

Egg release and settlement patterns of dioecious and hermaphroditic fucoid algae during the tidal cycle

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Abstract The timing of gamete release by fucoid algae, although known to be restricted to calm days is not clearly understood within a circadian time scale. The need for externally fertilizing species to avoid gamete dilution suggests that in wave-exposed areas spawning may occur during particular tidal phases. However, this may differ between mating systems, as selfing species may be less affected by gamete dilution. In this study, two different approaches were used to determine when egg release occurs during the tidal cycle in two sister species with different mating systems. First, egg settlement of *Fucus vesiculosus* (dioecious) and *Fucus spiralis* (selfing hermaphrodite) was quantified on removable substrates (egg

settlement disks) every day for 2 months and settlement patterns were used to statistically estimate the radius of a circle that would encompass 99% of each patch of settled eggs (the egg dispersal radius). Also, egg release was quantified every 2 h during the tidal cycle. A significantly larger egg dispersal radius ($P < 0.02$) was found for *F. spiralis* than *F. vesiculosus*, and this difference was somewhat site dependent with a greater difference between species in exposed sites. The egg dispersal radius was negatively correlated with significant wave height and positively correlated with sea surface temperature for both the species ($P < 0.05$), with a greater effect of both the factors for *F. spiralis* than for *F. vesiculosus*. Egg release during the tidal cycle was variable between species and experiments, with *F. vesiculosus* releasing more eggs, later in the day, and at a lower tide, than *F. spiralis*, which released fewer eggs, throughout the day and at all tides. The dioecious species, *F. vesiculosus*, may have developed a specific adaptation for timing the egg release to periods when emerged in exposed habitats to avoid rapid dilution of gametes that require outcrossing for fertilization. On the other hand, egg release for *F. spiralis*, which can self-fertilize, occurred both when emerged and immersed, suggesting this species has developed less synchrony with specific environmental factors. Site dependence also suggests local wave conditions can modulate timing of release. The data are consistent with the relaxation of the selective constraints of water motion on fertilization success in a selfing hermaphrodite, relative to an obligate outcrossing species. Results support the idea that species with different mating systems evolve different sensitivities to environmental cues for gamete release with specific implications for inbreeding and successful external fertilization in the ocean.

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Introduction

Externally fertilizing organisms are common in the marine environment. However, experimental studies and simulations predict that external fertilization is less successful in habitats characterized by high water motion (e.g., Pennington 1985; Denny and Shibata 1989; Levitan et al. 1992). Wave swept organisms that reproduce via external fertilization have developed mechanisms to increase the probability of gamete 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 Levitan and Petersen 1995; Brawley et al. 1999; Yund 2000; Santelices 2002). A greater probability of gamete encounters exists when self-fertilization is possible. Therefore, both monoecious and hermaphroditic species may be at an advantage over dioecious species under turbulent conditions (Denny and Shibata 1989; Pearson and Serrão 2006).

Fucacean algae fertilize externally in exposed environments, and sister species can have contrasting reproductive modes (self-compatible hermaphrodites vs. dioecious), allowing the unique opportunity to compare different reproductive strategies where most other characters do not differ. In this study, two such sister *Fucus* species are used as models. *Fucus vesiculosus* grows lower in the intertidal zone and is dioecious, with each individual producing either male or female gametes. *F. spiralis* grows higher in the intertidal zone and is hermaphroditic, with both male and female function in the same reproductive structure (conceptacle). In *Fucus*, reproductive tissue is found in specialized apices named receptacles, which contain numerous spherical conceptacles. Gametangia (oogonia with 8 eggs and/or antheridia with 64 sperm) are formed within the conceptacles and released through pores on the thallus surface (see review by Brawley et al. 1999 for a further description of *Fucus* reproduction).

Field data for fucoid algae show a high degree of successful external fertilization in turbulent environments (Brawley 1992; Pearson and Brawley 1996; Serrão et al. 1996; Berndt et al. 2002; Ladah et al. 2003). Fucoids restrict release of gametes to daytime, as photosynthesis-related mechanisms are required for gamete release (Serrão et al. 1996; Pearson et al. 1998). Gamete release is also restricted to calm periods using a water-motion sensing mechanism (Pearson and Brawley 1998; Pearson et al. 1998) that minimizes the negative effects of gamete dilution by water motion (see Pearson and Serrão 2006 for a review). For example, gamete release occurs during low tide periods for tide-pool populations, when they would be isolated from waves (Pearson and Brawley 1996), and at

slack high tide or low tide in intertidal populations (Berndt et al. 2002).

