots m^{-2}). Differences in shoot density between sites were not significant (One-Way ANOVA, $p = 0.3$; Table 2). The frequency of apical shoots ranged between 23 and 28% for deep and shallow plants, respectively, but differences between sites were not significant (One-Way ANOVA, $p > 0.05$; Table 2; Fig. 2C). Root density significantly differed between sites (One-Way ANOVA, $p < 0.01$; Fig. 2D, Table 2) with the highest values found in shallow site (6475 ± 658 SE roots m^{-2}), followed by the Near- and Far-reef 18 m sites (4790 ± 664 SE and 3083 ± 570 SE roots m^{-2}, respectively).

Percent cover in the Oranjestad Bay sites was found to be higher than what was reported from the Red Sea ($\sim 80\%$) and the average of data for

Fig. 2 Meadow characteristics in Oranjestad Bay and comparisons with previous studies. **A** Percent cover, **B** shoot density, **C** frequency of apical shoots, and (**D**) root density. Different small letters indicate statistical significant differences between study sites (Tukey HSD post hoc test, $p < 0.05$). No statistical analysis was done for the data collected from previous studies. The three left boxplots represent measured data while the three right boxplots represent published data. Boxplots represent the medians and quartiles (0.25, 0.75)
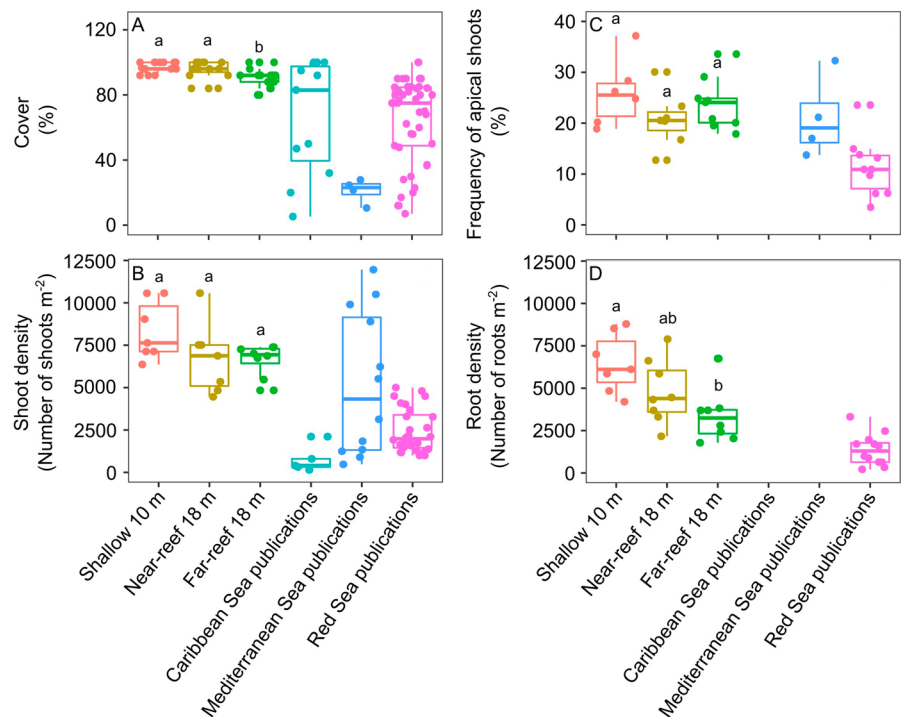
seagrass cover reported from the Mediterranean Sea (~ 25%) (Fig. 2A). Shoot densities reported here were much higher than values previously reported from the Caribbean Sea (Fig. 2B) but seem to be similar to some sites in the Mediterranean. This is the first report (as far as we know) of the frequency of apical shoots from the Caribbean *H. stipulacea*, and it shows that the occurance of apical shoots in the newy invasive Caribbean is slightly and much higher than previously reported from the Red and Mediterranean Seas, respectively (Fig. 2 C).

Biomass in Oranjestad Bay and comparisons with previous studies

Coherently with the percent cover and shoot density data, shallow plants from Oranjestad Bay had the highest above-ground (AG) biomass (Fig. 3A), significantly different from AG biomass of Far-reef plants (one-way ANOVA, $p < 0.05$, Table 2). Conversely, the differences detected among sites in values of below-ground (BG biomass and AG/

BG biomass ratios (Fig. 3B, C) were not significant (One-way ANOVA, $p = 0.731$ and $p = 0.505$, respectively; Table 2). Medians of both AG and BG biomass values from this study were higher than what was previously documented from the Mediterranean Sea and the Red Sea; previous studies from the Caribbean Sea have so far not reported on such parameters (Fig. 3A, B). AG/BG ratios published from the Red Sea seem to be higher than ratios from the Mediterranean and Carribean Seas (Fig. 3C).

