

SKewed SEX RATIOS, POLLEN LIMITATION, AND REPRODUCTIVE FAILURE IN THE DIOECIOUS SEAGRASS *PHYLLOSPADIX*

ANDREW OLAF SHELTON¹

Department of Ecology and Evolution, University of Chicago, 1101 E. 57th Street, Chicago, Illinois 60637 USA

Abstract. Dioecious mating systems in angiosperms, in which individuals are unisexual, require that pollen disperses between male and female individuals and may cause dioecious species to be vulnerable to reduced reproductive success via pollen limitation. This potential cost has rarely been documented, in part because of a dearth of studies investigating abiotic pollen dispersal, which tends to be associated with dioecy. I document widespread variation in reproductive success in an ecologically important, habitat-forming group, the seagrasses (marine angiosperms), using two species of the dioecious genus *Phyllospadix*. At multiple sites in the state of Washington, USA, I demonstrate pervasive male rarity (all sites <24% male by area). Male abundance is shown to predict female reproductive success at two scales. Within sites, the ratio of maturing seeds to total ovules declines rapidly with increasing distance to the nearest male, while among sites, mean seed : ovule ratios scale positively with male abundance. At some sites, less than 1 in 100 ovules mature into seeds. A field experiment conducted at a high pollen availability site shows that manipulating pollen availability could produce the range of seed sets observed across sites, but pollen limitation was not definitively demonstrated. Overall my results are consistent with pollen limitation in *Phyllospadix* and comprise the first strong evidence of this phenomenon in seagrass populations. In addition, seed production rates predicted local seedling recruitment, demonstrating a measurable demographic consequence of low pollen availability.

Key words: dioecy; *Phyllospadix*; pollen limitation; reproductive failure; seagrass; seed : ovule ratio; skewed sex ratio.

INTRODUCTION

In plants, many species produce far more ovules than successfully mature into seed (Weins 1984, Holland and Chamberlain 2007). Low levels of reproductive success, as measured by fruit : flower or seed : ovule ratios, have been hypothesized to result from both ecological and evolutionary causes (Sutherland and Delph 1984). On ecological scales, female plants may not receive sufficient pollen (reviewed in Ashman et al. 2004, Knight et al. 2005), may not receive enough compatible, high-quality pollen (Weins et al. 1987, Charlesworth 1989), or may not have sufficient resources to mature ovules into seeds (Lee and Bazzaz 1982, Griffin and Barrett 2002). These three causes are known as pollen limitation, pollen quality, and resource limitation, respectively, and each has been shown to limit reproductive success in natural systems. On evolutionary scales, the leading hypothesis for low reproductive success is that plants “bet-hedge,” overproducing flowers to take advantage of stochastic variation in pollen or resource availability across years (Sutherland 1986).

For dioecious plant species, where individuals are unisexual, the distance between male and female plants may be large and pollen receipt seems likely to limit

reproductive success. Adequate pollen may be particularly problematic if the sexes are spatially segregated (e.g., Eppley 2001) or males are rare (Lloyd 1974, Stehlik and Barrett 2006). Dioecious species have traits that appear to mitigate this potential pollen dispersal problem, including high pollen : ovule ratios relative to other plant breeding systems (Cruden 1977) and prevalence of abiotically dispersed pollen (Renner and Ricklefs 1995), which might ensure pollen delivery over wide areas (but see Davis et al. 2004).

Reports of low reproductive success in dioecious species with abiotic pollination modes are rare and generally limited to instances involving species at low density (e.g., Somanathan and Borges 2000, Steven and Waller 2007). However, relatively few studies have investigated reproductive success in abiotically pollinated plants.

The seagrasses are a polyphyletic group of marine monocots with an unusual prevalence of dioecy (~75% dioecious [Cox 1988] vs. ~6% in all angiosperms [Renner and Ricklefs 1995]), water-borne pollen, and a propensity for asexual reproduction. Asexual reproduction is thought to be important for the maintenance of many seagrass populations. Seagrasses play a vital ecological role in coastal marine and estuarine habitats worldwide and form the basis of some of the most productive ecosystems on earth (Mateo et al. 2006). For seagrasses and plants in general, the implications of dioecy and abiotic pollination for populations are virtually unstudied.

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¹ E-mail: ole@uchicago.edu

Here I explore the ecological causes of low reproductive success in two species of the seagrass genus *Phyllospadix* (the surfgrasses). I use field surveys at multiple sites over multiple years to examine how reproductive success varies within and among sites and to ask if this variation is predicted by male abundance. To determine if my observational methods were supported experimentally, I performed a field pollen manipulation. Finally, I ask if seed production rates determined seedling recruitment to assess the broader demographic effects of variation in reproductive success.

