

Observations on staminate flowers of *Cymodocea serrulata* in ex situ aquarium

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Abstract. Seagrasses are capable of forming meadows by clonal growth and sexual reproduction. *Cymodocea serrulata*, a common and widespread seagrass species that can be found throughout the tropical Indo-Pacific, was previously never documented flowering in Singapore. However, in early January 2020, *Cymodocea serrulata* staminate (male) flowers were observed in an ex situ aquarium. Here, we document the development of staminate flowers. We also present the environmental conditions in the tanks and provide a comparison to literature. This observational record can aid in seagrass assessment and monitoring programme efforts.

Key words. seagrass, *Cymodocea serrulata*, ex situ, staminate flowers, Singapore

INTRODUCTION

Seagrasses are marine angiosperms that provide high-value ecosystem services. These include providing shelter and nursery for fishes and invertebrates (Waycott et al., 2011), and providing food to other animals such as dugongs (Marsh et al., 2011) and turtles (Christianen et al., 2013). Seagrass meadows are also highly productive ecosystems (Duarte & Chiscano, 1999; Ow et al., 2015; Holmer, 2019) capable of capturing and storing organic carbon (Fourqurean et al., 2012). Despite their importance, seagrass meadows are experiencing a worldwide decline (Orth et al., 2006). Understanding their ecology and reproductive physiology is crucial for seagrass management and conservation.

The formation of dense seagrass meadows is a result of clonal growth and sexual reproduction (Tomlinson, 1974). In the island city of Singapore, there are 12 species of seagrass and seagrass beds cover about 0.04% of the Singapore coastline (Fortes et al., 2018). With the exception of two *Halophila* spp., all other seagrass species are dioecious (Bujang et al., 2006). To date, only flowers of *Enhalus acoroides*, *Halophila decipiens*, *Halophila ovalis*, *Thalassia hemprichii* and *Syringodium isoetifolium* have been recorded in Singapore (McKenzie et al., 2016; Ow et al., 2020).

Cymodocea serrulata is a successional species common throughout the tropical Indo-Pacific (Short et al., 2001). In Singapore, it occurs on coastal areas, offshore patch reefs and fringing reefs (McKenzie et al., 2016). Its flowers are hardly found in Malaysia (Bujang et al., 2006) and have never been observed in Singapore. Flowering of *Cymodocea serrulata* was observed in our experimental tanks from January to February 2020. The emergence of male flowers was observed across all experimental treatments, indicating that the initiation of flowering potentially occurred before the commencement of the experiment.

This paper provides a brief account of the characteristics of male *Cymodocea serrulata* flowers as observed in an ex situ aquarium. It describes the stages of staminate *Cymodocea serrulata* flower development to aid with identification during field monitoring. We also describe the environmental conditions in the ex situ aquarium when flowering occurred.

MATERIAL & METHODS

Cymodocea serrulata shoots were collected from Eagle Bay, Lazarus Island (1°13'33.55"N, 103°51'16.38"E), in September 2019. The shoots were brought to St. John's Island National Marine Laboratory, Singapore, and potted with an average of 11 shoots per pot in 1:1 sand/mud mixture. Two 3,870 mm², square-shaped pots were placed into each of the 12 glass tanks (length 485 mm, breadth 375 mm, height 330 mm) in a temperature-controlled aquarium. Seawater was supplied from the fast-flowing channel, at 10 m depth, off the western shore of St. John's Island, entering the tanks at a rate of 0.5 L min⁻¹. Prior to reaching the tanks, raw seawater was sand-filtered (400 µm) before undergoing protein skimming, UV exposure and further filtration (400 µm and 200 µm). Each tank was exposed to 12:12 hour dark: light cycle of approximately 162 µmol m⁻² s⁻¹ photosynthetic active radiation (PAR), which was measured with a PAR quantum sensor (Apogee, USA). Illumination was provided using LED lamps (Ocean Revive T247, China). Temperature, salinity, dissolved oxygen and pH were recorded using a hand-held temperature probe (logger 40d, Hach, USA) at least three times a week. The size of the flowers on photographs were analysed using Image J (version 1.52a).



Fig. 1. Developmental stages of male flowers of *Cymodocea serrulata*. A, development starts in the leaf sheath, which swells; B, leaf sheath splits; C, the male flower subtends from the swollen leaf sheath, with intact pollen and tightly closed anthers; D, the filament continues to extend from the sheath during anther dehiscence, and tannin cells became visible; E, anther walls thicken upon further maturation. All photographs were taken from different shoots. All ruler units are in cm.

OBSERVATIONS

Ten staminate flowers from *Cymodocea serrulata* were observed. The first flower was observed on 3 January 2020 in tanks with ambient temperatures (average of $27.8 \pm 0.29^\circ\text{C}$ [$\pm\text{SD}$]). 27 days later, the first flower was observed in tanks with heated seawater (average of $29.9 \pm 0.16^\circ\text{C}$ [$\pm\text{SD}$]). In both treatments, flowers continued to bloom until 10 February 2020. Seawater in the tanks with flowers had salinity between 31.6 and 32.5 psu, pH between 7.76 and 8.21, and dissolved oxygen content of 8.26 to 8.93 mg L⁻¹.