Biochemical and morphological results

Deeper *H. stipulacea* plants demonstrated classic photo adaptations to lower-light environments. Compared to the shallower plants, Near- and Far-reef 18 m plants, significantly increased their total chlorophyll and total carotenoid contents (One-way ANOVA, $p < 0.01$, Table 2; Fig. 4A, B). In addition to these biochemical adaptations to low light, deeper plants showed classic morphometric adaptations. Leaf surface area in shallow plants (240.6 ± 17.8 SE mm^2) was smaller than in deeper plants growing in the
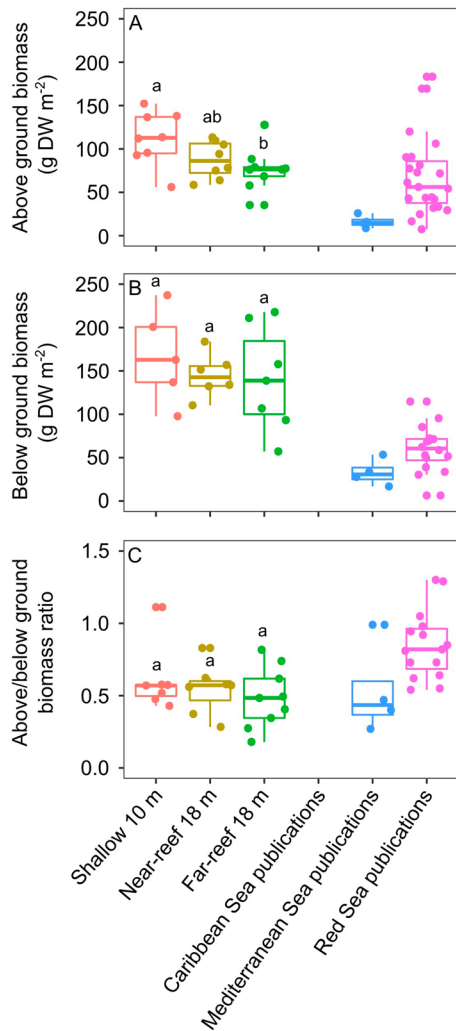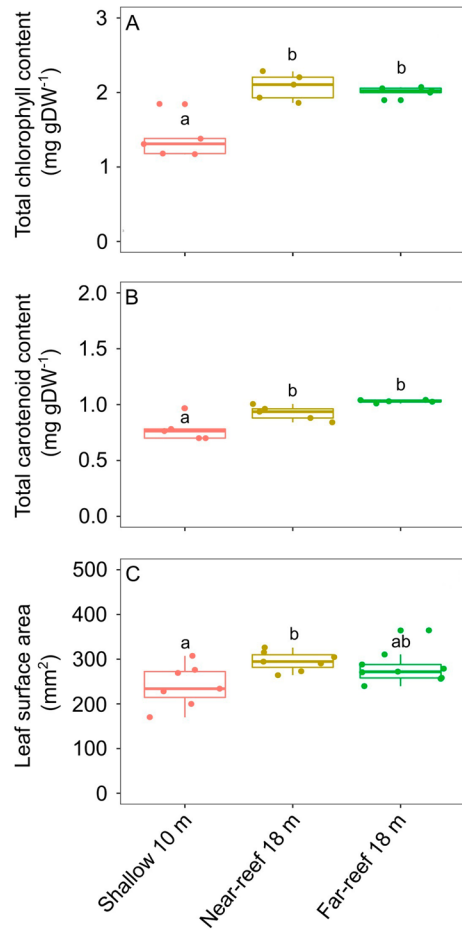
Fig. 3 Meadow biomass in Oranjestad Bay and comparisons with previous studies. **A** Above-ground (AG) biomass, **B** Below-ground (BG) biomass and (**C**) Above/below ground biomass ratios (AG/BG). Different small letters indicate statistical significant differences between study sites (Tukey HSD post hoc test, $p < 0.05$). No statistical analysis was done for the data collected from previous studies. The three left boxplots represent measured data while the three right boxplots represent published data. Boxplots represent medians and quartiles (0.25, 0.75)

Fig. 4 Biochemical and morphological measurements of samples from Oranjestad Bay. **A** Total chlorophyll, **B** and carotenoid content, and (**C**) leaf surface area. Different small letters indicate statistical significant differences between study sites (Tukey HSD post hoc test, $p < 0.05$). Boxplots represent medians and quartiles (0.25, 0.75)

near-reef (295.4 ± 8.2 SE mm^2) and Far-reef (281 ± 12 SE mm^2) sites (Fig. 4C), however, these differences were significant only for shallow plants vs. Near-reef plants (one-way ANOVA, $p = 0.029$; Table 2).

