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# Reproductive ecology of *Fucus distichus* (Phaeophyceae): an intertidal alga with successful external fertilization

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ABSTRACT: Patterns of natural gamete release and fertilization success are reported for the open-coast macrophyte *Fucus distichus* L. (formerly *Fucus distichus* ssp. *distichus*), which is restricted to tide pools in the upper intertidal zone. The release and settlement of eggs and zygotes of *F. distichus* occurred during daytime low tide periods (DLT), largely on days when the low tide fell between 10:00 and 14:00 h EST; very few gametes were released when high tides fell during the same time interval. Gamete release during nighttime low tides was very low. During the DLT series, gametes were released in all pools and this was better correlated with the time of day than with time of low tide per se. The timing of fertilization showed considerable variation both within and between pools during a single DLT. No relationship between either pool temperature or osmolality and gamete release was evident, and only small episodes of gamete release coincided with the times of full or new moons. The restriction of gamete release to periods of very low water motion, when tide pools are isolated by the low tide, prevents gamete dilution by water exchange and turbulent flows, and results in external fertilization success between 78 and 100% with low levels of polyspermy (1 to 5%). Dispersal of zygotes among tide pools may be facilitated by the low winter temperatures during reproduction, which retard adhesive production and zygote attachment. Our results demonstrate that intertidal organisms, living in habitats characterized by periodic, turbulent flow regimes, may achieve high levels of fertilization success by releasing gametes under optimal hydrodynamic conditions for sperm-egg encounters.

KEY WORDS: External fertilization success · Fertilization ecology · Fucoid algae · *Fucus distichus* · Gamete release · Polyspermy · Rocky intertidal zone

## INTRODUCTION

Temporal variation in the supply of propagules (e.g. zygotes and larvae) to a community can play a major role in determining its structure (e.g. Gaines & Roughgarden 1985; reviewed by Underwood & Fairweather 1989). A key component of propagule supply for many marine species is the success of external fertilization, a process that has long been a subject of scrutiny and debate (e.g. Mortensen 1938, Thorson 1950; reviewed by Levitan 1995). For example, recent modeling studies predict that external fertilization is poorly suited to turbulent environments such as the intertidal zone because of rapid dilution of gametes in the water column; fertilization success under these conditions may be less than 1% (Denny & Shibata 1989). Other studies suggest that even those eggs that are fertilized may still face damage from small-scale shear forces (Mead & Denny 1994). Experimental field studies conducted under less extreme hydrodynamic conditions have confirmed that fertilization success declines with increasing water velocity (e.g. Pennington 1985, Levitan et al. 1992; reviewed by Levitan & Petersen 1995 and Levitan 1995). However, to our knowledge, there have been no previous reports of natural patterns of gamete release and external fertilization success in an intertidal organism on exposed shores. Fertilization success during natural gamete release (spawning) in other habitats has been determined, i.e. in a few marine invertebrates (Babcock & Mundy 1992, Babcock et al. 1992, Sewell & Levitan 1992, Babcock et al. 1994), fish (Petersen 1991, Petersen et al. 1992), and

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macroalgae (Brawley 1992, Serrão et al. 1996). High levels of fertilization success (ca 75 to 100%) were observed in these studies, except during a proportion of the rougher days (Petersen 1991, Petersen et al. 1992), or when individuals spawned in temporal or spatial isolation (Babcock & Mundy 1992, Babcock et al. 1992). Fertilization success was 95 to 100% in the estuarine, intertidal alga *Fucus ceranoides*, which mostly releases gametes near slack high tide, and which has a semilunar periodicity of release (Brawley 1992). Continuously submerged populations of *Fucus vesiculosus* in the Baltic Sea also have high (>95%) fertilization success, and are very responsive to turbulent conditions, achieving high fertilization success by not releasing gametes when water velocities are high (Serrão et al. 1996). This suggests that spawning in marine organisms may be quite sensitive to water velocity. If so, tide pools on exposed shores offer a temporal refuge from water motion that is particularly predictable (Denny 1988).

