Frequent flowering but low sexual reproductive success of the dioecious seagrass Enhalus acoroides in Li'an Bay, China

Shuo Yu
Fourth Institute of Oceanography Ministry of Natural Resources
Lijun Cui
South China Sea Institute of Oceanology Chinese Academy of Sciences
Brigitta I. van Tussenbroek
Universidad Nacional Autónoma de México Facultad de Ciencias: Universidad Nacional Autonoma de Mexico Facultad de Ciencias
Yuchao Wu
South China Sea Institute of Oceanology Chinese Academy of Sciences
Fangchao Zhu
Fourth Institute of Oceanography Ministry of Natural Resources
Juan Diego Gaitan-Espitia
The University of Hong Kong
Kai Jiang
jiangkai@csnbgsh.cn

Shanghai Chen Shan Botanical Garden https://orcid.org/0000-0001-9795-9290

Short Report

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Abstract

Seagrasses are rapidly declining worldwide. To safeguard and restore their natural populations, it is fundamental to understand first the biological properties that influence seagrass ecological and demographic trends. Such characteristics are typically linked to the reproductive success of these flowering plants, modulating the genetic diversity and the adaptive potential of seagrass populations. Aiming to inform future restoration efforts, we investigated the reproductive phenology and fruit set of the dioecious tropical seagrass *Enhalus acoroides* in Li'an lagoon, Hainan Island, China. Although *E. acoroides* flowered year-round, the flowering intensity varied across seasons (highest in the summer and lowest in the winter), most likely mediated by sea surface temperatures and tides. Mature fruits occurred predominantly in fall and winter, but the frequency was low probably due to the influence of disturbance and algal blooms. Despite the high reproductive investment in terms of flower formation observed throughout the year in *E. acoroides*, there was limited sexual reproductive success evidenced by the low fruit formation. This study provides insight into the reproductive ecology of *E. acoroides*, which will be useful for the future conservation and restoration of this threatened seagrass.

Introduction

Seagrasses are a group of monocotyledonous submerged plants growing in marine coastal environments (den Hartog 1970; Short et al. 2007). These organisms provide valuable ecological and socio-economic services (Orth et al. 2006), that are imperiled due to the global decline of seagrass populations driven by anthropogenic pressures and climate change (Waycott et al. 2009; Dunic et al. 2021). Restoration of seagrass meadows may compensate for this loss (van Katwijk et al. 2016; Orth et al. 2020). As part of this effort, seed restoration appears as a promising approach due to its less destructive effect on the donor meadows and its potential to generate more genetically diverse restored populations (Sinclair et al. 2021). Harnessing the advantages of seed restoration requires a good understanding of the processes determining seed production, reproductive investment and success. Unfortunately, such knowledge is limited to a few species that are well-studied such as *Zostera marina* (Reusch 2003), *Posidonia oceanica* (Jahnke et al. 2015), and *Thalassia testudinum* (van Tussenbroek et al. 2008; 2016). This knowledge, however, cannot be extrapolated to all seagrass species as their reproductive characteristics are determined by the local environmental (e.g., temperature, light) and ecological (e.g., vertical zonation) conditions. Therefore, it is fundamental to develop population-specific and species-specific assessments of the reproductive phenology and investment (e.g., seed production) of seagrasses when planning restoration efforts.

Seagrass reproductive phenology and investment are highly influenced by temperature and light availability, factors that vary along vertical (von Staats et al. 2021) and latitudinal clines (Ito et al. 2021). In the tropics, these environmental drivers are more stable compared to sub-tropical and temperate zones, which may explain the year-round flowering pattern documented in most tropical seagrasses (Brouns and Heijs 1986; Walker et al. 2001; Rollón et al. 2003). However, even for tropical species, microgeographic environmental gradients can contribute to inter-population differences in reproductive

characteristics (Walker et al. 2001; Lee et al. 2007), potentially influencing the capacity of populations to respond to rapidly changing conditions (von Staats et al. 2021). For instance, intra-specific differences in flowering timing and duration have been documented in tropical populations of the seagrass *Halophila decipiens* in which flowering occurs in April for meadows in Thailand (Lewmanomont et al. 1996), while this process takes place between August and November in South Sulawesi (Verheij and Erftemeijer 1993). Similarly, in the tropical seagrass *Halodule wrightii*, flowering starts in early spring for populations in the Gulf of Mexico and Caribbean, and in late spring for those in the northern range of the species distribution (McGovern and Blankenhorn 2007). When flowering phenology is consistent across populations, other intra-specific differences may result from microgeographic differences in environmental conditions. In the Indo-Pacific, for example, populations of the seagrass *Enhalus acoroides* show geographic differences in flowering intensity correlated with spatial changes in light availably influenced by turbidity and water depth (Rollón et al. 2003).