Total fatty acid (TFA) content and FA profiles of *H. stipulacea* leaves harvested from the three study sites in the Caribbean are summarized in Table 1.

Plants from near-reef 18 m accumulated significantly more TFA (0.75 ± 0.1 SD, % DW) than plants from the Far-reef 18 m (0.54 ± 0.1 SD, % DW) or the Shallow 10 m (0.57 ± 0.1 SD, % DW; one-way ANOVA, $p < 0.001$, Table 2) sites. Plants from shallow 10 m had a significantly higher percentage of saturated fatty acid (SFA) than their deep counterparts (one-way ANOVA, $p < 0.01$, Tables 1 and 2) mostly explained by depletions in the production of C16:0 (Palmitic acid; Table 1). Plants from both deeper sites had a significantly higher percentage of polyunsaturated fatty acid (PUFA) than plants from the shallow site (one-way ANOVA, $p < 0.01$, Table 2). These

Table 1 Fatty acid content results. Data are means ($n=4$) $\pm$ SD. Small letters indicate statistical differences between sites (Tukey HSD post hoc test, $p < 0.05$)

		Shallow 10 m	Near-reef 18 m	Far-reef 18 m
TFA (% DW)		0.57 ± 0.1^a	0.75 ± 0.1^b	0.54 ± 0.1^c
Percent saturated FA	C14:0 Myristic acid	0.73 ± 0.1	0.64 ± 0.16	1.14 ± 0.33
	C15:0 Pentadecylic acid	0.5 ± 0.14	0.45 ± 0.09	0.79 ± 0.4
	C16:0 Palmitic acid	49.38 ± 3.3	34.8 ± 5.09	38.9 ± 2.49
	C17:0 Margaric acid	1.19 ± 0.94	0.58 ± 0.05	0.89 ± 0.33
	C18:0 Stearic acid	4.47 ± 0.41	3.35 ± 0.58	4.22 ± 0.32
	C20:0 Arachidic acid	0.93 ± 0.06	0.84 ± 0.47	1.07 ± 0.38
	Total SFA (% TFA)	57.2 ± 3.7^a	40.66 ± 6.12^b	47.04 ± 3.12^b
Percent monounsaturated FA	C16:1w9 Hexadecenoate	0.87 ± 0.3	0.48 ± 0.22	0.59 ± 0.13
	C16:1w7 Palmitoleate	2.5 ± 1.31	3.49 ± 0.41	3.05 ± 0.12
	C17:1w7 Heptadecenoic acid	3.07 ± 1.42	3.27 ± 0.91	3.92 ± 1.17
	C18:1w9 Oleic acid	2.89 ± 1.29	1.8 ± 0.3	1.96 ± 0.34
	C18:1w7 Vacecenic acid	0.29 ± 0.09	0.29 ± 0.1	0.4 ± 0.08
	Total MUFA (% TFA)	9.65 ± 1.18^a	9.34 ± 1.28^a	9.95 ± 0.9^a
Percent polyunsaturated FA	C16:3w3 α-Linolenic acid	1.46 ± 0.52	2.71 ± 0.38	2.88 ± 0.9
	C18:2w6 Linoleic acid	12.19 ± 3.18	12.8 ± 1.9	11.62 ± 1.4
	C18:3w3 Linolenic acid	19.49 ± 2.63	34.45 ± 5.2	28.48 ± 2.1
	Total PUFA (% TFA)	33.15 ± 3.89^a	50 ± 7.3^b	43 ± 3.3^b
	PUFA/SFA	0.58 ± 0.08^a	1.23 ± 0.26^b	0.91 ± 0.09^{ab}
Fatty acid percent of total FA	Percent w3 (Omega-3)	20.95 ± 2.91^a	37.15 ± 5.6^b	31.3 ± 2^b
	Percent w6 (Omega-6)	11.37 ± 3.18^a	11.98 ± 1.9^a	12.3 ± 1.4^a
	w3/w6	1.84 ± 0.58^a	3.10 ± 0.67^b	2.54 ± 0.33^b

higher PUFA levels are mostly driven by higher levels of n-3 PUFA (C16:3 n-3 and C18:3 n-3) than of n-6 PUFA (C18:2 n-6; Table 1). In terms of monounsaturated FA (MUFA), the levels were highly similar among sites (one-way ANOVA, $p = 0.821$, Table 1). Moreover, significant differences were assessed for PUFA/SFA, percent w3 (Omega-3) and (w3/w6 ratios (Table 1).

