Short communication

Kelcie L. Chiquillo*, Paul H. Barber and Demian A. Willette

Fruits and flowers of the invasive seagrass *Halophila stipulacea* in the Caribbean Sea

https://doi.org/10.1515/bot-2018-0052
Received 18 May, 2018; accepted 21 September, 2018

Abstract: While the seagrass *Halophila stipulacea* reproduces both sexually and asexually in its native range, reproduction is largely asexual in its invasive range in the Mediterranean and the Caribbean Seas. Here we make the first report of fruit-bearing *H. stipulacea* in the Caribbean. Although the lack of reports of *H. stipulacea* fruit could be the consequence of past survey effort, multiple recent reports of both flowers and fruit across the invasive range strongly suggest that introductions of *H. stipulacea* in the tropical western Atlantic and Caribbean included both sexes of this dioecious seagrass. This finding may have important implications for the future dispersal, survival, and maintenance of the non-native population.

Keywords: Caribbean; *Halophila stipulacea*; introduced species; seagrass; sexual reproduction.

Halophila stipulacea (Forsk.) Ascherson is a small, tropical, dioecious, euryhaline, subtidal seagrass species. It is widely distributed in its native range along the western parts of the Indian Ocean, Eastern Africa, Arabian Sea, Persian Gulf, and Red Sea (Lipkin 1975a), where it forms monospecific and polyspecific meadows (Lipkin 1979, Malm 2006). Following the opening of the Suez Canal in 1867, the first fragments of *H. stipulacea* were observed in Rhodes, Greece in 1894 (Fritsch 1895, Lipkin 1975a). Since this first invasion, referred to as "the historical invasion", *H. stipulacea* has flourished, expanding its range broadly throughout the Mediterranean, forming meadows with native seagrass and algal species. Subsequently, in 2002, *H. stipulacea* invaded the Caribbean. It was first reported on the island of Grenada (Ruiz and Ballantine 2004), but rapidly spread west to Bonaire, north to Puerto Rico and south to Venezuela, spanning a distance of over 700 miles and forming monospecific and/or polyspecific seagrass meadows at a range of depths and substratum types (Vera et al. 2014, Willette et al. 2014, Ruiz et al. 2017).

Reproduction in *Halophila stipulacea* varies greatly in its native and introduced ranges. In its native range, the flowering season of *H. stipulacea* begins in May and ends in October, producing both staminate (male) and pistillate (female) flowers at depths of 2.5–5 m (Malm 2006), with a strong bias towards female flowers between July and October (Nguyen et al. 2018). Recent discoveries show that the flowering season begins later and/or lasts longer in the Mediterranean than it does in the Red Sea (Nguyen et al. 2018). Reports of sexual reproduction in the western Mediterranean show a stronger bias towards male flowers (Gambi et al. 2009) and that flowering can occur much deeper (5–25 m; Procaccini et al. 1999, Gambi et al. 2009). Only twice have fruits been reported in the Mediterranean (Lipkin 1975a, Gerakaris and Tsiamis 2015). Little is known about sexual reproduction of invasive *H. stipulacea* populations in the Caribbean Sea beyond a single report of male flowers only in one locality (Vera et al. 2014).

Given the strong differences in flowering and seed production in its native and invasive ranges, it is hypothesized that the spread and expansion rate of *Halophila stipulacea* into the Mediterranean and Caribbean Seas have occurred exclusively via asexual reproduction. If sexual reproduction is occurring, however, it would have a significant impact on our understanding of the potential for dispersal and the genetic potential of introduced populations.

In this study, we surveyed a population of *Halophila stipulacea* in the Caribbean to determine whether sexual reproduction occurs in this invasive population. Specifically, we examined a monospecific *H. stipulacea* meadow in Great Lameshur Bay, St. John, U.S. Virgin Islands (18° 19′ 6.6″ N, 64° 43′ 18.48″ W) in April 2017 to determine the presence of flowers and fruiting bodies.

***Corresponding author: Kelcie L. Chiquillo,** Ecology and Evolutionary Biology Department, University of California Los Angeles, 610 Charles E. Young Drive, Los Angeles, CA 90095, USA, e-mail: Kelcie.L.Chiquillo@ucla.edu
Paul H. Barber: Ecology and Evolutionary Biology Department, University of California Los Angeles, 610 Charles E. Young Drive, Los Angeles, CA 90095, USA
Demian A. Willette: Biology Department, Loyola Marymount University, 1 LMU Drive, Los Angeles, CA 90045, USA

Surveys consisted of 10 predefined 1.02-m^2 removal plots at 5–6 m depth on sandy substrata with similar shoot density. Individual shoots were closely examined for reproductive structures (e.g. flowers and fruits). All flowers observed were collected, identified as female or male, individually photographed, and voucher specimens were frozen and stored at the University of California Los Angeles.

