Short communication

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Differences in flowering sex ratios between native and invasive populations of the seagrass Halophila stipulacea

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Abstract: Deviations from the 1:1 sex ratio are common in dioecious plants. The tropical seagrass Halophila stipulacea is among an extremely rare group of dioecious plants that are widely recognized as female-biased. Here we report on differences in sex ratios between native (Eilat, northern Red Sea) and invasive (Cyprus, Mediterranean Sea) populations. While H. stipulacea populations were female-biased in their native region, invasive populations were either male- or female-biased. The existence of both sexes simultaneously in the Mediterranean invasive populations might help its ongoing expansion in the Mediterranean, thereby threatening local seagrasses species.

Keywords: flowers; Halophila stipulacea; invasive; sex-ratio bias; sexual reproduction.

Dioecy, the separation between male and female individual plants, is a relatively uncommon reproductive system in flowering plants (only ~5% of angiosperms; Charlesworth 2002). Sex ratio is measured as the ratio of the number of individuals of one sex relative to the other sex, or the ratio of allocation in each (Sapir et al. 2008). An equal number of males and females is an evolutionarily stable strategy, led by frequency-dependent natural selection due to competition for mates among individuals of the same sex (Fisher 1930). The mechanisms governing sex determination include the existence of sex heteromorphic chromosomes or other genetic components (Charlesworth 2002, Hough et al. 2013). Deviations from the 1:1 sex ratio are common in dioecious plants, with female-biased being more common in dioecious plants with abiotic pollen dispersal, and less common in long-lived growth forms, with biotic seed dispersal and fleshy fruits (Field et al. 2013).

Demography and local mating environment, as well as ecological factors, affect differential allocation and survival of males and females, driving biased sex ratios (Graff et al. 2013, Pickup and Barrett 2013). While the role of ecological factors in determining sex ratio is intriguing (Shelton 2010), this role may still be obscured by other non-ecological factors such as genetic and evolutionary factors and the degree of sex chromosome differentiation (Decker and Pilson 2000, Field et al. 2013). The higher energy investment involved in producing female flowers (Obeso 2002), alongside a higher mortality rate of female flowers (Allen and Antos 1993), is probably the result of more male-biased flowering sex ratio in dioecious clonal plants (Field et al. 2013).

The seagrass Halophila stipulacea (Forssk.) Asch is a dioecious tropical plant species, growing in both shallow and deep environments (1–50 m depth; Sharon et al. 2011, Winters et al. 2017). It is native to the Red Sea, Persian Gulf and Indian Ocean (den Hartog 1970). Following the opening of the Suez Canal in 1869, this species became a Lessepsian immigrant (Lipkin 1975a), invading the eastern Mediterranean Sea (Procaccini et al. 1999, Gambi et al. 2009, Sghaier et al. 2011). In 2002, this seagrass species was also reported in the Caribbean Sea (Ruiz and Ballantine 2004) and, in just over 10 years, it has spread to most of the Eastern Caribbean island nations (Willette et al. 2014), and reached the South American continent (Venezuela; Vera et al. 2014). Studies from the Caribbean have alarmingly shown that H. stipulacea is physically displacing local Caribbean seagrass species (e.g. Syringodium filiforme). Willette and Ambrose (2012) experimentally demonstrated that in the Caribbean’s shallow
waters (3–5 m), *H. stipulacea* sends out lateral rhizomes that grow between the shoots of *S. filiforme*, growing all around *S. filiforme* and eventually (within a few months) pushing it out by monopolizing its space. This phenomenon is changing the Caribbean’s seagrass landscape (Steiner and Willette 2015).

Little is known about the flowering patterns and the sex ratios in seagrasses in general (Diaz-Almela et al. 2006, Kuo 2007, Shelton 2010) and particularly in *Halophila stipulacea* (Malm 2006).

Sex ratios differ between seagrass species. For example, while *Cymodocea nodosa* around the island of Ischia (Gulf of Naples, Italy) exhibited equal proportions of females to males (Buia and Mazzella 1991), the sex ratio of *Amphibolis antarctica* in Western Australia was 3.8:1 females to males (Waycott et al. 1996). While there is a growing interest in understanding the biology of invasive organisms in general (Rilov 2009), in invasive seagrass species, sex ratios have not been compared between native and invasive populations.

