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Sexual reproductive success in *Posidonia oceanica*

Elena Balestri*, Francesco Cinelli

Dipartimento di Scienze dell'Uomo e dell'Ambiente, Via Volta 6, 56100 Pisa, Italy

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Abstract

To identify the factors that might contribute to limiting the sexual reproduction of *Posidonia oceanica* we examined: (1) flowering and fruiting phenology; (2) variability in flowering frequency, seed production and reproductive success (i.e. the proportion of flowers setting mature fruits per unit area) over a 2-year period; and (3) losses of potential seed production to fruit abortion and/or pre-dispersal seed predation. The flowering frequency of *P. oceanica* varied among years, ranging between 7.9 and 19.8%. Despite the large number of flowers and ovules, few of these produced mature fruits owing to abortion. Moreover, about 84% of developing inflorescences were damaged by herbivores. Fruit production varied among years, ranging from 4.7 to 13.5 fruits per m², but reproductive success remained constant (2.0–2.4%). Exclusion of herbivores showed that abortion alone was responsible for the loss of about 87% of the reproductive potential. Since reduced fertilization did not completely explain the observed frequency of abortion, it was hypothesized that post-fertilization factors could affect seed formation. The higher reproductive success of protected plants (11.2%) compared to herbivore-exposed plants (3.1%) indicated that predation significantly reduced the number of seeds available for establishment. We concluded that pre-dispersal seed losses to abortion and predation may seriously reduce the reproductive success of the species.

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1. Introduction

Seagrasses can reproduce both vegetatively and sexually, although the frequency and intensity of sexual reproduction in some species can be highly variable (Duarte et al., 1997; Marbà and Walker, 1999). Little is known on the extent and controls of sexual reproductive success in seagrasses, despite their world-wide ecological importance in coastal and

* Corresponding author. Tel.: +39-50-500018; fax: +39-50-49694.

E-mail address: balestri@discat.unipi.it (E. Balestri).

estuarine environments (den Hartog, 1970; McRoy and Helfferich, 1977). Flowering is considered the most critical factor in limiting sexual reproduction in several seagrasses (Marbà and Walker, 1999), but estimates of the potential yield of seeds are often inconsistent with the number of established seedlings observed in the field, indicating that large losses occur during the seed or seedling stages. Williams (1995) estimated that a considerable percentage of the seeds produced in *Phyllospadix torreyi* S. Watson may be lost due to abortion and pre-dispersal seed predation. Other authors have identified physical disturbance and post-dispersal seed predator activities as additional important causes of seed losses in seagrasses (Orth et al., 1994; Fishman and Orth, 1996). Sexual reproduction, even at a low rate, could play an important role in colonization of new sites, recovery after disturbance and establishing new genotypes in existing seagrass populations (Alberte et al., 1994; Orth, 1999). Conservation and restorative efforts should, therefore, consider factors that affect seed production, and extensive studies on this research topic need to be conducted.

The present study focused on the reproductive ecology of *Posidonia oceanica* L. Delile, the dominant seagrass found in the Mediterranean Sea. The distribution of this species has declined in many areas due to anthropogenic activities (Marbà et al., 1996); efforts are thus being devoted both to conserving and restoring this habitat (Piazzi et al., 1998). To date, there is a paucity of studies that describe flowering and fruiting in this species, and the importance of pre- and post-dispersal seed factors in limiting reproduction has been largely ignored. *Posidonia oceanica* is a monoecious species with hermaphroditic and male flowers arranged in a terminal spike composed of three–four flowered spikelets. Production of flowers and fruits is considered episodic (Giraud, 1977; Caye and Meinesz, 1984; Pergent et al., 1989; Buia and Mazzella, 1991). The reproductive season typically starts in September–October in shallow stands, and in November in deep stands; fruit maturation takes approximately 4 months (Buia and Mazzella, 1991). The mature positively buoyant fruit consists of a single seed which lacks a thick-wall coat. The seed is negatively buoyant and lacks dormancy, germinating within a few days following fruit release (Caye and Meinesz, 1984). Despite the high germinability of seeds (Balestri et al., 1998a), reports on seedling establishment in nature are rare (Balestri et al., 1998b; Piazzi et al., 1999) suggesting that the success of sexual reproduction is low in this species. This hypothesis is also supported by the low degree of genetic variability in *P. oceanica* meadows of northwestern Italy (Procaccini et al., 1996). The low seedling recruitment is usually attributed to seed loss in sites unsuitable for establishment (Buia and Mazzella, 1991). However, the recent finding of aborted fruits and inflorescences with signs of predation (Buia and Mazzella, 1991; Piazzi et al., 2000) indicate that other factors such fruit abortion and pre-dispersal seed predation could play an important role.