We found four male flowers and 27 fruits on *Halophila stipulacea* from three of the 10 experimental plots in Great Lameshur Bay, representing the first report of sexual reproduction in the Caribbean. As reported by Ackerman (2007), flowers were solitary, highly-reduced naked flowers on short pedicels close to the base of the plant. Lipkin (1975b) described *H. stipulacea* flowers as (1) oval in shape, and whitish to greenish towards the apex with small black dots; (2) pedicels 1.5 cm in length; (3) translucent tepals measuring 4.0–5.0 mm in length, 2.5 mm in width; (4) anthers bearing three stamens 3.0–5.0 mm in length; and (5) borne beneath the sediment. Flowers of Caribbean *H. stipulacea* largely conformed to this description (Figure 1, Table 1).

Although no pistillate flowers were observed, a total of 27 developed fruits were recovered, indicating that Caribbean populations of *Halophila stipulacea* produce both male and female flowers. The genus *Halophila* exhibits geocarpy – ripening below the sediment (Ackerman 2007), which was consistent with our observations. The density of fruits (27 fruits from three 1.02-m^2 plots) is higher than reports from the native Indian Ocean range (e.g. Gulf of Aqaba) where only one flower and several fruits were discovered along two 50-m transects (Hulings and Kirkman 1982). Size of fruits (Table 1) was comparable to those reported for *H. stipulacea* in the native range (den Hartog 1970) and Mediterranean Sea (Lipkin 1975c).

Most seagrasses produce flowers that yield fruits and seeds during reproductive periods (Kuo and den Hartog 2007). *Halophila stipulacea*, however, produces fewer flowers than many other seagrass species, including other species of *Halophila*, and reproduction is primarily vegetative in its native range (den Hartog 1970). Because previous studies of *H. stipulacea* found limited evidence of flowering or fruiting in the Mediterranean and Caribbean Seas, it had long been assumed that these invasive seagrass populations reproduced exclusively asexually; the results of this study, however, indicate that this is not the case. Sexual reproduction of *H. stipulacea* in the Caribbean may indicate that environmental conditions in the Caribbean are more similar to those in the native Indian Ocean range as light and temperature influence flower production in *H. stipulacea* (Procaccini et al. 1999). In contrast, fluctuations in sea temperatures in the Mediterranean Sea (Martrat et al. 2004, Shaltout and Omstedt 2014) may act to control the spread and growth of *H. stipulacea*, potentially limiting the availability of energy resources required for flowering and fruit formation.

The different reproductive ecologies of the two invasive *Halophila stipulacea* populations may impact their ability to spread, resulting in different population dynamics and underlying genetic architecture. For

Table 1: Plant part measures for *Halophila stipulacea* fruits and male flowers from 10 1.02-m^2 plots.

	Count	Average length (mm)	SE	Range
Fruits	27	4.38	0.009	3.90–5.39
Flowers				
Pedicel	4	15.2	0.073	13.20–16.70
Tepal	4	3.80	0.039	3.28–4.97
Anther	4	3.48	0.044	2.54–4.64

Measurements include total number of fruits and flowers, as well as average, standard error, and range of fruits and flower parts (pedicel, tepal, and anther).

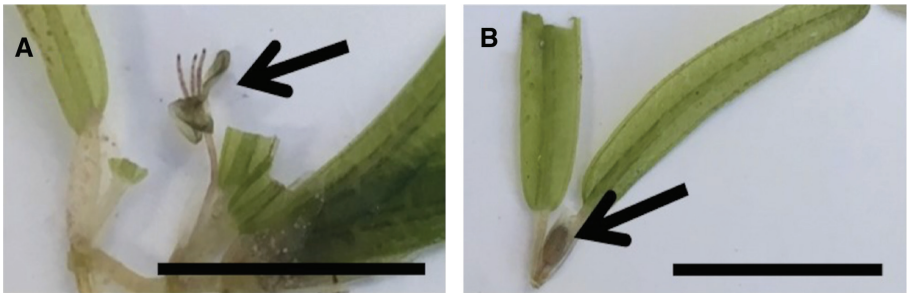

Figure 1: Flower and fruit of Halophila stipulacea from St. John, United States Virgin Islands; (A) male flower showing pedicel (B) a developed fruit.
Scale bar: 2 cm.

