

Female flowers of tropical seagrass *Syringodium isoetifolium* (Alismatales: Cymodoceaceae) in an ex-situ aquarium

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Abstract. Flowering is an important component of seagrass life cycle, but so far the flowering habit of seagrasses in the tropics remains largely understudied and/or under-reported. To date, only four out of the 12 seagrass species found in Singapore have limited phenological information described. A seagrass species, *Syringodium isoetifolium*, was observed to have flowered in an ex-situ aquarium. Only female flowers were produced and they lasted for ~five weeks before senescence. Flowering in *Syringodium isoetifolium* is postulated to be triggered by lowered temperatures in literature; however, that signal was absent in the environment of the ex-situ aquarium, which was monitored for temperature and nutrient levels in the prior nine months. Documentation of flowering habits of seagrasses grown in aquaria can provide useful information for field monitoring and conservation efforts for seagrass habitats.

Key words. Seagrass, *Syringodium isoetifolium*, reproduction, flowering

INTRODUCTION

Coastal modification of Singapore's shorelines has resulted in the loss of seagrass meadows (Lai et al., 2015); it is estimated that around 45% of seagrass has been lost during the last fifty years (Yaakub et al., 2014). Monitoring and conservation efforts are strongly needed to ensure the persistence of remaining seagrass beds and their associated ecosystem services. Even though seagrasses can propagate asexually, i.e. clonal expansion, sexual reproduction is paramount to genetic diversity and improved resilience to environmental stressors (Unsworth et al., 2015). Hence, understanding the reproductive phenology of seagrasses is important for their conservation.

The flowering phenology of tropical seagrass is largely understudied, particularly in Singapore. Existing data were compiled from observations made by monitoring groups and nature enthusiasts during sporadic visits to seagrass beds. With the exception of *Halophila beccarii* and *Halophila decipiens*, all seagrass species in Singapore are dioecious, i.e., having separate male and female flowers (Bujang et al., 2006). McKenzie et al. (2016) noted that between January 2000 and October 2014, there were 94 recorded independent observations of seagrass flowers (male or female) or seed-set (fruit with seed) from four seagrass species, namely *Enhalus acoroides*, *Thalassia hemprichii*, *Halophila ovalis* and *Halophila decipiens*. Compared to the four species that have been described so far (McKenzie et al., 2016), *Syringodium isoetifolium* is characterised as an opportunistic species with life history traits that are intermediate between colonizing (e.g., *Halophila* sp.) and persistent (e.g., *Enhalus acoroides*) species in the succession of seagrass meadows (Kilminster et al., 2015). *Syringodium isoetifolium* has leaves that are circular in cross-section and taper to a smooth pointed tip (Waycott et al., 2004). It is widely distributed across tropical and subtropical coastal marine environments in South Africa, Indo-Pacific, and Australia (Kuo & den Hartog, 2001).

Observations of *Syringodium isoetifolium* flowering have only been reported once in Singapore (L. McKenzie and R. Yoshida, 24 March 2007, herbarium record accessed from Seagrass-Watch [2015]). This paper provides a brief account of the characteristics of female flowers of *Syringodium isoetifolium* as observed in an ex-situ aquarium. Temperature and nutrient levels in the tank prior to the flowering event were documented here.

OBSERVATION

Syringodium isoetifolium flowering shoots were first observed on 7 January 2019. Flowering shoots were distinguished from non-flowering shoots by the presence of flowers arranged as distinct cymose inflorescences, known as a 'cyme' (Fig. 1A). Inflorescences were terminal at erect shoots (Kuo & den Hartog, 2001) and branched in one plane. Each cyme bore around 6–10 female flowers (Fig. 1B). The female flowers were observed to be sessile, with a pair of elongated carpels about 1–1.5 mm in diameter, each tapering into a short style which divides into two stout stigmata 6–8 mm long (Kuo & McComb, 1998) (Fig. 1C). *Syringodium isoetifolium* is dioecious. However, no male flowers, i.e. flowers with anthers, were observed. In total, 17 flowering shoots were observed, which we estimated to be less than 4% of total shoot density. By 12 February 2019, bracts of most of the mature but unfertilised flowers had turned brown, senesced and broke off at the nodes. Shoots that flowered appeared cropped and eventually senesced after the flowers

