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Spatio-temporal variation in sexual reproduction of the tropical seagrass *Enhalus acoroides* (L.f.) Royle in Cape Bolinao, NW Philippines

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Abstract

The Indo-Pacific seagrass *Enhalus acoroides* (L.f.) Royle can reproduce by vegetative growth as well as sexually, by the production of seeds. To assess the role of environmental conditions in the occurrence of sexual reproduction in this dioecious species, spatio-temporal variation in flowering was investigated in the reef flats off Cape Bolinao, north-western Philippines. Flowering occurred year-round, but the intensity varied temporally and correlated with mean water temperature. Spatially, differences in flowering intensity correlated with available light as affected by turbidity and water depth. Exposure duration of female flowers to air seems to be crucial for pollination and subsequent seed setting, resulting in higher numbers of fruits in shallower sites. Contrary to previous hypotheses, no effect of tidal levels on the release of male flowers was observed. It is suggested that production of gas bubbles in photosynthesis plays a key role. The great discrepancy between observed flowering and the presence of peduncle scars on the rhizomes at the deeper sites suggests that early abortion is common here. The use of these scars to quantify flowering will lead to an overestimation of reproductive effort in deep sites. The rare occurrence of flowering in deep sites supports the contention that light availability is a key factor in successful flowering in *E. acoroides*. Allocation of biomass and nutrients (N, P) over various plant components suggests that sexual reproduction involves high cost in *E. acoroides*.

Keywords: Flowering; Light; Depth; Reproduction costs; Age reconstruction

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1. Introduction

The seagrass *Enhalus acoroides* (L.f.) Royle, hereafter referred to as *Enhalus*, is widely distributed in the Indian Ocean and in the tropical parts of the Western Pacific (Phillips and Meñez, 1988). In the Philippines, *Enhalus* is one of the most prominent seagrass species in mixed seagrass beds (Vermaat et al., 1995). In the reef flats off Cape Bolinao, north-western Philippines, this species is able to colonize various habitat types (Vermaat et al., 1995; Rollón et al., 1998): muddy to coarse sandy substratum, turbid to clear waters and splash zones above zero datum to ca. 3 m depth. Across this range in environmental conditions, *Enhalus* shoots differ in morphology, biomass and density (Rollón, 1998).

Although the flowering biology of *Enhalus* has been studied extensively (Troll, 1931; Den Hartog, 1970; Pettit, 1984) little is known about the environmental factors involved in the relative importance of sexual and vegetative reproduction. According to Troll (1931), *Enhalus* flowers only when occasionally emerged and thus, no flowers may be found in deeper, permanently submerged meadows. In view of *Enhalus*' hydrophobic mode of pollination this hypothesis might apply to female plants that have to extend their flower peduncles to the water surface to capture pollen. However, for male plants this explanation will not suffice, as they release their tiny flowers underwater, which then ascend to the water surface, where they will be transported by wind and surface currents. Brouns and Heijs (1986) observed year-round flowering of *Enhalus* in Papua New Guinea. Based on reconstruction techniques (Duarte et al., 1994), Duarte et al. (1997) estimated that at Cape Bolinao, *Enhalus* produces 2.8 flowers per shoot per year on average and allocates up to 20% of its aboveground production to flowering and fruiting. Since reconstruction estimates are based on remaining peduncle scars, they are only an indirect measure of the completion of a full flowering cycle. They are overestimations that need to be verified. Furthermore, most published work has limited temporal coverage and is based on one or a few stations within a variable coastal environment.

The aim of the present study is to (1) assess the temporal and spatial variation in flowering in relation to environmental factors; (2) compare actual flowering intensity with the age-reconstruction method used by Duarte et al. (1997); and (3) estimate reproduction costs in terms of biomass and relative nutrient content (N, P).

2. Materials and methods

2.1. Study area

The study was carried out between 1994 and 1996 on the reef flat off Cape Bolinao, Pangasinan, NW Philippines (Fig. 1). Mixed seagrass beds cover approximately 37 km^2 of the total of 50 km^2 of reef flat area (Fortes, 1988, 1989; Vermaat et al., 1995). Six sites were selected differing in, a.o., depth and light attenuation coefficients (Fig. 1, Table 1).

2.2. Spatio-temporal variation in the intensity of flowering and fruiting

Flowering and fruiting of *Enhalus* were quantified in sites 1a, 1c, 4, 5 and 6 (see Fig. 1) on a monthly basis from April 1994 to February 1996. During each sampling, ca. 25 quadrats

