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Cover: Whale Shark *Rhincodon typus* and Reef - made with poster colours. © P. Kritika.

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# Flowering and fruiting of Tape Seagrass *Enhalus acoroides* (L.f.) Royle from the Andaman Islands: observations from inflorescence buds to dehiscent fruits

# Swapnali Gole 10, Sivakumar Kuppusamy 20, Himansu Das 30 & Jeyaraj Antony Johnson 40

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**Abstract:** Seagrass phenophases are crucial in understanding their reproductive biology but are seldom documented. We studied flowering and fruiting phenophases of *Enhalus acoroides* from a mixed-species intertidal seagrass meadow in Ritchie's archipelago, Andaman Islands, India. The estimated mean densities of pistillate and staminate flowers were  $16.0 \pm 12.0/$  m<sup>2</sup> and  $12.7 \pm 7.3/$  m<sup>2</sup>, respectively. We observed the bloom of free-floating male flowers (961.7 ± 360.4/ m<sup>2</sup>) during the spring low tides (at mean sea surface temperature ~30°C). Seagrass cover, shoot density, and canopy height of *E. acoroides*, along with flowering densities, showed a zonal variation within the sampled meadow. We report the first-time observations of several phenophases of *E. acoroides*, such as female inflorescence buds, male inflorescence, a bloom of released male flowers, pollination, and fertilized flowers from the Indian waters. We also report the prevailing threats to seagrass meadows, such as meadow scarring done by boat anchorage in the Andaman Islands.

Keywords: Mass bloom, meadow scarring, mixed-seagrass meadow, Swaraj Dweep.

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Author contributions: SG—Study design, field data curation, data processing and analysis, conceptualization, and drafting of the manuscript; SK—Funding acquisition, Study design, supervision, review, and editing of the manuscript; HD—Methodology design, supervision of work, validation of phenophases, review, and editing of the manuscript; JAJ—Funding acquisition, Supervision, review, and editing of the manuscript.

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## INTRODUCTION

Characterizing the demography of local seagrass populations is essential to understanding the phenology and ecological processes of seagrass species (Inglis 1999). Such information is critical to improving the knowledge and management of high ecological value species like Enhalus acoroides (L.f.) Royle that regulates the food web, primary production, & sediment dynamics and supports a diversity of benthic organisms & fish communities (Estacion & Fortes 1988; Komatsu et al. 2004; Yu et al. 2018). E. acoroides has a wide distribution range in the Indo-Pacific region, extending from the eastern coast of Africa to northern Australia (Waycott et al. 2004; Short & Waycott 2010). The species is dioecious and reproduces asexually (through clonal growth) and sexually (pollination). Pollination in E. acoroides is epihydrophilous, and fruiting and flowering occur throughout the year (Hartog 1970; Brouns & Heijs 1986; Ackerman 2006; Rattanachot 2008). The positively buoyant seeds (Hartog 1970) and released fruits have a higher potential for long-distance dispersal, thus facilitating the wider species distribution and ensuring succession (Lacap et al. 2002; Kendrick et al. 2012).

In the Indian waters, E. acoroides is known to occur on the southeastern coast, Andaman & Nicobar Islands (ANI), and Lakshadweep Islands (Jagtap 1991, 1992; Das 1996). In ANI, E. acoroides distribution is reported from the North Andaman (Paschim Sagar and North Reef), South Andaman (Tarmugli, Chidiyatapu, Wandoor, Dugong Creek, and Vivekandapur), Ritchie's archipelago (Kalapatthar, Vijay Nagar, Inglis, and Henry Lawrence), and Nicobar archipelago (Pilomilow, Camorta, Trinket, Nancowry, Katchal, and Great Nicobar) (Jagtap 1992; Das 1996; Thangaradjou et al. 2010; D'Souza et al. 2015; Ragavan et al. 2016; Savurirajan et al. 2018; Figure 1). Although the seagrass distribution, status, and associated fauna of E. acoroides are well documented, the reproductive phenology of this species was rarely observed from the Indian coastal waters including from ANI (Patankar et al. 2019).