In situ growth rates

The three study sites did not differ significantly in their in situ AG and BG biomass production rates and their rhizome elongation rates (Fig. 5, Kruskal–Wallis test, $p > 0.05$, Table 2).

Comparing the performance of native and invasive populations using a mesocosm experiment

Results from the mesocosm experiment showed that the maximum quantum yield of photosystem II (F_v/F_m) did not differ significantly between invasive and native populations, demonstrating that they were equally photo-acclimated to the light environment in the mesocosm (*t*-test, $p = 0.22$, Fig. 6A; Table 3). Nonetheless, for all other measurements (overall shoot formation rate, apical shoot formation rate, apical shoot frequency, rhizome elongation rate, and leaf formation rate), native plants exhibited significantly lower values compared with their invasive counterparts (Fig. 6B-F; Two Sample *t*-test and Welch Two Sample *t*-test: $p < 0.05$, Table 3).

Table 2 Results from one-way ANOVA or Kruskal–Wallis tests on different plant traits to evaluate the effects of the site (data from the field measurements); *df* degrees of freedom, *MS* mean square, *F*: F value from one-way ANOVA, χ^2: chi-squared from Kruskal–Wallis test. Significant codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

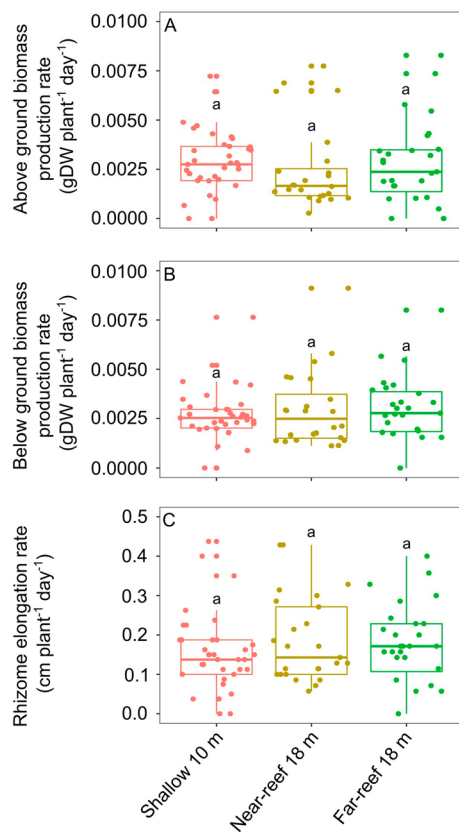| Measurement	Statistical analysis	df	MS	F	χ^2	p
Percent cover	One-way ANOVA	2	166.40	7.05		**
Shoot density		2	5,608,817	1.28		0.300
Frequency of apical shoots		2	52.66	0.87		0.435
Root density		2	17,954,465	5.96		**
Above-ground biomass (AG)		2	2805.20	4.20		*
Below-ground biomass (BG)		2	2728	0.32		0.731
AG:BG biomass ratio		2	0.03	0.71		0.505
Total chlorophyll content		2	0.74	19.76		***
Total carotenoid content		2	0.08	14.03		***
Leaf surface area		2	5775	4.25		*
Total fatty acid (TFA)		2	0.05	302.60		***
Percent Saturated FA (SFA)		2	281.72	13.51		**
Percent Monounsaturated FA (MUFA)		2	0.29	0.20		0.821
Percent Polyunsaturated FA (PUFA)		2	292.2	11.59		**
PUFA/SFA		2	0.49	10.30		**
Percent w3 (Omega-3)		2	259.54	18.77		***
Percent w6 (Omega-6)		2	1.314	0.30		0.752
w3/w6	Kruskal–Wallis test	2			8.35	*
Daily AG biomass production rate		2			1.55	0.462
Daily BG biomass production rate		2			1.20	0.550
Daily rhizome elongation rate		2			1.82	0.402

Discussion

The results of this study support our working hypothesis that the invasive population of *H. stipulacea* in Oranjestad Bay has superior growth traits compared to the native populations of the northern Red Sea, but also compared to the populations that invaded the Mediterranean Sea much earlier. This is an important finding because it can, at least partially, explain the rapid spread of this species in the Caribbean region compared to the much slower invasion in the Mediterranean Sea. It demonstrates trait differences (mostly related to growth) between the native and invasive populations of a marine invader. The results presented here point to similar "invasive traits" that were previously associated with invasive species, but on the invasive population level.

