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

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Abstract

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

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

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

1. Introduction

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

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

the Eastern Caribbean island nations (Willette and Ambrose 2012) and has even reached the South American continent (Vera et al. 2014). Studies from the Caribbean have demonstrated the invasiveness of *H. stipulacea* by showing that *H. stipulacea* is physically displacing local Caribbean seagrass species (e.g. *Syringodium filiforme*, *Halophila decipiens*, and *Halodule wrightii*; Willette and Ambrose 2012, Steiner and Willette 2015a), while in parallel it has also expanded into sand ‘halos’ and the margins of coral reefs, where other seagrasses usually do not grow (Steiner and Willette, 2015b). Taken together, these processes - This phenomenon has have transformed been changing the Caribbean’s seagrass landscapes (Steiner and Willette, 2015a).

~~through three different schemes including (1) “native strongholds” of sheer native seagrasses,~~

Considering the highly invasive character displayed by *Halophila stipulacea* in the Caribbean, the ongoing tropicalization of the Mediterranean Sea (Bianchi and Morri 2003) together with the recent expansion of the Suez Canal (Galil et al. 2015) and the increasing mortality of the Mediterranean's native seagrasses in an era of rapid global change (Jordà et al. 2012), there is growing concerns about the expansion of *H. stipulacea* in the Mediterranean and its potential effects on the native temperate seagrass species in this basin. Thus, it is surprising to know that only limited data are available regarding the year-round population dynamics of *H. stipulacea* in its original distributional ranges (e.g. within the Gulf of Aqaba [GoA]), where it is considered as the dominant seagrass species (Wahbeh 1988; Cardini et al. 2018), and no year-round data at all exists for *H. stipulacea* from the Mediterranean, where it has the potential to trigger significant changes to the local seagrass communities (Sghaier et al. 2014).

Lack of information regarding the year-round dynamics of both native and invasive populations of *Halophila stipulacea* limits our understanding of the current population dynamics of this seagrass species in both these sites but also hinders future conservation and management efforts directed at seagrasses in both habitats.

Here we report on the first-ever year-round quantitative comparison between native (Eilat, Israel, northern Gulf of Aqaba, northern Red Sea) and invasive (Limassol, Cyprus, eastern Mediterranean Sea) populations of *Halophila stipulacea*. ~~The R~~[Results presented here](#) provide knowledge about the population dynamics of *H. stipulacea* from both [its](#) native and invasive ranges. ~~This knowledge, also,~~ can serve as baseline data ~~to facilitate for~~ future monitoring efforts in [these](#) regions.

2. Methods

2.1. Setup of monitoring sites

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

2.2. Sea surface temperature

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

2.3. Seagrass measurements

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

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

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

Shoot density was calculated as the total number of shoots per m².

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

Data from replicated quadrats in each season were then averaged and normalized to m².

Thirty mature-undamaged leaves from each quadrat were digitally scanned (CanoScan LiDE 220, Canon U.S.A., Inc, USA) and images were processed with ImageJ (<https://imagej.nih.gov/ij/>) to estimate the leaf surface area (cm²).

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

2.4. Statistical analyses

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

3. Results and discussion

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

168 ~~It is interesting to see~~ ~~We also noticed~~ ~~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

(PERMANOVA, Pseudo-F=12.103, $p_{[perm]} = 0.0001$ and Pseudo-F=7.7689, $p_{[perm]} = 0.0003$, respectively). Changes in leaf area may help *Halophila stipulacea* plants to optimize their carbon balances. In Eilat where there are relatively small changes in water temperature throughout the year (21-27°C; Fig. 1h), changes in leaf area reflect the seasonal changes in light in this region (Winters et al. 2006). Minimal leaf areas in the Eilat's summer could indicate photoacclimation to the intense irradiance experienced during this season, while maximal leaf areas in the winter probably indicate attempts to compensate for the relatively low light in his season. In contrast, in the invasive population, smaller leaves during spring and winter can strengthen the ability of invasive *H. stipulacea* plants to cope better with the colder temperatures experienced in this region (17-18°C), as compared with warmer waters of Eilat (21°C). A similar mechanism has been demonstrated in terrestrial plants (Milford and Riley 1980). Indeed, results showed that the invasive plants increased their leaf size during autumn ($2.09 \text{ cm}^2 \pm 0.14 \text{ SE}$, Fig. 2f), when water temperatures were more favourable. Shoot density results (Fig. 2b) concur with the results from above-ground biomass and leaf surface area. Invasive plants produced a similar number of shoots as native plants during the spring and winter, but smaller leaf surface area year round (Fig. 2f) resulted in lower above-ground biomass (Fig. 2d).

Furthermore, our results demonstrate some of the different morphology that might be associated with the invasive characteristic of *Halophila stipulacea* in the Mediterranean ranges via a higher percentage of apical shoots and longer internode distances. Having significantly more apical shoots and longer internode distances (Fig. 2g,h; Eilat vs. Limassol, PERMANOVA, Pseudo-F=6.7344, $p_{[perm]} = 0.0178$ and Pseudo-F=15.74, $p_{[perm]} = 0.0015$, respectively) could contribute to the ability of the invasive plants to (i) rapidly occupy more space and (ii) escape from un-favourable new environments. These ~~invasive~~ morphological traits can potentially contribute to the invasiveness of *H. stipulacea* in the Mediterranean (Gambi et al. 2009) and Caribbean Seas (Steiner and Willette 2015).

Although *Halophila stipulacea* was included in the "100 Worst Invasive Alien Species in the Mediterranean" (Streftaris and Zenetos 2006), in this basin, evidence for its "invasive" characteristics are scarce. In the Mediterranean, *H. stipulacea* has been continuously spreading westwards and northwards (Lipkin 1975, Gambi et al. 2009, Sghaier et al. 2011) and was experimentally predicted to spread throughout the whole Mediterranean Sea in the coming future (Georgiou et al. 2006, Nguyen et al. *in review*). With the semi-enclosed Mediterranean Sea becoming warmer and saltier (Bianchi and Morri 2003), it has been predicted that the

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

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

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

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

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