STUDY ORGANISM AND STUDY AREAS

Five species in the genus *Phyllospadix* form beds in intertidal and shallow subtidal rocky shore habitats throughout the north Pacific ocean (Phillips 1979, Phillips and Menex 1988, Williams 1995). In this study I focus on *Phyllospadix scouleri* and *P. serrulatus*. *Phyllospadix scouleri* can be found in the subtidal and intertidal from Baja California to southern Alaska, whereas *P. serrulatus* ranges from Oregon to western Alaska in the low and middle intertidal zones (Phillips 1979). Surfgrasses are long-lived perennials but no estimates of genet longevity exist. Ovules are fertilized in the spring, mature over the summer, and disperse passively as seeds each fall and winter. Reproductive shoots in both species contain a single spike of male or female flowers termed the spadix (plural spadices). Pollen is released into the water column and pollination occurs on the water's surface as well as underwater (Cox et al. 1992). There are no known animal pollen vectors, fruits are uniovulate, and there is no long-lived seed bank (Orth et al. 2000). Unlike most plants, surfgrasses attach directly to rock and little or no sediment covers the rhizomes. The extent of surfgrass patches can be defined visually by their rhizome edges.

A striking feature of these dioecious species is the prevalence of male rarity. The sex ratio of flowers in two eastern Pacific species, *Phyllospadix torreyi* and *P. scouleri* are strongly biased toward females at all sites surveyed to date (Phillips 1979, Cox et al. 1992, Williams 1995). Despite observed male rarity, depressed seed production from pollen limitation has never been reported for this genus (Williams 1995).

I studied seven populations of *P. serrulatus* and four populations of *P. scouleri* on the outer coast of the Olympic Peninsula in the state of Washington, USA: Teahwhit Head (TH), Ozette (OZ), Shi Shi Beach (SS), Chibadehl Rocks (CR), and Kydaka Point (KD), and two sites on Tatoosh Island, Strawberry Draw (SD) and Main Beach (MB) (Appendix A). These populations span ~90 km on this outer coast where both species are abundant.

METHODS

Sex ratios

To estimate the sex ratio at each site, I established survey areas for each species on a series of rocky benches. Surge channels, tall rocky outcrops, and other

natural topographic features defined site edges, making sites vary in size (m^2). GPS coordinates and measuring tapes were used to calculate the total area within each site (hereafter "site area"; Appendix A). In spring 2006 I searched each site for male flowers by crawling through surfgrass patches and examining reproductive shoots. I define a patch as a continuous area of rock covered by rhizomes of surfgrass and separated from other surfgrass plants by bare rock. When I encountered a male patch I measured the rhizome area containing only male spadices and counted the total number of spadices present. I assumed that male patches separated by >1 m represented distinct individuals. Given the slow rhizome growth rate of surfgrasses (Williams 1995; and *unpublished data*), this assumption is reasonable. I also measured the cumulative area covered by all patches (including male, female, and nonflowering patches together) for each species at each site (hereafter "surfgrass area"; Appendix A).

Due to the difficulty of identifying distinct genets in asexually propagating species like surfgrasses, I used three methods to describe male abundance. First, I calculated the ratio of male to female flowering patches in terms of area. I measured the area occupied by male rhizomes and the total surfgrass area of each species directly. However, the non-male area at each site consists of both flowering female areas and nonflowering areas and their relative proportions were not directly measured. To account for uncertainty in the amount occupied by flowering female patches, I calculated a range for the sex ratio at each site using estimates for the proportion of area occupied by flowering females. Field observations suggested a majority of each site consisted of flowering females; thus I calculated sex ratios assuming 50% or 90% of the non-male area was flowering female, in order to establish a reasonable range for flowering area sex ratios.

Second, I used a patch-based metric to provide an upper estimate of the proportion of flowering male genets in each population. I estimated the total number of genets in the surfgrass area using a conservative assumption about the sizes of genets. I assumed all genets were as large as the largest male patch ever observed at any site ($0.5 m^2$ for *P. serrulatus* and $0.3 m^2$ for *P. scouleri*), and divided the surfgrass area by this size to estimate the total number of genets present at each site. I then divided the number of flowering male patches identified by my estimate of the total number of genets to generate an estimate of site sex ratios. Since this procedure results in a low estimate of the total number of genets, the patch-based sex ratio should overestimate the true proportion of males. Many isolated female patches smaller than the size of the male patch used in calculating the sex ratio were observed at all sites, making my estimates of the total number of genets low and my estimates of the proportion of males high.

I used the density of male spadices at each site as my final measure of male abundance. The number of spadices present in male patches varies nonlinearly with patch area (*unpublished data*). As a result, a single, large male patch has substantially more spadices than several small male patches even if both groups sum to the same total area. To account for differences in the size of male patches as well as differences in site area, I calculated the density of male spadices per m² by dividing the total number of spadices by the site area for each species. I used male spadix density as a proxy for pollen abundance.

Among site seed: ovule ratios

I tested whether the ratio of maturing seeds to total ovules (hereafter "S:O ratio") varied among sites and years and asked if variation in male abundance explained the S:O ratio at each site. The S:O ratio is the proportion of the sexual reproductive potential realized by an individual spadix and is an estimate of seed production. I haphazardly collected female spadices for each species in August 2005, 2006, and 2007. For *P. scouleri*, spadices were collected at four sites in each year (KD, MB, SD, and SS). *Phyllospadix serrulatus* spadices were collected from four sites in 2005 (KD, MB, SD, and SS), all seven sites in 2006, and five sites in 2007 (CR, KD, MB, SD, and SS). I counted the number of maturing seeds and total ovules in each spadix and calculated the S:O ratio. For each species, S:O ratios at sites sampled all three years (KD, MB, SD, and SS) were compared using a two-way ANOVA with site, year, and the site \times year interaction as factors. Tukey-Kramer hsd was used to make pairwise comparisons of mean S:O ratios between sites and years in the two-way ANOVA. Additionally, I compared *P. serrulatus* S:O ratios in 2006 among all seven sites using a one-way ANOVA and Tukey-Kramer hsd. For all analyses involving S:O ratios, model residuals were checked for deviation from a normal distribution using quantile-quantile plots. If deviations were detected, arcsine square-root transformations were applied. For the remainder of this study, I used arcsine square-root transformations on S:O ratios unless noted.