External fertilization and egg dispersal patterns in fucoids might also be affected by temperature, which affects the duration of the various stages in the reproductive processes. Higher temperatures can expedite gamete maturation and release, though gametes can still be released at very low temperatures (Pearson and Brawley 1996). Therefore, temperature could affect the number of eggs released and the sperm:egg ratio for encounters. Lower temperatures would allow for the oogonial sheath to remain intact for longer periods of time, most likely causing the eggs encased in the oogonium to disperse less and to settle in a more clumped pattern. Lower temperatures also delay zygote adhesion, which can take anywhere from 6 h at 15°C to more than 30 h near 0°C (Coleman and Brawley 2005b; Ladah et al. 2003; Pearson and Brawley 1996), thereby potentially allowing zygotes to disperse further prior to complete adhesion. Desiccation, which is somewhat related to temperature, is often used as a method for gamete release in the laboratory, yet it is unclear how desiccation affects gamete release in nature. Certainly, both desiccation and temperature can affect the photosynthetic and carbon signals required for potentiation and release of gametes in fucoids in nature (see Pearson and Serrão 2006 for a review).

Although the effects of physical factors have been studied in some fucoid populations, the timing of gamete release during the tidal cycle and the relative contribution of low tide spawning on wave exposed coasts is still not well understood. The timing of gamete release to different tidal heights would have both advantages and disadvantages. For example, at low tide, dioecious species would be less likely to combine eggs and sperm from separate individuals, and hermaphrodite species would most likely become inbred (Brawley et al. 1999). However, gamete mixing at low tide for dioecious species might be achieved as algae from different sexes often lie intermingled in dense wet stands, which could maximize the gamete concentration from both the sexes. These would be readily mixed in minimal water volumes (i.e., low dilution) as the incoming tide reaches them. At lower tides, however, eggs would be exposed to a greater range of temperatures and desiccation than when immersed. At higher tides, hermaphrodite species might gain an advantage from some dilution, as wave-induced water motion may prevent extensive inbreeding (Brawley et al. 1999). It is now understood that self-fertilization is a common reproductive mode in hermaphroditic *F. spiralis*, as shown by population genetic data (Coleman and Brawley 2005a, b; Engel et al. 2005; Perrin et al. 2007) and supported by sperm:egg ratios, which are an order of magnitude lower in

hermaphrodite (*F. spiralis*) than dioecious (*F. vesiculosus*) species (Billard et al. 2005).

In order to infer when, during the tidal cycle, egg release occurs in taxa with contrasting reproductive modes, egg settlement of *F. vesiculosus* (obligate outcrossing) and *F. spiralis* (self-compatible) was measured daily at three sites on removable substrates during a 2-month period in the spring recruitment season, and egg release was measured every 2 h during the tidal cycle for five different egg release periods. We hypothesized that these two species with different mating systems have evolved different sensitivity to environmental cues, or coupling strengths between signal input and response (gamete release) pathways, resulting in differences in the timing of egg release.

Methods

Egg settlement patterns

Egg settlement monitoring took place on the west coast of Southern Portugal at three sites south of Lisbon. Porto Covo (37.850°N, 8.795°W) is an exposed rocky shore, the beach of Sines (37.9473°N, 8.87°W) was used as a study site near the entrance of the Port to the exposed ocean, and Praia do Serro da Águia (hereafter Praia), just to the North of Porto Covo (37.8667°N, 8.80°W), is a rocky intertidal inlet where surge often occurs, but waves do not break. Egg settlement was measured in stands artificially created for both the species to be present at the same sites, thus subject to the same shore exposure, and to be at approximately the same density. The difference between these stands were the intertidal levels at which they were created, mimicking the natural levels of occurrence for these species (see below). The two species may differ slightly in morphology and size depending on location, with *F. spiralis* often smaller at the upper distributional limit, but in this particular collection site no major size difference was visually apparent between the individuals sampled for both the species (although not measured).

Approximately 100 individuals each of *F. vesiculosus* and *F. spiralis*, were collected from a nearby site where both the species grew attached to small rocks and were then attached with cement at low tide to the intertidal shelf of the study sites (see Ladah et al. 2003). At each site, the transplanted stand was at the natural height for each species in the intertidal zone (approximately 0.9–2.4 m above ELWS (extreme low water spring) for *F. vesiculosus* and 2.4–3.0 m above ELWS for *F. spiralis*). Transplants were arranged so that algae were at the highest possible density (i.e., rocks with plants attached were cemented as close together as possible) and that male and female (for *F. vesiculosus*) individuals were side by side in order to simulate ideal conditions for successful external fertilization. Approximately 20 large

individuals were used at each site for each species. Natural reproductive algae were removed within the area of the transplants. Directly below the transplanted algae, holes were drilled in the shelf to anchor screws. The screws held 6-cm diameter circular fiberglass disks made of food grade isophthamic resin with a rugose surface (equivalent to sandpaper, number 6 roughness), with a hole in the center. These disks were threaded onto an upside-down anchored screw and secured with a nut. The egg settlement disks could thus be easily removed and replaced with new ones during daily sampling.

