

1 **Title:** Seasonal dynamics of native and invasive *Halophila stipulacea* populations – a case  
2 study from the northern Gulf of Aqaba and the eastern Mediterranean Sea.

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19 **Short communication**

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33 **Abstract**

34 The tropical seagrass *Halophila stipulacea* is native to the Red Sea, Persian Gulf and the Indian  
35 Ocean. Following the opening of the Suez Canal, *H. stipulacea* became a Lessepsian  
36 immigrant, spreading to most of the eastern Mediterranean Sea. Its arrival in the Caribbean,  
37 where it has changed the local seagrass landscapes, has led to concerns about its potential  
38 effects on Mediterranean seagrass diversity. Surprisingly, morphological, growth, structural  
39 and demographic and ecological traits have never been quantitatively compared between native  
40 and invasive populations of *H. stipulacea*.

41 This study used a standardized methodology to provide the first quantitative comparison between  
42 populations of native and invasive *Halophila stipulacea* and sheds ~~a~~ light on the importance of  
43 long-term monitoring in both native (Red Sea) and invasive (Mediterranean and Caribbean  
44 Seas) regions. Results from our study are important for understanding the current population  
45 dynamics of *H. stipulacea* in both regions and could be used as baseline data ~~in~~ for future  
46 assessments.

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48 **Keywords:** *Halophila stipulacea*, seagrass monitoring, invasive species, seagrass cover

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59 **1. Introduction**

60 The seagrass *Halophila stipulacea* (Forsskal) Ascherson is a dioecious, small tropical species,  
61 native to the Red Sea, Persian Gulf, and Indian Ocean (Lipkin 1975). Soon after the opening  
62 of the Suez Canal in 1869, *H. stipulacea* became a Lessepsian migrant. Since its first report in  
63 the island of Rhodes in 1894, *H. stipulacea* has spread throughout most of the eastern and  
64 southern Mediterranean basins (Lipkin 1975, Gambi et al. 2009, Sghaier et al. 2011).

65 In 2002, ~~Halophila H.~~ *stipulacea* was reported for the first time in Grenada, in the Caribbean  
66 Sea (reviewed by Willette and Ambrose 2012). In just over 10 years, it has spread to most of

67 the Eastern Caribbean island nations (Willette and Ambrose 2012) and has even reached the  
68 South American continent (Vera et al. 2014). Studies from the Caribbean have demonstrated  
69 the invasiveness of *H. stipulacea* by showing that *H. stipulacea* is physically displacing local  
70 Caribbean seagrass species (e.g. *Syringodium filiforme*, *Halophila decipiens*, and *Halodule*  
71 *wrightii*; Willette and Ambrose 2012, Steiner and Willette 2015a), while in parallel it has also  
72 expanded into sand ‘halos’ and the margins of coral reefs, where other seagrasses usually do  
73 not grow (Steiner and Willette, 2015b). Taken together, these processes ~~–This phenomenon~~  
74 ~~has~~have transformed been changing the Caribbean’s seagrass landscapes (Steiner and Willette,  
75 2015a).  
76 ~~through three different schemes including (1) “native strongholds” of sheer native seagrasses,~~  
77 ~~On a side note, I have included a link to the original article by Willette and Ambrose (2012) for reference.~~  
78 Considering the highly invasive character displayed by *Halophila stipulacea* in the Caribbean,  
79 the ongoing tropicalization of the Mediterranean Sea (Bianchi and Morri 2003) together with  
80 the recent expansion of the Suez Canal (Galil et al. 2015) and the increasing mortality of the  
81 Mediterranean’s native seagrasses in an era of rapid global change (Jordà et al. 2012), there is  
82 growing concerns about the expansion of *H. stipulacea* in the Mediterranean and its potential  
83 effects on the native temperate seagrass species in this basin. Thus, it is surprising to know that  
84 only limited data are available regarding the year-round population dynamics of *H. stipulacea*  
85 *stipulacea* in its original distributional ranges (e.g. within the Gulf of Aqaba [GoA]), where it  
86 is considered as the dominant seagrass species (Wahbeh 1988; Cardini et al. 2018), and no  
87 year-round data at all exists for *H. stipulacea* from the Mediterranean, where it has the potential  
88 to trigger significant changes to the local seagrass communities (Sghaier et al. 2014).  
89 Lack of information regarding the year-round dynamics of both native and invasive populations  
90 of *Halophila stipulacea* limits our understanding of the current population dynamics of this  
91 seagrass species in both these sites but also hinders future conservation and management efforts  
92 directed at seagrasses in both habitats.  
93 Here we report on the first-ever year-round quantitative comparison between native (Eilat,  
94 Israel, northern Gulf of Aqaba, northern Red Sea) and invasive (Limassol, Cyprus, eastern  
95 Mediterranean Sea) populations of *Halophila stipulacea*. The Results presented here provide  
96 knowledge about the population dynamics of *H. stipulacea* from both its native and invasive  
97 ranges. This knowledge, also, can serve as baseline data ~~to facilitate for~~ future monitoring  
98 efforts in these regions.