To test for site-level effects of male abundance on seed production, I calculated pairwise correlations between the three estimates of sex ratio and back-transformed mean S:O ratio in 2006 for each species. Each site was treated as a replicate.

Within site seed: ovule ratios

If male rarity causes pollen to limit reproductive success, reduced seed production at increasing distances from pollen sources is expected. I surveyed S:O ratios as a function of distance from a single *P. serrulatus* male in the MB population and a cluster of male *P. serrulatus* patches found at CR to look for a signature of local pollen dispersal. In August of 2005, 2006, and 2007 at MB and in August 2007 at CR, I surveyed female *P.*

serrulatus patches surrounding the isolated males and calculated the S:O ratio in the first 12 spadices encountered in each patch. If fewer than 12 spadices were present, I surveyed all female spadices in the patch. The distance from the nearest edge of the male patch to each female spadix was measured to the nearest 0.1 m. A linear regression of S:O ratio as a function of distance (m) was performed at CR. I used a generalized linear model with distance, year (as class variable), and the distance \times year interaction as factors to analyze S:O ratios at MB.

At two sites with multiple pollen sources (TH for *P. serrulatus* and SD for *P. scouleri*), I used the spacing of males to compare seed set for female flowers that were close to (0–2 m) or far from (10–12 m) the nearest flowering male in August 2006. I haphazardly surveyed between 15 and 20 spadices in each distance category and used *t* tests to compare S:O ratios in each category for each species.

Pollen manipulation

To test the effect of pollen availability on S:O ratios, I performed a pollen manipulation experiment. Due to logistical difficulties associated with water-borne pollen, field manipulations of pollen availability commonly performed with terrestrial plants (e.g., Ashman et al. 2004) have never been conducted for seagrasses. The large size of surfgrass pollen (filaments \sim 1000 μ m long and 10 μ m in diameter) makes manipulating pollen feasible. To ensure sufficient pollen availability for hand-pollinated treatments, manipulations were performed at sites known to have abundant males (KD for *P. serrulatus* and MB for *P. scouleri*). In April 2006, 11 replicates of four adjacent spadices whose stigmas were just starting to mature were identified and assigned haphazardly to one of four treatments: (1) unmanipulated—the spadix was marked by tying a string around an adjacent rhizome but otherwise not manipulated; (2) exclusion—each spadix was shrouded in fine mesh (\sim 60 μ m diameter opening) and the bag was anchored to the plant by tying off the base of the bag to the rhizome with fly-fishing line backing; (3) reduction—each spadix was covered as in the exclusion treatment except that the mesh was perforated by multiple holes (6 mm in diameter) to reduce pollen availability from the unmanipulated treatment; and (4) hand-pollinated—each spadix was covered as in the exclusion treatment, but pollen was collected from a single local male plant using forceps and deposited within the bag before being secured around the spadix. The amount of pollen placed within bags was not quantified but was far more than the available number of ovules. Pollination was intended to occur as the incoming tide mixed pollen with receptive stigmas within the bag. True pollen addition treatments, where spadices have pollen added by hand but are left to receive naturally dispersed pollen as well, were not performed because of concerns that pollen might wash off focal spadices and impact nearby treatments.

TABLE 1. Estimates of male abundance for surfgrasses.

Sites	Male patches (<i>N</i>)	Flowering-area sex ratio	Patch-based sex ratio	Male spadix density (no./m ²)
<i>P. serrulatus</i>				
KD	20	0.032–0.059	0.23	0.63
CR	12	0.006–0.010	0.08	0.35
SD	0	0	0	0
MB	1	0.018–0.032	0.02	0.32
SS	4	0.002–0.004	0.03	0.11
OZ	3	0.006–0.012	0.08	0.06
TH	14	0.008–0.010	0.22	0.43
<i>P. scouleri</i>				
KD	13	0.022–0.039	0.10	0.65
SD	15	0.014–0.025	0.13	0.65
MB	31	0.017–0.031	0.10	0.48
SS	1	0.001–0.001	0.01	0.72

Notes: The total number of male patches identified, two estimates of sex ratio, and the density of male spadices are presented for each site. Sex ratios are represented as the proportion of male flowering individuals. Flowering-area sex ratios are presented as a range to account for uncertainty in the area in surfgrass beds that did not flower (see *Methods: Sex ratios* for details).

In late July, all spadices were collected and the S:O ratio for each spadix was calculated. Due to three instances of lost pollen manipulation bags for *P. serrulatus* only the eight replicates that contained all four treatments were included in the analysis. Differences between treatments were assessed using a one-way ANOVA with treatments as factors and Tukey-Kramer *h*_{sd}.