Seagrasses in the ANI are vulnerable to humaninduced (coastal modification and pollution) and natural stressors (tsunami and recurrent cyclones). These threats may vary in intensity and subsequently have caused habitat alteration or in worst-case scenario, a complete wipe-out of the local populations. For example, the 2004 tsunami in the Indian Ocean critically impacted several seagrass meadows and changed the species composition, with the local extinction of a few species (Thangaradjou et al. 2010). For recovering from such major disturbances through recolonization, sexual reproduction (seeds) has proven to be more effective than clonal expansion (ramets) in the seagrass restoration initiatives (Darnell & Dunton 2016). Thus, for directing local efforts for seagrass conservation and effective management of large-scale loss, documenting the sexual phases of seagrass species is a prerequisite (Short & Wyllie-Echeverria 1996).

Despite sexual reproductive strategies of species like E. acoroides contribute to the resilience of seagrass populations and genetic diversity (Duarte et al. 1997; Yu et al. 2018), these observations are scarcely reported from the Indian waters (Patankar et al. 2019). In this context, the present study aims to fill the existing research gaps in seagrass phenology of E. acoroides from the Indian waters and reports rare phenological phases from a mixed-species intertidal seagrass meadow of the Andaman Islands. Our study presents a detailed natural history observation on 10 different flowering and fruiting phenophases of E. acoroides, which provide a baseline for future research. Although opportunistic in nature, we believe our findings establishes improved knowledge of seasonality in phenology of the species, especially in the wake of E. acoroides gaining attention as a target species in global seagrass restoration initiatives (Lawrence et al. 2007).

# MATERIALS AND METHODS

As a part of pan-archipelago seagrass exploratory surveys, we sampled a mixed species intertidal seagrass meadow in Vijay Nagar, Swaraj Dweep Island of Ritchie's Archipelago (South Andaman; Figure 1), in January 2021 at the afternoon spring low tides. We mapped the seagrass meadow by walking around its fringes with a GPS and calculated the sampled area on Google Earth Pro version 7.3. Quadrats (0.5 X 0.5 m size; n = 18) were placed randomly in the selected seagrass meadow to document the species composition and seagrass cover (Duarte & Kirkman 2001). The shoot density (shoots/m<sup>2</sup>) of E. acoroides was calculated by counting all the shoots within the quadrat. Further, we randomly selected 20 shoots of E. acoroides from each sampling point and recorded canopy height using a measuring scale (cm). In addition, environmental variables such as sea surface temperature (SST), pH, and salinity were recorded at each sampling point using handheld multi-parameter testers (Eutech Oaklon- PCS Testr 35; refractometer- LABART).

We conducted field surveys for 12 consecutive days and studied different phenophases of flowering and fruiting of *E. acoroides*. We estimated the densities

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Figure 1. Study area map of Enhalus acoroides distribution and present sampling site at Vijay Nagar, Swaraj Dweep (Ritchie's archipelago), Andaman & Nicobar Islands, India.

of flowers and fruits within the quadrats across the sampling points. We measured peduncle length, sepal, and spathal leaf lengths of flowers using a measuring scale (cm). To study various stages of fruiting and seed development, we collected fruits of all phenophases (n = 5/ phase) except dehiscent fruits. Fruits were contained in seawater and immediately transported to the laboratory for further analysis. We dissected each fruit with a surgical blade and measured their diameter and length using a measuring scale (cm). Lastly, we recorded each fruit's seed development (immature/ mature seeds), the number of seeds, and morphometric measurements (seed length, seed base length).

We validated different flowering and fruiting stages by referring to published literature on the species (Bujang et al. 2006; Patankar et al. 2019) and through personal correspondences with seagrass experts.

## RESULTS

## Seagrass meadow characteristics

We observed six seagrass species from a continuous meadow spread across ~16.8 hectares in Vijay Nagar (Swaraj Dweep), viz: *Enhalus acoroides, Thalassia hemprichii* (Ehrenberg) Ascherson, 1871, *Halophila ovalis* (R. Brown) Hooker f., 1858, *Cymodocea rotundata* Asch. & Schweinf., *Halodule uninervis* (Forssk.) Asch, and *Syringodium isoetifolium* (Asch.) Dandy.