Ecophysiological traits of *H. stipulacea* meadows from St. Eustatius

Caribbean *H. stipulacea* plants from Oranjestad Bay showed morphological and biochemical adaptations to increasing depth (entailing reduced irradiance and wave exposure) as in other native and invaded habitats (Rotini et al. 2017; Beca-Carretero et al. 2019; Nguyen et al. 2020a) and other seagrass species (Olesen et al. 2002). Shallow-site (10 m) plants formed meadows with higher percent cover, shoot and root densities, and above- and below-ground biomasses than plants from deeper sites (18 m). The higher root densities found in the shallower site, compared with the deeper sites could be related to the higher hydrodynamics and storm effects at the shallow site. In the Caribbean Sea, tropical storms and hurricanes are regular disturbances with strong effects on shallow communities (Dorothy and Heck 2006). Similarly, the frequency of apical shoots was slightly higher in shallow plants than in their deeper

Fig. 5 In situ growth rates. **A** Above-ground biomass production rate, **B** Below-ground biomass production rate, and (**C**) rhizome elongation rate. Different small letters indicate statistical significant differences between study sites (Kruskal–Wallis test, $p > 0.05$)

summer, the below-ground biomass was higher in the shallow sites, but during the winter, the below-ground biomass was higher in the deeper sites.

We visited the Caribbean sites only once (Feb 2020), and do not have data from other seasons to compare with. However, it is possible that due to their geographical location (i.e., closer to the equator), seasonal changes (e.g., dry vs. wet season; Fig. S1) in the Caribbean may not have the same affect on local seagrasses as shown in other regions with more distinct seasons (e.g., GoA, the region of Beca-Carretero et al. 2020a's study). Further monitoring in Sint Eustatius and elsewhere in the region, will indicate if there is a seasonal pattern in the Caribbean.

As in other photophysiological studies (Schwarz and Hellblom 2002; Rotini et al. 2017), deeper plants tend to have larger leaves with higher pigment content (i.e., higher chlorophyll and carotenoid concentrations) and omega-3 PUFA than leaves of plants growing in shallow waters; this enables plants to compensate for the reduced irradiance at their deep habitat.

Fatty acid profiles of St. Eustatius' invasive *H. stipulacea* displayed a clear pattern with increases in PUFA relative to SFA with increasing depth and particularly, increases in omega-3 relative to omega-6 (i.e., increase in w3/w6 ratios). These results clearly show an increment in the unsaturation levels of *H. stipulacea* leaves with attenuation of irradiance. Similar patterns were previously reported in other marine primary producers including both tropical (*H. stipulacea*) and temperate (*Zostera marina*) seagrasses (Beca-Carretero et al. 2019, 2020a). Increases in the levels of unsaturated fatty acids with increasing depths have been suggested as a photo-physiological adaptive mechanism in primary producers; high levels of unsaturated fatty acids levels (n-3 PUFA) contribute to the fluidity of the thylakoid membranes and enhance the electron transport between photosystems, optimizing the photosynthetic performance under low light conditions (Klyachko-Gurvich et al. 1999). Besides, higher SFA in shallower populations in comparison with deeper plants can be associated with the capacity of primary producers to accumulate the excess energy produced in photosynthesis, under more favourable environmental conditions, as energetic reserves, mainly as triglycerides (TAG) which are also found in seagrass leaves (Koelmel et al. 2019). On the other hand, total fatty acid (TFA)

counterparts probably due to higher irradiance, as already reported in native plants (Azcárate-García et al. 2020), and/or the stronger hydrodynamics in this depth.

Finding that shallower plants displayed significantly higher above-ground biomass compared with their deeper counterparts is similar to some of the work by Beca-Carretero et al. (2020a). Working in the northern GoA, they showed that above-ground biomass in shallow habitats was higher than in their deeper sites, in both summer (July) and winter (February) months. In the study presented here, we did find slightly higher below-ground biomass in shallow vs. deeper plants as well, but this was not significant. Results by Beca-Carretero et al. (2020a) demonstrated that there might be a seasonal pattern for the below-ground plant compartment: during the