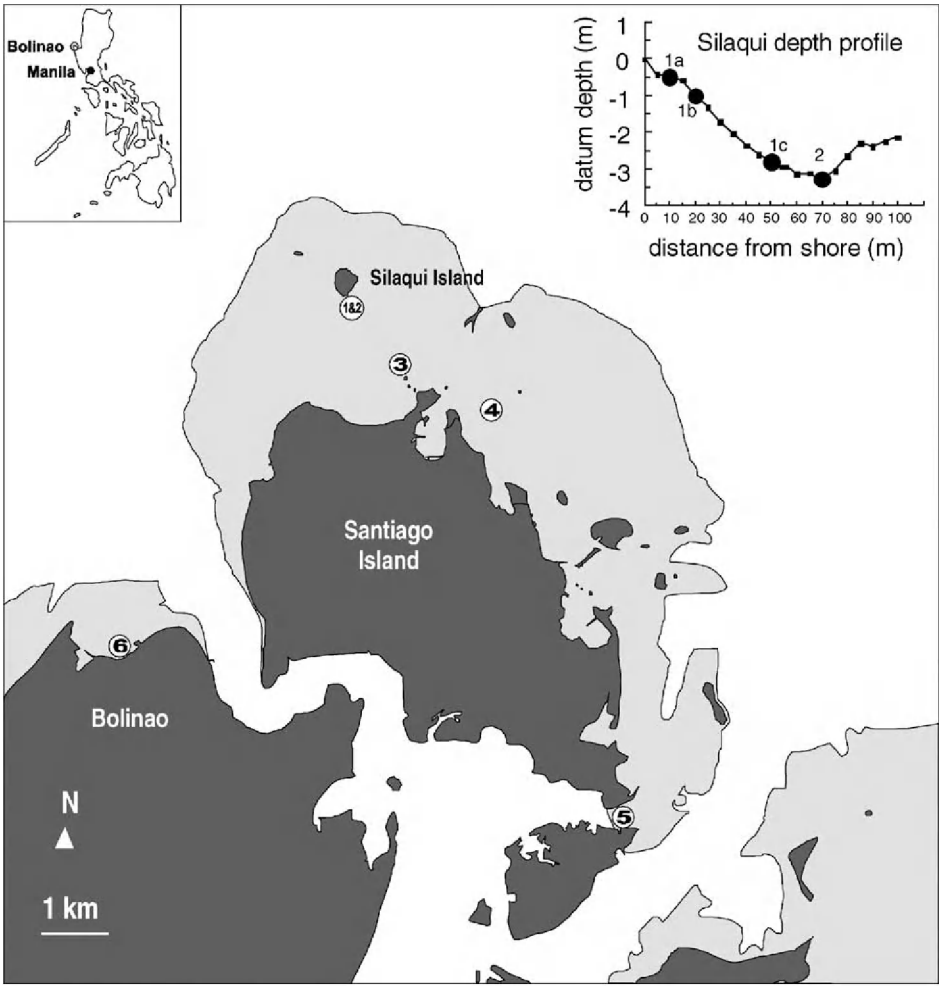

Fig. 1. Map showing the study area (Bolinao, Pangasinan, NW Philippines). Dark portion: land; light portion: reef flat. Numbers refer to study sites (cf. Table 1); site 2 is without *E. acoroides*.

Table 1
Representative environmental characteristics of the study sites; different letters indicate significant differences at $P < 0.05$ (Rollón, 1998)

Parameter	Site					
	1a	1b	1c	4	5	6
Relative cover *E. acoroides*	+++	++	+	+++	+	++
Datum depth (MLL water level) (m)	0.35	1.25	3.0	1.2	0.58	0.70
Light extinction coefficient (m^{-1})	0.31[a]	0.31[a]	0.31[a]	0.41[a]	0.58[b]	0.61[b]
Water temperature (°C)	25–33	25–33	25–33	27–34	27–33	26–32

Relative presence of *E. acoroides* (Rollón, personal observation): (+) <15; (++) 15–40; (+++) >40 shoots m^{-2}.

(50 cm × 50 cm) were randomly thrown, until at least 100 *Enhalus* shoots had been examined. Each *Enhalus* shoot was checked for the presence of flowers or fruits. For female flowers, four stages were distinguished, 1: emerging, young flower; 2: mature flower; 3: rotting, unfertilised flower; and 4: mature fruit. For male inflorescences, two stages were distinguished, 1: male spathe still full with flower buds; and 2: male flower buds had been released. The occurrence of these stages was expressed as percentage of the total number of shoots counted.

The frequency of flowering for an average shoot was determined by tagging 10 randomly chosen shoots of *Enhalus* at each of the sites 1a, 1b, 1c and 5. They were checked once every 1–2 months for the presence of flowers and fruits using the same criteria as above. When actually produced, inflorescence structures often remain with the plant for at least 2 months (Rollón, personal observation).

All observed events during which male flowers were released were recorded to test whether the timing of these events correlated with one or more of the tidal characteristics, i.e. level, rising/falling and time of day.

Trends in flowering intensities were correlated with ambient seawater temperatures (cf. Table 1) and light intensities. PAR at seagrass depth (cf. Rollón, 1998) was estimated by correcting PAR at the water surface for actual depth and light extinction by the water column. Variations due to tidal fluctuations, cloud cover and daylength were included to calculate mean values over the entire study period. To estimate PAR at the water surface, data from the nearest National Radiation Centre (Quezon City) were corrected according to Philippart (1995) and adjusted for the difference in latitude between Quezon City (14°N) and Bolinao (16°N).

Exposure duration to air of stage 2 female flowers (which are ca. equal to the height of the leaf tips; Rollón, personal observation) was calculated from the sinusoidal tidal curve of the day and the measured datum depths of flowers.

2.3. Scar-based quantification: ageing technique

Similar to leaf scars, peduncle scars occur on the rhizomes. Thus, flowering frequency may be quantified by age reconstruction of rhizome samples (Gallegos et al., 1992; Duarte et al., 1994, 1997). For this purpose, three clones of *Enhalus* were collected from each of the sites 1a, 1c, 5 and 6. All rhizome segments were aged using plastochrone interval (PI) values previously determined by Rollón et al. (1998): 31.2 ± 1.7; 26.9 ± 0.4; 27.1 ± 1.4; 26.2 ± 1.6 at sites 1a, 1c, 5 and 6, respectively. The minimum age of each clone was calculated by adding up all PIs with the leaves present along the longest axis. Flowering frequency of a rhizome segment was calculated as the number of flower marks in the segment divided by the time elapsed to attain the total segment length.