If pollen availability limits surfgrass seed production, S:O ratios were expected to be zero in the exclusion treatment, increase to low levels in the reduction treatment, and peak in the unmanipulated and hand-pollinated treatments. Furthermore, under pollen limitation, hand-pollinated S:O ratios are expected to be larger than unmanipulated treatments. The difference between hand-pollinated and unmanipulated treatments should indicate the strength of pollen limitation (Ashman et al. 2004). No differences among treatments would point to a failure to manipulate pollen abundance or the presence of asexual seed production (agamospermy). Agamospermy is uncommon and frequently affiliated with polyploidy (Richards 2003), and as a consequence is not suspected to occur in the diploid surfgrasses (Stewart and Rudenberg 1980).

Seedling recruitment

To determine if local seedling recruitment reflected local seed production, I assessed seedling recruitment rates in late April and early May 2006 (at KD, MB, SD, and SS) and in 2007 (all seven sites). I placed five, 0.04-m² quadrats at randomly generated, non-overlapping positions along 5-m transects and searched for newly recruited seedlings. Eleven transects were conducted in 2006 and 12 were conducted in 2007 (55 and 60 quadrats in total, respectively). I identified each seedling to species using a hand lens and identified its nurse plant to species or species group. Because seedlings might be limited by seed production as well as the availability of appropriate

habitat for establishment, I quantified the percent cover of algal and invertebrate species within each quadrat.

The abundance of seedlings per 0.04-m² quadrat was predicted using a generalized linear mixed model (GLMM). I used a log link (i.e., Poisson family) because the response was discrete. Fixed covariates were the untransformed mean S:O ratio from the previous fall at each site, year (as a class variable), and the combined percent cover of recruitment habitat. Recruitment habitat was defined as those species observed to receive *Phyllospadix* seedlings by Turner (1983; four groups of red algae) and *Phyllospadix* spp. Site and transect nested within site were included as random grouping effects. The percent cover of recruitment habitat was arcsine square-root transformed before analysis.

RESULTS

Sex ratios

Searches for males during May and June of 2006 yielded few reproductive males at all sites. The ratio of male to female flowering areas showed that flowering males covered no more than 6% of the flowering area at any site (Table 1). Assuming that 50% or 90% of the non-male area consisted of flowering females did not affect the conclusion that flowering males make up a small proportion of the surfgrass area. Patch-based sex ratio estimates ranged from <0.01 to 0.23 male and, with one exception (*P. serrulatus* at MB), were substantially less biased toward females than flowering sex ratios (Table 1). Male spadix density averaged less than one male spadix per m². In accordance with previous studies of surfgrasses, all metrics of male abundance show that flowering male plants are exceedingly uncommon.

Among site seed:ovule ratios

At all sites S:O ratios were dramatically lower than the potential reproductive output of 1.0. Mean S:O

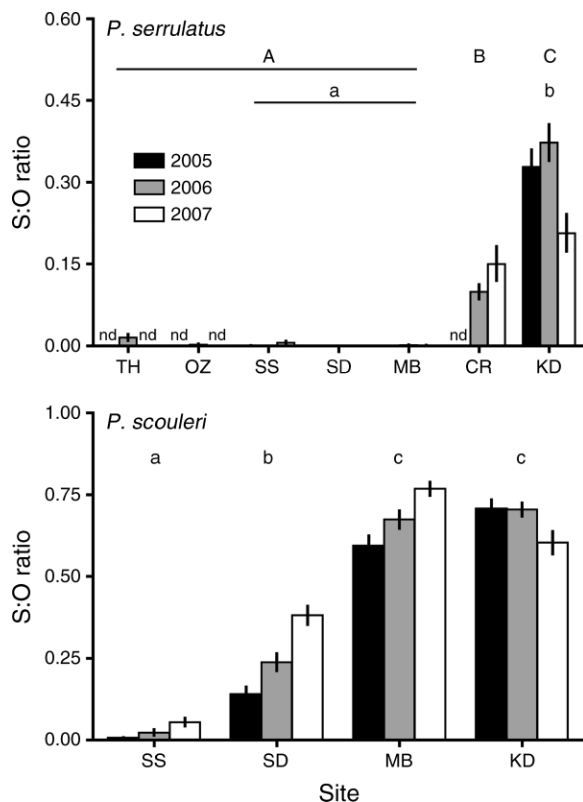


FIG. 1. Seed:ovule (S:O) ratios for the surfgrasses *Phyllospadix serrulatus* and *P. scouleri* at different sites on the Olympic Peninsula, Washington, USA. Back-transformed means \pm SE are shown. Note the different y-axis scales for the two species. (Upper panel) *P. serrulatus* ratios. Significant site differences ($P < 0.05$) determined by Tukey-Kramer hsd at the four sites included in the two-way ANOVA are indicated by different lowercase letters. Uppercase letters show Tukey-Kramer hsd for all seven sites in 2006; "nd" indicates that no data were available. (Lower panel) *P. scouleri* ratios. Significant site differences determined by Tukey-Kramer hsd calculated from the two-way ANOVA are indicated by different lowercase letters. See *Study organism and study areas* or Appendix A for site codes.

