Short communication

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Inconclusive evidence of sexual reproduction of invasive *Halophila stipulacea*: a new field guide to encourage investigation of flower and fruit production throughout its invasive range

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Abstract: The dioecious seagrass species *Halophila stipulacea* reproduces mainly through fast clonal growth, underlying its invasive behavior. Here, we provide morphological evidence to show that the first findings of fruits in the Caribbean were misidentified. Consequently, *H. stipulacea* reproduction is likely still only asexual in the Caribbean. Therefore, we introduce an identification key of *H. stipulacea* reproductive structures to encourage careful identification and quantification throughout its invasive range. Until large-scale seed production in invaded habitats is reported, the apparent low rate of sexual reproduction needs to be considered in current studies investigating the invasion capacity of this species.

Keywords: dichotomous key; exotic macrophyte; *Halophila stipulacea*; invasion potential; sexual reproduction.

Native to the Red Sea and Western Indo-Pacific, the seagrass *Halophila stipulacea* (Forsk.) Ascherson, was reported to have invaded the Mediterranean Sea in 1894 (Lipkin 1975) and the Caribbean in 2002 (Ruiz and Ballantine 2004), after which it spread successfully in both regions (Winters et al. 2020). Similar to many invasive macrophytes, *H. stipulacea* expands mainly through asexual clonal growth and fragmentation (Lipkin 1975; Smulders et al. 2017), a feature believed to characterize the colonization of multiple Caribbean islands (Willette et al. 2014).

Sexual reproduction increases the genetic variability and dispersal potential of seagrasses, which is important for long-term stability of populations under dynamic change (Ackerman 2006). In its native range, *Halophila stipulacea* flowers predictably with both female and male flower production, followed by seed formation within 1–2 months after fertilization (Dural 2020; Malm 2006; Nguyen et al. 2018 see Figure 1A and B). Male flowering has commonly been reported throughout its invasive range; both in the Mediterranean as well as in the Caribbean (Chiquillo et al. 2019; Dural et al. 2020; Gambi et al. 2018; Procaccini et al. 1999; Vera et al. 2014). In contrast, documentation of female flowers and fruits is rare, and was limited to four studies in the Mediterranean (Dural et al. 2020; Gerakaris and Tsiamis 2015; Lipkin 1975; Nguyen et al. 2018), until a recent report of fruits in the US Virgin Islands (Chiquillo et al. 2019).

In 2017, male flowers and fruits of *Halophila stipulacea* were reported in the US Virgin Islands, implying the existence of sexual reproduction of this species in the Caribbean (Chiquillo et al. 2019). Here we reevaluate the morphological features of the proposed male fruits presented in Chiquillo et al. (2019) and compare them with reproductive structures from Caribbean *H. stipulacea* that flowered in a controlled laboratory environment (Figure 2). The images of the proposed fruit (Chiquillo et al. 2019, Figure 1B) did not match the description of previous published work on *H. stipulacea* fruit morphology. Gerakaris and Tsiamis (2015) and Nguyen et al. (2018) published images that show that the

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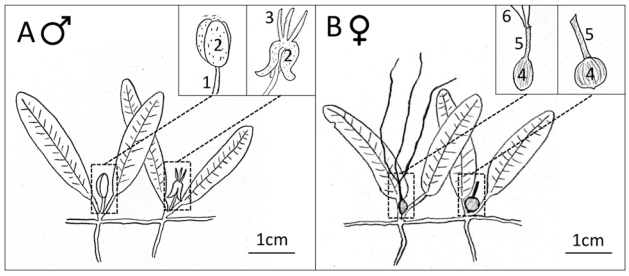

Figure 1: *Halophila stipulacea* reproductive structures of (A) male and (B) female plants. Structures of the male flower bud and male flower are 1: pedicel, 2: tepals, 3: stamens. Structures of the female flower and developing seed are 4: ovary, 5: hypanthium, 6: styles.

fruit of *H. stipulacea* is spherical, attached to the base of the shoot, and that the hypanthium (enlargement of the flower receptacle) remains attached to the fruit (see Figure 1B). Images by Vohník et al. (2017) show that female flowers and fruits mature progressively at each node along the rhizome and they discuss the possibility of confusion with a fungal phytomixid infection.

Contrary to these accounts, the photograph of Chiquillo et al. (2019, Figure 1B) shows an oblong shaped flower, attached to the base of the shoot by a stem. Additionally, the distinctive female hypanthium, attached to mature fruits (Figure 1B), is missing and no female flowers at various stages of development are documented. The proposed fruits in Chiquillo et al. (2019) are more likely to be male flower buds, which have similar dimensions to fruits, but are more oval in shape (see Figures 1A, 2A, 2B and dichotomous key in Appendix 1). The male flower bud is attached to a stem (pedicel) of varying length, similar to the image in Chiquillo et al. (2019). The flower buds presented in Figure 2 have all been observed to develop in male flowers (Figures 2C and D).