The exceptional occurrence of flowering and fruiting over two consecutive years in a *P. oceanica* meadow of north-western Mediterranean allowed us the opportunity to describe several aspects of the reproductive ecology of this species. Specifically, we examined: (1) flowering and fruiting phenology, (2) variability in flowering frequency, seed production and reproductive success (i.e. proportion of flowers setting mature fruits per unit area) over two consecutive years, and (3) whether and to what extent, fruit abortion and/or pre-dispersal seed predation affect seed production of this species.

2. Materials and methods

2.1. Study site, flowering and fruiting phenology

The study was conducted at the upper limit (3–4 m deep) of a *P. oceanica* meadow located on the south coast of Livorno (43°30'N, 10°19'E; NW Italy). To examine the reproductive phenology of *P. oceanica*, 10 intact reproductive shoots were randomly collected at monthly intervals throughout the reproductive season 1993–1994. The following variables were recorded: rhizome length, number of standing leaves (adult, intermediate and juvenile) and maximum leaf length, total length of the inflorescence, number of spikelets per inflorescence, number of male and hermaphroditic flowers per inflorescence and per spikelet, and number of developing or aborted fruits per inflorescence. Aborted fruits were recognisable as they were dark green and smaller compared to developing fruits. Since each hermaphroditic flower of *P. oceanica* has the potential to produce one normal well-developed fruit containing one seed, fertilization success was quantified as the proportion of hermaphroditic flowers that initiated fruits per inflorescence. The position of fertilized fruits within the inflorescence (lower, middle and upper spikelet) was recorded 30 days after fertilization. At fruit maturation, all fruits on inflorescences were dissected and examined under a dissecting microscope to categorize mature fruits as those containing fully-developed embryos. The number of mature fruits per inflorescence was counted, and the size and mass of mature fruits and seeds determined. Additional fruits were collected at the site and used to assess seed viability: two replicates of 25 seeds were placed in aquaria (30 cm × 20 cm) containing seawater (37‰ of salinity), equipped with an air supply and maintained at 18 °C and under 30 mmol/(m² s) photon irradiance and 12-h photoperiod (Balestri et al., 1998b). Germination percentage was calculated after 15–20 days. The viability of pollen extracted from anthers at anthesis was also tested by staining with 0.1% (w/v) vital neutral red stain (three replicate samples).

2.2. Inter-year variability in flowering, seed production and reproductive success

In September 1993 and 1994, the density of vegetative and reproductive shoots of *P. oceanica* (i.e. number of vegetative and flowering shoots per m²) and flowering frequency (i.e. number of flowering shoots/total number of shoots) were determined by using ten 0.25 m² quadrats randomly placed within the meadow. In October 1993 and 1994, the number of inflorescences and hermaphroditic flowers was counted on 10 randomly selected flowering patches permanently marked with 1 m² quadrats. The maximum potential seed production per unit area (1 m²) was estimated in both the years by multiplying the mean number of hermaphroditic flowers per inflorescence by the mean density of flowering shoots. Inflorescence development was monitored monthly until fruit maturation, and the proportion of inflorescences and flowers developing fruits was determined. At fruit maturation, the number of mature fruits per m² and the proportion of flowers producing mature fruits were calculated. In each year, final fruit and seed production (per m²) was estimated by multiplying the mean number of mature fruits per inflorescence by the mean density of inflorescences producing mature fruits. The proportion of hermaphroditic flowers producing mature fruits was used as a measure of reproductive success.

