

Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit

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ABSTRACT: Near its southern limit in the Northeastern Atlantic, the dioecious brown alga *Fucus vesiculosus* is absent from the exposed coast yet it is abundant in estuaries and coastal lagoons. In contrast, the phylogenetically and ecologically related hermaphroditic species *F. spiralis* occurs along the open coast, though often in low abundance. We hypothesized that the absence of *F. vesiculosus* from exposed shores near its southern limit was due to reduced external fertilization success, as its gametes may be diluted beyond the level required for successful fertilization, in contrast with its hermaphroditic, self-compatible congener. To test this hypothesis, individuals of both species were transplanted to 3 exposed sites near their southern limit in the Northeastern Atlantic. Egg settlement and fertilization success (% of eggs fertilized) were evaluated daily during the main reproductive season. Recruitment was evaluated at the end of the reproductive season, and recruit mortality was evaluated using outplants of laboratory-cultured embryos. On the exposed shores near their southern limit, transplanted adults of both species survived and released eggs, and fertilization success was unexpectedly high. However, recruitment and recruit survivorship of *F. vesiculosus* was significantly lower than *F. spiralis*. Our results suggest that *F. vesiculosus* is restricted to low water-motion environments because of recruitment failure and recruit mortality on exposed bare shores near its southern limit, and not because of inability to fertilize eggs in turbulent environments. This study does not support our hypothesis of a role for dioecy/hermaphroditism in explaining the distribution of externally fertilizing marine organisms in high water-motion environments.

KEY WORDS: Reproductive ecology · Mating system · Gamete release · Post-settlement mortality · *Fucus* · recruitment · External fertilization · Brown algae · Distributional limits

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INTRODUCTION

Marine species with external fertilization rely on the bulk mixing of water to bring male and female gametes together. If mixing is turbulent, gamete dilution may occur rapidly, therefore reducing the probability of egg fertilization (Denny 1988). Modeling approaches and experimental field data (with induced spawning) show that fertilization success of organisms with external fertilization is greatly reduced with increasing water motion (e.g. Pennington 1985, Denny & Shibata 1989, Levitan et al. 1992, Oliver & Babcock 1992). However, in dioecious organisms with separate

male and female individuals, some mixing may be necessary for gamete encounter and fertilization (Denny & Shibata 1989). Because of the greater probability of gamete encounters when self-fertilization is possible, both monoecious species, in which the male and female gametes are released from different reproductive structures in the same individual, and hermaphroditic species, where eggs and sperm occur in the same reproductive structure, may be at an advantage over dioecious species under turbulent conditions. Regardless of reproductive mode, wave swept organisms that reproduce via external fertilization often have mechanisms to increase the probability of gamete

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encounters, such as synchronous spawning, release of gametes under optimal conditions for encounters, morphological and physiological adaptations, and chemical cues for gamete location (e.g. reviews by Giese & Kanatani 1987, Brawley & Johnson 1992, Levitan 1995, Levitan & Petersen 1995, Brawley et al. 1999, Yund 2000, Santelices 2002).

Fucus spp. reproduce exclusively by external fertilization, with gamete release occurring synchronously during calm periods and during the daytime (Pearson & Brawley 1996, Serrão et al. 1996). Fucoids use a water-motion sensing mechanism (Pearson & Brawley 1998, Pearson et al. 1998) to minimize the negative effects of gamete dilution by restricting gamete release to calmer periods during the reproductive season. Gamete release often occurs on days with lower water motion for subtidal populations (Serrão et al. 1996, 1999a), during low-tide periods for tide-pool populations when they would be isolated from waves (Pearson & Brawley 1996), and at slack high-tide in intertidal populations (Berndt et al. 2002). Gamete release is restricted to daytime since photosynthetic signals are required for gamete release (Serrão et al. 1996b, Pearson et al. 1998). In all natural furoid populations studied to date, fertilization success has been very high (Brawley 1992, Pearson & Brawley 1996, Serrão et al. 1996, Berndt et al. 2002), except near the northern limit of distribution of *Fucus vesiculosus* in the Baltic Sea (Serrão et al. 1999a).

During reproduction, gametangia are released from conceptacles in the reproductive tissue (the receptacles). Furoid eggs are released from oogonia (8 eggs per oogonium in *Fucus* spp.) and the sperm from antheridia (64 sperm per antheridium), either in the water column or soon after reaching the substrate. Eggs, oogonia, and antheridia sink, and the sperm are negatively phototactic. The sperm are attracted to the eggs by a pheromone (Müller & Gassman 1978). Eggs can reach the bottom (i.e. settle) either before or after fertilization. Because there is no planktonic stage, settlement in *Fucus* spp. is directly related to gamete release and defined as the shower of eggs and zygotes that have fallen to the bottom. Adhesion to the substrate is not simultaneous with settlement. Instead, settlement occurs within minutes of release, whereas attachment occurs a few hours after fertilization. Only zygotes attach; unfertilized eggs do not secrete adhesive material. Attachment can therefore be used as an assay to distinguish unfertilized eggs from zygotes. Another post-fertilization event that can also be used to evaluate fertilization is germination, which results in the appearance of a rhizoid (an elongation of 1 zygote pole), approximately 1 d post-fertilization. Recruitment is defined as the appearance of established individuals of a certain predefined size/

stage. In this study, any stage beyond a fertilized egg was considered a recruit.

Fucoids can have either a hermaphroditic or dioecious reproductive mode. *Fucus vesiculosus* is dioecious, with each individual producing only 1 type of gamete (i.e. separate males and females). *F. spiralis*, like several other hermaphroditic fucoids, has been, in our opinion, incorrectly referred to as monoecious, even in our own studies (e.g. Serrão et al. 1999b). Although the term monoecious has been defined for flowering plants, it refers to genets in which the male and female function are separated (i.e. separate male and female flowers in the same individual) (Richards 1997), which is not the case for *F. spiralis* if a flower and a conceptacle are considered to be analogous. *F. spiralis* is clearly a hermaphroditic, a genet with both male and female function (Richards 1997). The reproductive organs (e.g. flowers, or conceptacles in the case of *Fucus* spp.) of hermaphroditic individuals can be either monoecious (each has a single sex) or hermaphrodite (both sexes in the same conceptacle, which is the case for *F. spiralis*).