example, *H. stipulacea* populations in the Mediterranean are ephemeral, with meadows shrinking in winter, and expanding in summer (Proccaccini, pers comm). In contrast, populations of *H. stipulacea* in the Caribbean seagrass are spreading rapidly, having expanded more than 700 km in 15 years (Willette and Ambrose 2012, Willette et al. 2014, Ruiz et al. 2017); seed dispersal in Caribbean could contribute to the fast rate of spread in this region. In addition to aiding dispersal, sexual reproduction could be facilitating spread in the Caribbean because outcrossing results in novel genetic combinations on which natural selection can act, a process that may be more limited in the Mediterranean. These regional differences in fruiting and flowering of *H. stipulacea* across its invasive range highlight the opportunistic nature of this invasive species, and could contribute to its ability to successfully invade new habitat. However, other environmental conditions such as high nutrient levels that promote dense growth of *H. stipulacea* in the Caribbean (Van Tussenbroek et al. 2016) could also facilitate rapid growth in this region.

Acknowledgments: This work was supported by NSF Bridge to Doctorate Award HRD-1400789, NSF GRFP Award DGE-1650604, NPS Cooperative Research award to P13AC00676 to DAW, and UCLA EEB Travel funds to KLC. We thank P. Jobsis, E. Cruz- Rivera, S. Habtes, I. Byrne, W. Sears, A. Jensen, K. Wilson-Grimes, E. Ryznar (dcp), P. Fong, M. Joaquin, E. Chiquillo, R. Chiquillo, and L. Chiquillo for their assistance.

References

Ackerman, J.D. 2007. Sexual reproduction of seagrasses: pollination in the marine context. *In:* (A.W.D. Larkum, R.J. Orth and C.M. Duarte, eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht. pp. 89–109.

den Hartog, C. 1970. *The seagrasses of the world*. North Holland, Amsterdam. pp. 275.

Fritsch, C. 1895. Uber die auffindung einer marinen Hydrocharidee im Mittelmeer. *Verh. Zool. Bot. Ges. Wien. 45*: 104–106.

Gambi, M.C., F. Barbieri and C.N. Bianchi. 2009. New record of the alien seagrass *Halophila stipulacea* (Hydrocharitaceae) in the western Mediterranean: a further clue to changing Mediterranean Sea biogeography. *Mar. Biodiv. Rec. 2*: e84. (published online). http://journals.cambridge.org/article_S175526720900058X.19/09/2011.

Gerakaris, V. and T. Tsiamis. 2015. Sexual reproduction of the Lessepsian seagrass *Halophila stipulacea* in the Mediterranean Sea. *Bot. Mar. 58*: 51–53.

Hulings, N.C. and H. Kirkman. 1982. Further observations and data on seagrasses along the Jordanian and Saudi Arabian coasts of the Gulf of Aqaba. *Tethys 10*: 218–220.

Kuo, J. and C. den Hartog. 2007. Seagrass morphology, anatomy, and ultrastructure. *In:* (A.W.D. Larkum, R.J. Orth and C.M. Duarte, eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht. pp. 51–87.

Lipkin, Y. 1975a. *Halophila stipulacea*, a review of a successful immigration. *Aquat. Bot. 1*: 203–215.

Lipkin, Y. 1975b. *Halophila stipulacea* in Cyprus and Rhodes. *Aquat. Bot. 1*: 309–320.

Lipkin, Y. 1975c. On the male flower of *Halophila stipulacea*. *Israel J. Bot. 24*: 198–200.

Lipkin, Y. 1979. Quantitative aspects of seagrass communities, particularly of those dominated by *Halophila stipulacea*, in Sinai (Northern Red Sea). *Aquat. Bot. 7*: 119–128.

Malm, T. 2006. Reproduction and recruitment of the seagrass *Halophila stipulacea*. *Aquat. Bot. 85*: 345–349.

Martrat, B., J.O. Grimalt, C. Lopez-Martinez, I. Cacho, F. Sierro, J.A. Flores, R. Zahn, M. Canals, J.H. Curtis and D.A. Hodell. 2004. Abrupt temperature changes in the Western Mediterranean over the past 250,000 years. *Science. 306*: 1762–1765.