2.3. Incidence of fruit abortion and pre-dispersal seed predation

During the reproductive period 1994–1995, the relative losses of potential seed production to abortion and pre-dispersal seed predation were estimated by comparing the reproductive success per unit area of plants that were protected from and naturally exposed to herbivores. The herbivore exclusion experiment was carried out in two areas (approximately 100 m apart) within the meadow. In October 1994, nine 35 cm × 35 cm plots containing flowering shoots were randomly established within each area. Three of these plots were then randomly assigned to one of the following treatments: (1) exclusion of herbivores; (2) natural exposure to herbivores (control); (3) procedural control. In the first treatment macro-herbivores were excluded from plots by using 1-cm mesh-wire cages (35 cm × 35 cm × 40 cm) fixed at the corners with metal bars buried in the substratum. In the second treatment, plots were left open to herbivores and kept as controls. In the third treatment, cages as in the exclusion treatment but lacking the top and one side were placed on each plot to test for possible effects of the cage on microenvironment conditions. Prior to allocation of treatments, the number of inflorescences and hermaphroditic flowers per inflorescence was determined in each plot, and any possible herbivore was removed. The cages were maintained clean throughout the experimental period to minimise the effects of fouling on water currents and light attenuation. Cages were removed prior to natural shedding of mature fruits and all inflorescences from the plots were collected.

To quantify fruit abortion, the total number of aborted fruits present on inflorescences protected from herbivores was determined. The loss of the estimated potential seed production to abortion was calculated as the proportion of fruits aborted from the initial number of hermaphroditic flowers. To evaluate the effect of predation on reproductive success, the proportion of flowers producing mature fruits in experimental and control plots on each area was calculated. We also recorded the proportion of undamaged inflorescences that had at least one mature fruit, aborted inflorescences and inflorescences damaged by herbivores versus the initial number of inflorescences for control and experimental plots.

2.4. Statistical analysis

Differences among the reproductive years 1993–1994 and 1994–1995 in flowering frequency, fruit and seed production, and proportion of flowers producing mature fruits were tested by one-way analyses of variance (ANOVAs), with the year treated as fixed factor. A two-way ANOVA was performed to test the effects of treatments on the proportion of flowers producing mature fruits, with plant treatment as fixed factor and area as random factor. Two-way ANOVAs were also used to test for the effects on the proportion of aborted, mature and herbivore damaged inflorescences. Finally, a two-way ANOVA was used to test for the effect of small-scale spatial differences among treatments and areas on initial number of inflorescences. Homogeneity of variances was tested by Cochran's *C*-test, and data were appropriately transformed before ANOVAs, when necessary to improve normality and homoscedasticity (Sokal and Rohlf, 1995; Underwood, 1997). When necessary, the Student–Newman–Keuls test (SNK) was used to identify specific differences between pairs of treatments (Sokal and Rohlf, 1995).

3. Results

3.1. Flowering and fruit phenology

In September 1993, each sampled reproductive shoot of *P. oceanica* had one and occasionally two inflorescences (1.7%). The size of rhizomes of reproductive shoots was highly variable, ranging from 6.4 (± 1.5 S.E.) to 12.3 (± 4.9 S.E.) cm. Anther dehiscence was observed in October 1994. The pollen was trinucleate, filamentous (8–10 μm in diameter and 70–90 μm long) and filled with starch granules. Pollen viability ranged from 89 to 95%, with a mean of $91.3 \pm 3.2\%$. Data concerning the vegetative and reproductive characteristics examined are reported in Table 1.