*Halophila stipulacea* belongs to the family Hydrocharitaceae, in which male-biased sex ratios are abundant (Field et al. 2013). While reports of dioecy in *H. stipulacea* found in the Red Sea date back to 1975, sex ratios were not recorded (Lipkin 1975b). The only survey of sex ratios in *H. stipulacea* was carried out in the northern GoA (May, June and August 2002; Eilat, Israel) and it found that populations were female-biased across all depths of the species’ distribution (2.5–15 m; Malm 2006). Although signs of sexual reproduction in *H. stipulacea* were found in invasive populations in the Mediterranean, sex ratios were never quantified. Procaccini et al. (1999; Eolian Islands, Sicily) and Gambi et al. (2009; harbor of Palinuro, western Italy) reported on male-only flowers in *H. stipulacea* plants growing in the western Mediterranean. Lack of female flowers led Procaccini et al. (1999) to conclude that clonal propagation was probably the dominant reproductive mode in *H. stipulacea* colonizing new environments in the Mediterranean Sea. Similarly, Gambi et al. (2009) proposed that the introduction of *H. stipulacea* into the Mediterranean was of male genotypes only, or alternatively, that female flowers were unable to develop under the Mediterranean environmental conditions.

Nonetheless, Gerakaris and Tsiamis (2015) reported mature seed capsules (i.e. female plants) from the island Chios (Greece) in 2012 but did not report the fraction of flowering plants. Thus, while the existence of both sexes of *Halophila stipulacea* in its invasive Mediterranean

![Figure 1](image-url)
distribution has been recorded (but not in the same site or period), no data are available to determine whether this sex ratio is similar to that of the native habitat (e.g. the Red Sea, that is, female-biased), or whether it differs due to the different environmental conditions in the invasive range.

Here we report a quantitative comparison of sex ratios in the native range and in two invasive populations of *Halophila stipulacea* (Figure 1A–C). We hypothesized that due to ecological differences (mainly the amplitude of temperature fluctuations differs between sites; Figure 1D), sex ratios in the invasion range (Mediterranean) will be male-biased, as opposed to female-biased sex ratio found in the native region (northern Red Sea, Eilat; Malm 2006). Furthermore, we hypothesized that sex ratios will be more variable among sites in the invasive range, either due to multiple colonizations or due to differences among invasion sites.

We sampled plants and quantitatively recorded flowering in both the native population (the northern Red Sea – Eilat, Israel; Figure 1A and C) and two invasive populations (Mediterranean – Limassol, Cyprus; Figure 1A and B) during the 2017 flowering season (July–October; Figure 1E–J). Even though we might have missed the onset of the flowering season, which probably started in May (Malm 2006, Figure 2A), the results show a significant female-biased sex ratio in the native (Eilat) population (Figure 2D), as previously reported (Malm 2006) compared to the significant male-biased sex ratio in the invasive Dream Café population (generalized linear model with binomial distribution: $\chi^2 = 23.5$, $p < 0.001$; Figure 2E). We

![Graphs showing sex ratio and flowering distribution](image)

Figure 2: Percentage of flowering and sex ratio in *Halophila stipulacea* plant segments.

In one native (Eilat, northern Gulf of Aqaba, Red Sea, Israel; A, D) and two invasive sites: Dream Café beach (B, E) and Port of Limassol (C, F) (Limassol, eastern Mediterranean Sea, Cyprus). (A–C) Proportion of plant segments with flowers. (D–F) Proportion of plant segments with male vs. female flowers. Sampling was conducted between July and October 2017. Total number of individual plant segments (A–C) and total number of male/female plant segments (D–F) collected at each site are shown above each column. Plant segments of *H. stipulacea* were collected by either snorkeling (Eilat and Dream Café sites) or SCUBA-diving (Port site). In order to avoid collecting plant segments from the same clone, plant segments were located at least 2 m away from each other. Collected plant segments were transferred to the laboratory in zip-lock bags with seawater, and examined visually for signs of male/female reproductive organs.
also found a significant difference between the two invasive populations ($\chi^2_1 = 87.0, p < 0.001$), where the sex ratio in the invasive Port site (Figure 2F) was more similar to that in the native population in Eilat (Figure 2D). One potential explanation could be resource limitations or environmental stresses that resulted in male-biased sex ratios (Obeso 2002). An alternative (but not mutually excluded) explanation is that male-biased sex ratios are expected in recently-colonizing populations with a greater proportion of non-reproductive individuals which consume less of the available resources (Lloyd 1973). Additionally, observations of other Halophila stipulacea populations in the Mediterranean suggested that female flowers hardly develop under the Mediterranean environmental conditions (Procaccini et al. 1999, Gambi et al. 2009). Thus, the invasive H. stipulacea population in the Dream Café site (Figure 1B and E) confirms our hypothesis and exhibits the expected male-biased sex ratio.

However, in the second invasive site in Limassol Port, only 12 km away from the male-biased Dream Café population (Figure 1B), we found a different sex ratio. In a slightly deeper and denser Halophila stipulacea meadow in this site, we found an extreme female-biased sex ratio, similar to that in Eilat (Figure 2D). Moreover, while the flowering season in Eilat had practically ended in the last survey (October 3rd, 2017), with only one flowering plant (female) found (0.46% females; n = 217; Figure 2A), nearly a quarter of the sampled plants in the Port site (23%; n = 113) were still flowering, and all were females (Figure 2C). This suggests that the flowering season in the invasive range in the Mediterranean is longer and/or starts later than that in the Red sea, as was hypothesized by Lipkin (1975a). However, there could also be local forces at this site that are causing a female-biased sex ratio that needs to be further investigated.