## 99 2. Methods

100 2.1. Setup of monitoring sites

101 50-meter long permanent transects were set up at 3-4 m depth at both the native site (Eilat,  
102 northern Gulf of Aqaba, northern Red Sea, Israel; 29°34'48"N, 34°57'33"E; Fig. 1c,e,g) and at  
103 the invasive site (Limassol, eastern Mediterranean Sea, Cyprus; 34°42'20"N, 33°07'24"E; Fig.  
104 1b,d,f). Transects were marked with labelled plastic poles and floats for easy identification,  
105 allowing for revisiting once every three months. The transects (one in each site) were visited  
106 seasonally in 2017 as follows: spring (Eilat: 18<sup>th</sup> March; Limassol: 9<sup>th</sup> April), summer (Eilat:  
107 3<sup>rd</sup> July; Limassol: 9<sup>th</sup> July), autumn (Eilat: 03<sup>rd</sup> October; Limassol: 29<sup>th</sup> September) and winter  
108 (Eilat: 25<sup>th</sup> December; Limassol: 2<sup>nd</sup> December) (Fig. 1h).

109 2.2. Sea surface temperature

110 To compare environmental temperatures between study locations in Eilat and Limassol, we  
111 obtained daily average sea surface temperatures for the period of 2017 from the NOAA  
112 dOISST.v2 dataset at [www.ngdc.noaa.gov](http://www.ngdc.noaa.gov). We used Advanced Very High-Resolution  
113 Radiometer (AVHRR) only data, due to its longer temporal span and because it has been shown  
114 to out-perform other datasets in coastal areas (Lima and Wethey 2012).

115 2.3. Seagrass measurements

116 Roca et al. (2016) showed that in small seagrasses (e.g. *Halophila* sp.), morphological, growth,  
117 structural and demographic traits were particularly responsive to both a suit of stressors and  
118 the recovery from them. Following these traits provides a basic understanding of the  
119 population's dynamics, seasonal changes, alongside basic population characteristics and  
120 general environmental quality (Roca et al. 2016). These traits also tend to be relatively cheap  
121 and easy to apply, providing an opportunity for citizen science programs to join future  
122 monitoring of *H. stipulacea*.

123 Seagrass cover was assessed in replicated photo-quadrats (50x50 cm, n=10) every 5 m along  
124 the 50 m transects and the taken photos were processed using the CoralNet Platform (Beijbom  
125 et al. 2015) by applying 100 random points per quadrat.

126 Plant materials were collected from 25x25 cm quadrats (n=4 in each site and season), placed  
127 along the transect (~10 m away from each other). Samples were collected and transported to  
128 the laboratory in zip-lock bags filled with seawater for further measurements.

129 Shoot density was calculated as the total number of shoots per m<sup>2</sup>.

130 Fresh plant material was separated into above-ground (leaves) and below-ground (rhizomes  
131 and roots) compartments and oven-dried at 70°C for 24 hours to obtain above- and below-  
132 ground dry biomass (g DW m<sup>-2</sup>) and their ratios.

133 Data from replicated quadrats in each season were then averaged and normalized to m<sup>2</sup>.

134 Thirty mature-undamaged leaves from each quadrat were digitally scanned (CanoScan LiDE  
135 220, Canon U.S.A., Inc, USA) and images were processed with ImageJ  
136 (<https://imagej.nih.gov/ij/>) to estimate the leaf surface area (cm<sup>2</sup>).