There are advantages and disadvantages associated with the different reproductive modes of hermaphroditism versus dioecy (e.g. as reviewed by Bawa 1980, Thomson & Brunet 1990, Freeman et al. 1997, Barrett 1998, Charlesworth 1999). Hermaphrodites that are capable of self-fertilization can pass their entire genome to the offspring, reduce the likelihood of disrupting locally co-adapted gene combinations through recombination, and guarantee the possibility of reproduction at least by self-fertilization. This is particularly true when the likelihood of encounters with gametes from different individuals is very low, as is predicted to be the case for externally fertilizing organisms on open coasts (e.g. Denny 1988). Dioecious reproduction provides the selective advantages of obligatory outcrossing, including avoidance of inbreeding depression, and promoting genetic variability, which is important for adaptation to variable ecological conditions. Dioecy and hermaphroditism have evolved independently several times within the Fucaceae family (Serrão et al. 1999), as well as in many different taxonomic groups, indicating that there may often be a thin and easily crossed line dividing and balancing the selective advantages of these contrasting reproductive modes.

The furoid algae *Fucus vesiculosus* and *F. spiralis* are good model species on which to test the role of mating systems on fertilization success because the 2 species are similar in their ecology and morphology and, although evolutionarily very closely related (Serrão et al. 1999b), they differ in their reproductive modes (dioecious vs. hermaphroditic). The dioecious *F. vesiculosus* has a much higher sperm-to-egg allocation than the hermaphroditic *F. spiralis* (Vernet & Harper 1980).

F. spiralis allocates much more biomass to eggs than sperm, which is suggestive of a high degree of inbreeding (Vernet & Harper 1980). Lower investment into male function by *F. spiralis* than for dioecious *F. vesiculosus* (Vernet & Harper 1980) also suggests that it is much more efficient at achieving successful external fertilization in the sea than dioecious species.

Both *Fucus vesiculosus* and *F. spiralis* are common in the intertidal zone in cold temperate environments in the Northern Atlantic. Their southern limit in the Northeastern Atlantic is found in the biogeographic transition zone between Southwest Europe and North Africa, between a cold temperate and a warm temperate environment (Lüning 1990). Near its southern limit along the Portuguese and Moroccan coasts, *F. vesiculosus* is restricted to growing in estuaries and coastal lagoons (i.e. areas with minimal wave motion, where it occurs in locally abundant stands) and it does not occur on the open coast as it does in more northern environments. On the other hand, *F. spiralis* does occur on open rocky shores near its southern limit, often at low abundance in canopy-devoid rocky areas separated by long stretches of sandy beaches.

This study addresses the question of whether the reproductive system, in particular hermaphroditism versus dioecy, can be a factor determining the distribution of marine species with external fertilization, in particular in exposed habitats where, once spawning takes place, the probability of gamete encounters is predicted to be very low due to rapid gamete dilution. In comparing the role of reproductive mode in the reproductive success of these 2 species, we hypothesized that (1) the absence of *Fucus vesiculosus* (dioecious) on exposed shores near its southern limit is due to reduced fertilization success in the high water-motion environment there, and that (2) *F. spiralis* (hermaphroditic), by being able to release both male and female gametes from the same individual, increases the probability of sperm-egg encounters, and can thus achieve high fertilization success even on these exposed shores or in turbulent environments. In this study, our hypothesis was tested by moving both species to the exposed shores near their southern limit and determining fertilization success during the reproductive season of 2001. The expected outcome was that *F. vesiculosus* would not be able to fertilize its eggs in the turbulent environment and would have a much lower fertilization success than its hermaphroditic congener.

MATERIALS AND METHODS

Study sites. In Northern Portugal, *Fucus vesiculosus* and *F. spiralis* coexist in dense stands on the open coast. One of the southernmost locations where both

species coexist on the open shore is Viana do Castelo (Fig. 1), where offshore rocky bluffs reduce wave action in many inner sites where both *Fucus* species are abundant. This site was the source of all transplants. The 3 sites separated by 15 km to which both *Fucus* species were transplanted were approximately 500 km south, in the area around Sines, and were representative of the exposed shores near the southern limit (Fig. 1).

Viana do Castelo has a cool temperate climate; air temperatures average $14.3 \pm 2.9^\circ\text{C}$ (SD) over the year and annual precipitation averages 1444 mm (Instituto de Meteorologia, Portugal, data available from www.meteo.pt). At this site, population density of *Fucus* spp. can exceed 600 ind. m^{-2} in some areas with many small recruits, with other intertidal canopy-forming species such as *Ascophyllum nodosum* and *Pelvetia canaliculata*. In most areas, canopy cover is 100% (unpubl. data).

In Sines, average air temperature is approximately 2°C warmer than in Viana do Castelo, with much lower average annual precipitation at 492 mm (Instituto de Meteorologia, Portugal, data available from www.meteo.pt). Sines has long stretches of sandy