2.4. Biomass and nutrient allocation to reproduction

As spatial differences in flowering might be due to carbon or nutrient (N, P) limitation (cf. Agawin et al., 1996; Duarte et al., 1997), the cost of producing sexual reproductive structures was estimated as C, N and P allocation. At site 1a where flowering plants were abundant, 30

shoots with reproductive structures (10 with male 1 inflorescence, 10 with female 2 flowers and 10 with fruits) were collected. Reproductive structures included inflorescence peduncle, spathe, flowers, pericarp and seeds. Ten shoots each of *Enhalus* without reproductive structures were randomly collected at sites 1a, 1b, 1c, 4, 5 and 6, as non-reproductive baseline. After washing-off periphyton and attached sediment and separating leaves, roots, rhizomes (about six internodes) and reproductive structures, samples were oven-dried at 70 °C until weights were constant (24–48 h).

For total nitrogen and phosphorus, plant samples, after powdering using mortar and pestle, were digested with H_2SO_4/Se/salicylic acid and H_2O_2 (Knight, 1996; following procedures in Kruis, 1995). Ash-free dry weight (AFDW) was determined by combustion at 550 °C for 6 h. Organic carbon was assumed to be 40% of AFDW (Hootsmans, 1994). Values are expressed on a per shoot basis, i.e. one shoot composed of ca. five to six leaves, six rhizome internodes and their associated roots.

To quantify the variability in the number of seeds per fruit, 190 fruits were harvested and the seeds per fruit were counted. Fruits were collected in July 1994, November 1994 and December 1995 from a wider area but with conditions similar to site 1a (datum depth ca. 0.5 m; multi-species meadows). The mean number of seeds obtained from these separate collections did not significantly vary (Tukey test, $P > 0.05$) and samples were pooled to obtain an overall mean.

2.5. Data analysis

After data transformations to control normality and homogeneity of variance, spatio-temporal differences in various parameters were tested using ANOVA. When multiple comparisons were necessary, the Tukey test was used. Differences were considered significant when $P < 0.05$. Correlations of two variables (e.g. temperature versus flowering; PAR versus flowering) were tested by regression.

3. Results

3.1. Spatio-temporal variation in the intensity of flowering and fruiting

Two-way ANOVA indicated significant effects of sampling site, period, and their interaction for total female inflorescences, fruits, male inflorescences and total flowering (Fig. 2, Table 2). In all cases, the variance component for site was greater than for period, although the variance component for these factors was small (2–11%) compared with the variation between quadrats (80–88%). For both male and female shoots, flowering intensity at the shallow/clear water sites 1a and 4 was higher than at the deepest/clear water site 1c, with intermediate positions for the turbid but relatively shallow sites 5 and 6 (Table 3).

Of the 10 tagged shoots at sites 1a and b, nine shoots flowered (six male, three female, one died and four male, five female, one died, respectively), at site 5, five (all male), and at site 1c only one (female) did so. The frequency with which shoots flowered ranged from 0.5 to 3.4 per year. The average value at the deepest site (1c: 0.1 ± 0.1) was significantly