137 Percentage of apical shoots (shoot apical meristems) and internode distance was assessed  
138 throughout all collected samples.

#### 139 2.4. Statistical analyses

140 ~~Statistically,~~ Permutational multivariate analysis of variance (PERMANOVA) was performed  
141 to assess the significant difference between native vs. invasive sites, seasons (i.e. spring  
142 [March-April 2017], summer [July 2017], autumn [Sep-Oct 2017], and winter [Dec 2017]) and  
143 the interaction between sites and seasons. Data were analyzed on the resemblance matrix  
144 (created by S17 BrayCurtis similarity) on Primer 6 v.6.1.16 + PERMANOVA v. 1.0.6  
145 (Anderson et al. 2008) with site and season treated as fixed factors and 9999 permutations.  
146 ~~Following PERMANOVA was followed by a pair-wise test was performed to detect~~  
147 ~~significant differences between seasons of each population separately.~~

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### 149 3. Results and discussion

150 Results show that percent of seagrass cover (Fig. 2a) was higher year-round in the native site  
151 (Eilat) compared with its invasive site (Limassol; PERMANOVA, Pseudo-F =155.6,  $p_{(perm)} =$   
152 0.0001). This is not surprising since *Halophila stipulacea* is the dominant and often the only  
153 seagrass species in Eilat ~~where (i.e. it is found)~~ growing in a monospecific meadows ~~(Fig. 1g;~~  
154 Winters et al. 2017) while in Limassol, *H. stipulacea* has to compete with other native species  
155 ~~and is usually (here it is found) growing in a mixed meadows (Fig. 1f).~~ ~~In the native population,~~  
156 ~~the highest percent of *H. stipulacea* cover was found in the summer, for the native population,~~  
157 while ~~in~~ the Cyprus ~~invasive~~ population, ~~percent of cover developed to a maximum relatively~~  
158 ~~was highest~~ during autumn (Fig. 2a). This could be explained by two possible reasons, (1) the  
159 invasive *H. stipulacea* could be limited by the fast-growing neighbouring Mediterranean  
160 *Cymodocea nodosa* which reaches a maximum during the summer (Cancemi et al. 2002) and  
161 (2) the much colder winter (~16°C) in Limassol than in Eilat (~21°C; Fig. 1h) might extend  
162 the recovery time of the invasive plants before flowering takes place during the summer months  
163 (Nguyen et al. 2018). As a result, we observed significant differences in season  
164 (PERMANOVA, Pseudo-F =4.5192,  $p_{(perm)} = 0.0041$ ) as well as the interaction between  
165 seasons and sites (PERMANOVA, Pseudo-F =13.815,  $p_{(perm)} = 0.0001$ ) in percent of cover.  
166 ~~(Eilat vs. Limassol; PERMANOVA, Pseudo-F =4.5192,  $p_{(perm)} = 0.0041$  and Pseudo-F~~  
167 ~~=13.815,  $p_{(perm)} = 0.0001$ , respectively).~~

168 ~~It is interesting to see~~ ~~We also noticed~~ ~~note~~ that shoot density was ~~somehow~~ similar between both populations during spring, autumn  
169 and winter ~~while but very different in the summer due to the~~ extremely higher shoot density in the  
170 native population during summer time (Fig. 2b) that leads to the significant difference between  
171 sites (Eilat vs. Limassol; PERMANOVA, Pseudo-F =4.979,  $p_{[perm]} = 0.019$ ).

172 Results for the above-ground biomass (Fig. 2c) reflected what was found in the percent cover.  
173 Above-ground dry biomass was significantly higher year-round in plants from Eilat compared  
174 with their invasive counterparts (Fig. 2c, PERMANOVA, Pseudo-F =24.741,  $p_{[perm]} =$   
175 0.0001), with highest above-ground biomass found in Eilat during the summer, but in ~~October~~  
176 ~~the autumn~~ for the Cyprus population.