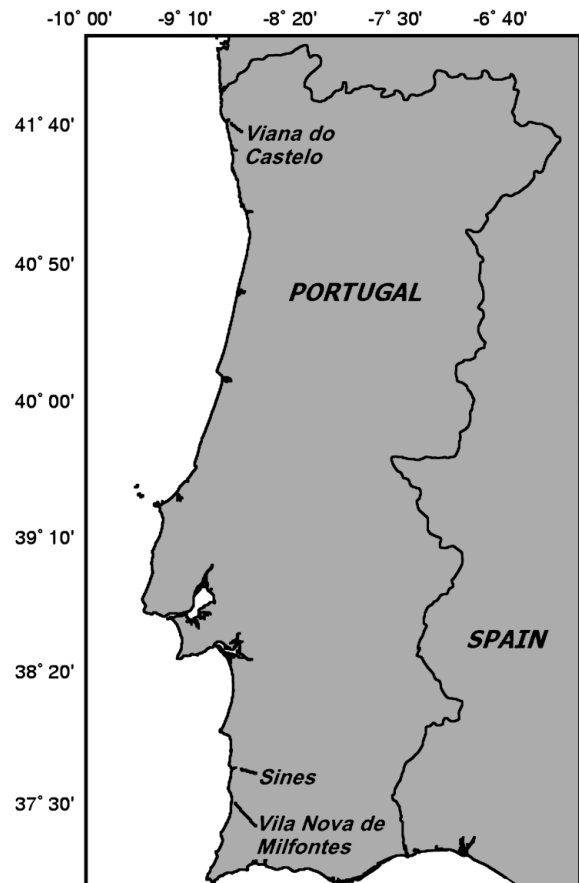


Fig. 1. Map of study sites

beach interspersed with rocky outcrops that are exposed to wave action. On rocks, stunted forms of *Fucus spiralis* exist in low-abundance stands; they are reproductive during spring/summer. *F. vesiculosus* is absent on the open coast, but occurs nearby in an estuary (Vila Nova de Milfontes, see Fig. 1).

Transplants. Reproductive individuals of *Fucus vesiculosus* and *F. spiralis*, which were naturally attached to small rocks, were collected in March 2001 in Viana do Castelo and transported to Sines (still attached to rocks). The following day at low tide, half of the rocks were transplanted to the 3 wave-exposed sites near Sines (Porto de Sines, Porto Covo, and Praia de Serro da Águia), and attached to the bedrock using a small amount of cement (Sika grout quick drying cement, Industrias Quimicas). The other half were extra boulders left in a nearby intertidal pool for replacements, as necessary, when cement failed and rocks were lost throughout the season. This way, replacements could be made with algae exposed to the same conditions as the original transplants. Stands were transplanted to the same shore levels occupied by the 2 species in the intertidal zone of Viana: 0.9 to 2.4 m above ELWS (extreme low water spring) for *F. vesiculosus* and 2.4 to 3.0 m above ELWS for *F. spiralis*, and rocks with numerous algae naturally attached were arranged side-by-side so that algae were at the same density as in the source location, and so that males and females (of *F. vesiculosus*) were side-by-side in order to simulate stands in ideal conditions for successful external fertilization. Two stands of approximately 20 large reproductive individuals each were made for each species in 2 of the 3 sites (in Porto Covo and in Praia de Serro da Águia). In the third site (Porto de Sines), only 1 stand of each species was made, and the area was netted to exclude a grazing fish (*Sarpa salpa*) which is capable of removing a large part of the adult *Fucus* biomass (pers. obs.). Controls were performed by transplanting algae back to their original site (as part of a student's thesis, Monteiro unpubl.) and showed no effects of transplantation.

Zygote adhesion assay. In order to determine fertilization success in the field, an inexpensive method to distinguish fertilized eggs from non-fertilized eggs was developed. Because only fertilized eggs attach to the substrate, the number of eggs that were attached versus those that were not could be used as a proxy for the percentage of eggs fertilized in the field. In order to do this, the time necessary to give fertilized eggs a chance to attach after collection, under laboratory conditions, needed to be established, since in field experiments the eggs could have been fertilized shortly before collection and still might not have had enough time to attach. Eggs of *Fucus vesiculosus* and *F. spiralis* from Viana do Castelo were released and fertilized at high

sperm:egg ratios in the laboratory in separate batch cultures of seawater at ambient temperature, with glass slides placed at the bottom of each container. At various time intervals after fertilization, which usually occurs immediately after gamete release in enclosed environments in culture, 3 glass slides for each species were collected, rinsed gently with seawater, and the numbers of attached zygotes were counted with a dissecting microscope (Zeiss). This was repeated at intervals for the first 10 h after fertilization, and a final evaluation was performed again after 24 h. This experiment was performed 3 times for *F. spiralis* and twice for *F. vesiculosus*. The time after which no significant increase of attached zygotes occurred was chosen as the minimum time required for zygote adhesion. All field collected samples of settled eggs were then allowed more than this amount of time to attach, after which time they were considered unfertilized if they were not attached or had not formed a polar rhizoid-thallus axis.

Egg settlement. Egg settlement was monitored using circular fiberglass collecting disks of 6 cm in diameter made from food-grade isophthalmic fiberglass resin (ALMO, Produtos Quimicos). In order to retain settled eggs, the surfaces of the disks were made rugose by casting the fiberglass in a latex rubber mold with sandpaper (number 6 roughness) at the bottom. Once dry, isophthalmic resin is non-toxic; a preliminary experiment in which eggs were settled on the disks confirmed this (they fertilized, attached, and developed normally). These disks were drilled in the center (5 mm diameter drill bit). A larger hole was drilled into the intertidal bedrock and a stainless steel bolt (5 mm diameter, 50 mm length) was fixed head-down in the hole with quick-drying cement (as used for the transplants). The perforated disks were placed onto the fixed bolt, and fastened with a nut. The disks were able to withstand strong wave exposure, yet were quick and easy to remove during sampling at low-tide periods.

At least 3 disks in each stand for each species were collected daily at each low tide that occurred during daylight hours (twice a day during extreme low tides) in each of the 3 sites, from April to June 2001. In these natural stands some reproductive algae can be found all year round, but there is a large peak when nearly all individuals are reproductive during the spring, and this period is designated as the reproductive season. Night sampling was not performed because fucoids require light for potentiation of gamete release (Serrão et al. 1996, Pearson & Brawley 1998, Pearson et al. 1998), and have been shown not to release gametes at night (Pearson & Brawley 1996). The number of eggs per disk was counted with a dissecting microscope (Leica MZ6).