At the population level, changes in reproductive phenology and investment can be related to ecological and temporal trends (e.g., seasonality) that are part of the life histories of these plants (Turschwell et al. 2021). Beyond these natural dynamics, changes in reproductive characteristics can be related to the demographic impacts of rapid and large-scale population declines (Baranano et al. 2022; de los Santos et al. 2019). Such declines typically result in disturbance and the reduction of reproductive success due to pollen limitation (van Tussenbroek et al. 2016). In China, the distribution area and density of *E. acoroides* have drastically declined over the last decades, and some populations have become fragile due to pollution and physical disturbances (Herbeck et al. 2014; Chen et al. 2015). Furthermore, this dioecious seagrass is a surface-pollinated seagrass species with a lower pollen-ovule ratio of 10:1 (Ackerman 2006). This may result in limited propagule production and plant material to support seagrass habitat recovery. Although *E. acoroides* has a continuous flowering pattern in other areas of the South Asia region (e.g., Indonesia; Brouns and Heijs 1986), this reproductive process has rarely been noticed in China (Yu et al. 2019). Considering the use of sexual propagules for *E. acoroides* restoration, we aimed to assess the reproductive phenology of *E. acoroides* and the effects of fragmentation on the fruit set in Hainan.

Methods

Study site

Li'an lagoon is located in Lingshui County, on the southeast coast of Hainan Island, China (Fig. 1). This area has a tropical maritime monsoon climate, with an annual average temperature of ca. 25°C. The semi-closed lagoon is connected to the South China Sea through a 60 m wide channel with an irregular full-day mixed tidal regime (Yang et al. 2016). In this lagoon, the seagrass meadow is dominated by *E. acoroides*, forming monospecific or mixed meadows with other seagrass species, such as *Halophila ovalis*, *Thalassia hemprichii* and *Cymodocea rotundata* (Chen et al. 2015). The seagrass meadow area has decreased from 207 ha (2009) to 142 ha (2016) due to physical disturbance (e.g. clam digging and fishing) and pollution by domestic sewage and aquaculture. Flowering and fruiting phenology for this *E.*

acoroides population has not been previously documented remaining unclear the potential impacts of the diverse anthropogenic pressures on the reproductive traits of local seagrasses.

Field observations

We established two 20 m × 20 m plots in monospecific meadows nearby the tidal inlet with relatively better water quality (Yu et al. 2019; Fig. 1). The size of these plots followed Brouns and Heijs (1986), aiming to enhance the detection of flowers and fruits. Flowering frequency was assessed by daily observations of the seagrass meadow from May 24th to July 21th in 2017. Subsequently, we visited the studied site twice per month at low tide and recorded the flowering occurrence from July 2017 to June 2018 at low tide and recorded flowering occurrence.

We randomly marked 30 male inflorescences and 30 female flower buds that had emerged from the sheath in June 2017 to estimate the floral longevity. The female flower longevity was defined from opening up until pollination had occurred. The male flower longevity was defined from the opening-up of the bracts until the release of the florets. The number of florets (per male inflorescence; n = 8), ovules (per female flower; n = 9), and pollens per floret (n = 15) were counted by dissecting microscope.

In our study, Plot A was in a patchy area with low meadow coverage (41.43 ± 7.02%), and the average cover of the meadow of Plot B was 85.21% (± 20.02%) (Yu et al. 2019). Based on the observation of female floral longevity, three stages were distinguished for female flowers (Fig. 2a, b, c, d). These stages include emerging young flowers, unfertilized-mature flowers, and newly-fertilized flowers (fertilized several days ago). The female flowers per stage were counted, representing recent flower production. The newly fertilized female flowers have a flattened ovary and a slightly spiraled peduncle, while the young fruits are swollen and the peduncle presents several spirals (Fig. 2e). Fruiting shoots were excluded, because of their long ripening period of 3–5 months (Rollón 1998). As the male inflorescences decay quickly after opening within one week (Fig. 3a, b), all the male inflorescences (buds and those that had released florets) were counted at each visit.

Finally, the fruit set and fruit size were recorded between September 2017 and February 2018. The diameter (2r) and height (h) of the fruits were measured monthly using a Vernier caliper.

Sea surface temperatures (SST) in Li'an lagoon were supplied by Haikou Marine Environmental Monitoring Center Station, State Oceanic Administration, China) from June to December 2017.

Data analysis

To assess the effect of sea surface temperatures (SST) on the flowering abundance, correlation analysis was conducted in R 4.1.2 (R Development Core Team, 2021). The difference in flowering pattern between Plot A and Plot B was tested by Paired t-test in R 4.1.2.

Sex ratio ($\mathrm{R_s}$) was defined as male shoot number/female shoot number. The pollen-ovule ratio ($\mathrm{R_{po}}$) was calculated by the formula: $\mathrm{R_{po}} = \frac{\mathrm{NP}}{\mathrm{NO}} * \mathrm{R_s}$. NP is the pollen number of one male inflorescence, NO

is the number of ovules per female flower. The fruit set rate was the total number of mature fruits/the total number of female flowers.

Based on observations on fruit development, five size classes were distinguished according to the fruit development period, as follows: 1) Extra small (< 4 cm^3; one-month-old fruits); 2) Small (4–12 cm^3; two-month-old fruits); 3) Middle (12–25 cm^3; three-month-old fruits); 4) Large (> 25 cm^3; mature fruits). The fruit size was represented by the volume (v), which was calculated using the cone volume formula ($v = 1/3\pi r^2 h$).

Results

Population flowering phenology

Monthly blooms were observed for *E. acoroides* between July 2017 and June 2018 in this population. The flowering peak took place in August, and the flower abundance decreased sharply in winter (Fig. 4), aligned with changes in the surface temperature ($r = 0.84$, $df = 7$, $P < 0.01$). In this population, the flowering of females and males was synchronized except in winter. While the anthesis of female flowers lasted only one day, this process lasted 1–3 days in male inflorescences.