177 In the invasive population, below-ground biomass (Fig. 2d) started to increase from the autumn  
178 and reached the highest value during spring (PERMANOVA, Pseudo-F =2.4767,  $p_{[perm]} =$   
179 0.0261). This phenomenon corresponds with the hypothesis that the invasive plants were using  
180 their below-ground energy to survive the cold Mediterranean winter (Figure 1h). The year-  
181 round above- to below-ground biomass ratios in Eilat plants were much higher than that of  
182 their Cyprus counterparts (never less than 0.7; Fig. 2e), indicating that relatively, in Eilat plants,  
183 more biomass was being accumulated above-ground compared with the Cyprus population.  
184 The significant interaction between sites and sampling seasons found for the above- to below-  
185 ground biomass ratios (PERMANOVA, Pseudo-F =~~4.15882.565~~,  $p_{[perm]} =$ ~~0.00420.0235~~)  
186 confirms that both of these populations modified their above to below-ground biomass ratios  
187 but at different seasons (Figure 2e). Above- to below-ground biomass ratios in invasive plants  
188 were less than 0.5 most of the year (spring, summer, and winter) indicating that majority of the  
189 biomass of these plants during most of the year was below ground. The accumulation of  
190 underground biomass could potentially help these plants store energy in their “underground  
191 storage” to better overcome the cold winter (Marín-Guirao et al. 2018). The year-round above-  
192 to below-ground biomass ratios in Eilat plants were much higher than that of their Cyprus  
193 counterparts (never less than 0.7), indicating that relatively, more biomass was being  
194 accumulated above ground compared with the Cyprus population. Highest above- to below-  
195 ground ratios were found in Cyprus in the autumn (close to 1.0) compared with maximal ratios  
196 in the Eilat plants that were measured in the summer (close to 1.2).

197 Although found growing in similar depths, in terms of leaf surface area (Fig. 2f), native plants  
198 were found to be significantly larger year-round than leaves from invasive plants  
199 (PERMANOVA, Pseudo-F =123.21,  $p_{[perm]} = 0.0001$ ). The fact that leaf area might be affected  
200 by different environmental parameters at the different sites might be indicated by the  
201 significant differences between seasons and a significant season×site interaction

202 (PERMANOVA, Pseudo-F = 12.103,  $p_{[perm]} = 0.0001$  and Pseudo-F = 7.7689,  $p_{[perm]} = 0.0003$ ,  
203 respectively). Changes in leaf area may help *Halophila stipulacea* plants to optimize their  
204 carbon balances. In Eilat where there are relatively small changes in water temperature  
205 throughout the year (21-27°C; Fig. 1h), changes in leaf area reflect the seasonal changes in light  
206 in this region (Winters et al. 2006). Minimal leaf areas in the Eilat's summer could indicate  
207 photoacclimation to the intense irradiance experienced during this season, while maximal leaf  
208 areas in the winter probably indicate attempts to compensate for the relatively low light in his  
209 season. In contrast, in the invasive population, smaller leaves during spring and winter can  
210 strengthen the ability of invasive *H. stipulacea* plants to cope better with the colder  
211 temperatures experienced in this region (17-18°C), as compared with warmer waters of Eilat  
212 (21°C). A similar mechanism has been demonstrated in terrestrial plants (Milford and Riley  
213 1980). Indeed, results showed that the invasive plants increased their leaf size during autumn  
214 ( $2.09 \text{ cm}^2 \pm 0.14 \text{ SE}$ , Fig. 2f), when water temperatures were more favourable. Shoot density  
215 results (Fig. 2b) concur with the results from above-ground biomass and leaf surface area.  
216 Invasive plants produced a similar number of shoots as native plants during the spring and  
217 winter, but smaller leaf surface area year round (Fig. 2f) resulted in lower above-ground  
218 biomass (Fig. 2d).  
219 Furthermore, our results demonstrate some of the different morphology that might be  
220 associated with the invasive characteristic of *Halophila stipulacea* in the Mediterranean ranges  
221 via a higher percentage of apical shoots and longer internode distances. Having significantly  
222 more apical shoots and longer internode distances (Fig. 2g,h; Eilat vs. Limassol,  
223 PERMANOVA, Pseudo-F = 6.7344,  $p_{[perm]} = 0.0178$  and Pseudo-F = 15.74,  $p_{[perm]} = 0.0015$ ,  
224 respectively) could contribute to the ability of the invasive plants to (i) rapidly occupy more  
225 space and (ii) escape from un-favourable new environments. These ~~invasive~~ morphological  
226 traits can potentially contribute to the invasiveness of *H. stipulacea* in the Mediterranean  
227 (Gambi et al. 2009) and Caribbean Seas (Steiner and Willette 2015).  
228 Although *Halophila stipulacea* was included in the "100 Worst Invasive Alien Species in the  
229 Mediterranean" (Streftaris and Zenetos 2006), in this basin, evidence for its "invasive"  
230 characteristics are scarce. In the Mediterranean, *H. stipulacea* has been continuously spreading  
231 westwards and northwards (Lipkin 1975, Gambi et al. 2009, Sghaier et al. 2011) and was  
232 experimentally predicted to spread throughout the whole Mediterranean Sea in the coming  
233 future (Georgiou et al. 2006, Nguyen et al. *in review*). With the semi-enclosed Mediterranean  
234 Sea becoming warmer and saltier (Bianchi and Morri 2003), it has been predicted that the