ratios over the three years ranged from 0.02 to 0.70 for *P. scouleri* and from 0.004 to 0.37 for *P. serrulatus* (Fig. 1). For both species, site, year, and their interaction all significantly predicted S:O ratios in the two-way ANOVA (Appendix B). Site differences explained the most variation in S:O ratios. Sequential sums of squares (i.e., Type 1) from the two-way ANOVA showed site had a partial R^2 of 0.54 and 0.58 for *P. scouleri* and *P. serrulatus*, respectively (Appendix B). Year and site \times year had less than one-fifth the explanatory power of site (partial $R^2 < 0.09$ for both terms in both species). While this type of partial R^2 does not account for the order in which factors are added to the ANOVA and thus may overestimate the explanatory power of the factors added to the model first, it provides an approximate measure of each factor's explanatory power and clearly demonstrates that site differences determine much of the

variation in S:O ratios. Tukey-Kramer hsd showed significant differences among years for *P. serrulatus* and *P. scouleri* with 2005 significantly lower than both 2006 and 2007 in each species (Appendix B). The one-way ANOVA on S:O ratios for *P. serrulatus* sites in 2006 showed dramatic differences in S:O ratios across sites as well ($P < 0.0001$; Fig. 1; Appendix B).

Pairwise correlations between the three estimates of male abundance and mean S:O ratios were uniformly positive, but not always significant. *Phyllospadix scouleri* correlation coefficients were $r = 0.61$ ($P = 0.39$) and $r = 0.51$ ($P = 0.48$) for flowering and patch-based sex ratios, respectively. Similar coefficients were observed in *P. serrulatus* with $r = 0.82$ ($P = 0.02$) for flowering and $r = 0.65$ ($P = 0.11$) for patch-based sex ratios. Density of male spadices correlated strongly with S:O ratio in both species ($r = 0.93$, $P = 0.07$ and $r = 0.76$, $P = 0.05$ for *P. scouleri* and *P. serrulatus*, respectively; Fig. 2). Despite the relatively small number of sites included in these analyses and marginal significance values, the magnitude of correlation coefficients indicates a substantial site-level effect of male abundance on average S:O ratios.

Within site seed:ovule ratios

S:O ratios declined significantly with increased distance from pollen sources at all sites surveyed (Fig. 3). At MB, *P. serrulatus* S:O ratio declined significantly with distance from the male patch ($P < 0.0001$; Fig. 3 [top panel]; Appendix C; $n = 120, 90$, and 82 spadices for 2005, 2006, and 2007, respectively). The year and year \times distance terms in the model were both not significant (Appendix C). No spadix had more than two developed seeds.

Similarly S:O ratios decline with distance from the cluster of males at CR (Fig. 3B [middle panel]; $P < 0.0001$; $n = 125$; Appendix C). Unlike MB, developing

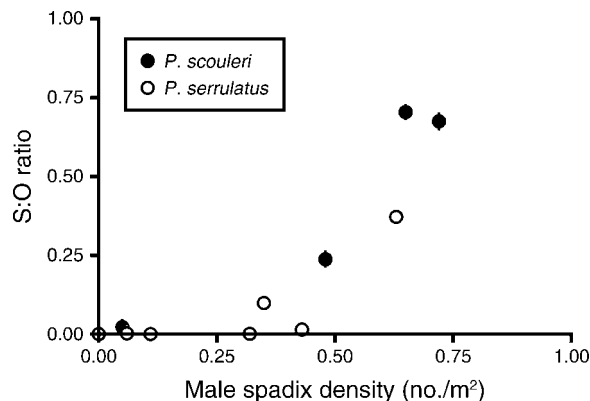


FIG. 2. Seed:ovule ratios as a function of male spadix density for the two surfgrass species in 2006. Each point shows the density of male spadices and back-transformed mean \pm SE for seed:ovule ratios at each site. Sample sizes were 41 spadices or greater for each point (see Appendix B for all sample sizes). Error bars on the y-axis may be smaller than points.