Flowering density in plots

From June 2017 to February 2018, the monthly flowering abundance of Plot A was significantly different from Plot B ($t = -2.37$, $df = 8$, $P = 0.045$; Fig. 4). Plot A presented 970 female flowers and 2,238 male inflorescences. The average density of female flowers per month was 0.27 ± 0.28 flowers m^{-2}, and the maximum density was 0.78 ± 0.27 flowers m^2 in July. The average density of male inflorescences per month was 0.62 ± 0.68/m^2, and the maximum density was 2.06/m^2 in August. In Plot B, there were 1,316 female flowers and 2,694 male inflorescences. The average density of female flowers per month was 0.37 ± 0.27/m^2, and the maximum density was 0.68/m^2 in August; the average density of male flowers per month was 0.75 ± 0.59/m^2, and the maximum density was 1.91/m^2 in August.

The sex ratio was male-biased ranging from 1.27 to 4.91 for these two plots with an average of 2.25 ± 1.05 from June to December 2017. Only three female flowers were found in the two plots without male inflorescences in January and February 2018. Females had on average 17.22 ± 3.49 ovules, while male inflorescences contained 544 ± 27 florets, each of them with 97.2 ± 17.92 pollen. The pollen-ovule ratio was ca. 0.98×10^4 :1 in June 2017.

Fruit set

The fruit density was low in plots A and B from June 2017 to February 2018. The average fruit density per month was 0.22 ± 0.047/m^2 in Plot A. The fruit density per month of Plot B fluctuated greatly from 0.01/m^2 in September to 0.32/m^2 in December with a monthly average of 0.16 ± 0.14/m^2.

From June 2017 to February 2018, we found in total 528 and 374 fruits in Plot A and Plot B, respectively. From these, only 77 fruits were mature in Plot A, while no mature fruits were found in Plot B. Therefore, the mature fruit set rate was 3.4% in the Li'an population during this observation period. Most ripe fruits were found in November 2017 (Fig. 5).

Discussion

Seagrass reproductive phenology, investment and success are highly influenced by local environmental conditions (e.g., temperature, light) and habitat health (e.g., pollution). Negative changes which are linked to seagrass coverage reduction and fragmentation can trigger temporal and spatial differences in the timing and production of flowers and fruits (Rollón et al. 2003; Vermaat et al.,2004; van Tussenbroek et al. 2016). Our findings suggest that despite the high reproductive investment throughout the year (in terms of flower formation), there is limited sexual reproductive success (low fruit formation). Consequently, demographic dynamics of local populations of *E. acoroides* are most likely modulated by asexual reproduction through plant fragmentation and clonal growth, with a minor contribution of seeds in recent years. In this case, the natural recovery of *E. acoroides* will be very difficult in this population. Because the rhizome elongation rate of this species is very slow (Marbà and Duarte 1998), while sexual reproduction is the main means for its spread (Yu et al. 2019).

The flowering pattern of Enhalus acoroides

In our study, we recorded nine months' data for the flower abundance of *Enhalus acoroides*, because the plots were destroyed in March in 2018. However, we also found the flowers in March to June 2018 out of the plots in this population. The flowers and fruits of *Enhalus acoroides* were present all year round in Li'an lagoon. Permanent flowering for this species has been also observed in other regions including Papua New Guinea (Brouns and Heijs 1986), Gulf of Carpentaria in Australia (Kenyon et al.1997), Cape Bolinao in north-western Philippines (Rollón et al. 2003), Haad Chao Mai National Park in Thailand (Rattanachot and Prathep 2011). Contrary, flowering intensity shows temporal patterns and geographic differences, with unimodal flowering peaks in summer for populations in Li'an and Cape Bolinao, and bimodal maximal flowering (spring and summer) in Papua New Guinea (Brouns and Heijs; 1986). Such differences in seasonal flowering intensity are probably related to temporal changes in the sea surface temperatures or irradiance. The optimal water temperature for the growth and photosynthesis of *E. acoroides* ranges between 24–33℃ (Agawin et al. 2001). Brouns and Heijs (1986) and Rattanachot and Prathep (2011) found that leaf growth and production of new shoots were higher during spring and summer than during fall and winter. In Li'an lagoon, the highest intensity of flowering was in summer (June to August), whilst the lowest took place in winter when the water temperature was below 24℃.

Most females and males bloom on the same days in Li'an lagoon, coinciding with spring tides as was already noted in early studies on this species (Brouns and Heijs 1986; Rollón 1998). Such synchronization may be common for dioecious seagrass species as documented in the tropical seagrasses *S. filiforme* (Samper-Villarreal et al. 2020)d *testudinum* (van Tussenbroek et al. 2009). For

these species, flowering synchronization has been suggested to be a strategy to maximize fertilization success. Synchronization is also aligned to tidal dynamics, as pollination of *E. acoroides* occurs on the water surface and thus, during low tides. During this period, the male florets float to the surface while the peduncle of the sessile female flower can elongate rapidly (up to 50 cm) lifting the floral buds rapidly to the water surface just before anthesis (Yu S., unpublished data).