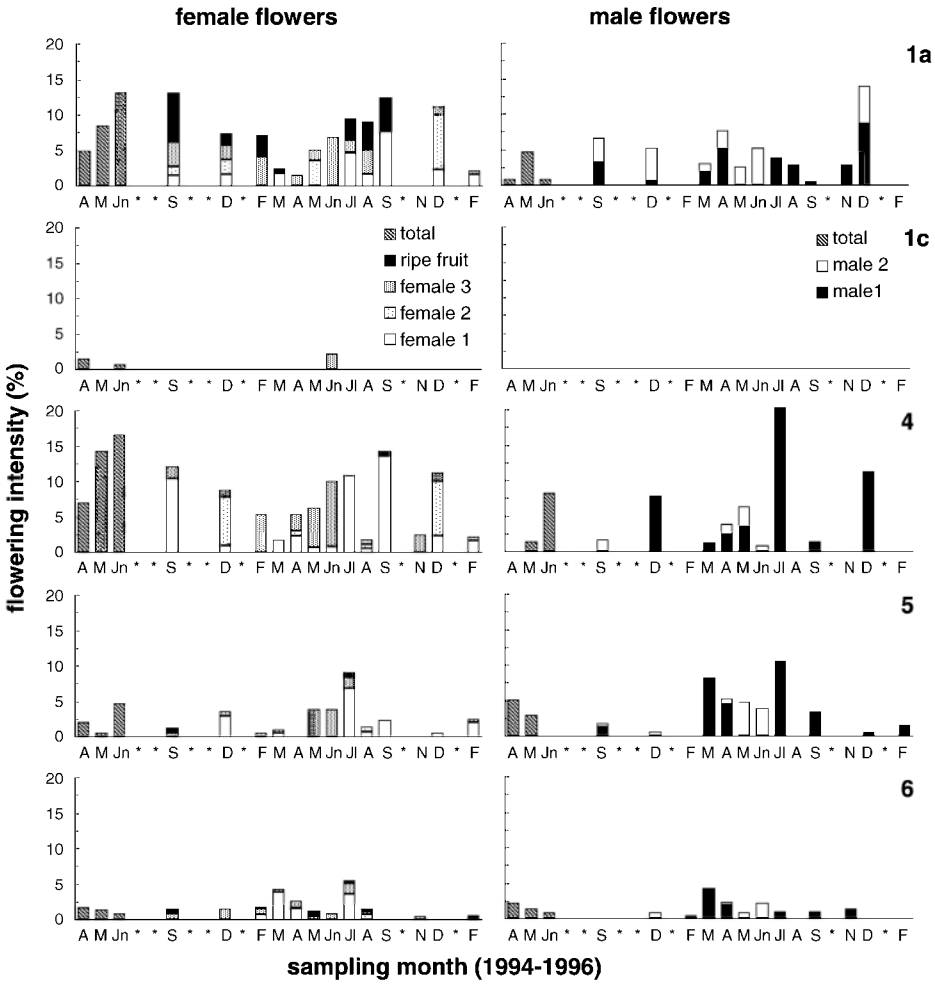

Fig. 2. Percentage of *E. acoroides* plants with flowers or fruits in random quadrats at the study sites 1a, 1c, 4, 5 and 6 in the period April 1994 to February 1996 ($n \geq 100$). Total: different flower categories were not distinguished (i.e. during first three sampling months); for flower categories, see Section 2.

lower than that observed at the shallower sites 1a (1.1 ± 0.3) and 1b (1.3 ± 0.2). The most turbid, shallow site 5 (0.7 ± 0.4) was intermediate, not differing from all other sites.

Although no continuous observations were made, the synchronous release of male flowers was observed year-round without a clear pattern in the occurrence of major (reef flat-wide large aggregates of flowers) and minor events. Mass release of male flowers occurred during both spring and neap tides, during rising and falling tides, at various tidal levels, and independent of the minimum water level of the day during the event. In addition, mass release occurred in all phases of the moon.

Table 2
Two-way ANOVA testing the effects of site and sampling month on the abundance of *E. acoroides* sexual reproductive structures in randomly selected quadrats at sites 1a, 1c, 4, 5 and 6 in the reef flat off Cape Bolinao between April 1994 and February 1996; $n = 25$ (quadrats)

	P-value	Variance component (TSS%)
Total female flowers		
Site	<0.001	8
Sampling month	<0.001	2
Interaction	<0.001	2
Basic error (i.e. between quadrats)	<0.001	88
Fruits		
Site	<0.001	6
Sampling month	<0.001	2
Interaction	<0.001	9
Basic error (i.e. between quadrats)	<0.001	83
Total male flowers		
Site	<0.001	3
Sampling month	<0.001	2
Interaction	<0.001	9
Basic error (i.e. between quadrats)	<0.001	86
Total flowers		
Site	<0.001	11
Sampling month	<0.001	3
Interaction	<0.001	6
Basic error (i.e. between quadrats)	<0.001	80

Variance component values indicate the variance percentage explained by the corresponding source of variation and are calculated as factor SS/total SS.

3.2. Environmental factors affecting sexual reproduction

3.2.1. Temperature

The intensity of flowering in the randomly collected shoots correlated positively with temperature for female flowers stage 3 at sites 4 and 5 and for male flowers stage 2 at sites 5 and 6 (Table 4). When considering total flowering (male plus female), significant correlations with temperature were observed at the sites with most intense flowering, i.e. at sites 1a and 4. At the other sites, such correlations were not found.

3.2.2. PAR

In general, *Enhalus* flowering intensities in the darker environments (deep and/or turbid) were lower than at sites with higher levels of PAR (Figs. 2 and 3). When leaving out the intertidal site 1a, this trend correlated significantly with available PAR for the marked shoots (Fig. 3b). For the randomly selected shoots (Fig. 3a) this correlation was not significant.

3.2.3. Exposure duration

Fig. 4 combines the estimated time female flowers are exposed to air at the water surface with the observed frequencies of fruits. The exceptional flowers at site 1c remain covered by water all the time, which prevents pollination and explains the absence of fruits at this

Table 3
Mean percentage ± S.E. of *E. acoroides* plants at the study sites with flowers in the period April 1994 to February 1996

Flower category	Site				
	1a	1c	4	5	6
Female					
1	1.52 ± 0.64^{b}	0.00 ± 0.00^{a}	3.54 ± 1.34^{c}	1.22 ± 0.54^{ab}	0.75 ± 0.38^{ab}
2	0.93 ± 0.47^{b}	0.00 ± 0.00^{a}	1.22 ± 0.75^{b}	0.05 ± 0.05^{a}	0.06 ± 0.06^{a}
3	2.06 ± 0.56^{bc}	0.16 ± 0.16^{a}	2.29 ± 0.77^{c}	0.92 ± 0.38^{ab}	0.62 ± 0.14^{a}
4	1.88 ± 0.63^{b}	0.00 ± 0.00^{a}	0.06 ± 0.06^{a}	0.10 ± 0.07^{a}	0.25 ± 0.09^{a}
Total	6.85 ± 1.08^{c}	0.27 ± 0.16^{a}	8.13 ± 1.21^{c}	2.31 ± 0.58^{b}	1.60 ± 0.37^{ab}
Male					
1	2.25 ± 0.71^{b}	0.00 ± 0.00^{a}	3.68 ± 1.70^{c}	2.30 ± 0.97^{c}	0.76 ± 0.34^{ab}
2	1.92 ± 0.60^{c}	0.00 ± 0.00^{a}	0.52 ± 0.52^{ab}	0.82 ± 0.44^{b}	0.32 ± 0.18^{ab}
Total	3.78 ± 0.89^{b}	0.00 ± 0.00^{a}	4.02 ± 1.41^{b}	3.03 ± 0.79^{b}	1.15 ± 0.28^{a}
Total	10.64 ± 1.46^{d}	0.27 ± 0.16^{a}	12.15 ± 2.19^{d}	5.35 ± 1.19^{c}	2.74 ± 0.56^{b}