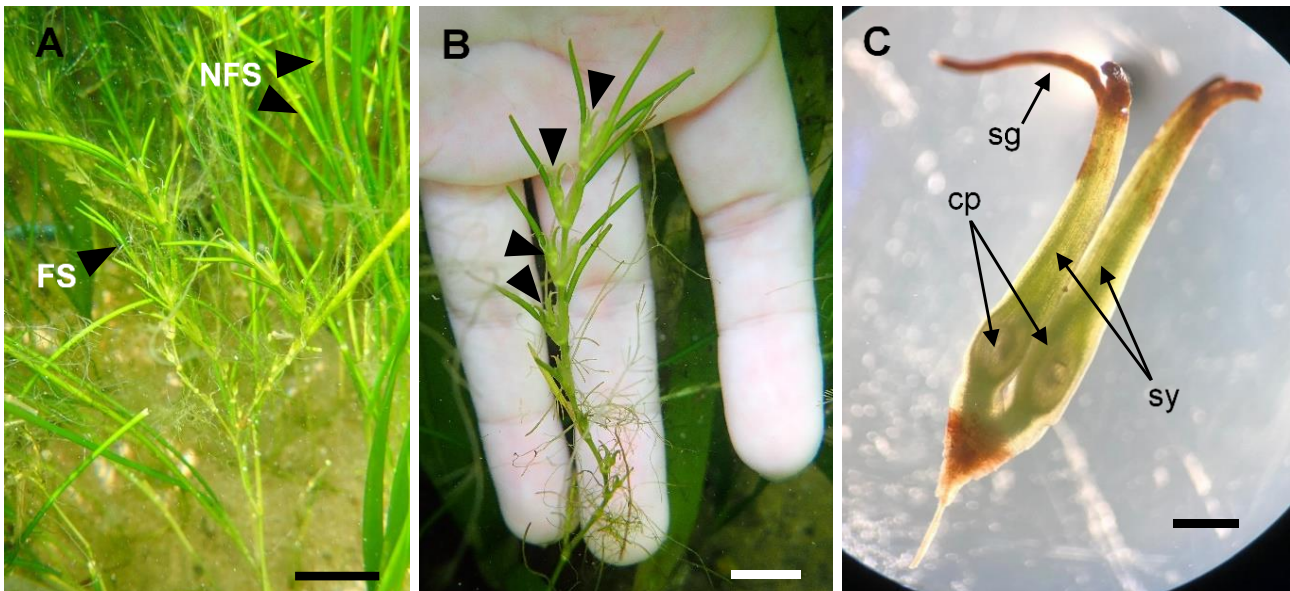


Fig. 1. (A) Inflorescences of female flowers of *Syringodium isoetifolium*. Flowering shoots (FS) are distinguished from non-flowering shoots (NFS) by the presence of terminal inflorescences. (B) A cyme, with arrows pointing at individual female flowers. (C) Individual female flower, with carpels (cp), styles (sy) and stigmata (sg). The stigmata were very delicate and broke off easily. Scale bars = 20 mm [A], 20 mm [B], 2 mm [C].

were shed. During flowering, we also noticed leaf shortening and shedding of non-flowering *Syringodium isoetifolium* shoots. After the flowering event, there was an estimated overall ~10% reduction in shoot density.

The ex-situ outdoor aquarium that housed the *Syringodium isoetifolium* plants was part of the facility on Saint John's Island National Marine Laboratory. *Syringodium isoetifolium* was collected from Eagle Bay, Lazarus Island (1°13' 33.55"N, 103°51'16.38"E), and planted in the aquarium tank in a 1:1 sand/mud mixture in May 2017. The aquarium tank holds ~500 L of seawater, flowing through at a rate of 2 L min⁻¹, cumulating in 5.76 times of seawater turnover per day. Seawater was pumped from the fast-flowing channel, at 10 m depth, off the western shore of St John's Island, and sand-filtered (400 µm) before being supplied to the tank. The tank was covered with 2 layers of shade nets (50% shading), which meant the plants were exposed to 25% of down-welling solar irradiance.

Available temperature and nutrients data of seawater in the outdoor aquarium for the prior nine months are presented in Fig. 2. Temperature was recorded using a hand-held temperature probe (probe LDO101, logger 40d, Hach, USA), and nutrients were assayed from weekly water samples using assay kits for total nitrogen (ammonia and nitrates) and phosphates (Hach ammonia reagents, NITRAVER5 and PHOSVER3). In the nine months prior to the observation of flowers (July to December 2018), average temperature was 29.52 ± 0.36°C (±SD). Average levels of total nitrogen (ammonia and nitrate) and phosphates (July to December 2018) were 1.03 ± 0.23 mg l⁻¹ and 0.03 ± 0.02 mg l⁻¹ (±SD) respectively.