The staminate flower development is depicted in Fig. 1. The first visual cue of a flower began from within the leaf sheath. The leaf sheath swelled (Fig. 1A) before it split (Fig. 1B). The solitary male flower, consisting of a dithecous anther attached by a filament to the shoot, subtended from the swollen leaf sheath (Fig. 1C). Anther dehiscence occurred (Fig. 1D) and pollen was released, with specs of tannin cells becoming visible in the anther. As the flower continued to mature, the walls of the anther thickened and became opaquer (Fig. 1E). All flowers were solitary and found on the terminal end of the shoots. Flowers were observed to develop even in the absence of leaves. The average anther length ($n = 6$) was 7.88 ± 0.63 mm ($\pm\text{SD}$), anther width ($n = 6$) 3.00 ± 0.82 mm ($\pm\text{SD}$), and filament length that was extended from the sheath ($n = 5$) 5.88 ± 1.69 mm ($\pm\text{SD}$). One flower did not develop a pedicel that extended beyond the sheath (Fig. 1C). In general, staminate flower development was observed over a span of 5–7 days, from the swelling of leaf sheath (Fig. 1A) to the maturation of flower (Fig. 1E). Post-maturation, staminate flowers senesce within 3–4 days.

DISCUSSION

In situ records of staminate *Cymodocea serrulata* flowers have been made in Moreton Bay, Australia (Kirkman, 1975); New Guinea (Johnstone, 1982); coast of Tamil Nadu, Southern India (Parthasarathy et al., 1991); Magnetic Island, Australia (Birch & Birch, 1984); tropical northeastern Australia (Waycott et al., 2004); and Okinawa Island, Japan (Nonaka & Yonaha, 2010). The only record of environmental conditions was collected from ex situ observations of staminate flowers from Kenya (McMillan, 1980b) and Queensland, Australia (McMillan, 1982). This is the first record of staminate *Cymodocea serrulata* in Singapore, observed in an ex situ aquarium. It provides details of the emergence stages of staminate *Cymodocea serrulata* flowers and the environmental conditions which can be used to aid field monitoring and seagrass conservation efforts.

Temperature is a crucial factor for the triggering of flower development in seagrasses (McMillan, 1982). This effect could be site- or region-specific as flowers were observed blooming in different seasons. Peak flowering and seed setting of *Cymodocea serrulata* in tropical northeastern Australia occurs from May to August (median range of 23.3 to 25.1°C [Australian Government, Bureau of Meteorology, 2020b]), which coincides with dry and cool winter temperatures (Waycott et al., 2004). Kirkman (1975) suggested that *Cymodocea serrulata* flowering season in Queensland occurs from February to March, in the late summer and early autumn season (mean maximum temperature of 25.8 to 26.6°C [Australian Government, Bureau of Meteorology, 2020a]). In Tamil Nadu, male and female flowers were found in February (Parthasarathy et al., 1991) at the end of winter, with the average temperature estimated to be 26.8°C (Stat World, 2020). In Okinawa island, Japan, male *Cymodocea serrulata* flowers were found mainly in October (autumn), when average temperatures were between 24 and 28°C (Time and Date, 2020), and continued until February (winter) (Nonaka & Yonaha, 2010) when average temperatures were between 15 and 20°C (Time and Date, 2020). It is suggested that the peak flowering season of seagrasses in Singapore happens between February and May (McKenzie et al., 2016). However, *Cymodocea serrulata* flowers had never been observed in Singapore. Our flowers were observed between 3 January 2020 and 11 February 2020, which coincides with the northeast monsoon in Singapore. The flowers were found

in temperatures as low as 27.4°C and as high as 30.1°C, which is similar to the temperatures where flowering was observed in other ex situ cultures (McMillan, 1980b, 1982).

Variations of *Cymodocea serrulata* staminate flower morphology were noticed upon comparison to literature (Kirkman, 1975; McMillan, 1980b; Nonaka & Yonaha, 2010). The flower characteristics that were previously described and photographed (see Kirkman, 1975: fig. 1B; McMillan, 1980b: figs. 1–3; Nonaka & Yonaha, 2010: fig. 2), in particular, the colour and flower structure with the ditheous anther, resemble what we observed in our tanks (Fig. 1). The anther-bearing region of the flower ranged from 6 mm (McMillan, 1980b) to 22 mm (Kirkman, 1975) in length. Nonaka & Yonaha (2010) described the stages of *Cymodocea serrulata* flower development in situ. What was unique to our ex situ observation was that the swollen leaf sheath split before the anther subtended. In addition, *Cymodocea serrulata* staminate flowers continued to mature even after the pollen was released, through the extension of the filaments above the sheath (Fig. 1C, D). This phenomenon was first recorded by McMillan (1980a). Flowers described by Kirkman (1975) stood out, as they did not extend past the sheath (Kirkman, 1975: fig. 1B). It is suggested that staminate flowers held within the sheaths are possibly a consequence of lower and more stable temperatures (27°C) (McMillan, 1980b). A dominant feature present in male flowers was dense tannin cells (Fig. 1D). The presence of these visible black cells on the surface of the anther was not described by Kirkman (1975). There have also been reports of more than one male flower developing from a leaf sheath (McMillan, 1980b; Nonaka & Yonaha, 2010). All 10 of our flowers were solitary.

All of our flowers were male. McMillan (1980b) found that pistillate (female) flowers bloomed when they were cultured between 27 and 31°C and between 24 and 26°C. When the same pot of plants was heated from 24–26°C to 27–31°C, under 13 h photoperiod, with between 70 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of white fluorescent light, both male and female flowers developed. Further investigation found that more flowers bloomed when salinity was reduced to 25 ppt (McMillan, 1980a, 1980b). It is not clear if our male-only observations were a result of environmental factors or the disproportionate number of male plants at the collection site. Future work is needed to explore developmental features of seagrass flowers and their environmental conditions. With so little known about Singapore's seagrass propagation in and ex situ, any additional information would aid in seagrass assessment and monitoring programme efforts.

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