Fertilization success. Fertilization success was evaluated on days when substantial egg settlement was observed (>50 eggs disk^{-1}). Two assays were used to determine fertilization: zygote attachment, and rhizoid formation, both of which only occur in fertilized eggs. Adhesion of fertilized eggs to the substrate takes place some hours (see 'Zygote adhesion assay' above) after fertilization. Unfertilized eggs settle and become trapped in the disks' crevices but do not adhere. The number of eggs on the disk upon collection was recorded. Then the disks were rinsed gently with seawater and loose eggs were kept in a Petri dish. After 24 h, the number of eggs attached to the Petri dish plus the number of eggs that were originally attached to the disk were counted as fertilized. This number was then divided by the number of eggs originally collected on the disk to give the percentage of settled eggs that were fertilized, and to calculate fertilization success. The 24 h period was sufficient for all fertilized eggs to adhere. The attachment assay was confirmed with the germination assay: formation of a rhizoid.

Recruitment. Recruitment during the reproductive season was estimated by placing permanent collecting disks (as above) under the algae, interspersed with the daily collecting disks. These were collected at the end of the sampling period. The number of recruits on the disks was evaluated by counting under a dissecting microscope. In this study, any attached individual in the field at the end of the study that had reached a post-settlement size/stage was considered a recruit. This number was divided by the total number of eggs settled on the disks (the sum of all the eggs on the 3 disks over the entire collection period) in that site during the reproductive season to estimate the percentage of settled eggs that recruited (i.e. attached and survived until the end of the reproductive season) and by the number of eggs that were fertilized on the disks (the sum of all the fertilized eggs on major days of release on the 3 disks over the entire collection period).

Recruit mortality. Embryo mortality was estimated by outplanting 1 mo old laboratory-cultured embryos to the field sites. Disks were seeded with fertilized eggs in batch culture in the laboratory and grown under natural daylight at ambient temperature in natural seawater which was replaced weekly. After 1 mo, the number of recruits on the disks was evaluated and then the disks were outplanted to 2 of the transplant sites (Porto de Sines and Praia do Serro da Águia). The outplant experiment followed a factorial design to test for the effects of canopy (2 levels) and grazers (3 levels) on recruit mortality (1 mo old embryos in this case). The effect of canopy was tested by placing outplants underneath *Fucus spiralis* transplants which had had their reproductive structures (receptacles) trimmed (to avoid interference from egg release), versus placing the out-

plants on bare rock. The effect of grazers was tested by using grazer exclusion cages, built from plastic mosquito mesh sewn around a cage of plastic-coated wire. The cages were intended to exclude snails and limpets, which are abundant on the Portuguese open coast, and grazers of *Fucus* spp. embryos. Sea urchins and large amphipods were also excluded. This treatment was compared with a cage control treatment (cage with the wire but no mesh) and a treatment where no cage or wire was used. Three replicate disks were used for each treatment, for each site (Porto de Sines and Praia do Serro da Águia). The factors were: sites ($n = 2$), species ($n = 2$), cages ($n = 3$, cage, cage-control, and no cage), and canopy ($n = 2$, presence or absence). The number of recruits surviving per disk was evaluated after 1 mo in the field, and percent survivorship was calculated. The number of embryos outplanted per disk (28.3 cm^2) ranged from 1000 to 1500.

Statistical analyses. ANOVA was used to compare means, followed by Student-Newman-Keuls (SNK) multiple comparisons, using $\alpha = 0.05$ in both cases. If variances were not homogeneous (Cochran's test), they were log-transformed to comply with homoscedasticity. The percentage data were arcsine-transformed before analyses.

RESULTS

Zygote adhesion time

The time for zygotes to adhere to glass slides in culture ranged from 7 to 8 h post fertilization (Fig. 2), showing that an overnight culture period for eggs that were not attached upon collection from the field was sufficient time for fertilized eggs to adhere.

Egg settlement

Egg settlement of both species occurred with a periodicity of ca. 2 wk (Fig. 3). The periodicity of egg release did not appear to be exclusively related to lunar phases. Few eggs were collected on days with high wave action, but wave activity was measured offshore, and shore morphology and orientation obviously strongly influenced the exposure observed at the transplant sites. In 2 of the 3 sites, there was greater egg settlement for *Fucus vesiculosus* than for *F. spiralis* (Figs. 3 & 4a), and settlement of both species was always very patchy (see large SE in Fig. 3). Taking into account only major settlement events (>500 eggs total per site), 14 of the 16 events occurred on days when the low tide was later in the day (between 13:00 and 21:00 h, Fig. 5).

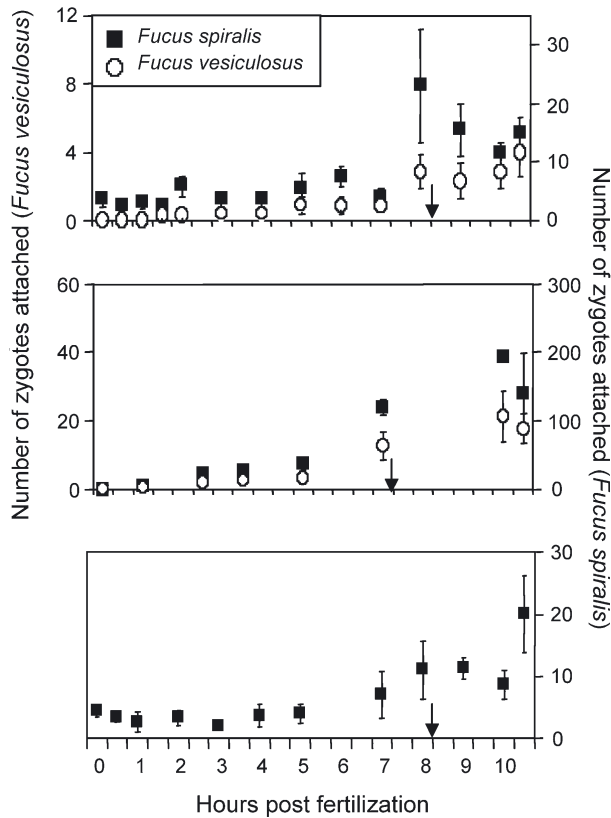


Fig. 2. *Fucus vesiculosus* and *F. spiralis*. Zygote adhesion timing. Arrows mark time after which no significant increase in number of zygotes attached occurred (Student-Newman-Keuls [SNK] multiple comparisons, after ANOVA, $p < 0.05$, confirmed there was a significant difference among times). Last sample at 24 h post fertilization (not to scale). Values are means \pm SE. Panels represent experiment repetitions