The pollination of Enhalus acoroides

Like other abiotic pollinated plants, pollen-ovule ratios in seagrasses are in general high within a range of 10^4–10^5:1 (Ackerman 2006). In contrast, the pollen-ovule ratio documented for *E. acoroides* is considered low (10:1) (Ackerman 2006, Kausik 1941). This low ratio, however, does not account for the number of florets per male inflorescence and also omits the shoot sex ratio, which should be considered when estimating pollen-ovule ratios for dioecious species (Yakimowski and Barrett 2014). By including these characteristics, the pollen-ovule ratio of *E. acoroides* was ca. 0.98×10^4 in June 2017, which is within the range reported for other seagrass species.

Enhalus acoroides is considered as an obligate surface-pollinated plant as its freshwater relative, *Vallisneria* (Cox 1988). Male florets detach underwater and float to the water's surface and encounter the female flower at the water's surface. Interestingly, we also found that wind pollination occurred in *E. acoroides*. When the tide is below 60 cm in Li'an lagoon, the male inflorescence of *E. acoroides* was fully exposed to the air, and the male florets were dispersed by wind. Other aquatic macrophytes also have pollen dispersal by air, such as *Hydrilla verticillate*, which has male flowers that are released underwater, but that catapult pollen through the air into the floating female flowers (Cook et al. 1982). However, we are not sure whether the wind pollination in *E. acoroides* is effective.

Low fruit output of Enhalus acoroides

Excessive disturbance by fishing activities has decreased the density and cover and also increased the shoot mortality of *E. acoroides* in Li'an lagoon (Chen et al. 2015). The total number of flowers in patchy Plot A was much lower than in Plot B. Vermaat et al. (2004) found that meadow fragmentation affected reproductive success and that fruit set of *E. acoroides* was higher with about 50% meadow cover in Bolinao. In our study, Plot A (41% cover) had a slightly higher fruit set than Plot B (85% cover). Therefore, fragmentation was not the main reason resulting in the very low fruit output. The fruits of *E. acoroides* take 3–4 months to mature. During this process, the fruits face several threats. Firstly, the flowers and the young fruits of *E. acoroides* (1–2 months) were eaten by fish and crabs (Yu et al. unpublished data). Similar herbivore pressure on exposed young fruits of *Thalassia testudinum* was also common (Troyo et al. 2021). Physical and chemical disturbances can also impact fruit maturation and reproductive success. For instance, strong winds and waves can break the long peduncle of *E. acoroides*, particularly in summer (Yu et al. unpublished data). Similarly, water pollution can be detrimental to the physiology of the seagrasses causing fruit loss. In Hainan, intense eutrophication is induced by aquaculture activities leading to high epiphyte loads and sulfide poisoning of local seagrass meadows (Herbeck et al. 2014; Chen et al. 2015). This may explain the high proportion of rotten young fruits and dead shoots found

year-round in our study, in which we detected excessive cover of epiphyte and algal on fruits and leaves of *E. acoroides*. Nevertheless, it should be pointed out that our study only observed one year's data in Li'an, and it does not rule out that there are inter-annual or regional differences in the flowering pattern of *E. acoroides*.

Declarations

Author contribution

SY and KJ: designed and analyzed the work and wrote the manuscript with the collaboration of BivT and JDGE; LJC, YCW, and FCZ: collected the data in the field.

Declaration of Competing Interest

The enclosed work has not been published or accepted for publication. There are no financial, personal, or professional interests that could be construed to have influenced the paper.

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References

1. Ackerman JD (2006) Sexual reproduction of seagrasses: pollination in the marine context. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: biology, ecology and conservation. Springer, Dordrecht, The Netherlands, pp 89–109.
2. Agawin NSR, Duarte CM, Fortes MD, Uri, JS, et al. (2001) Temporal changes in the abundance, leaf growth and photosynthesis of three co-occurring Philippine seagrasses. J Exp Mar Biol Ecol 260:217–239.
3. Baranano C, Fernández E, Morán P, Urbieta P and Méndez G (2022) Population dynamics of a fragmented subtidal Zostera marina population affected by shell fishing. Estuar Coast Shelf S 269:107818.
4. Brouns JJWM, Heijs FML (1986) Production and biomass of the seagrass *Enhalus acoroides* (L.f.) Royle and its epiphytes. Aquat Bot 25:21–45.
5. Chen SQ, Wang DR, Wu ZJ, Zhang GX, et al. (2015) Discussion of the change trend of the seagrass beds in the east coast of Hainan Island in nearly a decade. Mar Environ Sci 34:106–113 (In Chinese).
6. Cook CDK, Luond R (1982) A revision of the genus *Hydrilla* (Hydrocharitaceae). Aquat Bot 13:485-504.