Each month ≥100 shoots were checked for the presence of flowers and fruits. Different letters (superscript) attached to values indicate significant (Tukey test, $P < 0.05$) differences across sites, ns: not significant; nd: no data; female, 1: young, emerging; 2: ready for pollination; 3: rotting, unfertilized; 4: fruit; male spathe, 1: full of flower buds; 2: empty spathe.

site. Due to its depth, the number of female flowers that can reach the water surface at site 4 is small, which might be responsible for the low occurrence of fruits, in spite of relatively abundant flowering. Female flowers at the other, shallower, sites are more often exposed at the water surface, increasing the chances for pollination and the development of fruits.

3.3. Scar-based quantification of reproductive effort

Flowering frequencies established from peduncle scars at sites 1a, c, 5 and 6 were different from those recorded on the tagged shoots (Table 5). With averages of 3.6 ± 0.5 (site 1a) to 4.5 ± 0.5 (1c), there were no significant differences between the sites sampled. Differences between ramets, however, were considerable.

Table 4
Single linear regressions of the percentage of the different *E. acoroides* flower categories in the randomly selected quadrats with water temperature over the period April 1994 to February 1996

Flower category	Site	n	R^2	P-value	Slope	Intercept
Female 3	4	13	0.33	0.04	0.010 ± 0.004	-0.28 ± 0.13
	5	13	0.44	0.013	0.006 ± 0.002	-0.16 ± 0.06
Male 2	5	13	0.47	0.009	0.007 ± 0.002	-0.20 ± 0.07
	6	13	0.31	0.049	0.003 ± 0.001	-0.08 ± 0.04
Total flowering	1a	16	0.38	0.011	0.018 ± 0.006	-0.46 ± 0.18
	4	16	0.36	0.014	0.0196 ± 0.007	-0.51 ± 0.21

Only the regressions that are significant ($P < 0.05$). See Section 2 for codes of flower categories.

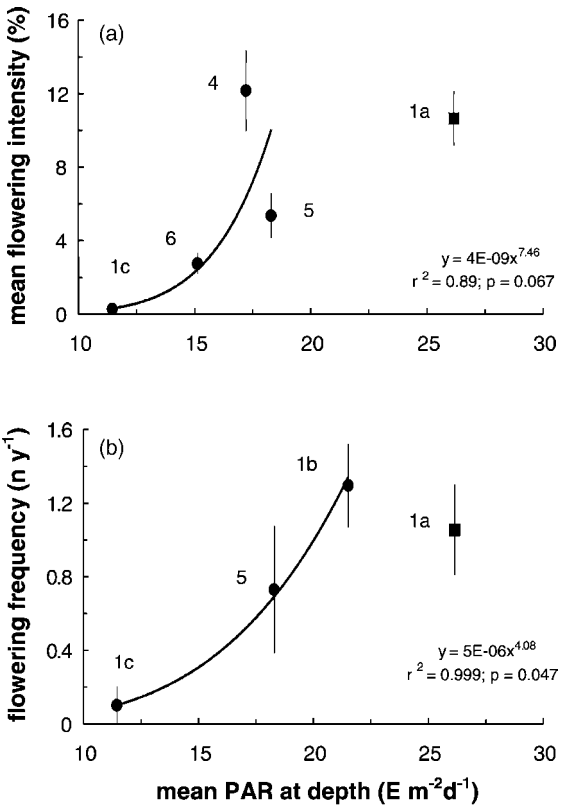

Fig. 3. Mean total (female + male) flowering intensity of *E. acoroides* in the random quadrats (a) and mean total annual flowering frequency of the 10 tagged *E. acoroides* shoots randomly selected (b) plotted against the mean PAR at seagrass depth at the respective sites. Vertical lines are standard errors of flowering. Site 1a is left out of the regression analyses (see Section 3 for explanation).

The high frequency of scars found at site 1c was remarkable compared with the actually low flowering observed at this site (Fig. 2, Tables 3 and 4). The number of disintegrating young inflorescences (smaller than stage 1, male or female) was also high at site 1c. Apparently, flower primordia were produced regularly, but did not develop into mature flowers, probably as a consequence of the low level of PAR reaching this site.

3.4. Biomass and nutrient allocation to reproduction

At site 1a, the biomass of non-reproductive structures (leaves plus rhizomes and roots) of flowering female shoots was higher than that of non-flowering shoots ($P < 0.05$; Table 6). For male shoots the difference was not significant.