Fertilization occurred in late October 1994. Virtually all inflorescences (96–99%) were fertilized. The mean rate of fertilization within the inflorescence was 85.3% (± 24.4); the proportion of developing fruits was higher in the middle spikelet ($63.3 \pm 28.3\%$) when compared with the lower- ($16.6 \pm 27.2\%$) and upper-spikelets ($23 \pm 22\%$). Fruit maturation took approximately 4 months and a progressive decline in the number of developing fruits due to abortion was observed during this period. At maturation, there was just one mature fruit or exceptionally three fruits per inflorescence, while the remaining fruits were aborted. Aborted fruits were smaller compared with mature fruits, ranging from 0.6 ± 0.2 cm in length and 0.3 ± 0.1 cm width. Mature fruits typically contained one seed per fruit, but two abnormal fruits were found; the first fruit bore two seeds developed side by side while the latter lacked seed. Average length, width and mass of mature seeds were $1.8 (\pm 2.3)$ cm,

Table 1
Vegetative and reproductive variables of *P. oceanica* flowering shoots during the reproductive season 1993–1994

Variable	September 1993	October 1993	November 1993	December 1993	January 1994	February 1994
Nr. leaves per shoot:	5.3 ± 0.8	7.6 ± 0.6	6 ± 0.9	6.1 ± 0.6	7 ± 2.0	5.6 ± 0.5
Adults	2.6 ± 0.5	4.3 ± 0.7	5.6 ± 0.5			
Intermediate	1.9 ± 0.5	2.7 ± 1.0	0.6 ± 0.5			
Juvenile	0.9 ± 0.5	0.6 ± 0.5	0			
Maximum leaf length (cm)	20.9 ± 4.1	32.6 ± 3.6	29.3 ± 5.0	30.3 ± 3.0	30.6 ± 3.5	34.1 ± 0.1
Spike length (cm)	3.4 ± 0.5	3.2 ± 0.3	3.5 ± 0.6	3.3 ± 1.1	4.4 ± 1.4	5.2 ± 0.4
Nr. spikelets/inflorescence	3	3	2.8 ± 0.3	3	3	3
Nr. hermaphroditic flowers/inflorescence	4.2 ± 0.7	4.1 ± 0.6				
Nr. ovules/spikelet	1.3 ± 0.3	1 ± 0.4				
Nr. male flowers/inflorescence	2.6 ± 0.8	2.4 ± 1.0				
Nr. developing fruits/inflorescence			3 ± 1.1	3.1 ± 0.8	2.5 ± 0.9	1.1 ± 0.4
Nr. developing fruits/spikelet			0.8 ± 0.4	1.1 ± 0.2	0.8 ± 0.7	0.3 ± 0.4
Developing fruit length (mm)		6	8.2 ± 2.4	10.4 ± 3	19.3 ± 1.1	23.8 ± 2.1
Developing fruit width (mm)		4	4.2 ± 1.2	5.0 ± 2.5	11.6 ± 0.5	14.8 ± 0.6
Developing fruit weight (g)			0.3	0.4 ± 0.1	0.6 ± 0.1	1.1 ± 0.4

Data are means (\pm S.E.), $n = 10$.

Table 2

Variables describing sexual reproduction of *P. oceanica* during the reproductive periods 1993–1994 and 1994–1995

Variable	1993–94	1994–95
Flowering frequency (%)	7.9 ± 3.8	19.8 ± 7.1
Inflorescence density (shoots per m ²)	55.6 ± 31.2	136.8 ± 46.8
Hermaphroditic flowers (number per m ²)	233.4 ± 131	574.2 ± 196.6
Mature inflorescences (number per m ²)	4.4 ± 6.3	10.8 ± 7
Proportion flowers producing fruits (%)	2 ± 2.2	2.4 ± 2
Mature fruits per inflorescence	1.1 ± 0.4	1.2 ± 0.4
Mature fruits or seeds (number per m ²)	4.7 ± 6.9	13.5 ± 10.6

Data are means (±S.E.) with $n = 10$.

0.9 cm and 0.4 (± 0.1) g, respectively. The majority of seeds were viable, as demonstrated by the germination rate (86.5 ± 9.2%).