7. de los Santos CB, Krause-Jensen D, Alcoverro T, et al. (2019) Recent trend reversal for declining European seagrass meadows. Nat Commun 10:3356.
8. Cox PA (1988) Hydrophilous Pollination. Annu Rev Ecol Syst 19:261–279.
9. den Hartog C (1970) The sea-grasses of the world. North Holland, Amsterdam.
10. Dunic JC, Brown CJ, Connolly RM, Turschwell MP, et al. (2021) Long-term declines and recovery of meadow area across the world's seagrass bioregions. Glob Chang Biol 27(17): 4096-4109.
11. Herbeck LS, Sollich M, Unger D, Holmer M, et al. (2014) Impact of pond aquaculture effluents on seagrass performance in NE Hainan, tropical China. Mar Pollut Bull 85:190–203.
12. Ito MA, Lin HJ, Connor MI, and Nakaoka M (2021) Large-scale comparison of biomass and reproductive phenology among native and non-native populations of the seagrass *Zostera japonica*. Mar Ecol Prog Ser 675:1-21.
13. Jahnke M, Pages JF, Alcoverro T, Lavery PS, et al. (2015) Should we sync? Seascape-level genetic and ecological factors determine seagrass flowering patterns. J Ecol 103:1464–1474.
14. Kausik SB (1941) Structure and development of the staminate flower and the male gametophyte of *Enalus acoroides* (L. fil.), Steud. Proc Indian Acad Sci - Sect B 14:1–16.
15. Kenyon RA, Conacher CA, Poiner IR (1997) Seasonal growth and reproduction of *Enhalus acoroides* (L.f.) Royle in a shallow bay in the Western Gulf of Carpentaria, Australia. Mar Freshw Res 48:335–342.
16. Lee K, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. J Exp Mar Bio Ecol 350:144–175.
17. Lewmanomont K, Deetae S, Srimanobhas V (1996) Seagrasses of Thailand. pp 21-26. In: JSS Kuo, R Phillips, DI Walker, H Kirkman (eds.) Seagrass Biology: Proceedings of an international workshop, Rotmest Island, Western Australia, 25-29th January 1996. Faculty of Science, University of WA.
18. Marbà N, and Duarte CM (1998) Rhizome elongation and seagrass clonal growth. Mar Ecol Prog Ser 174:269-280.
19. McGovern TM and Blankenhorn K (2007) Observation of fruit production by the seagrass *Halodule wrightii* in the northeastern Gulf of Mexico. Aquat Bot 87(3): 247-250.
20. Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, et al. (2006) A Global Crisis for Seagrass Ecosystems. Bioscience 56:987–996.
21. Orth RJ, Lefcheck JS, McGlathery KS, Aoki L, et al. (2020). Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Sci Adv 6, eabc6434.
22. R Development Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
23. Rattanachot E, Prathep A (2011) Temporal variation in growth and reproduction of *Enhalus acoroides* (L.f.) Royle in a monospecific meadow in Haad Chao Mai National Park, Trang Province, Thailand. Bot Mar 54:201–207.

24. Reusch TBH (2003) Floral neighbourhoods in the sea: how floral density, opportunity for outcrossing and population fragmentation affect seed set in *Zostera marina*. J Ecol 91:610–615.
25. Rollón RN (1998). Spatial variation and seasonality in growth and reproduction of *Enhalus acoroides* (L.F.) Royle populations in the coastal waters off Cape Bolinao, NW Philippines. Ph.D. Thesis, IHE-Delft and Wageningen Agricultural University, The Netherlands. 135 pp.
26. Rollón RN, de Ruyter van Steveninck ED, Vierssen W Van (2003) Spatio-temporal variation in sexual reproduction of the tropical seagrass *Enhalus acoroides* (L.f.) Royle in Cape Bolinao, NW Philippines. Aquat Bot 76:339–354.
27. Short FT, Carruthers TJB, Dennison WC, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. J Exp Mar Bio Ecol 350:3–20.
28. Sinclair EA, Sherman CDH, Statton J, Copeland C, et al. (2021) Advances in approaches to seagrass restoration in Australia. Ecol Manag Restor 22(1):10-21.
29. Turschwell MP, Connolly RM, Dunic JC, Sievers M, et al. (2021) Anthropogenic pressures and life history predict trajectories of seagrass meadow extent at a global scale. Proc Natl Acad Sci USA 118(45): e2110802118.
30. Troyo A, Jiménez-Duran K, van Tussenbroek BI, Márquez-Guzmán J, et al. (2021) Fruit development in the seagrass *Thalassia testudinum*: Possible relationships between structure, physiology and defense. Aquat Bot 174: 103418.
31. van Katwijk MM, Thorhaug A, Marba N, Orth RJ, et al. (2016) Global analysis of seagrass restoration: the importance of large-scale planting. J Appl Ecol 53:567–578.
32. van Katwijk MM, van Tussenbroek BI, Hanssen SV, Hendriks AJ, et al. (2021) Rewilding the sea with domesticated seagrass. BioScience 71(11):1171-1178.
33. van Tussenbroek BI, Guzmán JM, Wong JGR (2009) Phenology of marine angiosperms seagrasses: reproductive synchrony in the sea. Functional Diversity of Plant Reproduction, 2009: 17-46 ISBN: 978-81-308-0360-9 Editors: Alicia Gamboa-deBuen, Alma Orozco-Segovia and Felipe Cruz-Garcia.
34. van Tussenbroek BI, Valdiviacarrillo T, Rodriguezvirgen IT, Sanabriaalcaraz SNM, et al. (2016) Coping with potential bi-parental inbreeding: limited pollen and seed dispersal and large genets in the dioecious marine angiosperm *Thalassia testudinum*. Ecol Evol 6:5542–5556.
35. van Tussenbroek BI, Wong J, Marquezguzman J (2008) Synchronized anthesis and predation on pollen in the marine angiosperm *Thalassia testudinum* (Hydrocharitaceae). Mar Ecol Prog Ser 354:119–124.
36. Verduin J, Backhaus JO, Walker D (2000) Estimates of pollen dispersal and capture within submerged *Amphibolis antarctica* meadows. Biol Mar Medit 7:152–155.
37. Verheij E and Erftemeijer PLA (1993) Distribution of seagrasses and associated macroalgae in South Sulawesi, Indonesia. Blumea 38: 45–64.
38. Vermaat JE, Rollón RN, Lacap CDA, Billot C, et al. (2004) Meadow fragmentation and reproductive output of the SE Asian seagrass *Enhalus acoroides*. J Sea Res 52:321–328.