The production costs for a female flower in dry weight (567 ± 96 mg DW), nitrogen (14.8 ± 1.1 mg N) and phosphorus (2.3 ± 0.6 mg P) were significantly higher than those

Table 5
Frequency of peduncle scars on the rhizome of *E. acoroides* and the number of developed flowers found in between leaf bases

Site	Estimated minimum age of clone (year)	Frequency ± S.E. (*n* flower scars per year)	Number of standing shoots	Number of developed flowers	Mean *n* developed flowers standing per shoot
1a	9.9	4.81 ± 0.62	2	1	0.50
	6.8	6.32	1	0	0.00
	8.6	2.95 ± 0.60	6	13	2.17
1c	5.3	6.27 ± 0.16^{b}	3	8	2.67
	3.1	1.76 ± 0.84^{a}	5	2	0.40
	5.8	6.05 ± 0.28^{b}	4	9	2.25
5	10.9	5.49 ± 0.50^{b}	7	16	2.29
	5.1	1.01 ± 0.21^{a}	8	20	2.50
	4.1	6.25 ± 0.32^{b}	6	11	1.83
6	7.8	1.37 ± 0.77^{a}	2	0	0.00
	6.4	4.51 ± 0.48^{b}	9	17	1.89
	8.6	3.78 ± 0.64^{ab}	11	20	1.82
Overall					
1a		3.55 ± 0.52	9	14	1.52
1c		4.50 ± 0.51	12	19*	1.58
5		3.92 ± 0.42	22	47	2.24
6		3.72 ± 0.40	21	37	1.68

Different letters (superscript) attached to values indicate significant differences (Tukey test, $P < 0.05$) between clones per site. The overall values did not differ significantly between sites. Standing shoots: living shoots at the time of harvest; developed flowers: very young developed flowers found in between leaf bases (asterisk means that all flowers at site 1c showed signs of disintegration).

Table 6
Shoot weight and relative nutrient content (N, P, C) of non-reproductive *E. acoroides* plants collected at sites 1a, 1b, 1c, 4, 5 and 6 at the reef flat off Cape Bolinao and of reproductive plants sampled at site 1a

	Dry weight (mg per shoot)				N pooled (%)	P pooled (%)	C pooled (%)
	Leaves	Roots	Rhizomes	Reproductive structures			
Non-reproductive shoots various sites							
1a	459 ± 44	84 ± 20	225 ± 42	–	1.7 ± 0.2	0.37 ± 0.03	31
1b	1038 ± 120	149 ± 40	581 ± 78	–	1.3 ± 0.1	0.32 ± 0.02	31
1c	2066 ± 247	189 ± 17	803 ± 128	–	2.1 ± 0.1	0.41 ± 0.02	31
4	1825 ± 162	176 ± 23	878 ± 78	–	1.9 ± 0.1	0.30 ± 0.01	32
5	2078 ± 113	190 ± 63	723 ± 93	–	1.8 ± 0.1	0.37 ± 0.02	30
6	1387 ± 190	66 ± 13	548 ± 83	–	1.9 ± 0.1	0.30 ± 0.02	31
Reproductive shoots site 1a							
Male 1	738 ± 87	65 ± 25	330 ± 64	128 ± 14	2.1 ± 0.1	0.40 ± 0.02	33
Female 2	1329 ± 195	64 ± 19	401 ± 61	567 ± 91	2.2 ± 0.1	0.45 ± 0.00	32
Fruits	1005 ± 145	149 ± 39	370 ± 74	4860 ± 281	1.5 ± 0.1	0.34 ± 0.02	33

Shoot weight values are presented separately for leaves, roots and rhizomes; nutrient contents are pooled over these structures. Error values are standard errors; $n = 10$.