Nguyen, H.M., P. Kleitou, D. Kletou, Y. Sapir and G. Winters. 2018. Differences in flowering sex ratios between native and invasive populations of the seagrass *Halophila stipulacea*. *Bot. Mar. 61:* 337–342.

Procaccini, G., S. Acunto, P. Famà and F. Maltagliati. 1999. Structural, morphological and genetic variability in *Halophila stipulacea* (Hydrocharitaceae) populations in the western Mediterranean. *Mar. Biol. 135*: 181–189.

Ruiz, H. and D.L. Ballantine. 2004. Occurrence of the seagrass *Halophila stipulacea* in the tropical West Atlantic. *Bull. Mar. Sci. 75*: 131–135.

Ruiz, H., D.L. Ballantine and H. Sabater. 2017. Continued spread of the seagrass *Halophila stipulacea* in the Caribbean: documentation in Puerto Rico and the British Virgin Islands. *Gulf Caribb. Res. 28*: SC5–SC7.

Shaltout, M. and A. Omstedt. 2014. Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. *Oceanologia. 56*: 411–443.

Van Tussenbroek, B.I., M.M. van Katwijk, T.J. Bouma, T. van der Heide, L.L. Govers and R.S.E.W. Leuven. 2016. Non-native seagrass Halophila stipulacea forms dense mats under eutrophic conditions in the Caribbean. *J. Sea. Res. 1*: 1–5.

Vera, B., L. Collado-Vides, C. Moreno and B.I. van Tussenbroek. 2014. *Halophila stipulacea* (Hydrocharitaceae): a recent introduction to the continental waters of Venezuela. *Caribb. J. Sci. 48*: 66–70.

Willette, D.A. and R.F. Ambrose. 2012. Effects of the invasive seagrass Halophila stipulacea on the native seagrass, Syringodium filiforme, and associated fish and epibiota communities in the Eastern Caribbean. *Aquat. Bot. 103*: 74–82.

Willette, D.A., J. Chalifour, A.O.D. Debrot, M.S. Engel, J. Miller, H.A. Oxenford, F.T. Short, S.C. Steiner and F. Védie. 2014. Continued expansion of the trans-Atlantic invasive marine angiosperm *Halophila stipulacea* in the Eastern Caribbean. *Aquat. Bot. 112*: 98–102.

Bionotes

Kelcie L. Chiquillo
Ecology and Evolutionary Biology Department, University of California, Los Angeles, 610 Charles E. Young Drive, Los Angeles, CA 90095, USA,
Kelcie.L.Chiquillo@ucla.edu

Kelcie L. Chiquillo is a PhD candidate of the Ecology and Evolutionary Biology Department at the University of California, Los Angeles (UCLA). Her research is broadly interested in using multidisciplinary approaches to understand the origins and ecological dynamics of invasive marine organisms, and will use this information to advance marine conservation and biodiversity. Kelcie hopes to become a professor and continue to do research and mentor diversity students to increase minority participation in marine science.

Paul H. Barber
Ecology and Evolutionary Biology Department, University of California, Los Angeles, 610 Charles E. Young Drive, Los Angeles, CA 90095, USA

Paul H. Barber is a professor of the Ecology and Evolutionary Biology Department at the University of California, Los Angeles (UCLA). Paul's research program integrates genetics, genomics, ecology, and oceanography to understand the evolution of marine biodiversity, and uses this information to promote marine conservation. He was recently named HHMI Professor, and continues to integrate this research in the context of educational programs, like The Diversity Project, to promote and increase minority participation in marine science.

Demian A. Willette
Biology Department, Loyola Marymount University, 1 LMU Drive, Los Angeles, CA 90045, USA

Demian A. Willette is an assistant professor of ecology at Loyola Marymount University, USA. His research interests include applied ecology, biological invasions, conservation biology, and fisheries management. He is a Fulbright Global Scholar and conducts research across the Pacific and Caribbean regions.

Graphical abstract

Kelcie L. Chiquillo, Paul H. Barber and Demian A. Willette

Fruits and flowers of the invasive seagrass *Halophila stipulacea* in the Caribbean Sea

https://doi.org/10.1515/bot-2018-0052
Botanica Marina 2018; x(x): xxx–xxx

Short communication: This is the first report of fruit-bearing *Halophila stipulacea* in the Caribbean.

Keywords: Caribbean; *Halophila stipulacea*; introduced species; seagrass; sexual reproduction.