3.2. Inter-year variability in flowering, seed production and reproductive success

Density of reproductive shoots was highly variable (Table 2). Results of ANOVAs showed that flowering frequency and final seed production were significantly higher in the 1994–1995 reproductive period than in 1993–1994 ($F_{1,18} = 21.25$, $P < 0.05$; $F_{1,18} = 4.70$, $P < 0.05$), but the proportion of flowers setting mature fruits per unit area did not differ among the years ($F_{1,18} = 0.25$, $P > 0.05$), indicating that reproductive success was constant. Based on the mean density of flowering shoots and the mean number of flowers per inflorescence (Table 2), the estimated maximum potential seed production in the reproductive season 1993–1994 ranged from 84 to 487 seeds per m², but varied between 340 and 945 seeds per m² in the subsequent season 1994–1995. However, the proportion of inflorescences retaining developing fruits decreased progressively during the fruit-growth phase as consequence of abortion and herbivore attack (Fig. 1). Thus, only a small percentage of the initial marked

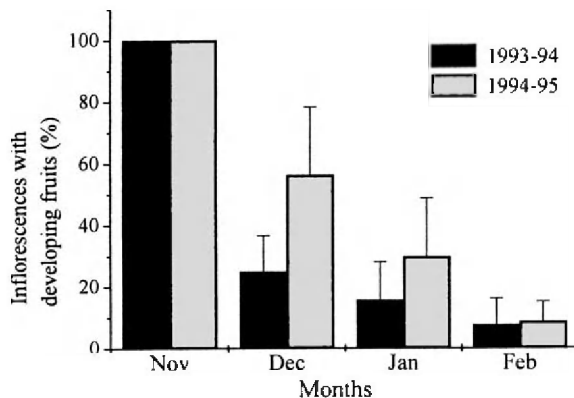


Fig. 1. The proportion of inflorescences of *P. oceanica* retaining developing fruits at each survey during the reproductive periods 1993–1994 and 1994–1995. Data are means (±S.E.), $n = 10$.

Table 3
Comparison of variables describing sexual reproduction of *P. oceanica* between treatments (a) and results of ANOVAs for differences between treatments in the proportion of mature, aborted or damaged inflorescences, and of flowers producing mature fruits (b)

Variable	Plant treatment												
	Herbivores	No herbivores	Procedural control										
(a)													
Nr. Inflorescences per plot	16.6 ± 3.3	13 ± 0.5	12.9 ± 0.9										
Nr. hermaphroditic flowers per plot	70 ± 13.8	54.6 ± 1.9	54.6										
Nr. mature inflorescences per plot	1.5 ± 0.2	4.9 ± 0.4	0.8 ± 0.4										
Nr. mature fruits or seeds per inflorescence	1.1 ± 0.6	1.2 ± 0.7	1.1 ± 0.2										
Nr. mature fruits or seeds per plot	1.9 ± 0.9	6.3 ± 0.9	1.1 ± 0.2										
Nr. aborted fruits per plot	47.3 ± 6.3												
Sources of variation	d.f.	Mature inflorescences			Aborted inflorescences			Eaten inflorescences			Flowers setting mature fruits		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
(b)													
Plant treatment = <i>P</i>	2	1711.44	54.87	0.01	5545.43	38.36	0.02	13457.75	39.45	0.00	146.50	37.01	0.02
Area = <i>A</i>	1	56.25	0.97	0.34	130.68	2.64	0.13	22.85	0.65	0.43	13.10	2.26	0.15
<i>P</i> × <i>A</i>	2	31.19	0.539	0.59	144.54	2.92	0.09	96.50	2.77	0.10	3.95	0.68	0.52
Error	12	57.80			49.46			34.79			5.78		
SNK test		Exposed = control < protected			Exposed = control < protected			Exposed = control > protected			Exposed = control < protected		

Data are means (±S.E.) for the two areas with n = 6.

inflorescences produced fruits (Table 2). Reproductive success ranged from 2% in 1994 to 2.4% in 1995 and mean final seed production varied from 4.7 to 13.5 seeds per m², respectively (Table 2).

3.3. Pre-dispersal seed losses due to abortion and predation

At the start of the experiment there was no significant difference in the number of inflorescences of *P. oceanica* per plot between treatments ($F_{2,12} = 1.49$, $P > 0.05$; Table 3) or areas ($F_{1,12} = 0.19$, $P > 0.05$). At fruit maturation, experimental and control plots varied significantly in the proportion of mature, aborted or damaged inflorescences and in reproductive success, but there were no significant area effects (Table 3). The absence of significant artefact effects (Table 3) indicated that the exclusion did not affect fruit maturation.