DISCUSSION

Documented observations of *Syringodium isoetifolium* flowers in the region are rare, with sporadic records from Pulau Tinggi, Malaysia (Bujang et al., 2006), Caramoan, Philippines (Clores and Agoo, 2013), Manora Palk Bay, India (T. Thangaradjou, 2011, herbarium record from Seagrass-Watch [2015]) and Cyrene Reef, Singapore (L. McKenzie and R. Yoshida, 2007, herbarium record from Seagrass-Watch [2015]). All these observations were documented from January to October across different years and were not detailed with measurements of in-situ environmental conditions. Here, an observation of *Syringodium isoetifolium* flowering in an aquarium tank that was monitored for water conditions could provide some insight into the flowering phenology of this species and aid in field surveys.

Flowering of *Syringodium isoetifolium* has been known to be induced by lowered temperatures (3–6°C), and possibly by a change in nutrients in ex-situ experimental cultures (McMillan, 1980, 1982). In tropical northeast Australia, peak flowering and seed-set in *Syringodium isoetifolium* typically coincides with the dry and cool austral winter season (August to October), when seawater temperatures are generally lower and underwater irradiance levels are high (Waycott et al., 2004; McKenzie et al., 2015). In the aquarium tank, where temperature and nutrients level tend to mirror that of the intake seawater, there were a few consecutive weeks prior to flowering where temperatures dropped below the average (three weeks in July, four weeks in September, and two weeks in October 2018). However, the drop was less than 0.5°C and it remains unclear if that alone is sufficient to trigger flowering. It was noted that total nitrogen