Fig. 6 Comparisons between native (Eilat) and invasive (St. Eustatius) *H. stipulacea* plants growing in the mesocosm. **A** maximum quantum yield of photosystem II (F_v/F_m) (**B**) daily shoot, **C** apical shoot formation rates, **D** apical shoot frequency, **E** daily rhizome elongation, and **F** leaf formation rates. Different small letters indicate statistical significant differences between populations from the results of the two-sample student *t*-test or Welch two sample *t*-test ($p < 0.05$)
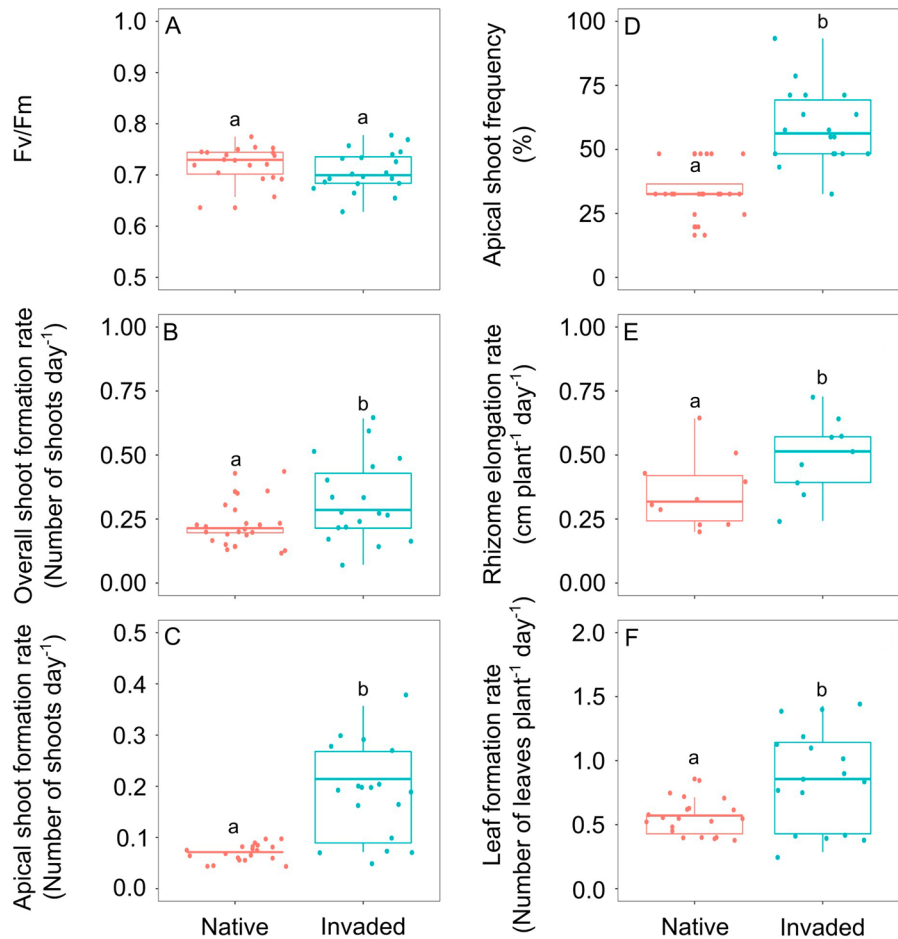
Table 3 Results from either two sample students *t*-tests or Welch two sample *t*-tests that were used to test the effects of populations (native [Eilat] and invasive [St. Eustatius]) on different plant traits measured in the mesocosm experiment. Degrees of freedom (df) and *t*-values are shown. Significant codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Measurement	Statistical analysis	df	*t*	*p*
Maximum quantum yield of photosystem II (F_v/F_m)	Two sample *t*-test	38	1.25	0.220
Overall shoot formation rate	Welch two sample *t*-test	24	− 2.29	*
Apical shoot formation rate	Welch two sample *t*-test	17	− 6.51	***
Apical shoot frequency	Two sample *t*-test	36	− 6.15	***
Rhizome elongation rate	Two sample *t*-test	17	− 2.11	*
Leaf formation rate	Welch two sample *t*-test	19	− 3.19	**

contents of Caribbean *H. stipulacea* were found to be similar across the three sites sampled at St. Eustatius, their values ranging between 0.54 and 0.75 ± 0.1 SD % DW.

Although we hypothesized that proximity to the coral reef might affect neighbouring seagrass (by the movement of grazers between habitats), we found no significant differences, in any of the parameters we measured, between the deeper 18-m Near-reef and Far-reef populations *H. stipulacea*.

Comparisons of native-invasive plants growing under controlled conditions confirmed the superior growth traits of invasive plants

Invasive (St. Eustatius) and native (Eilat) *H. stipulacea* plants, acclimated and growing under the same conditions (e.g., light, sediment, water temperature, and salinity), did not differ in their photo physiological capacity. However, the Caribbean plants were found to have a higher percentage of apical shoots than the Eilat ones, confirming field results showing the same pattern. While there is a lack of data from other sites in the Caribbean to conclude if this important trait is shared among *H. stipulacea* populations in the entire Caribbean region, interestingly, invasive *H. stipulacea* plants from Cyprus also had a higher percentage of apical shoots and longer internode distances compared to native plants from the northern GoA growing at similar depths (Nguyen et al. 2020a).