On average, in control plots and in procedural controls only 6.7–10% of the original inflorescences showed mature fruits; most of the original inflorescences (79.5–84.4%) were

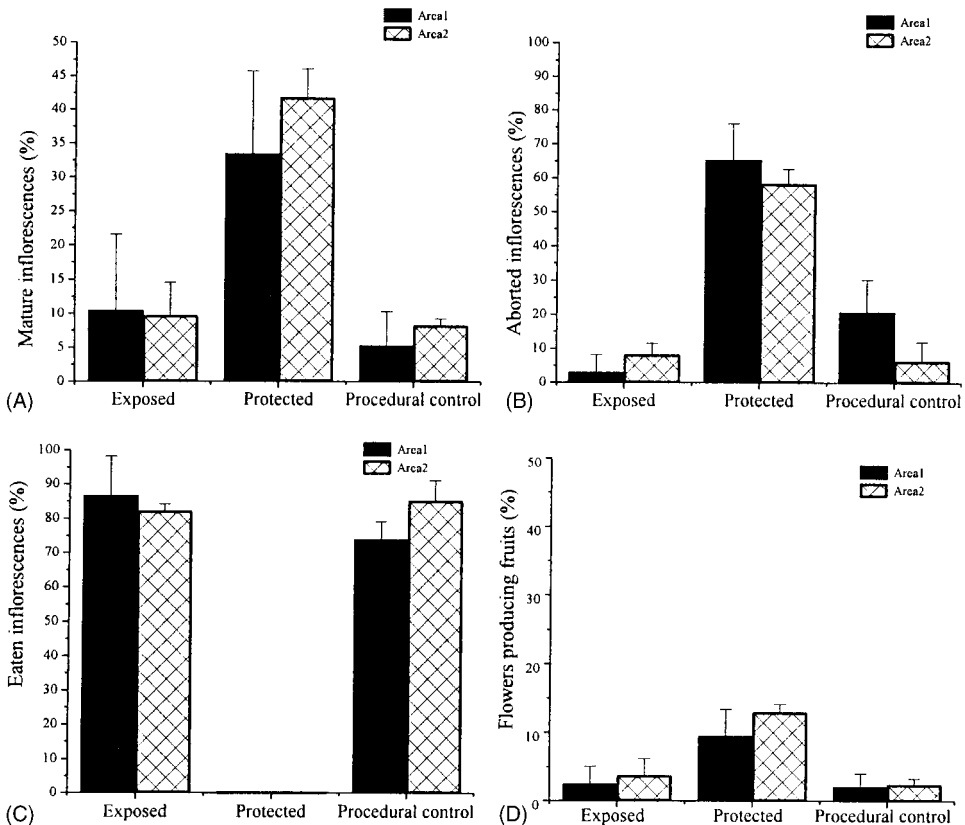


Fig. 2. Proportion of inflorescences (A) mature, (B) aborted and (C) damaged by herbivores, and (E) proportion of flowers producing mature fruits in patches of *P. oceanica* exposed to vs. protected from herbivores vs. procedural control for each area. Data are means (\pm S.E.), $n = 3$.

damaged by herbivores, while the remaining fraction of inflorescences were aborted (Fig. 2). The proportion of flowers producing mature fruits was 3.1% and mature inflorescences had from 1 to 3 mature fruits (Table 3). Thus, approximately 97% of potential seed production was lost due to predation and abortion of fruits on undamaged inflorescences.

In herbivore protected plots, the proportion of inflorescences producing mature fruits increased significantly when compared with controls and procedural controls, being on average 37.5% (Fig. 2). The proportion of aborted inflorescences (61.6%) and the proportion of mature fruits (11.2%) also increased in the two areas (Fig. 2). The total number of mature fruits produced per plot in the two areas varied between 5, 6 and 7 (Table 3). Therefore, fruit abortion alone accounted for a mean loss of 86.5% of potential seed production. The higher number of inflorescences aborted in the absence of predation suggests that herbivores destroyed most of the inflorescences that would have aborted under natural conditions.