39. von Staats DA, Hanley TC, Hays CG, Madden SR, et al. (2021) Intra-meadow variation in seagrass flowering phenology across depths. Estuaries Coast 44:325-338.
40. Walker D, Olesen B, Phillips RC (2001) Reproduction and phenology in seagrasses. Glob Seagrass Res Methods:59–78.
41. Waycott M, Duarte CM, Carruthers TJB, Orth RJ, et al, (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci USA 106:12377–12381.
42. Yakimowski SB, Barrett SCH (2014) Variation and evolution of sex ratios at the northern range limit of a sexually polymorphic plant. J Evol Biol 27:1454–1466.
43. Yang Y, Gao S, Zhou L, Han Z, et al. (2016) Analysis of tides and P-A relationships of Xincun and Li-an lagoons, southeastern Hainan Island. Quaternary SCI 36:163-172.
44. Yu S, Wu YC, Serrao EA, Zhang JP, et al. (2019) Fine-scale genetic structure and flowering output of the seagrass *Enhalus acoroides* undergoing disturbance. Ecol Evol 9:5186–5195.
45. Samper-Villarreal J, Loría-Naranjo M, van Tussenbroek BI, Cortés J. (2020) Synchronized sexual reproduction of the seagrass *Syringodium filiforme* (Cymodoceaceae) in a tropical reef lagoon on the Caribbean Coast of Costa Rica. Rev Mar Cost 12 (1): 49-68.

Figures

Figure. 1

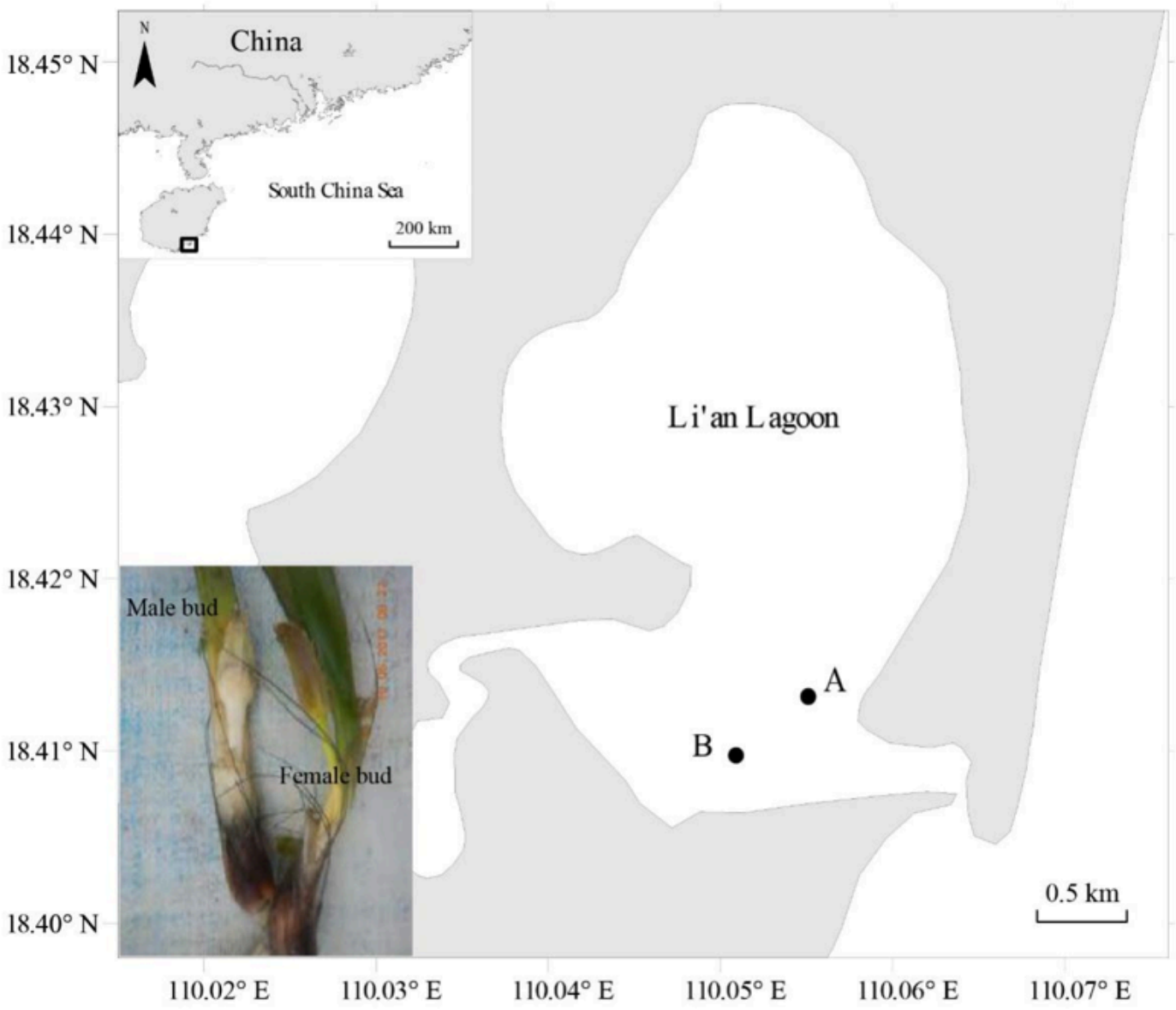

Figure 1

Sampling sites of *Enhalus acoroides* in Li'an Lagoon, Hainan Island, South China Sea.