Fertilization success

Fertilization success, when taken as an average over the whole reproductive season, was close to 100% for all 3 transplant sites for both species (Fig. 4b). When analyzed on separate days, fertilization success varied between <1 and 100%, but was high (>80%) on most days (Fig. 6). Days with fertilization success below 50% were only observed for *Fucus vesiculosus* (Fig. 6b). The lowest days of fertilization success were, in particular, on April 6 and 7 (major release of *F. vesiculosus* only), and April 16, 18, and 19 for both species. On April 7, one of the largest oceanic swells occurred during the sampling period, causing strong wave activity. Smaller wave events occurred on April 18 and 19. Thus, low fertilization success usually coincided with days of high wave activity, although the wave height data are measured offshore and do not correlate precisely with local water motion at the sites. Fertilization success was higher for both species during the later half of the sampling period (Fig. 6).

Recruitment

Recruitment occurred in 2 of the 3 transplant sites. In the Porto de Sines site, recruitment could not be estimated as the stands were vandalized during the experiment and recruitment disks were lost. Significantly more recruits were found at the end of the reproductive season for *Fucus spiralis* than for *F. vesiculosus*, and there was also a significant difference between sites (ANOVA, $p < 0.05$, for the effect of species $F_{1,37} = 6.78$, and for site $F_{1,37} = 7.80$) (Fig. 7a). When recruitment was analyzed as a percentage of the total number of eggs that had settled

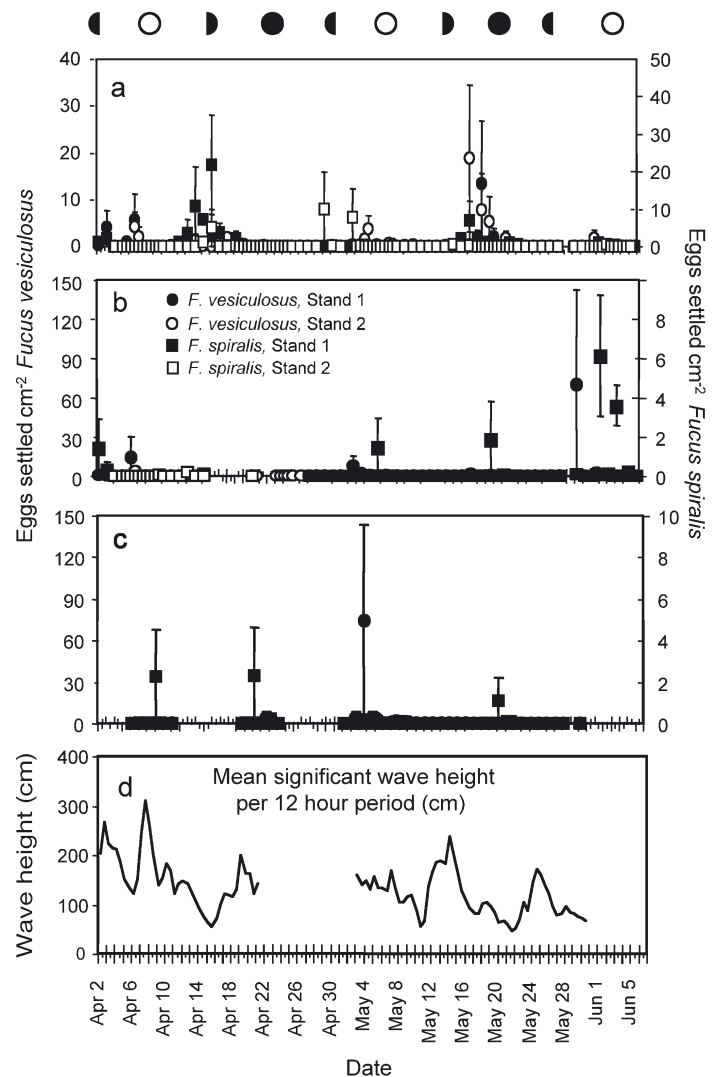


Fig. 3. *Fucus vesiculosus* and *F. spiralis*. Daily egg settlement (mean \pm SE, $n = 3$ to 5 disks per low tide) in 3 sites near Sines: (a) SA = Praia do Serro da Águia, (b) PC = Porto Covo, and (c) PS = Porto de Sines. (d) Significant wave height at a buoy directly offshore of Sines. Note different scales for *F. vesiculosus* and *F. spiralis*. Lunar phases are shown above the graph