*Enhalus acoroides* was the dominant of all species with the highest mean cover, followed by *T. hemprichii* and *C. rotundata* (Table 1). Seagrass species exhibited spatial variation in distribution within the meadow. In the high tide zone, *S. isoetifolium* and *H. uninervis* occurred in a mixed substratum of very fine sand and silt (Table 1). The distribution of *C. rotundata* was patchy across the mid-tide edges, and the species preferred fine sand.

Seagrass species	Mean seagrass cover (%)	Shoot density (shoots/ m²)	Shoot length (cm; n=20)	Substratum	Species distribution within the meadow	
Enhalus acoroides	36 ± 39.3	289.9 ± 103.9	35.3 ± 12.1	Fine sand mixed with silt and clay	high and mid-tide zones	
Thalassia hemprichii	10.9 ± 4.8	70.3 ± 43.6	10.7 ± 4.8	Coarse sand and rubble	mid and low-tide zones	
Cymodocea rotundata	6.5 ± 23.1	30.3 ± 18.5	6.0 ± 3.6	Fine sand	mid-tide zone	
Halophila ovalis	5.9 ± 17.1	29.3 ± 17.9	0.4 ± 0.6	Sand and rubble	mid and low-tide zones	
Halodule uninervis	1.9 ± 5.7	46.9 ± 41.6	8.5 ± 2.6	Very fine sand mixed with silt	high-tide zone	
Syringodium isoetifolium	1.3 ± 4.6	53.3 ± 46.0	8.2 ± 3.0	Very fine sand mixed with silt	high-tide zone	
SST (°C)- 30.1 p	oH- 7.7 Sa	linity (ppt)- 30.9				

Table 1. Meadow characteristics, species' substratum preference, and spatial distribution of seagrasses at Vijay Nagar, Swaraj Dweep.

Halophila ovalis and T. hemprichii occupied coarse sand and rubble in the meadow's mid and low-tide edges (Table 1). Distribution of *E. acoroides* was spread across high and mid-tide zones, where the species was found either as monospecific strands in fine sand mixed with silt and clay or co-occurred with *C. rotundata*, *H. uninervis*, *S. isoetifolium*, and *T. hemprichii*.

Seagrass cover, shoot density, and canopy height for *E. acoroides* varied considerably within the high and midtide zones of the sampled meadow. The total mean cover of *E. acoroides* was estimated as  $36 \pm 39.3$  % (Table 1), but we observed a reduced species coverage from high (64.8  $\pm$  33.5 %) to mid tide zone (19.3  $\pm$  33 %). Similarly, overall shoot density for *E. acoroides* was 289.9  $\pm$  103.9 shoots/ m<sup>2</sup> (Table 1); however, mean densities in the high and mid tide zones varied as 144.9  $\pm$  130.8 shoots/ m<sup>2</sup> and 30.3  $\pm$ 55.2 shoots/ m<sup>2</sup>, respectively. We observed longer shoots of *E. acoroides* in the high tide patches (33. 9  $\pm$  10.1 cm). Shoots in the mid-tide zone were comparatively shorter (19. 4  $\pm$  7.2 cm), with signs of herbivory.

#### Flowering phases and natural history

In the present study, we recorded different stages of both pistillate and staminate flowers of *E. acoroides*—female inflorescence bud, pistillate flower at anthesis, male inflorescence, the bloom of free-floating male flowers, empty male spathe (post-release of male florets), pollination (released male florets attached to female inflorescence), and fertilized flowers (Table 2). Like species characteristics, a significant zonal variation was observed in flowering densities of *E. acoroides* within the sampled meadow. Densities of pistillate flowers in high and mid tide zones were  $22.8 \pm 13.4/m^2$  and  $4 \pm 1.4/m^2$ , respectively. Similarly, densities of staminate flowers were much higher (17.7 ± 10.4/m<sup>2</sup>) towards the high tide shore than in the mid-tide region ( $4 \pm 1.1/m^2$ ).