Figure. 2

Figure 2

Three stages of female flower and the fruits for *Enhalus acoroides.* a-b. young female flowers, c. mature flower, d. newly-fertilized flower, e. fruits.

Figure. 3

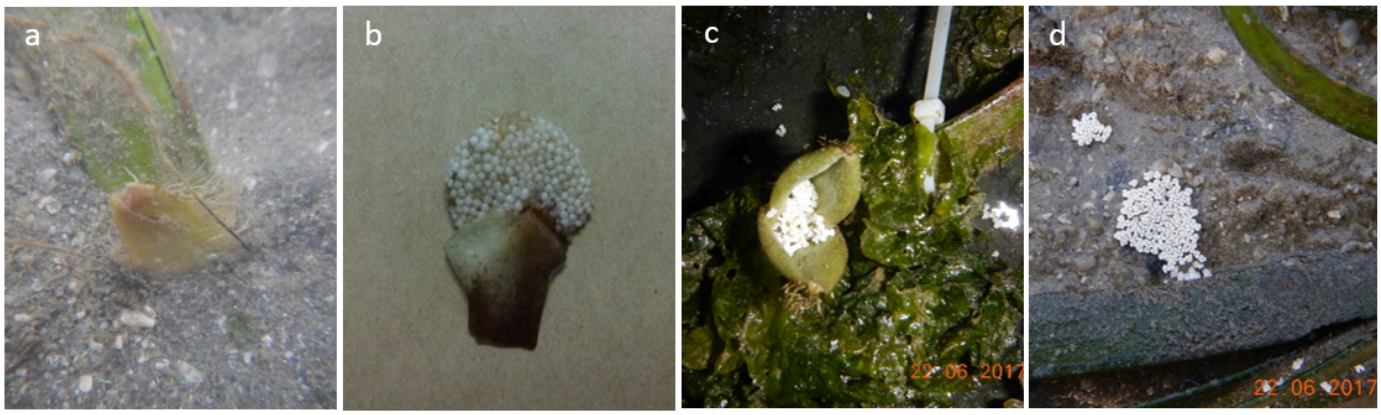

Figure 3

The stages of male flower for *Enhalus acoroides*. a. male bud, b. unopened flower inflorescence, c. mature inflorescence, d. small florets