Our mesocosm comparisons also showed that plants from St. Eustatius had higher horizontal elongation and shoot formation rates than their native counterparts, suggesting that invasive plants grew faster and in many more directions in parallel, potentially enhancing the invasive success of *H. stipulacea* in St. Eustatius. It should be noted that seagrasses are clonal plants, whereby the plant modules are "repeated" via cell division at the apical rhizome meristem (i.e., apical shoots are the basis for seagrass clonal growth; Sintes et al. 2006). The enhanced clonal shoot formation found here, along with the demonstrated ability of fragments to settle (Smulders et al. 2020), might be one of the key traits to its invasiveness and rapid spread of *H. stipulacea* in the Caribbean. Moreover, the high frequency of apical shoots might be particularly advantageous since they grow mostly horizontally, unlike other seagrass species, such as *Posidonia oceanica* and *Zostera marina*, where most of the biomass growth is concentrated in the vertical axis. Taken together, these traits could contribute to the invasive plant's abilities to (*i*) rapidly occupy more space and (*ii*) escape from unfavorable new environments. This trait might be of particular importance especially since sexual reproduction has so far never been documented in the Caribbean (Smulders et al. 2020), surprising given the high rate of recruitment and expansion. Similarly, evidence of sexual reproduction of *H. stipulacea* in

the Mediterranean is limited to reports on the presence of female flowers (Gerakaris and Tsiamis 2015), or the presence of both female and male flowers side by side (Nguyen et al. 2018), with no reports so far on fruits. The reports from the Mediterranean might indicate that sexual reproduction in its historic invasive habitat is rare, as is the situation in the Caribbean. These morphological traits can potentially compensate for the lack of sexual reproduction and at the same time contribute to the invasiveness of *H. stipulacea* in the Mediterranean – its historic invasive habitat (Gambi et al. 2009; Thibaut et al. 2022) and Caribbean Seas – its recent invasive habitat (Steiner and Willette 2015b, a).

Comparisons with other studies: invasive plants showed superior growth traits

Although *H. stipulacea* has been growing in Oranjestad Bay for only a decade (Maitz et al. 2020), it has developed meadows that are higher in density and percent cover than native and historically invaded habitats (Table 4).

The differences in root densities between depths found here but also in native meadows from the GoA at similar depths (Beca-Carretero et al. 2020a) suggest that increased root densities and underground biomass, could be general traits of shallow, wave-exposed meadows.

Overall, the values of meadow traits from St. Eustatius were also higher than those measured in the shallow waters (3–4 m) of the historically invasive *H. stipulacea* populations growing in the eastern Mediterranean Sea (Limassol, Cyprus; Table 4, 5–25% cover, 800–1800 shoots m^{-2}, 5–25 g DW m^{-2} and 18–55 g DW m^{-2}, respectively; Nguyen et al. 2020a). However, the values of meadow traits from St. Eustatius were closer to newly colonized invasive habitats in the central Mediterranean Sea (Salerno, Italy) where Gambi et al. (2009, 2018) reported on shallow (2–4 m) newly formed *H. stipulacea* mono-specific meadows with shoot densities of $6{,}230 \pm 1{,}644$ (June 2007; Gambi et al. 2009) and $10{,}500 \pm 2700$ (October 2017; Gambi et al. 2018).

Interestingly, the frequency of apical shoots in Oranjestad Bay's *H. stipulacea* plants (ranging between 23 and 28%) was higher than the frequency measured in Red Sea sites (Azcárate-García et al. 2020; Nguyen et al. 2020a) but closer to the frequency

of apical shoots in their historic invasive habitat of Cyprus (ranging year-round 15–33%; Nguyen et al. 2020a). Thus, in both its new and historic invasive habitats, *H. stipulacea* plants were characterized by higher frequencies of apical shoots, compared with plants from the native habitat.

Leaves from Caribbean plants were larger (ranging from 240.6 to 295.4 mm^2 across the three sites) and with higher chlorophyll concentration than leaves growing in GoA's pristine sites at similar depths and sampled during the equivalent season (July, ranging from 150 to 185 mm^2; Beca-Carretero et al. 2019, 2020a, b). These adaptations of *H. stipulacea* plants growing around St. Eustatius could be due to lower irradiance received compared to their counterparts growing at similar depths in both the Red and Mediterranean Seas (Supplementary data, Fig. S1). At least for the summer (dry) months, this was also demonstrated by the daily GHI data acquired from CAMS (Supplementary data, Fig. S1). Indeed, Winters et al. (2003) pointed out the very low percent of annual cloudiness (< 15%) in the arid GoA, which would explain the much higher year-round average GHI (5799 W m^{-2} day^{-1}), compared to that in the much cloudier tropical Caribbean, where year-round average GHI was much lower (5375 W m^{-2} day^{-1}). When comparing across different regions, particularly notable was the relatively high carotenoid concentrations in the St. Eustatius sites, compared to measurements from native plants growing in Eilat's sites (Rotini et al. 2017). It was indeed surprising to find that carotenoid content was ten times higher in invasive *H. stipulacea* plants compared to the values previously measured in native plants (Rotini et al. 2017; Beca-Carretero et al. 2019). Studies on other seagrass species showed an increase in carotenoid content under thermal stress (i.e. *Z. noltii*, Repolho et al. 2017) or low light (*T. testudinum*; Cayabyab and Enríquez 2007).