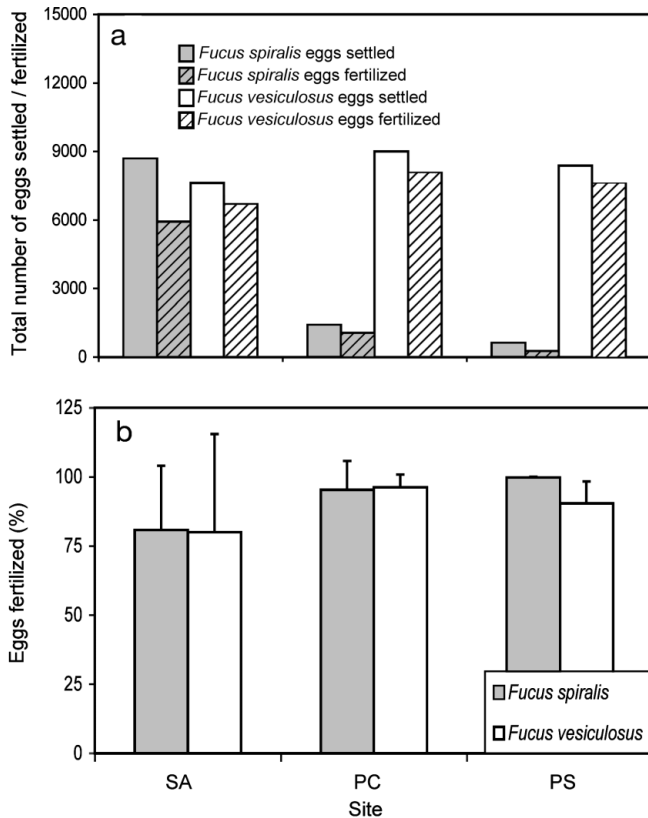


Fig. 4. *Fucus vesiculosus* and *F. spiralis*. (a) Total number of eggs settled over reproductive season, and number that were fertilized (for days of major release) over reproductive season in each transplant site, and (b) average fertilization success over total sampling period (SA = Praia do Serro da Águia, PC = Porto Covo, PS = Porto de Sines). Values for (b) are means \pm SE, for all days with major release as defined in text

on disks in that site over the reproductive season, or as a percentage of the total number of eggs on days of major release that were fertilized on disks over the reproductive season, the same pattern was observed (Fig. 7b,c).

Recruit mortality

In outplant experiments (Fig. 8), 1 mo old embryos of *Fucus spiralis* showed significantly greater survivorship than *F. vesiculosus* under all conditions, except in the more wave-protected of the 2 sites (Porto de Sines) where outplants of *F. vesiculosus* outsurvived *F. spiralis* only if there was canopy present (ANOVA, interaction between site, canopy, and species; $p = 0.007$, $F_{1,48} = 7.84$). Regardless of the site, if there was no canopy present, *F. spiralis* had significantly greater survivorship. There was no effect of caging and grazer exclusion.

DISCUSSION

The hypothesis that *Fucus vesiculosus* is absent from the exposed shores near its southern limit because it is unable to fertilize its eggs in environments with high water motion was rejected. High overall levels of external fertilization success for both *F. spiralis* and *F. vesiculosus* showed that dioecious species of fucoids can achieve as high a fertilization success as hermaphroditic species, even in exposed environments. However, the absence of *F. vesiculosus* on exposed shores near the southern limit of *Fucus* spp. in the NE Atlantic, where rocks have no significant canopy-forming species, may be explained by the reduced recruitment of *F. vesiculosus* in exposed sites, and the low survivorship of *F. vesiculosus* embryos (compared to *F. spiralis*) in exposed areas without canopy. Whether recruitment and recruit survivorship of *F. vesiculosus* is greater in less exposed areas is a question that will be addressed in future experiments.

Gamete release during the experiments was often patchy and was not well correlated with the lunar cycle. The patchiness found may represent either a patchy release pattern among different plants, or a small scale hydrographic or hydrodynamic effect. This type of patchiness in fucoid egg settlement was previously found in the Baltic Sea in a subtidal population (Serrão et al. 1996, 1999a), as well as in an intertidal population on the western Atlantic (Pearson & Brawley, 1996). The correlation of *Fucus* gamete release with the lunar cycle has been found in natural sheltered populations (Brawley 1992) and in the laboratory (Andersson et al. 1994), but is often obscured by other environmental conditions in the field (Pearson & Braw-

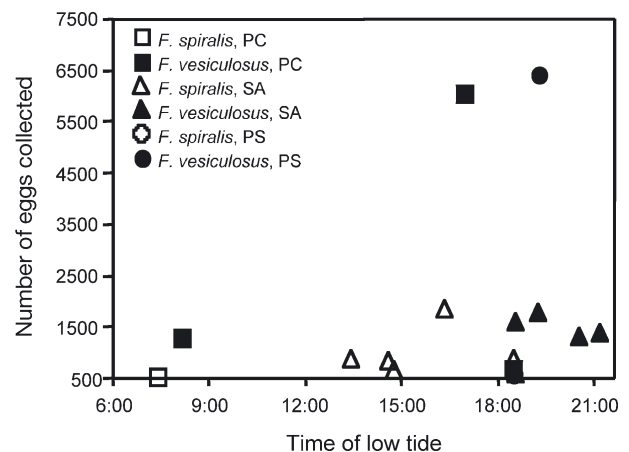


Fig. 5. *Fucus vesiculosus* and *F. spiralis*. Time (h) of low tide on days of substantial egg release (>500 eggs, released) and total number of eggs collected. SA = Praia do Serro da Águia, PC = Porto Covo, PS = Porto de Sines