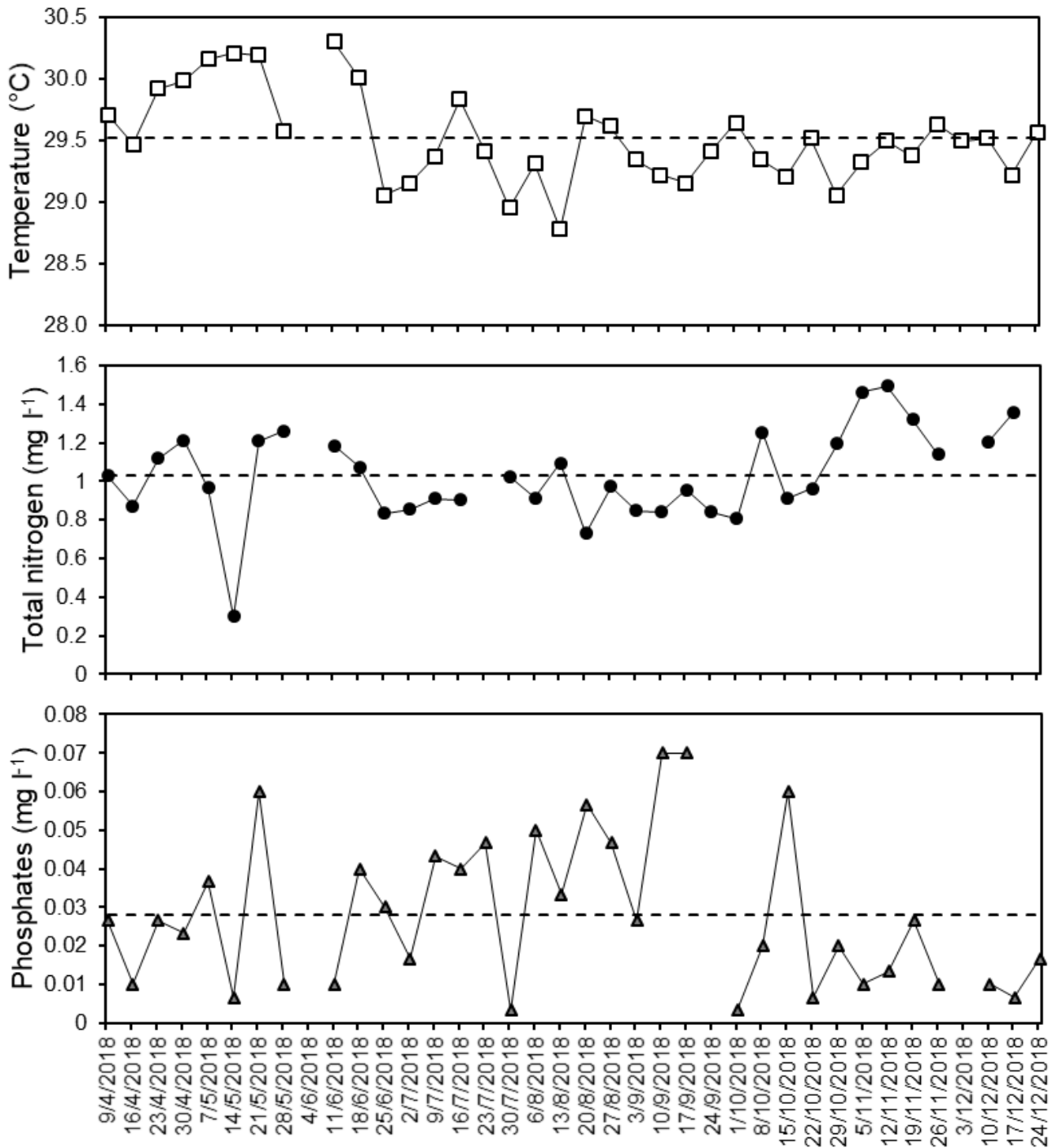


Fig. 2. Environmental data in aquarium tank. Weekly-averaged (temperature, total nitrogen and phosphates) data were plotted from April to December 2018. Dotted line represents the averaged value for each parameter for the period of April to December 2018.

levels increased while phosphates levels decreased from end October to December 2018, but the role of nutrient availability on seagrass flowering remained unknown. Since *Syringodium isoetifolium* has a wide distribution range (Kuo & den Hartog, 2001), it is possible that flowering cues might exhibit some variation across regions (Blackman, 2017) and a wide range of environmental conditions were involved in the flowering cue for this species (McMillan, 1980). Future work should aim to narrow down the environmental cues for reproduction in this species, as well as other seagrass species that have some phenological data described in the region (McKenzie et al., 2016).

Female flowers of observed *Syringodium isoetifolium* were morphologically similar to that of its twin species *Syringodium filiforme* (Kuo & McComb, 1998). Detailed aspects of floral morphology and development of *Syringodium filiforme* flowers can be found in Tomlinson and Posluszny (1978). No male flowers were found before, during or after the period when female flowers were observed. As there has been no literature to suggest that flowers of different sexes develop under different environmental conditions, this suggests that *Syringodium isoetifolium* observed in the tank were mainly female. So far, there has been only one documented study of the female-to-male flower ratio in

a seagrass meadow—that study reported a high ratio of female-to-male *Syringodium isoetifolium* flowers (~9:1) in a sub-tidal seagrass meadow in the Philippines (Clores & Agoos 2013). Whether this skewed female-to-male flower ratio is an environmental response, or simply a consequence of the distribution of dioecious flowers requires corroboration of further field observations.

Syringodium isoetifolium flowers are not easy to observe in the field. The short ~5-week period between the observation of flowers to their senescence in the aquaria tank points to the difficulty in seeing seagrass flowers, at least of this species, in the field if survey intervals are longer than one month. Furthermore, the female flowers of *Syringodium isoetifolium* were rather delicate—they break off at the nodes with some agitation—and inconspicuous (cymes were 6–10 cm long) within the canopy. This meant that *Syringodium isoetifolium* could be vulnerable to stress from human visitors to seagrass beds, i.e. trampling on seagrasses, during their flowering period. Keeping aquarium cultures of seagrasses can be a complementary approach to field surveys in documenting flowering habits (for e.g., McMillan, 1980, 1982).

Information on the phenology of seagrasses is important for seagrass management efforts. In Singapore, opportunistic and ad hoc observations suggest that January to late March could be the peak flowering and seed-set period for Singapore's seagrass, with a possible second period in November (McKenzie et al., 2016). Species which form persistent seed banks, such as *Syringodium isoetifolium* (Inglis & Waycott, 2001), are important for the recovery of seagrass meadows following strong disturbances (Unsworth et al., 2015). Quantification of reproductive success is also essential in understanding dispersal and recruitment characteristics of different seagrass species (McMahon et al., 2014). For seagrass restoration efforts that want to use sexual propagules, knowledge of seagrass reproductive biology is indispensable. Therefore, future studies should aim to fill our current knowledge gaps on seagrass reproductive phenology through field observations and aquaria experiments.

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