During the 2-month spring recruitment season from April to June 2001, *F. vesiculosus* and *F. spiralis* eggs were counted every day (or twice a day on days with two daytime low tides) on the egg settlement disks using a dissecting microscope. The average number of eggs per disk per site is shown in Ladah et al. 2003, and because in that study, eggs were counted in subsampled quadrants of 0.5 × 0.5 cm, further analysis of how the eggs were distributed on the disk (i.e., whether they were clumped or evenly distributed; see egg dispersal radius below) is presented here. Between three and six disks per site and per species were recovered for each collection. Any disk that had 50 eggs or more was considered a disk with significant egg settlement for egg radius calculation (see below), as this threshold represented approximately 1% of the maximum egg settlement measured during the study period.

Egg dispersal radius calculation

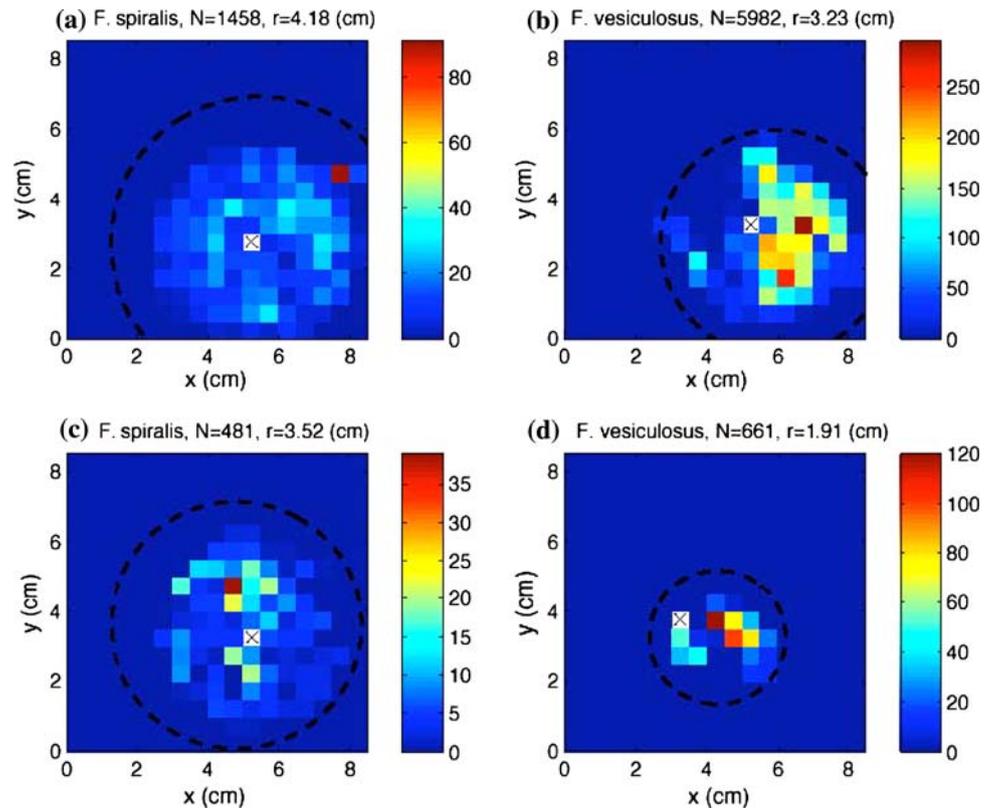
The patterns of egg settlement observed on the removable disks were used to statistically estimate the radius of a circle encompassing 99% of the settled eggs. This statistic, termed the egg dispersal radius, gives an idea of how clumped or dispersed the egg settlement pattern was. This could also provide an indication of tidal height at release considering a simple hydrodynamic model of eggs falling through the water column and dispersing (or releasing into a thin film of water at low tide).

During each collection, eggs were enumerated in 0.5 × 0.5 cm quadrant bins on the egg settlement disk giving a (2D) two-dimensional (*x*- and *y*-axis) histogram of egg concentration. Examples of these 2D histograms are shown in Fig. 1. The egg distribution is often Gaussian shaped (e.g., Fig. 1a) but is at times skewed (e.g., Fig. 1b). For each disk, the settled egg center of mass location (\bar{x} , \bar{y}) is the first moment of the egg concentration histograms $C(x, y)$, i.e.,

$$\bar{x} = \frac{\iint xC(x, y)dx dy}{\iint C(x, y)dx dy}$$

where the integrals are over the entire disk. The egg concentration variances (σ_x^2 and σ_y^2) are then calculated in the standard way, i.e.,