We observed solitary female inflorescence buds on

the terminal shoots. Peduncles of female buds were shorter than pistillate flowers at anthesis (Table 2; Image 1A). Female inflorescence appeared as solitary flowers on the terminal shoots, with visible sepals, petals, and pistils/ styles (Image 1B). Petals (3) were pink and had 2–3 longitudinal ridges with folded margins, enclosing 5–6 styles. Long peduncles aided the pistillate flowers to sway in the tidal waters, with petals wide open, floating at the surface. The male inflorescence had multiple white male flowers on the spadix enclosed at the base of widely open spathal leaves (Table 2; Image 1C). All male inflorescences we observed were submerged in the water column with shorter peduncles ( $5.2 \pm 1.1 \text{ cm}$  above the substratum) than the female inflorescence at anthesis ( $26.1 \pm 8.0 \text{ cm}$ ; Table 2).

A noteworthy observation in the present study was the mass bloom of released male florets free-floating in the high tide zone. Male florets (white) were 0.2 ± 0.1 cm long, with 2-3 stamens and 5-6 tepals. We observed released male florets in masses (961.7 ± 360.4/ m<sup>2</sup>; n=3 quadrats) along the sandy coastline (~1.5 km), floating on the water surface and trapped in seagrass blades (Image 1D). After the release of male flowers, a male spathe with two valves and a barren spadix (light orange) was visible. Shorter peduncles supported empty spathes filled with sand in the seagrass meadow (Image 1E). Further, we observed released male florets attached to the pistil of a wide-open female inflorescence on the water surface (2-8 male flowers/ female inflorescence; Image 1F). Lastly, a fertilized inflorescence observed had shed its petals, and the ovary was swollen, indicating the beginning of fruit formation (Image 1G).

# Fruiting phases, seed development, and natural history

Based on the stages of seed development, we categorized the fruits observed as immature, mature, and dehiscent fruits. Immature fruits were fleshy,



Image 1. Stages of flowering of *Enhalus acoroides*: 1A—Female Inflorescence bud (only sepals visible) | 1B—Pistillate flowers at an anthesis at the water surface (visible sepals, petals, pistils/ styles) | 1C—Male inflorescence with spathal leaves (SP) enclosing multiple white flowers (indicated by white arrow) | 1D—Released male flowers trapped in seagrass blades | 1E—Empty spathe filled with sand, post-release of male flowers | 1F—Pollination; male flowers attached to the pistil of female inflorescence at anthesis (indicated by white arrow) | 1G—Fertilized inflorescence. © Swapnali Gole and Ajay Kumar.

Table 2. Different phenophases of flowering and fruiting of *Enhalus acoroides* reported from the sampled seagrass meadow in Andaman Islands, India.

Stages of flowering	Density/ m <sup>2</sup>	Peduncle length (cm)			Sepal/ Spathal leaf length (cm)				
Female inflorescence bud	3.2 ± 1.8	23.2 ± 7.8			3.9 ± 0.7				
Pistillate flower at anthesis	16.0 ± 12.0	26.1 ± 8.0			3.9 ± 0.8				
Male inflorescence	12.7 ± 7.3	5.2 ± 1.1			4.2 ± 0.3				
Male spathe (Post-release of male flowers)	3.3 ± 1.5	6.4 ± 1.6			4.1 ± 0.1				
Pollination (Male flowers attached to female inflorescence)	10 ± 1.3	26.4 ± 8.0			3.9 ± 0.7				
Fertilized flower	2.2 ± 1.0	20.3 ± 4.9			4.0 ± 0.6				
Stages of fruiting	Density/ m <sup>2</sup>	Diameter (cm; n = 20)	Fruit length (cm; n = 20)		No. of seeds/ Fruit (cm)	Seed length (cm)	Seed base (cm)		
Immature fruits (Seeds still developing)	7.3 ± 2.0	4.6 ± 2.2	5.2 ± 0.8		11.3 ± 1.5	0.7 ± 0.1	0.8 ± 0.1		
Mature fruits (Developed seeds)	8.0 ± 3.9	9.2 ± 2.8 6.7 ± 1.0			11.8 ± 3.3	0.9 ± 0.2	1.1 ± 0.1		
Dehiscent fruits	2.2 ± 0.9	12.6 ± 0.7	-		-	-	-		