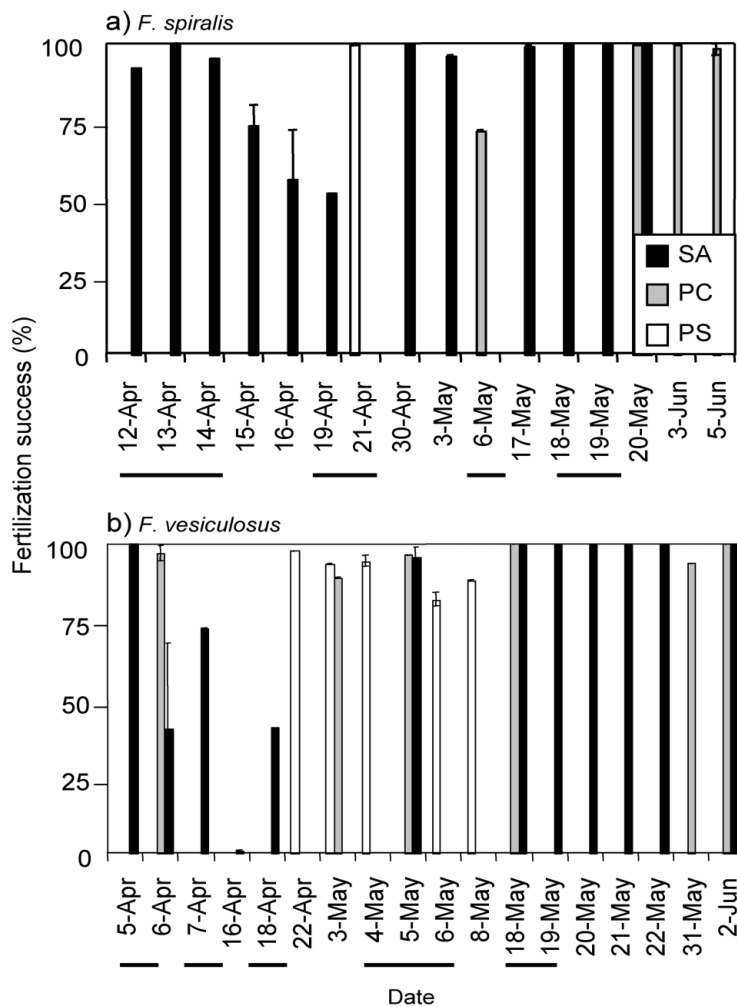


Fig. 6. *Fucus vesiculosus* and *F. spiralis*. Fertilization success of settled eggs in transplanted stands of (a) *F. spiralis*, and (b) *F. vesiculosus* on days of higher egg settlement, in 3 sites near Sines: Praia do Serro da Águia (SA), Porto Covo (PC), and Porto de Sines (PS). Values are means \pm SE, (data with no error bars had release on only 1 disk). Lines below graph represent days which had significant wave height >100 cm measured offshore

ley 1996, Serrão et al. 1996, 1999a). This reflects the response of algae to factors other than tidal or lunar cycles. Laboratory and field experiments showed strong inhibition of gamete release by water motion in all fucacean species tested: *Fucus distichus*, *F. vesiculosus* (Pearson & Brawley 1996, Serrão et al. 1996, Pearson et al. 1998, Serrão et al. 1999a), *Ascophyllum nodosum* (Serrão 1996), and *Pelvetia compressa* (now *Silvetia compressa*, Serrão et al. 1999b) (Pearson & Brawley 1998, Pearson et al. 1998). In our experiments, minimal gamete settlement was observed on days with high wave activity, even when the lunar cue of a full or new moon occurred.

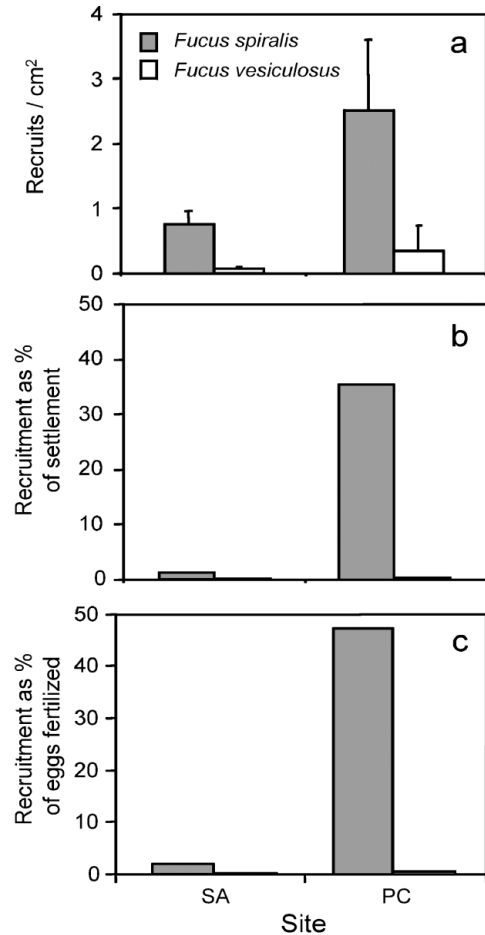


Fig. 7. *Fucus vesiculosus* and *F. spiralis*. Recruitment as (a) mean number of recruits per area ($n = 4$ to 15 recruitment disks per site), (b) % of total eggs settled during the reproductive season that recruited, and (c) % of total eggs fertilized (measured on days of major release) during reproductive season that recruited. Sites listed at the bottom refer to sites of transplants: SA = Praia do Serro da Águia, PC = Porto Covo

In this study, most eggs or zygotes settled on days when the low tide was later in the day, similar to that found for *Fucus distichus* in tide pools (Pearson & Brawley 1996), and for *F. vesiculosus* in the Baltic Sea, where peaks of gamete release always occurred towards the end of the day (Serrão et al. 1996). This may be due to the requirement of a period of photosynthesis for potentiation of gamete release to occur in the field. In the laboratory, a photosynthetic inhibitor reduced or eliminated gamete release in *Silvetia compressa* and *F. vesiculosus* (Serrão et al. 1996), and carbon limitation during photosynthesis was shown to be a signal for gamete release in *F. distichus* and *S. com-*

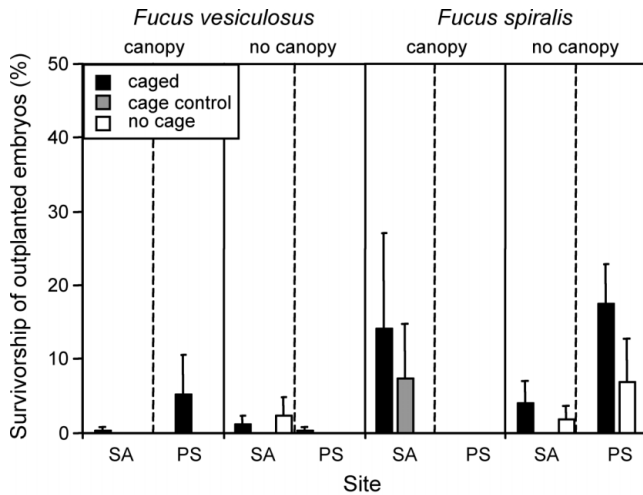


Fig. 8. *Fucus vesiculosus* and *F. spiralis*. Survivorship of outplanted recruits (% per disk) when canopy was present vs absent, and herbivores excluded vs controls, in 2 sites near Sines (SA = Praia do Serro da Água, PS = Porto de Sines). Values are means \pm SE, $n = 3$ disks per treatment

pressa (Pearson et al. 1998). Because, overall, gamete release was periodic, the data suggest that there is a combination of rhythmic cues (e.g. tidal or lunar) and local factors, such as periods of exposure to light and calm conditions, necessary for the induction of gamete release.