Fig. 1 Examples of egg settlement histograms on the settlement disks together with dispersal radius calculations. *Fucus spiralis* with relatively high (a) and low (c) egg settlement, and b, d show the same for *F. vesiculosus*. The species, number of eggs settled per disk (N), and calculated dispersal radius (r) are shown at the top of each panel. The dotted line represents the circle encompassing 99% of eggs settled, with radius r . The color legend shows the number of eggs settled in each 0.5×0.5 -cm quadrant on the 6-cm diameter egg settlement disk. The X marks the hole for the screw to attach the egg settlement disk to the bottom during deployment



$$\sigma_x^2 = \frac{\iint (x - \bar{x})^2 C(x, y) dx dy}{\iint C(x, y) dx dy}$$

The integrals were calculated from the egg concentration histograms using the trapezoidal rule. Assuming a Gaussian and independent (in x and y) distributed egg settlement histogram, the histogram is exponentially distributed with radius squared. Thus, it is expected that 99% of the eggs will fall within the radius, r , defined by

$$r = 3\sqrt{\sigma_x \sigma_y}.$$

The circle defined by this radius r (thick black dashed circles in Fig. 1) typically encompasses almost all the eggs. For all the disks, the radius, r , varied between 0.75 and 4.23 cm, and was approximately uniformly distributed. A smaller dispersal radius would be associated with a clumped egg settlement pattern (see Fig. 1b, d), whereas a larger dispersal radius would be associated with a broader and even settlement pattern (Fig. 1a, c). As the dispersal radius is a statistical measure that assumes a Gaussian egg distribution pattern, the estimated dispersal radius can be larger than the 3-cm radius of the settlement disk. This statistic, r , will be used in the dispersal analysis in the “Egg release experiments”.

Egg release experiments

When natural *Fucus* populations in the area were releasing gametes, the timing of egg release during the

tidal cycle was measured using artificially created plants at the site of Sines. Artificial plants were created by cutting five hermaphrodite receptacles from *F. spiralis* or five female and five male receptacles from *F. vesiculosus* (to provide both male and female gametes in the artificial algae in case this would affect the release of eggs). The receptacles were placed in nytex mesh bags (mesh size of $40 \mu\text{m}$), made of transparent plastic with nytex windows, and attached to strings mimicking the stipes of algae in natural stands. The mesh bags allowed water motion around the receptacles but were also able to trap the eggs when released. The mesh bags allowed plenty of light through (though not measured), and have been shown previously to correspond well with patterns in natural populations (Serrão et al. 1996). Thirty nytex bags were attached to 30 separate strings for each species, about 0.5-m long, which were tied to rocks in the intertidal zone at their respective tidal height for natural populations. The artificial plants were prepared and placed at their appropriate respective tidal heights the night prior to the experiment.

Egg release was evaluated on five different days in April and May. Five nytex bags were collected for each species every 2 h during the tidal cycle during daylight hours. After collection, the nytex bags and the receptacles were rinsed and eggs that had been released were counted.

Environmental data

Significant wave height (H_s , average height for the 1/3 highest waves) and sea surface temperature (SST) were obtained from the Maritime Institute in Portugal (<http://www.hidrografico.pt>) using a directional wave buoy offshore of the Port of Sines (37.95°N, 8.87°W). Daily averages of both the parameters were calculated for each egg settlement disk deployment period.

Plaster disks to estimate relative wave activity and water motion were made of plaster of paris and water (1:1) in molds producing disks about 10-cm wide (per Thompson and Glenn 1994). Five plaster disks were deployed at each site for a complete tidal cycle (24 h) and a control was left for the same amount of time in a seawater bucket on two different occasions during the spring recruitment season. Plaster disks were completely dried at 60°C and weighed prior to and after deployment and percentage erosion was calculated by subtracting the final weight from the initial weight divided by the initial weight, $\times 100$.

Statistics

Statistica v. 6.0 was used to run correlations, factorial fixed effect ANOVA's, U Mann-Whitney, Chi-square and t -tests, as well as Cochran's test for homogeneity of variances. Post hoc comparisons were performed using the Tukey Honest Significant Difference test with an alpha of 0.05. All error bars in figures are standard errors. Where an interaction term had $P > 0.25$, the test was repeated after pooling/eliminating this term, but in no case did this make a difference, thus pooling results are not presented.

Results

Environmental conditions, the number of eggs settled, and the calculated dispersal radius, are shown in Fig. 2. Egg settlement occurred on days with a daily average H_s as low as 48 cm and as high as 197 cm. *Fucus vesiculosus* released eggs on days of significantly greater H_s ($H_s = 122 \pm 4.7$ cm, $n = 49$) than *F. spiralis* ($H_s = 103 \pm 5.6$ cm, $n = 33$) ($F_{1,80} = 6.82$, $P = 0.01$). There was no correlation between the number of eggs settled and the calculated dispersal radius for *F. vesiculosus* ($R = 0.17$, $P = 0.21$) or for *F. spiralis* ($R = 0.004$, $P = 0.98$).