It is likely that sampling early in the flowering season (mid-June 2007), might have caused Gambi et al. (2009) to miss the female flowers, which, judging from our own study in Eilat (presented here), probably appear after male flowers (Figure 2A). Procaccini et al. (1999) sampled in September (1997), and we believe that was already towards the end of the flowering season. Hence, the fact that Procaccini et al. (1999) did not find any female flowers, could be due to the Mediterranean environmental conditions. Another possibility is that Halophila stipulacea changes its flowering sex ratios and/or timing of these ratios, as it gets further away from its original point of invasion (Port Suez). However, the fact that Gerakaris and Tsiamis (2015) found female flowers in August (2012) in Greece, not far from the sites of Procaccini et al. (1999) and Gambi et al. (2009), suggests otherwise.

The seagrass Halophila stipulacea was suggested to be highly adapted to a wide range of physiological conditions, such as salinities, water temperatures, light intensities, and nutrient levels (Por 1971, Gambi et al. 2009, Sharon et al. 2009, 2011, Oscar et al., 2018). The ongoing tropicalization of the Mediterranean Sea (Bianchi and Morri 2003), accompanied by the recent expansion of the Suez Canal (July 2015), could contribute to the spreading of H. stipulacea in the Mediterranean, potentially threatening the local Mediterranean seagrass species (Marbá et al. 2010). Variation in sex ratio may be associated with the possibility of this species to either outcompete local species (a process that is already taking place in the Caribbean; Steiner and Willette 2015) or take up new available habitats following mortality of local Mediterranean species under heat stress (Marbá et al. 2010, Jordà et al. 2012). Further mesocosm based studies may facilitate our understanding of the association of heat stress expected from climate change with sex ratio and reproduction (Nguyen et al. unpublished). Knowledge of differences in sex ratios between native and invasive regions will contribute to our understanding of the processes that invasive plants may undergo during colonization of new habitats. Our study, as well as long-term monitoring in both native and invasive meadows, may provide a better understanding of the factors affecting the invasiveness of H. stipulacea.

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References


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Periklis Kleitou graduated from the University of Brighton (UK) in 2014 (Environmental Biology and Education) and since then he is working at Marine and Environmental Research (MER) Lab Ltd in Cyprus. Concurrently, he attended a distance MSc in Sustainable Aquaculture at the University of St-Andrews (UK) (2014–2017). Since 2018, he also started working part-time for the University of Plymouth (UK) on marine invasive species, and specifically on lionfish. He participated in several research projects related to the marine ecosystem, biodiversity assessments, fisheries, and aquaculture. His interests focus on the marine conservation, ecology, and blue growth.

Demetris Kletou received his BSc (2005) and MSc (2007) in Marine Biology from the Department of Biological Sciences, Florida Atlantic University. Upon returning to Cyprus (2008) he founded Marine and Environmental Research (MER) Lab Ltd, where he is the Director and Principle Investigator. He did his PhD (2011–2018) at the University of Plymouth studying the anthropogenic impacts to marine oligotrophic ecosystems. His interests include sustainable development of human activities and aquaculture, marine ecological characterization and assessments. He is currently the scientific coordinator of the LIFE+ Project titled RELIONMED aiming to set the first line of defense against the lionfish invasion in the Mediterranean.

Yuval Sapir studied in the Hebrew University of Jerusalem, Israel. His MSc dealt with *Iris* morphological taxonomy, while in his doctorate he studied pollination ecology of the *Oncocyclus* irises. In his postdoc research, he studied ecological genetics and pollination of sunflowers in Indiana University (USA). Yuval was appointed as a director of the Tel Aviv University Botanical Garden and joined School of Plant Sciences and Food Security as a faculty member in 2012. His research interests include evolution of plants under climate changes, plant’s mating systems, ecological speciation, and the effect of pollinators’ behavior on the evolution of flowers.

Gidon Winters received his PhD in Molecular Biology and Ecology of Plants from Faculty of Life Sciences, Tel Aviv University, Israel (2009). He was a Post-Doctoral research fellow at the Institute for Evolution and Biodiversity, Westfälsche Wilhelms-Universität, Germany (2008–2010), working on effects of thermal stress on *Zostera marina*. Since his return to Israel, he has been a researcher at the Dead Sea and Arava Science Center (ADSSC). His research interests include seagrass mapping, studying the effects of salinity and climate change on seagrasses, and biotechnology applications of seagrasses. He teaches a seagrass dedicated course at the Inter-University Institute for Marine Science in Eilat.