Fertilization success of *Fucus vesiculosus* at exposed sites was higher than we predicted. On only a few days during the reproductive season was fertilization success lower than 50%, and this often coincided with higher wave activity. In other experiments on wave exposed shores, permanently submerged fucoids concentrated gamete release on days or tidal phases with low water motion, thereby achieving high fertilization success (Pearson & Brawley 1996, Serrão et al. 1996, and see Santelices 2002 for a review). Fucoids that are emersed at low tide on exposed shores can also adjust their timing of gamete release to take advantage of periods of limited water motion in order to release and fertilize their gametes, thereby increasing the chances for fertilization and recruitment (Berndt et al. 2002, this study).

High fertilization success has been found in all fucacean populations studied to date (Brawley 1992, Serrão et al. 1996, Pearson et al. 1998, Berndt et al. 2002), except for *Fucus vesiculosus* at their northern distributional limit in the Baltic Sea (Serrão et al. 1999a). However, studies of fertilization success in intertidal (non-tide pool) fucoid populations on exposed shores are rare (but see Berndt et al. 2002). Our discovery of high fertilization success in exposed shore intertidal populations of fucoids with contrasting reproductive modes raises new questions concerning

timing of gamete release during the tidal cycle. Further experiments will address questions such as: (1) at what phase of the tidal cycle does gamete release occur for self-compatible hermaphrodite versus dioecious species, and (2) when during the tidal cycle do signals controlling gamete release operate?

Recruitment was low in both species, though it was significantly lower in *Fucus vesiculosus* than in *F. spiralis*, in spite of greater egg settlement and a greater absolute number of eggs fertilized at the sites. This larger settlement of *F. vesiculosus* might be due to its larger egg production per individual at our sites (unpubl. data). This is due to adults of *F. vesiculosus* achieving large sizes with many receptacles, and does not necessarily represent higher biomass allocation to sex. In fact, *Fucus spiralis* has been found to produce more eggs per weight than *F. vesiculosus*, although investment in sperm is much lower than for dioecious fucoids like *F. vesiculosus* (Vernet & Harper 1980).

The low recruitment of *Fucus vesiculosus* measured in this study is likely to have been caused by failure of zygotes to survive after settlement and attachment, rather than dislodgment, since we found higher numbers of zygotes of *F. vesiculosus* than *F. spiralis* on settlement disks. Recruits of *F. vesiculosus* may be more sensitive to temperature, wave motion, high light, UV, desiccation, or a combination of these factors, than *F. spiralis*. Although air temperatures in the coastal lagoons and estuaries where *F. vesiculosus* occurs at the same latitude are similar to those on the exposed coast, desiccation and photoinhibitory light stress may actually be lower in estuarine and coastal lagoon environments because the fucoids in such habitats are partially covered by a protective muddy film during low tide. However, soft sediment habitats have been shown to be unfavourable for recruitment of *Fucus* (Albrecht 1998, Chapman & Fletcher 2002).

The survivorship of outplanted 1 mo old recruits was also significantly reduced for *Fucus vesiculosus* in this study compared to *F. spiralis*, particularly where no canopy was provided, which is the natural case on the exposed shores at the southern limit. It appears that, in general, recruits of *F. vesiculosus* are less tolerant than *F. spiralis* recruits. Recruitment has been found to be a bottleneck stage for fucoid algae in other intertidal habitats (e.g. Burrows 1964, McLachlan 1974, Gunnill 1980, Vadas et al. 1990, Brawley & Johnson 1991, Johnson & Brawley 1998). Canopy presence is reported to have both beneficial and detrimental effects on survival of early post-settlement stages of fucoid algae. Brawley & Johnson (1991) and Johnson & Brawley (1998) showed greater survivorship of outplanted zygotes and embryos of *Silvetia compressa* underneath canopy and in turf, which they attributed to protection from desic-

cation. In contrast, reduced survivorship of zygotes and recruits of *Ascophyllum nodosum* and *Fucus* spp. underneath canopy has been reported by several authors (e.g. Chapman 1989, 1990, Vadas et al. 1992, Åberg & Pavia 1997, Jenkins et al. 1999), and is commonly attributed to whiplash and/or competition. In our study, both canopy presence and low water motion seemed to be crucial for zygotes of *F. vesiculosus* to survive.

In summary, the experiments in this study have shown high fertilization success in exposed environments, synchronous gamete release during low water-motion periods, and surprisingly low recruitment and recruit survivorship for intertidal populations of both dioecious *Fucus vesiculosus* and hermaphroditic *F. spiralis* transplanted near their southern limit of distribution. For both species, on days when settlement peaks occurred, fertilization success was high; thus recruitment was not limited by gamete dilution and fertilization failure, even in exposed areas. Rather, the establishment and survival of the early stages of both species was very low on exposed shores, shown by low recruitment and embryo survivorship, and was significantly lower for *F. vesiculosus*, particularly in the absence of canopy.

We conclude that the absence of *Fucus vesiculosus* from exposed shores where the hermaphroditic *F. spiralis* occurs, is not caused by fertilization failure of dioecious species in exposed environments. The absence of *F. vesiculosus* on exposed shores near its southern limit in the Northeast Atlantic, where rocks have no significant canopy-forming species, may be explained by the reduced recruitment, and the low survivorship of recruits in exposed areas without canopy. We have shown that establishment (i.e. attachment and/or survival) of *F. vesiculosus* recruits is very low or absent on shores that have both high wave exposure (on the open coast) and high desiccation stress (at their southern limit), particularly where no canopy is present. Future studies should test the hypothesis that low establishment and/or survival of early post-settlement stages on the open coast determine the confinement of *F. vesiculosus* to estuaries and coastal lagoons at its southern limit.

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