4. Discussion

In this paper, we provide accurate information on the reproductive success of *P. oceanica* over two consecutive reproductive seasons. Results indicate that the flowering frequency of this species is rather variable, at least in the period investigated. According to previous findings (Caye and Meinesz, 1984; Buia and Mazzella, 1991; Piazzini et al., 2000), only a small percentage of inflorescences of *P. oceanica* produce mature fruits and seeds. Although significant variations in fruit and seed abundance are observed, the fraction of flowers producing mature fruits remains constant. These findings indicate that the species is capable of a considerable reproductive effort, but most of the investment may be lost during the fruit-growth phase. Based on these results it can be speculated that 12–13 inflorescences are necessary for the production of one mature viable fruit in this species. Comparison of herbivore protected and control plants demonstrates that abortion alone was responsible for the loss of ca. 87% of the reproductive material, with pre-dispersal consumption contributing a further 10%. Thus, fruit formation represents a significant investment of energy for *P. oceanica*, most of which was subsequently lost from the population through abortion.

There is very little information about the causes of fruit mortality occurring between fertilization and seed production in seagrasses (Williams, 1995). Several factors that can act in the pre- or post-fertilization phase, such as limited pollination, resource limitation, adverse environmental conditions and inbreeding depression could account for fruit abortion in *P. oceanica*. Reduced fertilization per se cannot explain the high level of abortion since 73% of ovules within *P. oceanica* inflorescences were initiated. On the other hand, the decline in number of developing fruits with increasing inflorescence age suggests that post-fertilization factors (such as competition for resources or unfavourable local environmental conditions) may be responsible for abortion. Because of self-pollination within the same floral axis, fragmented clonal patches may occur in *P. oceanica* populations of the northwestern Mediterranean (Sandmeier et al., 1999), so that it cannot be excluded that abortion results from inbreeding depression. In addition, the production of seedless fruits of *P. oceanica*, a phenomenon also occurring in *Halophila engelmannii* Aschers. (McMillan and Jewett-Smith, 1988), could indicate problems with the pollen tube growth and, thus, failure to fertilize the ovules.

Large scale abortion of flowers and fruits is a common phenomenon in hermaphroditic terrestrial plants (Lloyd, 1980; Sutherland, 1987; Wiens et al., 1987; Ehrlén, 1992). Abortion of fruits could be of adaptive significance in that elevated abortion rates provide a reserve of maturing into fruits if unpredictable resources become abundant, or reduce the risk of loss of mature fruits to predation (Lloyd, 1980; Stephenson, 1981; Sutherland, 1987; Ehrlén, 1991). Clearly, the reasons for the high level of abortion found in *P. oceanica* needs to be elucidated.

Our results also demonstrated that predation significantly reduced the potential *P. oceanica* seed production, but most destroyed fruits would otherwise be aborted, so never would have contributed to the population. Although numerous studies have addressed the role of herbivore control on seagrass production (Tayer et al., 1984; Zieman et al., 1984; Pergent et al., 1997; Valentine and Heck, 1999), very few studies have been designed specifically to evaluate the effects of herbivores on seagrass reproduction (Williams, 1995; Fishman and Orth, 1996; Piazzzi et al., 2000). Fruit and seed predation is a serious hazard for decline in plant populations, especially those of rare and endangered species (Louda, 1989). Therefore, further studies on other *P. oceanica* populations are needed to elucidate the extent and generality of *P. oceanica* seed predation and examine whether environmental conditions and habitat type might influence pre-dispersal predation levels. It also remains to be examined whether and in what conditions this species can compensate for herbivore-induced seed loss.

In conclusion, the present study showed that high levels of abortion, and to a lesser extent predation, have the potential to reduce dramatically the reproduction success of *P. oceanica*. Therefore, management strategies could benefit from improved fruit production through decreased seed abortion to restore *P. oceanica* meadows. Other factors not considered here, such as fruit dispersal, post-dispersal predation, and availability of safe sites for seedling establishment may contribute to enhanced restoration success, and should form the object of future research.

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