235 ongoing tropicalization of the Mediterranean Sea might be causing declines in local  
236 Mediterranean temperate seagrasses species (Jordà et al. 2012), while favouring the expansion  
237 of the tropical invasive *H. stipulacea* (Georgiou et al. 2016, Gambi et al. 2009). Evidence for  
238 the invasive characteristics of *H. stipulacea* in the Mediterranean includes observations by  
239 Sghaier et al. (2014) that showed in Tunisia, that introduced *H. stipulacea* was taking over the  
240 meadows of the local Mediterranean seagrass species (i.e. *Cymodocea nodosa*). Work by  
241 Chiquillo et al. (*in prep.*) has recently experimentally shown that both in the Caribbean and the  
242 Mediterranean Seas, *H. stipulacea* grows better with local native species than by itself, hinting  
243 to the potential mechanism of *H. stipulacea*'s success in its new invasive habitats.

~~244 Indeed, the limited data available for year round population dynamics of *Halophila stipulacea*  
245 within the Gulf of Aqaba, where it is considered the dominant and sometimes only (Winters et  
246 al. 2017) seagrass species (Wahbeh 1988, Cardini et al. 2018) is worrying.~~

247 On the other hand, tropicalization (Bianchi and Morri 2003) of the Mediterranean invasive  
248 habitats, accompanied by the recent doubling of the Suez Canal (Galil et al. 2015), could  
249 potentially (i) facilitate the further spreading of *Halophila stipulacea*, (ii) enhance its ability to  
250 outcompete local seagrass species (Sghaier et al. 2014) or (iii) broaden its stability to occupy  
251 newly available habitat following predicted extirpation of local Mediterranean seagrass species  
252 (Jordà et al. 2012). Although *H. stipulacea* has yet been on the main agenda of seagrass  
253 research and monitoring efforts in Mediterranean waters, we emphasize that now is the time to  
254 put more effort into studying and monitoring this seagrass species.

255 It is important to note that in this study, we used only one population from each basin and  
256 visited each site only once per season. Considering the exponentially growing human pressures  
257 on coastal areas, specifically in the crowded shores of Mediterranean and the northern GoA,  
258 we highlight the need for coordinated monitoring (e.g. this study) and mapping efforts (e.g.  
259 Winters et al. 2017) that will focus on recording changes over time and space in *Halophila*  
260 *stipulacea* and associated communities in multiple sites both its native and invasive ranges. ~~In  
261 addition to field based efforts, simulated mesocosm studies answering the question about the  
262 future of the seagrass *H. stipulacea* both natively and invasively are incredibly crucial in an  
263 era of rapid global change (Oliver et al. 2018).~~

264  
265 **Funding:** This work was partially funded by a short term scientific mission (STSM) awarded  
266 to GW and DK through COST Action CA15121 (Advancing marine conservation in the  
267 European and contiguous seas = MarCons). We thank the Arava International Center for  
268 Agriculture Training (AICAT) for giving Hung Manh Nguyen financial support during his

269 M.Sc. We thank the Manna Center Program for Food Safety & Security, at the School of  
270 Plant Sciences and Food Security Faculty of Life Sciences Tel Aviv University (TAU) for  
271 their ongoing support for the co-op between TAU and ADSSC. [We thank the Israeli Ministry  
272 of Science and Technology \(MOST\) for their continued support of the ADSSC.](#)  
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