The total fatty acid (TFA) contents of Caribbean *H. stipulacea* invasive plants were less than half that of native plants (Red Sea, Eilat) growing at similar depths (ranging from 1.23 to 1.60 ± 0.01 SD; Beca-Carretero et al. 2019). Both SFAs, Palmitic acid (Fatty acid C16:00) and Stearic acid (Fatty acid C18:00), were higher in Caribbean shallow vs. deeper *H. stipulacea* plants, and interestingly, both acids had higher concentrations in these populations than in

the native plants from Eilat growing at similar depths (Beca-Carretero et al. 2019). Besides, PUFA contents and particularly n-3 PUFA (omega-3) from Caribbean plants (~ 28% n-3 PUFA of TFA was more related to other tropical or subtropical species including species belonging to the family *Halophila*, whereas, the composition of plants from the Red Sea (~ 46% n-3 PUFA of TFA) were more likely related to temperate seagrasses (Beca-Carretero et al. 2019, 2022). These differences can reflect different diverse in situ environmental conditions, but also can indicate that the Caribbean and the Red Sea plants are different lineages. In this line, the use of fatty acid signatures was implemented to differentiate marine primary producers to differentiate among phyla, orders, and families (Galloway et al. 2012).

The comparison of the in-situ growth rates from Oranjestad Bay with those recently reported by Azcárate-García et al. (2020) for native *H. stipulacea* plants growing in the GoA at 5 and 14 m, indicated that the Caribbean plants might be faster growing than their native counterparts. This hypothesis was further confirmed when we compared the Caribbean and native plants from the northern GoA (Eilat) under controlled conditions (see above).

Lastly, in the marine realm, it is surprising how few studies have compared traits of invasive versus native populations (Google Scholar search terms "marine native and invasive populations"; but see Wright (2005)). Recent work has compared native *H. stipulacea* from the Red Sea, with historically invasive populations from the Mediterranean Sea, both in the field (Nguyen et al. 2018, 2020a, 2020b) and in mesocosm conditions (Nguyen et al. 2020b; Wesselmann et al. 2020). To date, these Red vs. Mediterranean comparisons have shown differences in flowering patterns, a shift in thermal tolerance of the invasive plants to colder (winter) water temperatures, and a higher tolerance to thermal stress compared with native plants. In parallel, recent work on *H. stipulacea* in the Mediterranean Sea, where it was so far widespread but highly fragmented, has modelled and projected rapid expansion of *H. stipulacea* as the result of the ongoing tropicalization of this basin (Beca-Carretero et al. 2020b). The indices used in St. Eustatius were shown to be relevant both at regional and local scales (Kirkman 1996; Prathep et al. 2010;

Pergent et al. 2015) and together they provide information on several different trait levels from individual to population.

Conclusions

To the best of our knowledge, the study presented here is the first-ever comparison of Caribbean invasive and Red Sea native populations of *H. stipulacea*, and one of very few that compared traits between native and invasive marine organisms. Our results are further evidence of the ecological plasticity of *H. stipulacea*. The pattern shown here, vigorous growth rates and more apical shoots in the invasive plants, is similar to patterns shown in terrestrial plants, supporting the idea that increased growth vigor one of the main "invasive traits" that characterize successful invasions of plants.

While our findings suggest a trait advantageous to the invasive *H. stipulacea* population (see also Nguyen et al. 2020b), we acknowledge that for both the mesocosm and the field sampling parts of this study, we used plants from only one invasive Caribbean site (Oranjestad Bay), and one native site (north beach, Eilat). To test whether the traits described here are indeed widespread (i.e., across the Caribbean) or rather a localized phenomenon (e.g., only the population from Oranjestad Bay vs. the only north beach population), we call for future studies to include more populations from both invasive (e.g. Caribbean and Mediterranean Seas) and native (Red Sea, Indian Ocean, Persian Gulf) habitats.

Finally, we encourage and recommend the long-term coordinated monitoring of *H. stipulacea* both in its native and invaded habitats using the methodology shown here, which would improve our predictions on the future invasiveness of this species (e.g., is it likely to spread throughout the Caribbean or expand into the western Mediterranian) and its impacts on local habitats.

Declarations

Conflict of interest All authors have no relevant financial or non-financial competing interests to disclose.

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