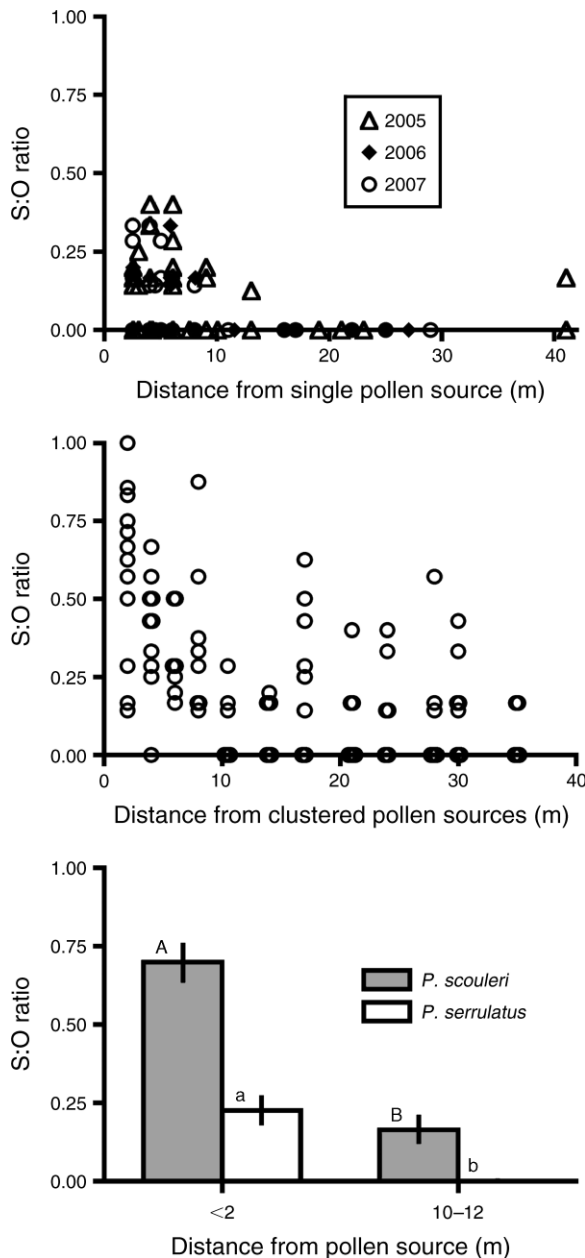


FIG. 3. (Top panel) Seed:ovule ratios in *P. serrulatus* spadices as a function of distance from the single pollen source at Main Beach (MB), Olympic Peninsula, Washington, USA, over three years. (Middle panel) Seed:ovule ratios in *P. serrulatus* spadices as a function of distance from the cluster of pollen sources at Chibadehl Rocks (CR) in 2007. Data points are slightly offset to avoid overlap. (Bottom panel) Seed:ovule ratios in spadices at two distance classes away from the nearest pollen source. Back-transformed means \pm SE are shown. Significant differences ($P < 0.05$) within species using Tukey-Kramer hsd are indicated by uppercase and lowercase letters for *P. scouleri* and *P. serrulatus*, respectively.

seeds were noted at every distance sampled, including the most distant patch at 35 m.

At sites with scattered male plants (TH for *P. serrulatus* and SD for *P. scouleri*), the S:O ratio declined

sharply with distance in both *P. serrulatus* ($n = 19$ for <2 m and $n = 36$ for 10–12 m; $t = 5.91$, $P < 0.001$), and *P. scouleri* ($n = 16$ for <2 m and $n = 21$ for 10–12 m; $t = 6.92$, $P < 0.0001$; Fig. 3C [bottom panel]). In all three analyses, S:O ratios declined with distance from the nearest pollen source. My results are consistent with a pattern expected for S:O ratios if pollen is limiting and only disperses over short distances.

Pollen manipulation

S:O ratios differed significantly between pollen manipulation treatments for both species (Fig. 4). S:O ratios in *P. scouleri* spadices where pollen was excluded declined $\sim 90\%$ compared with unmanipulated spadices, while the reduction treatment had an S:O ratio midway between the two (ANOVA; $F = 19.6$, $df = 3, 40$, $P < 0.0001$). The hand-pollinated treatment produced a slight but not significant increase in S:O ratio relative to the unmanipulated treatment (Fig. 4), indicating that the hand-pollinated treatment was successful. However, the lack of a significant difference between hand-pollinated and unmanipulated treatments suggests that fertilization could not be enhanced and that pollen is not strongly limiting *P. scouleri* at MB.

Experimental results for *P. serrulatus* were less clear. *Phyllospadix serrulatus* exhibited a $\sim 80\%$ decline in S:O ratio between unmanipulated and the three manipulation treatments (ANOVA; $F = 14.6$, $df = 3, 28$, $P < 0.0001$), but the reduction, exclusion and addition treatments were statistically indistinguishable (Fig. 4). My results do not indicate pollen limitation for *P. serrulatus* at KD.

Seedling recruitment

Seedling recruitment varied among sites with mean recruitment rate ranging from 0.4 to 13.3/m² for *P. scouleri* and from 0 to 1.3/m² for *P. serrulatus*. Across all sites and years, only six seedlings were found for *P.*

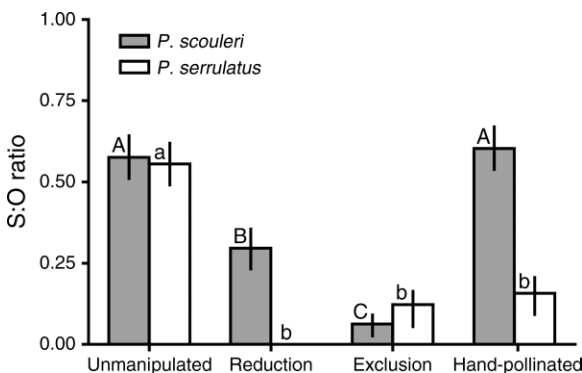


FIG. 4. Seed:ovule ratios in the pollen manipulation experiment. Back-transformed means \pm SE are shown for *P. scouleri* and *P. serrulatus*. Significant differences ($P < 0.05$) within species using Tukey-Kramer hsd are indicated by uppercase and lowercase letters for *P. scouleri* and *P. serrulatus*, respectively.

TABLE 2. Results for a generalized linear mixed model (GLMM) predicting the abundance of *P. scouleri* seedling recruits in surveyed quadrats.

Parameter	Fixed effects				Random effects	
	Estimate	SE	Z	P	Variance	SD
Intercept	-3.64	0.48	-7.60	<0.0001
S:O ratio	2.90	0.73	4.02	<0.0001
Habitat	1.09	0.31	3.55	0.0004
Year	-0.29	0.28	-1.05	0.29
Site	0.04	0.20
Site(transect)	0.70	0.83

Notes: Fixed effects are the average S:O ratios at a site, the proportion of area occupied by surfgrass recruitment habitat, and year. Random effects are site and transect nested within site.

serrulatus (three at CR in 2007, one at KD in 2006, and two at KD in 2007). Due to low *P. serrulatus* recruitment, I performed the GLMM on *P. scouleri* only.