When egg settlement collections were performed twice daily at both the low tides, the two species showed a similar number of collection disks above the egg settlement threshold (>50 eggs/disk) for the a.m. low tide ($\chi^2 = 0.05$, $P = 0.81$), whereas there were significantly more disks above the egg settlement threshold for *F. vesiculosus* than

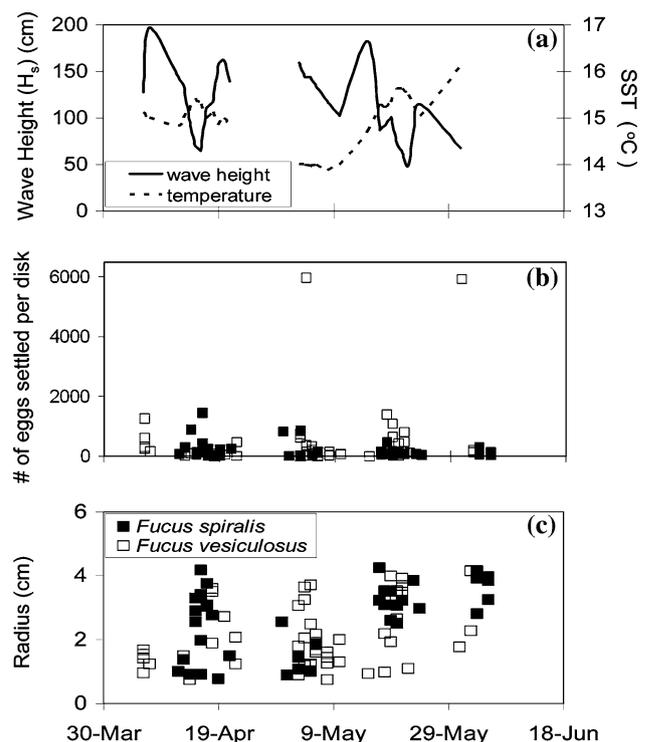


Fig. 2 **a** Significant wave height (H_s , solid) and sea surface temperature (SST, dashed) measured offshore of the Port of Sines (see “Methods”) for the study period, **b** number of eggs settled on disks during daily semidiurnal or diurnal collections for *Fucus spiralis* and *Fucus vesiculosus*, and **c** dispersal radius calculated from egg settlement patterns (see “Methods”) for *F. spiralis* and *F. vesiculosus*

for *F. spiralis* during the p.m. low tide ($\chi^2 = 9.9$, $P < 0.001$) (Fig. 3). However, collection time (a.m. vs. p.m.) had no effect on the number of eggs settled (Table 1a) or on the calculated dispersal radius (Table 1b).

Egg release in nytex bags at the Sines site was extremely variable between experiments and species, and in general *F. vesiculosus* released more eggs, later in the day and at a lower tide than *F. spiralis* (Fig. 4). Egg release for *F. vesiculosus* was significantly correlated with the time of day, showing greater release later in the day ($R = 0.91$, $R^2 = 0.82$, $P = 0.002$), and showed negative correlation with tidal height, having greater release at lower tidal heights ($R = -0.76$, $R^2 = 0.582$, $P = 0.13$). *F. spiralis* egg release showed no correlation with time ($R = -0.08$, $R^2 = 0.006$, $P = 0.85$) or with tide ($R = -0.39$, $R^2 = 0.15$, $P = 0.52$) (Fig. 4a, b).

The calculated egg dispersal radius (r) differed significantly between species (Table 2b), with no species effect on the number of eggs settled (Table 2a). On average, the dispersal radius for *F. spiralis* ($r = 3.0 \pm 0.37$ cm, $n = 41$) was significantly larger ($P = 0.016$) than for *F. vesiculosus* ($r = 1.99 \pm 0.17$ cm, $n = 52$). The

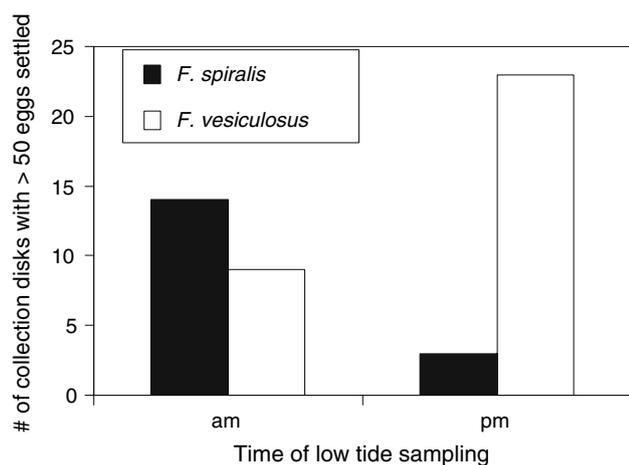


Fig. 3 Number of egg settlement disks with significant egg settlement (>50 eggs settled per disk) during a.m. versus p.m. low tide collections for *Fucus spiralis* and *Fucus vesiculosus* on days when both the low tides were sampled. Differences are significant for the p.m. period (Chi-square, $P < 0.001$)

Table 1 Factorial ANOVA for the effect of species and semidiurnal occurrence (a.m. vs. p.m.) of low tide collections on **a** the number of eggs enumerated on egg settlement disks, and **b** the dispersal radius calculated for each egg settlement disk, for *Fucus spiralis* ($n = 17$) and *Fucus vesiculosus* ($n = 32$)