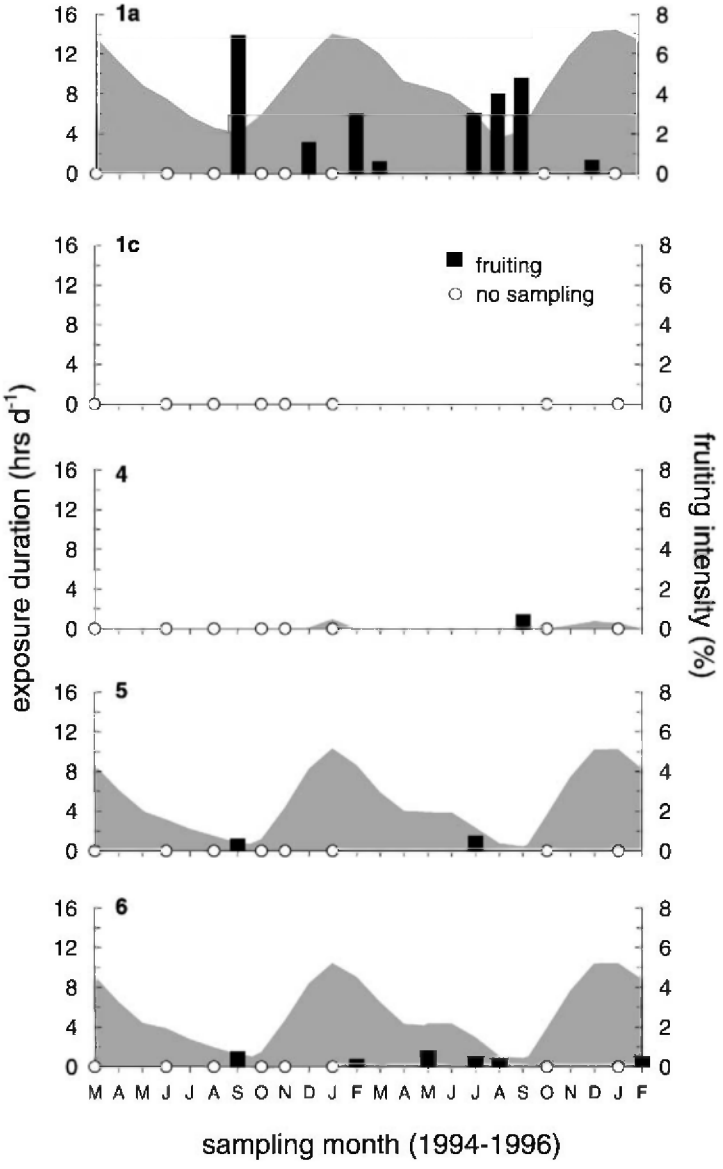

Fig. 4. Percentage of *E. acoroides* plants with fruits (bars) plotted with the exposure duration of the female flowers to air at the water surface (area graph).

for a male inflorescence (128 ± 15, 3.8 ± 0.3 and 0.8 ± 0.3, respectively). For fruits these values increased to 4890 ± 296, 63.2 ± 4.9 and 14.6 ± 0.5 for dry weight, nitrogen and phosphorus, respectively (Table 6).

The number of seeds per fruit ranged from 3 to 15 with a mean of 9.1 ± 2.5 ($n = 190$).

4. Discussion

Flowering in *Enhalus* was observed year-round. For other seagrass species, flowering has been shown to be a distinctly seasonal event, e.g. *Thalassia testudinum*, *Zostera marina* (Gallegos et al., 1992), *Thalassia hemprichii* (Duarte et al., 1997; Rollón, personal observation), *Zostera noltii* (Vermaat and Verhagen, 1996) and *Cymodocea nodosa* (Caye and Meinesz, 1985; Duarte et al., 1994). Still, within the observed range (Table 1), the intensity of flowering in *Enhalus* correlated with fluctuating water temperatures. However, in view of the lack of significant differences in average seawater temperatures between sites, it is unlikely that temperature played a role in the observed spatial variation in *Enhalus* flowering and our data suggest a decisive role of light in providing sufficient energy to induce flowering in *Enhalus*. Differences in available PAR between sites, as a consequence of differences in depth and turbidity, can be used to explain differences in flowering frequencies between sites. This is further supported by the only observation of flowering at the deepest site 1c, which was preceeded by a 2-month period of total daily PAR exceeding 15 E m^{-2} per day. Extrapolation of the correlation between available PAR and flowering frequency results in a minimum of 12 E m^{-2} per day necessary to enable flowering of *Enhalus*. However, the relatively low frequency at site 1a (shallow/intertidal, highest PAR) suggest that other factors (e.g. desiccation, wave energy) may be important in explaining flowering in *Enhalus* populations as well.

Flowering of *Enhalus* below a temperature of 31 °C is in contrast with the findings of McMillan (1980, 1982), who failed to induce flowering in *Enhalus* in his culture experiments (temperature range 20–31 °C) and suggested that the temperature range for flowering of this species would be above 31 °C. From the present study light limitation seems to be more plausible to explain McMillan's (1980, 1982) findings. The artificial light used in his experiments was provided by cool, fluorescent bulbs with photon flux densities from 70 to 200 μE m^{-2} s^{-1} (McMillan, 1980). Even under continuous light this corresponds to only 6–17 E m^{-2} per day (about 12 E m^{-2} per day), a light environment that approximates, at the most, the actual situation at our site 1c where fruit development was never observed.

Considering the high construction costs for female flowers and fruits, nutrient limitation could also be a relevant factor in explaining differences in flowering frequencies between sites (cf. Agawin et al., 1996; Duarte et al., 1997). As there were no significant differences in nutrient concentrations (both water column and pore water) between the various depths at site 1 (Knight, 1996; Rollón, 1998), nutrient limitation does not appear to be decisive for flowering in this transect. Moreover, the relatively large plants at site 1c contain sufficient nutrients to sustain flowering. In addition, the fact that *Enhalus* showed low flowering intensities at the relatively turbid sites 5 and 6, where nutrient levels occasionally increase from river run-off and/or wastewater, does not support the lack of nutrients as a critical factor limiting *Enhalus* flowering. Rather, it is hypothesised that differences in light intensities across sites is the key. Assuming that (1) the production of male and female flowers takes 2 months, and production of a fruit takes a total of 5 months (Brouns and Heijs, 1986; Rollón, 1998) and (2) shoots at the various sites produce the same biomass of reproductive structures as at site 1a (Table 6) this would result in the photosynthetic

Table 7
Relative carbon requirements for the production of reproductive structures and leaves and the specific carbon production at the various sites

Site	Male flower (% per day)	Female flower (% per day)	Fruit (% per day)	Leaf SGR (% per day)	Specific C production (% per day)	Available for non-leaf plant parts (% per day)
1a	0.46	2.06	7.06	2.31 ± 0.07	3.00 ± 0.01	0.69
1b	0.21	0.91	3.12	2.15 ± 0.05	2.64 ± 0.01	0.49
1c	0.10	0.46	1.57	2.09 ± 0.25	1.89 ± 0.01	−0.20
4	0.12	0.52	1.78	2.16 ± 0.08	2.45 ± 0.01	0.29
5	0.10	0.45	1.56	2.75 ± 0.09	2.53 ± 0.01	−0.22
6	0.15	0.68	2.34	2.57 ± 0.11	2.27 ± 0.01	−0.30