Based on these observations, the existence of female flowers and fruits of *Halophila stipulacea* in the Caribbean Sea is open to question. Consequently, the rapid spread of this species is currently likely to be limited to asexual reproduction: clonal growth and fragmentation. Why male plants of this species seem to be more successful in colonizing new areas outside its native range compared to female plants, and why female plants do not flower regularly outside the native range, remain important open questions.

Collecting improved pictures of sampled or *in situ* fruits and female flowers of *Halophila stipulacea* would increase our understanding of sexual reproduction in the Caribbean as would more frequent monitoring of plants for reproductive structures and, once they are located *in situ*, monitoring of flowers approximately every 2 weeks (Dural et al. 2020). In-depth studies of male and female flowering rates and ratios, seed production per area and seed viability within the invasive range would increase our knowledge on the invasion capacity of *H. stipulacea*. To facilitate such studies, we provide a field guide with a dichotomous key for identifying the reproductive structures of *H. stipulacea* (Appendix 1).

Understanding reproduction of *Halophila stipulacea* in its invasive range is critical to managing this species. For example, sexual reproduction may increase physical dispersal capacity (Ackerman 2006). Increased genetic diversity associated with sexual reproduction could also amplify the adaptive capacity of this tropical species, for instance, to colder water conditions in subtropical regions. Furthermore, the development of seed banks could increase the resilience of *H. stipulacea* to physical disturbance such as storms, or seasonal differences (Unsworth et al. 2015). Sexual reproduction therefore adds an understudied dimension to seagrass invasive potential. The only

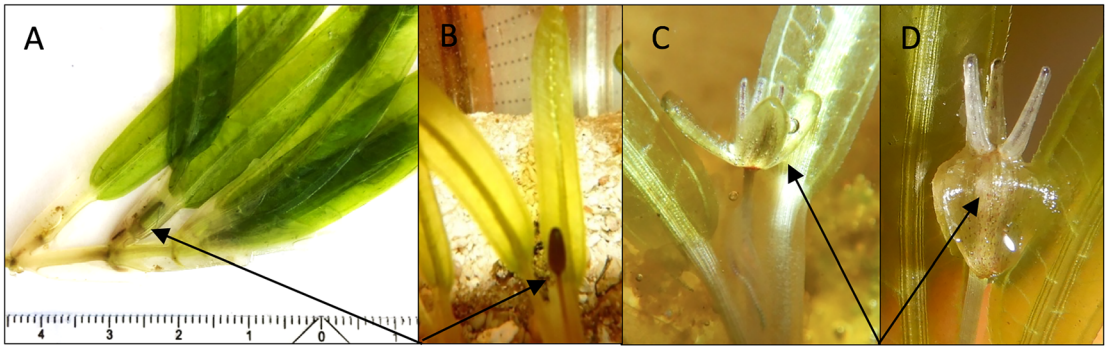

Figure 2: *Halophila stipulacea* male flower buds in different stages of development. The arrows in (A) and (B) depict flower buds attached with a pedicel to the base of the shoot. The arrows in (C) and (D) depict male flowers emerging from flower buds, with three stamens that release pollen grains. *Halophila stipulacea* male flower buds and flowers shown were harvested on Bonaire, Dutch Caribbean and grown in the laboratory on coral sand, at 25 °C and a salinity of 35. The plants were observed flowering in January 2020 in three separate mesocosms (length × width × height: 0.6 × 0.3 × 0.25 m).

other transoceanic invasive marine angiosperm, *Zostera japonica*, is believed to be transported by seeds in shipments of Japanese oysters to the northeast Pacific, where it has spread along the west coast of the USA (Harrison and Bigley 1982; Williams 2007). High reproductive output is suggested to be one of the main reasons for the invasion success of *Z. japonica*, and increased output following disturbance events was found to provide a temporal and/or spatial escape, facilitating its invasion (Bando 2006; Henderson and Hacker 2015; Ruesink et al. 2010). Therefore, monitoring the occurrence and rate of sexual reproduction is vital in understanding invasive seagrass dynamics and this knowledge needs to be taken into account in further studies on dispersal, genetic diversity and resilience of these species in invasive habitats.

We strongly encourage new monitoring efforts that target *Halophila stipulacea* flowers and fruits across its invasive range, as shifts in temperature and disturbance regimes may be accelerating the plant's growth and spread (Nguyen et al. 2020; Willette et al. 2020). Until future studies quantify the production of invasive *H. stipulacea* fruits, we should take into consideration that sexual reproduction is either absent or largely insignificant throughout its Caribbean invasive range.

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