Using the GLMM, S:O ratio ($Z = 4.02$; $P < 0.0001$) and recruitment habitat ($Z = 3.55$; $P = 0.0004$) were significant predictors of the abundance of *P. scouleri* seedling recruits (Table 2). Year was not a significant effect. The strong significance of the S:O ratio shows a strong effect of seed set from the previous fall on seedling recruitment and shows that local seed production predicts local recruitment. There was no evidence that the availability of recruitment habitat varied among sites or years (Appendix D).

DISCUSSION

Despite theoretical arguments that pollen receipt should not limit seed production (e.g., Haig and Westoby 1988), recent reviews have demonstrated that plant seed set is often pollen limited (Ashman et al. 2004, Knight et al. 2005). In most studies, the mechanism driving pollen limitation involves plant–pollinator interactions. Species with abiotic pollen dispersal, and therefore without plant–pollinator interactions, have been largely ignored in the pollination literature. Because they lack the complications of plant–pollinator interactions, species with abiotic pollination provide an opportunity to study plant reproductive biology in a simplified, single-species framework.

As seen for many other plant species (Weins 1984), the ratios of maturing seeds to total ovules (i.e., S:O ratios) were less than the theoretical potential of 1.0 in both surfgrasses at all sites (Fig. 1). Among plants for which S:O ratios have been examined, it is unusual to have so many sites and years with S:O ratios close to zero. S:O ratios changed with pollen abundance at two scales. Within sites, seed set of individual spadices declined rapidly with distance to the nearest male (Fig. 3). Among sites, three measures of male abundance were positively correlated with average S:O ratios (Fig. 2). My data also show site variation in S:O ratio as a function of distance from a pollen source (Fig. 3). While S:O ratios within sites may vary slightly under the influence of local factors such as hydrodynamic conditions or the pollen production of individual males (e.g.,

Fig. 3, top vs. middle panel), the strong relationship between S:O ratios and male abundance at two scales in two species leads me to conclude that pollen availability drives S:O ratio variation and spadices are commonly pollen limited. My results, and the local pollen dispersal reported for the monoecious seagrass species *Zostera marina* (Ruckelshaus 1996, Reusch 2003), suggest that pollen dispersal may be a general hurdle for sexual reproduction for seagrasses (but see Ackerman 2002).

Experimental pollen manipulation produced results consistent with pollen availability driving reproductive success in *P. scouleri*. As expected, spadices of both species that were bagged to reduce pollen availability had significantly smaller S:O ratios. However, I could not increase S:O ratios with hand pollination, suggesting that pollen is saturated and pollen limitation did not occur at this site (Fig. 4). A more powerful test for pollen limitation would have been to manipulate pollen at a site with few males or to add pollen on multiple occasions to hand-pollinated spadices.

Results for *P. serrulatus* did not provide a strong test of pollen limitation. S:O ratios in hand-pollinated treatments were less than the unmanipulated treatment and not statistically different from the exclusion or reduction treatments, indicating a failure of the hand-pollination treatment. I speculate that the most likely cause of this result is that stigmas of *P. serrulatus* were not receptive to pollen when the addition occurred. However, several other alternatives, such as stigmas being clogged by too much pollen or that the manipulation bag disrupted seed development, are also possible explanations.

Note that in both species small S:O ratios were present in the exclusion treatments (range: 0–0.43 and 0–0.37 for *P. scouleri* and *P. serrulatus*, respectively; Fig. 4). I ascribe this result to the difficulty in maintaining a tight seal around the base of exclusion bags in this high wave energy environment. While most spadices in the exclusion treatment had no maturing seeds, occasional strands of pollen may have entered through the bottom of some bags and resulted in low average S:O ratios. Because fertilized seeds were present in all treatments, agamospermy cannot be ruled out, though it is very unlikely to produce the range of S:O ratios observed in natural populations.

The role of the two other ecological causes of low S:O ratios, resource availability and pollen quality, remain speculative. Resources, in terms of nutrients and sunlight, are not expected to differ among these sites and thus drive the variation documented in S:O ratios. The nutrient environment is characterized well for Tatoosh Island and nitrogen is relatively abundant (Pfister et al. 2007) and does not limit growth in kelp species that co-occur with surfgrasses (Pfister and Van Alstyne 2003). Reduced reproduction as a result of pollen quality remains a possibility because genetic variation, inbreeding rates, and pollen compatibility are entirely unknown for surfgrasses. The only evolutionary cause of low S:O ratios applicable to surfgrasses, "bet-hedging" on stochastic pollen or resource availability, receives tentative support from the significant difference in S:O ratios across years (Appendix B). However, whether pollen or resource availability drives variation in S:O ratios is unknown.

Interestingly, low S:O ratios also result in reduced seedling recruitment (Table 2). While the implications of reduced seedling recruitment for populations depend upon the degree to which surfgrasses are seed limited (e.g., Turnbull et al. 2000), my data indicate a strong effect of male rarity on seed production and recruitment. Local seed dispersal for surfgrass is consistent with experimental evidence from the related seagrass *Zostera marina* (Orth et al. 1994).