Values expressed as mean ± standard deviation; (- not recorded).

greenish-brown in color, with an uncoiled peduncle. Solitary fruits were erect on terminal shoots of the peduncle and concealed 10–13 spherical white seeds still developing (Images 2A & 2B). Mature fruits were large, ovoid-shaped, and fleshy, with a pointed tip. The

fruit cover was greenish, with longitudinal rows of brown spikes, and the coiled peduncle positioned above the substratum supported the fruits (Image 2C). We found 8–14 fully developed, germinating seeds per mature fruit (Image 2D). A membranous white seed coat concealed

Flowering and fruiting of Tape Seagrass from the Andaman Islands



Image 2. Stages of fruiting of *Enhalus acoroides*: 2A—Young, immature fruit | 2B—Dissected immature fruit with developing seeds | 2C— Mature fruit with a coiled peduncle | 2D—Dissected mature fruits with developed seeds | 2E—Germinated seed with shoot bud visible | 2F—Dehiscent fruit. © Swapnali Gole and Ajay Kumar.

the seeds. Seeds were conical, yellow at the base and dark green at the apex. We observed visible shoot buds with a length of  $1.2 \pm 0.3$  cm in each germinating seed (3 shoot buds/ seed; Image 2E). Dehiscent fruits were observed at the base of plant shoots, right above the ground (Image 2F). Fleshy fruit cover (mean diameter  $12.6 \pm 0.7$  cm; Table 2) was broken into 6–7 halves post-release of seeds.

# DISCUSSION

The lack of information on the phenology of *E. accoroides* from the Andaman Islands and Indian waters limits our understanding of the species' reproductive phases and seasonality. Densities of shoots, fruits, and flowers, in the present study (post-monsoon) were higher than previous reports in pre-monsoon (Patankar et al. 2019), possibly due to different sampling seasons. Additionally, no male flowers (inflorescence or released), pollination event, or fertilized flowers were recorded by Patankar et al. (2019). Since both the studies were opportunistic in nature, our findings supplement and strengthen the previous observations Patankar et al. (2019) made on *E. accoroides* phenology in the Andaman Islands.

In the present study, no correlation can be established between zonal variation in meadow characteristics and flowering densities of *E. acoroides*, given limited data. However, this aspect credits detailed investigation as studies have highlighted the role of meadow characteristics (seagrass cover, shoot density, and canopy height) and herbivory on the reproductive success of *E. accoroides* (Vermaat et al. 2004; Rattanachot 2008). Novel observations on the mass bloom of released male flowers (at SST ~30°C; mean) align with similar notes reported for the species (Hartog 1970; Rollon 1998).

In conclusion, based on higher densities of multiple phenophases observed in the present study (from buds to dehiscent fruits) as compared to previous reports (Patankar et al. 2019), we presume that January could be a critical period for *E. acoroides* phenology at a local scale, but this needs further validation through seasonal studies. Furthermore, the fruit ripening period for *E. acoroides* is long (2–3 months; Rollon 1998), after which the seeds are released. Thus, we assume that pollination is somewhere in October–November for the fruits observed in the present study. Thus, we recommend long-term seasonal monitoring studies to understand the peak flowering and fruiting season of *E. acoroides* and assess local drivers influencing the species' phenology in the Andaman Islands.

Lastly, our observations also report meadow scarring of the seagrass bed, as the study site is a fishing transit lane used for boat anchorage by local fishers (personal observations). Also, the entire inhabited coastline of Swaraj Dweep is known for gleaning activities and recreational and commercial fishing using 'khevla/ feka

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jaal' (cast net) and 'taana jaal' (shore seine). Moreover, anecdotal reports (from local fishers) and our field observations (direct encounters) suggest that these seagrass beds are important to support threatened species like green sea turtles and dugongs. Hence, detailed baseline information on the seagrass meadow, including its biodiversity, needs to be established to emphasize its management and conservation and to understand the species' natural history. Therefore, although our observations have provided detailed documentation of the meadow and natural history notes on different phenophases for *E. acoroides*, this baseline needs to be supplemented with future research and long-term studies.

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