The difference between specific C production and Leaf SGR remains for plant parts other than leaves (i.e. flowers and rhizomes and roots). See Section 4 for further explanation.

requirements presented in Table 7. From the calculation in Table 7 it can be concluded that:

(1) Energy requirements for the production of male flowers are much less than those for female flowers and fruits. This may explain the lack of variation in male flowering between sites (3% of the total variance, Table 2).
(2) Only the largest shoots at site 1a allocate sufficient energy to produce female flowers; this explains why shoots with female stage 2 flowers or fruits have significantly higher biomass than non-reproductive shoots (Table 6).
(3) Producing a fruit implies a significant trade-off in resource allocation or physiological adjustment.

Based on the present calculations (Table 7), shoots at sites 1c, 5 and 6 would have a negative photosynthetic balance, which implies that *Enhalus* is not able to grow at these sites. A more accurate estimation of photosynthetic parameters (P_{max}, K_m and R) under local environmental conditions should lead to a more reliable measure of the carbon balance. However, the present results support the hypothesis that a lack of light is the major factor in limiting flowering at sites 1c, 5 and 6.

The effectiveness of flowering for sexual reproduction ultimately depends on pollination success. For *Enhalus* this means that the female flowers have to reach the water surface to be able to catch the pollen that are released under water, but are transported over the water surface. For female plants this implies that the lowest water depth should at least equal the length of the peduncle to allow pollination. At deeper sites this is not always the case. Besides limiting light conditions, this could explain the absence of fruits at site 1c. At site 4, with assumingly sufficient light and abundant flowering, the water appears too deep for frequent pollination. The mechanism of male flower discharge is also subject to discussion (Troll, 1931) and existing hypotheses are not supported by the present study. Male flower discharges were observed in any tidal condition, which makes the pressure-reduction hypothesis (reduction of water pressure during low tide enables flower buds to rise to the surface) as an explanation for the mass release of flowers unlikely. Also, in the present study the release of male flowers was observed in relatively deep sites (1b, 4: sites never shallower than 0.75 m). This makes the tide-pool hypothesis, in which a significant temperature rise of 10 °C

is considered as the main trigger for flower release, doubtful as well. A plausible explanation for the mass release of male flowers could be the production of gas bubbles. Some studies (Troll, 1931; Verhoeven, 1979; De Cock, 1980) reported the production of gas bubbles in association with flowering. Although the function of gas bubbles may vary with different species (in some cases no function: van Vierssen et al., 1982), gas bubbles have been observed to support the inflorescence of *Ruppia cirrhosa* to reach the water surface (Verhoeven, 1979). If gas bubbles play a similar role for *Enhalus* (as was suggested by Troll, 1931), this could explain several observations: (1) no release occurs in the early morning (Rollón, personal observation), because gas storage has been exhausted from dark respiration; (2) gas saturation inside tissues occurs around noontime till late afternoon, hence, male flower discharge is most likely to occur around this period (Brouns and Heijs, 1986; Rollón, personal observation); and (3) since gas production is primarily light dependent, the effect is about the same everywhere within areas/depths with comparable availability of PAR, facilitating synchronous release (Troll, 1931; Brouns and Heijs, 1986; Rollón, personal observation).

Although attractive as a substitute for labour-intensive fieldwork, the present study demonstrates that care is needed with reconstruction techniques for the estimation of annual flowering (e.g. Duarte et al., 1994, 1997). The frequency of peduncle scars does not necessarily correspond to the number of developed flowers, let alone the number of successfully pollinated female flowers. In view of the limited number of initiated flowers that reach full maturity, the reconstruction technique indicates an upper 'flowering potential'. The fact that the largest discrepancy (Table 5) between flower scars and actual flowering was observed at the deepest site 1c further supports the hypothesis that light energy is a major limiting factor in the flowering of *Enhalus*.

Combining the present data on reproduction of *Enhalus* with densities and recolonisation at the shallow site 1a results in the following estimation of successful sexual reproduction. Density of *Enhalus* is ca. 40 shoots m^{-2} (Rollón et al., 1998). Based on a mean female shoot density of $0.64 \times 40 = 25.6$ shoots m^{-2} (Table 3), a flowering frequency of 1.05 times per year (Table 4), a ratio of 0.3 of the flowers that produce fruits (Table 3), a seed content of 9.1 ± 2.5 (Rollón, 1998), and a mean settling success of ca. 0.35 per year in pot experiments (Rollón, 1998), we estimate a potential annual seedling establishment of 27 m^{-2} per year. This is more than an order higher than actually measured seedling establishment rates of 0.7 m^{-2} per year (Rollón et al., 1998). This large discrepancy suggests that survival in the pot experiments must be higher than in situ, probably because losses due to wave disturbance and herbivory are lower. Alternatively, long-distance export outside natural *Enhalus* stands may be considerable, since both mature fruits (when dislodged) and seeds have the capacity to float for days and hours, respectively (Lacap et al., 2002). Since only shallow-water stands of *Enhalus* produce substantial quantities of seeds, the extensive occurrence of this species in deeper waters must reflect colonisation by seedlings from elsewhere. Thus, seed export from shallow stands, like site 1a, should be quantitatively significant.

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