Figure. 4

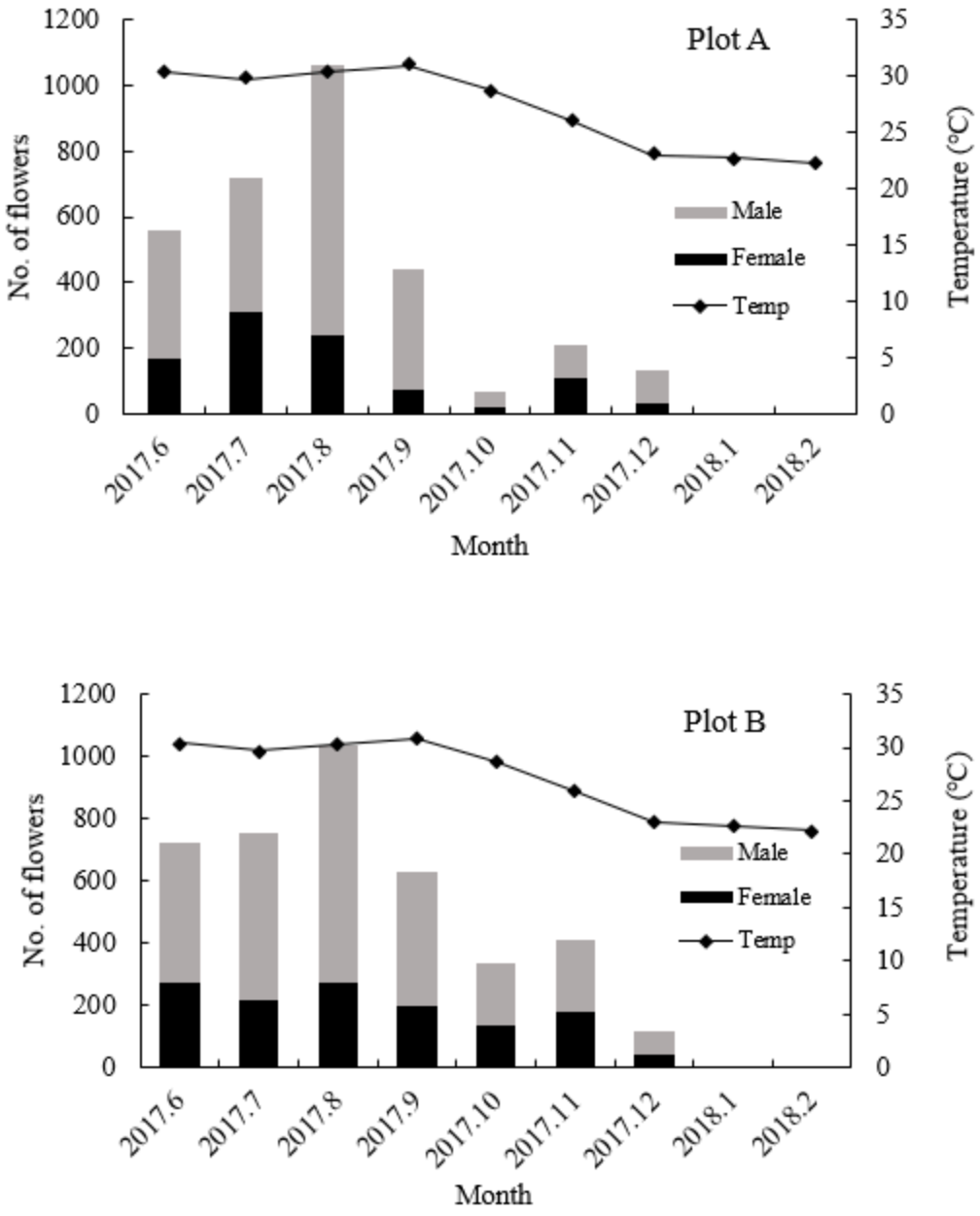

Figure 4

Flower number of *Enhalus acoroides* in the two plots during June in 2017 to February in 2018.

Figure. 5

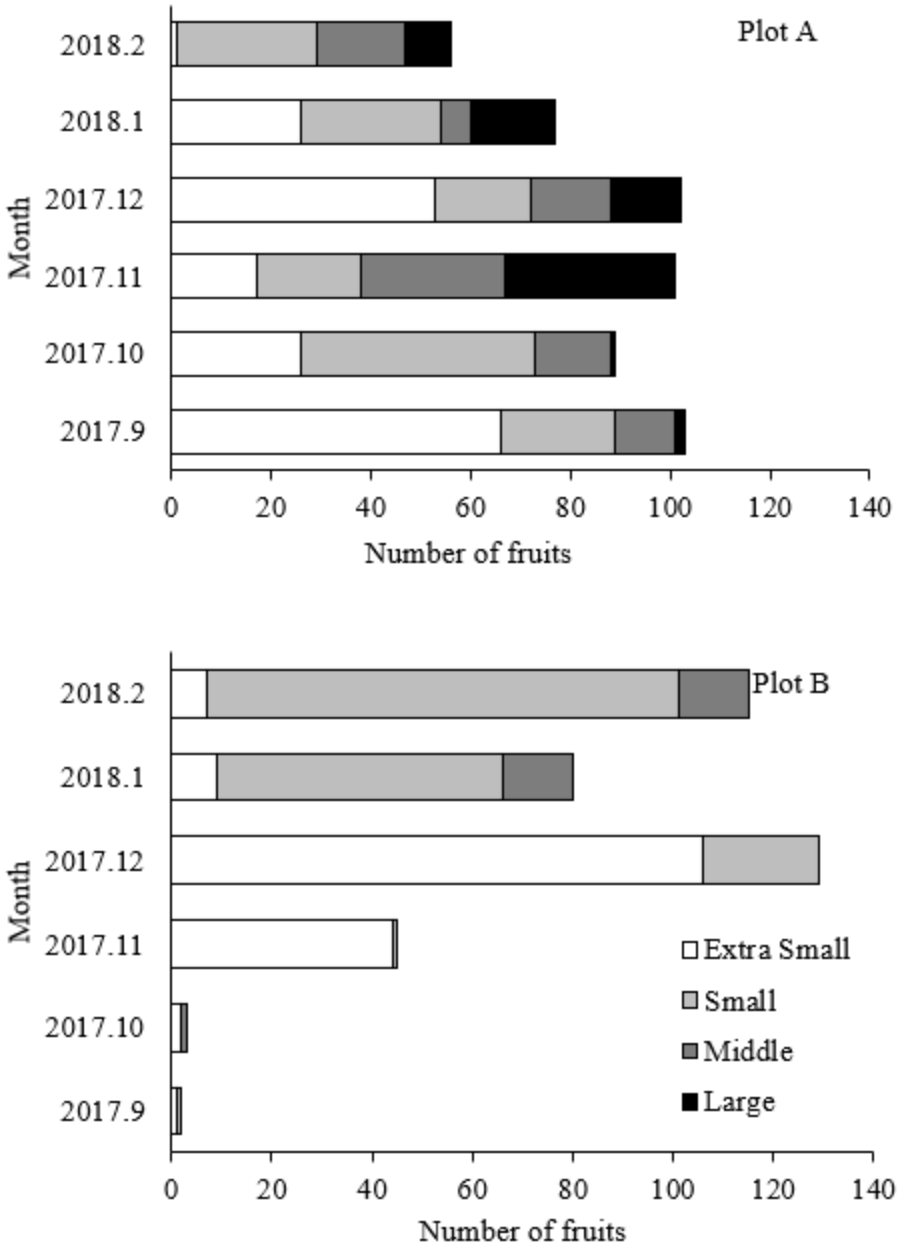

Figure 5

Fruit output of *Enhalus acoroides* for plots A and B from September 2017 to February 2018, respectively. Extra small (< 4 cm^3; one-month-old fruits); Small (4–12 cm^3; two-month-old fruits); Middle (12–25 cm^3; three-month-old fruits); Large (> 25 cm^3; mature fruits).