Effect	df	MS	F	p
Number of eggs enumerated on egg settlement disks				
Species	1	158554	2.19	0.145
a.m. versus p.m.	1	3935	0.054	0.816
Species \times a.m. versus p.m.	1	6428	0.089	0.766
Error	45	72192		
Dispersal radius calculated for each egg settlement disk				
Species	1	4.37	4.39	0.042
a.m. versus p.m.	1	0.21	0.21	0.648
Species \times a.m. versus p.m.	1	1.63	1.64	0.206
Error	45	0.99		

Significant factor is shown in bold

dispersal radius was negatively correlated with Hs and positively correlated with SST for both the species, with a greater effect of Hs and SST for *F. spiralis* than for *F. vesiculosus* (Fig. 5; Table 3).

Significantly greater erosion of plaster disks was found in Porto Covo, with no significant difference found between Sines and Praia ($F_{2,15} = 47.23$, $P < 0.001$) (Fig. 6a). Erosion of control plaster disks was negligible (0.16%). In Porto Covo, where plaster disk erosion was greatest, *F. vesiculosus* showed a significantly smaller dispersal radius than *F. spiralis* (Tukey HSD, $P = 0.038$), with no difference found between the two species in Sines or Praia (Fig. 6b).

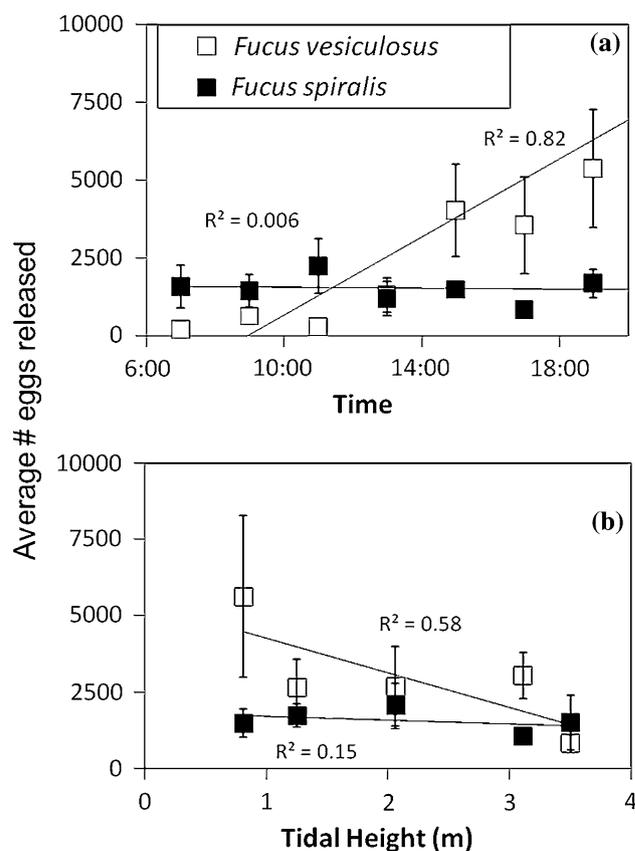


Fig. 4 Number (mean \pm SE) of *Fucus spiralis* and *Fucus vesiculosus* eggs released in nytex bags by five receptacles versus **a** time, and **b** tidal height, for the five different experiments in April and May. Correlation R^2 values are shown, significant ($P < 0.05$) for *F. vesiculosus* and not for *F. spiralis*

Table 2 Factorial ANOVA for the effect of species and site on **a** the number of eggs enumerated on egg settlement disks, and **b** the dispersal radius calculated for each egg settlement disk, for *Fucus spiralis* ($n = 17$) and *Fucus vesiculosus* ($n = 32$)

Effect	df	MS	F	p
Number of eggs enumerated on egg settlement disks				
Species	1	1709729	5.98	0.137
Site	2	708748	2.25	0.396
Species \times site	2	1224294	0.93	0.205
Error	87	757392	1.61	
Dispersal radius calculated for each egg settlement disk				
Species	1	6.43	6.0	0.016
Site	2	0.48	0.45	0.637
Species \times site	2	2.91	2.7	0.072
Error	87	1.07		

Significant factor is shown in bold

Discussion

The results show that time of day, tidal height, and wave action all play a role in the timing of *Fucus* egg release,