While exact sex ratio estimates are impossible without genetic information, my estimates of surfgrass sex ratios suggest that these species have some of the most extreme female-biased sex ratios known (Lloyd 1974, Stehlik and Barrett 2006). Such skewed sex ratios prompt the question: Why is male rarity present in all known surfgrass populations? Skewed sex ratios in flowering plants can arise from two sources: (1) seeds are produced with skewed sex ratios, or (2) the sexes experience differential mortality during maturation. Surfgrass sex appears to be chromosomally, and not environmentally, determined (Stewart and Rudenberg 1980, Uchiyama 1993) and evidence currently suggests that surfgrass sex ratio bias is driven primarily by sex differences in mortality (*unpublished manuscript*). Males of a congener, *P. torreyi*, are more prone to wave-driven disturbance, the most important source of mortality in surfgrasses (Williams 1995).

If sex differences in mortality drive skewed sex ratios, surfgrasses are consistent with the general prediction that male and female offspring should be produced in equal proportions (Fisher 1930, Hardy 2002). Selection is then expected to increase survivorship, not to modify the sex ratio of offspring (Hardy 2002). Because so few flowering surfgrass males are present, males who survive sire many seeds and will disproportionately pass on their genes. Assuming traits determining survivorship are heritable, this should produce strong selection for increased survivorship of male plants. Despite this prediction, male rarity has been observed in all surfgrass

populations surveyed to date (Williams 1995). Two mechanisms that are not mutually exclusive may underlie male rarity. First, males and females may have different life histories. Males might mature quickly, invest heavily in sexual reproduction, and die young, while females expend few resources in sexual reproduction and have extensive asexual growth and long life spans. Under this scenario and consistent with my results, surveyed populations would have few flowering males. If this case proves true, surfgrasses would be an exception to the widely held assertion that females experience higher costs of reproduction in plants (Delph 1999).

A second possibility is that the presence of asexual reproduction in surfgrasses may modify the strength of selection acting on survivorship. As in all marine angiosperms, surfgrasses can reproduce vegetatively through rhizome fragmentation as well as reproduce sexually. If asexual reproduction contributes substantially more to fitness than sexual reproduction, differences in seed production may not translate into substantial differences in total fitness among males, reducing selection for increased survivorship. The prerequisite for this scenario (i.e., a large contribution to population growth rate from asexual, relative to sexual, reproduction) has been shown for some asexually reproducing species (McFadden 1991, Damman and Cain 1998), but is by no means universal (Wepler et al. 2006). While the forces generating skewed sex ratios are myriad and interact in complex ways (reviewed in Hardy 2002), the presence of asexual reproduction may contribute to the maintenance of skewed sex ratios in surfgrasses. To my knowledge, the interaction of multiple reproductive modes and sex ratio has not been explored.

Arguments for the dominance of vegetative reproduction in surfgrasses may be undermined by the observation that seeds are required for dispersal. Fragments of adult plants cannot reattach to the substratum once dislodged, so the colonization of new areas must arise through seed dispersal. Reaching an isolated rocky outcrop requires a seed if the outcrop is meters or kilometers away. Therefore, seeds have a clear fitness benefit, even if dispersal occurs rarely. Even without considering the possible benefits of genetic recombination or diversity, the necessity of dispersal should ensure the maintenance of sexual reproduction.

Given the foundational role of seagrass in coastal marine ecosystems, understanding processes that mediate sexual reproduction and seedling recruitment in populations is vital for assessing conservation threats as well as informing restoration efforts (Williams 2001). The populations studied here appear to have varying but generally limited potential for sexual reproduction and thus may be vulnerable to changing disturbance and climatic regimes. Indeed, it is striking to note that relatively small numbers of male patches (~10–20 patches; Table 1) coincide with large variation in S:O

ratios. The scale of abiotic pollen dispersal appears to mediate the success of sexual reproduction for these populations. Understanding how genetic diversity and the relative frequency of asexual and sexual reproduction interact to affect population persistence in seagrasses and other habitat-forming species remains a pressing, practical question in ecology.

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APPENDIX A

A map of the study area (*Ecological Archives* E089-175-A1).

APPENDIX B

Two-way ANOVA tables, partial R^2 , and year effect Tukey-Kramer hsd tables for S:O ratio in *P. scouleri* and *P. serrulatus*; one-way ANOVA table for *P. serrulatus* at all seven sites in 2006; and sample sizes of *P. serrulatus* and *P. scouleri* species collected over three years and seven sites (*Ecological Archives* E089-175-A2).

APPENDIX C

S:O ratio as a function of distance from (1) MB male and (2) the CR male cluster (*Ecological Archives* E089-175-A3).

APPENDIX D

Number of *Phyllospadix serrulatus* and *P. scouleri* seedlings found recruited to algae and seagrass, years 2006 and 2007 combined; ANOVA table for the percent cover of *Phyllospadix* spp. found in surveys for seedling recruitment conducted in April and May 2006 and 2007, at four sites; and ANOVA table for the percent cover of articulated coralline algae, *Neorhodamela larix* and *Odonthalia* spp., found in surveys for seedling recruitment conducted in April and May 2006 and 2007, at four sites (*Ecological Archives* E089-175-A4).