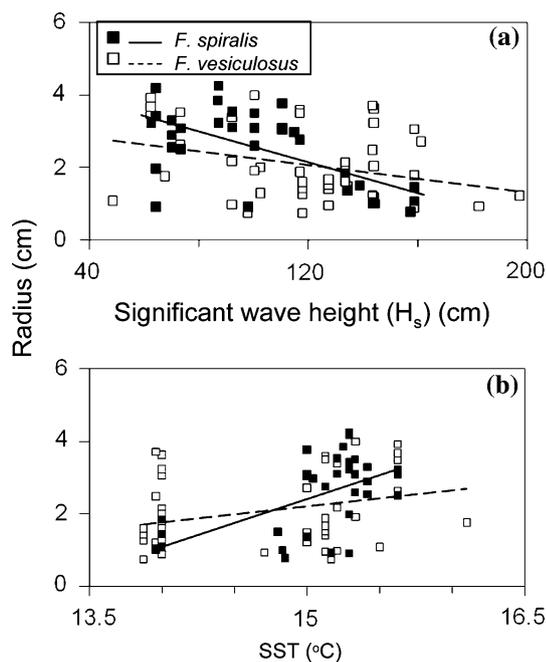


Fig. 5 Correlation of calculated dispersal radius with **a** significant wave height (H_s) and **b** sea surface temperature (SST) on the day of egg settlement for *Fucus spiralis* and *Fucus vesiculosus* ($P < 0.05$ for all correlations, see Table 2 for correlation matrix)

Table 3 Correlation matrix for calculated dispersal radius from egg settlement data on egg settlement disks and physical factors for *Fucus spiralis* and *Fucus vesiculosus*

Species	Factor	<i>n</i>	<i>F</i>	<i>R</i>	<i>p</i>
<i>F. spiralis</i>	Wave height	33	19.76	-0.62	0.0001
<i>F. spiralis</i>	Water temperature	33	13.83	0.55	0.0008
<i>F. vesiculosus</i>	Wave height	49	5.06	-0.31	0.029
<i>F. vesiculosus</i>	Water temperature	49	5.12	0.32	0.028

and that egg release, settlement and dispersal differ between a dioecious and hermaphroditic furoid species, particularly in more wave exposed areas. Species level differences in egg dispersal radius were reduced in wave protected sites, suggesting that wave exposure modulates *Fucus* gamete release. These differences suggest that species with different reproductive modes have evolved different sensitivities to environmental cues for gamete release, and that these differences vary depending on the local wave exposure conditions of each site. Species level differences in the coupling strength between sensory input and response pathways would have specific implications for successful external fertilization, dispersal, and population genetic structure. The data are consistent with a relaxation of the selective constraints of water motion on fertilization success in a selfing hermaphrodite, relative to an obligate outcrossing species. In this model system it is, however, not possible to disentangle the effects of mating

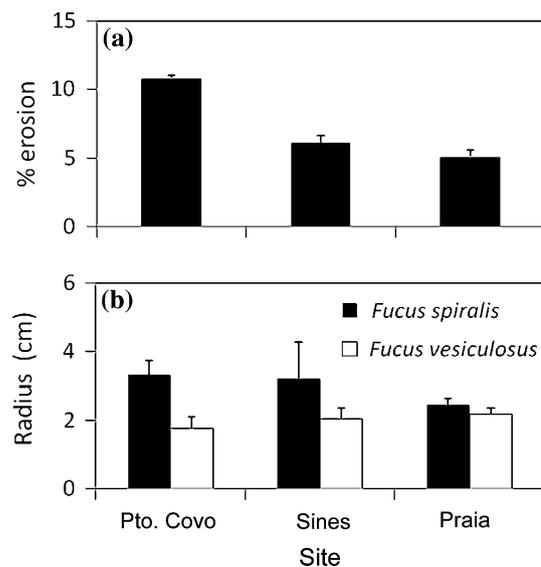


Fig. 6 Mean and standard error at each site of **a** percent plaster disk erosion and **b** dispersal radius for both *Fucus spiralis* and *Fucus vesiculosus* (Factorial ANOVA for species \times site, $P = 0.07$, Post hoc comparisons using Tukey honest significant differences test, $P = 0.038$ between species for Porto Covo site)

system and tidal height, given that both the effects may affect the selective pressures for gamete release patterns. It would thus be of great interest in future work to replicate this study with more taxa having contrasting reproductive modes but opposite or totally overlapping vertical distribution ranges.

Natural gamete release for *Fucus* spp. occurs exclusively in the light (Pearson and Brawley 1996) and requires active photosynthesis (Serrão et al. 1996). Gamete release is regulated by flow conditions via a water motion sensing mechanism, which detects dissolved inorganic carbon through a photosynthetic signaling pathway (Pearson and Brawley 1998; Pearson et al. 1998). Inorganic carbon-limited photosynthesis and low-flow conditions would both occur during afternoon low tides on sunny days if exposed algae were hydrated and photosynthetically active (Kawamitsu and Boyer 1999). Therefore, the general pattern of low tide release following an extended period under light found in this study for *F. vesiculosus* is consistent with this mechanism (reviewed in Pearson and Serrão 2006). Midday or afternoon low tide release has also been found for tide-pool furoids (Pearson and Brawley 1996), for a green alga (Togashi and Cox 2001), for a hydroid (Belorustseva and Marfenin 2002), and for a sea anemone (Marshall et al. 2004).

Predominant high tide gamete release has been reported for *Fucus* species (Brawley et al. 1999; Berndt et al. 2002). Berndt et al. (2002) found that release of eggs from *F. vesiculosus* at both slack high and low tide in Maine, and hypothesized that gamete release at low tide occurred only

when high tide release was inhibited by wave action, an effect that can explain results inferred in this study. We found that *F. spiralis* released gametes when immersed on days of lower wave height, results in a greater dispersal radius on these days. However, the calculated egg dispersal radius for *F. spiralis* on days of high wave action was lower than on days with lower wave height, suggesting release into less water. This can be explained if on days of greater wave height, immersed release of *F. spiralis* gametes is inhibited, then release on these days occurs at low tide. This would result in *F. spiralis* having a more similar dispersal radius to *F. vesiculosus*, during strong wave events. *F. spiralis* may therefore risk greater inbreeding potential as a trade-off for fertilization success, rather than risk a lower probability of gamete encounters during strong wave exposure. This suggests that the triggers for gamete release in *F. spiralis* are less sensitive to wave exposure than *F. vesiculosus*, and that greater wave heights are necessary to inhibit *F. spiralis* release.

The dispersal radius for *F. vesiculosus* showed lower, but still significant, correlation with wave exposure, although egg settlement occurred on days with larger significant wave heights than *F. spiralis*. This suggests that *F. vesiculosus* generally releases during low tides, which provide a refuge from direct wave exposure on more exposed shores (depending on how much water actually reaches them during low tide wave events), thereby increasing gamete encounters due to low water volume. Frequent low tide release would result in a smaller dispersal radius for *F. vesiculosus*, most probably from falling through less water, than for *F. spiralis*. This could explain why *F. vesiculosus* is less correlated with wave exposure and SST, as at low tide egg settlement is independent of these factors. Egg release by *F. spiralis* during immersion likely results in greater gamete dispersal, as released gametes falling through the water column have more time to be affected by turbulence. No correlation of calculated egg dispersal radius with the number of eggs settled was found, also suggesting the dispersal radius is most likely the result of environmental conditions affecting egg dilution and dispersal.

The species level differences detected in this study were somewhat site dependent. The hypothesis that *F. spiralis* restricts release to low tide only during heavy water-motion periods is supported by data showing a greater difference between species' dispersal radius in wave exposed sites and little difference in wave protected sites. Under calmer conditions or in more protected sites, these species may behave more similarly as the factors modulating timing of release may become less important for reproductive success, which presumably requires both high fertilization rates and low zygote dispersal away from the intertidal (see Pearson and Serrão 2006).

Selfing species may not need to be as sensitive to wave exposure as dioecious species to guarantee gamete encounters. Supporting this hypothesis, *F. spiralis* populations were shown genetically to reproduce primarily through selfing on the exposed coasts in Maine (Coleman and Brawley 2005a), and in France and Portugal (Engel et al. 2005; Perrin et al. 2007), and had close to 100% fertilization success on this same shore (Ladah et al. 2003). Inbreeding may be advantageous in extreme environments such as the upper intertidal, e.g., to maintain adaptive gene complexes.

Gamete release at low tide in the intertidal zone, although precluding immediate mixing and exposing gametangia to potential desiccation stress, allows efficient mixing of gametes in small water volumes as the incoming tide reaches the algae. Occasional observations of low tide gamete release are well-known in some dioecious fucoid taxa where widespread gamete release at low tide on certain days is highly visible due to the copious release of bright orange spermatangia (e.g., *Ascophyllum nodosum*, *Hormosira banksii*, in the northern and southern hemispheres, respectively).

The specific environmental conditions resulting in gamete release have not been closely studied for *F. spiralis* or *F. vesiculosus*. However, a related fucoid, *Silvetia compressa*, has been shown to have a potentiation phase dependent on light and photosynthesis, followed by a rapid gamete expulsion phase that is triggered by darkness (Jaffe 1954; Pearson and Brawley 1998; reviewed in Pearson and Serrão 2006), although gamete release also occurs in blue light, possibly linked to high tide release in nature (Pearson et al. 2004). The mechanism for gamete release in *Fucus* spp. is probably similar to that proposed for *Silvetia*, yet there are also important differences, e.g., a lack of induction of gamete release by light to dark shifts. At present, it is not possible to say whether the interspecific differences detected in the patterns of egg release and settlement in this study might be due to different thresholds for photosynthetic potentiation (i.e., shorter exposure to sunlight necessary for release for *F. spiralis*), differences in sensitivity to light quality and/or quantity (which would be affected by the degree of immersion), or other factors such as wave action which on this coast would also correlate with tides.

In this study, we show that time of day, tidal height, and wave exposure affect egg release and settlement patterns in fucoid algae, with differing patterns for a hermaphroditic versus a dioecious species. Further study of species-specific mechanisms and sensitivity to small-scale local environmental conditions would shed light on how and why the different species respond the way they do and help to better explain their present day ecology and distribution.

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