In this paper we describe natural gamete release and fertilization success in the fucoid alga *Fucus distichus* L., which is a monoecious species found exclusively in tide pools in the upper littoral zone of moderately to very exposed rocky shores. Transplant experiments with microscopic early stages have demonstrated that this species is physiologically incompetent to survive on emergent rock, except in the lowest regions of the intertidal zone (Chapman & Johnson 1990), which is not its natural distribution. Male and female gametangia (antheridia and oogonia) are released from conceptacles within the reproductive tips (receptacles) of the alga; conceptacles are spherical, sub-epidermal chambers connected via a pore to the surface, and each receptacle contains numerous conceptacles (Evans et al. 1982). Reproduction is seasonal, and the onset of receptacle formation occurs in late autumn in response to short days (Bird & McLachlan 1976). Gamete release occurs during the winter and early spring. Given the variations in temperature and salinity in tide pools during the winter in New England, USA, and eastern Canada (Edelstein & McLachlan 1975, this study), it is of interest to determine whether gamete release follows the lunar or semilunar periodicity found in several other macroalgae (Williams 1905, Fletcher 1980, Brawley 1992; references in Brawley & Johnson 1992), or occurs in response to different endogenous or environmental factors. Further, the synchrony of gamete release between individuals of monoecious species may be lower than that in dioecious species (Brawley 1992) if they are capable of self-fertilization, as monoecious fucoids are (Pollock 1970, S. H. Brawley pers. obs.). Our results show that fertilization is successful in *F. distichus* due to avoidance of gamete release under turbulent conditions at high tide.

## MATERIALS AND METHODS

**Study site and preparation of pools.** A total of 8 tide pools were studied between January and April 1995, 4 in each of 2 moderately exposed sites at Chamberlain, Maine, USA (44° N, 69° 30' E). Further studies of 2 of these pools were conducted during March 1996. Site A was situated on the eastern side of a rocky promontory (Long Cove Point), and Site B was located ca 50 m further east across the small, south facing bay (Fig 1). Both sites were characterized by granitic rock on seaward-facing slopes of gentle to medium grade, with the pools containing *Fucus distichus* (formerly *F. distichus* ssp. *distichus*; Rice & Chapman 1985) being found just above the *Fucus spiralis* zone. Observations of immersion time at high tide revealed that the pools at Site A were flushed by waves for approximately 1 h longer (ca 4 h) than those at Site B. During neap tides coincident with calm seas, pools were sometimes observed to be isolated from tidal input for 2 to 3 d. Preparation of the sites was carried out between September and October 1994, prior to the onset of receptacle formation by the adult plants. A tape measure was placed along the long axis of each pool, and at 5 or 10 cm intervals (depending on the size of the pool) the distance to either edge was measured perpendicular to this axis; water depth was also determined at this time.

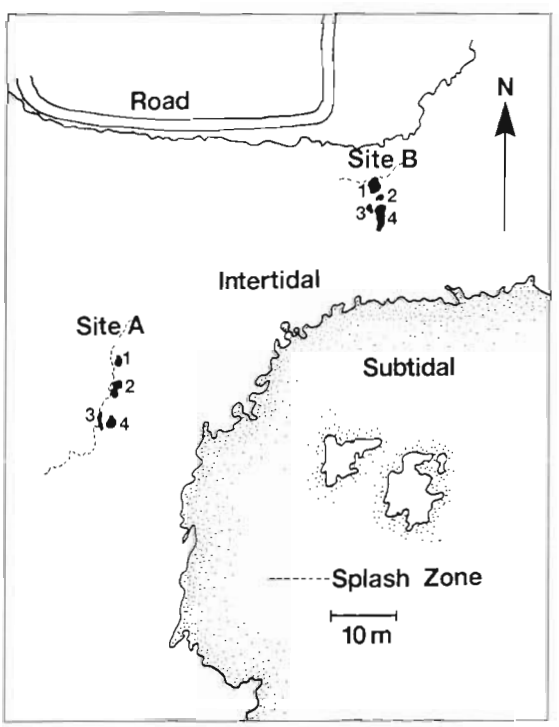


Fig. 1. Study area at Chamberlain, Maine, USA (Long Cove Point), showing the positions of tide pools containing *Fucus distichus* at Sites A and B

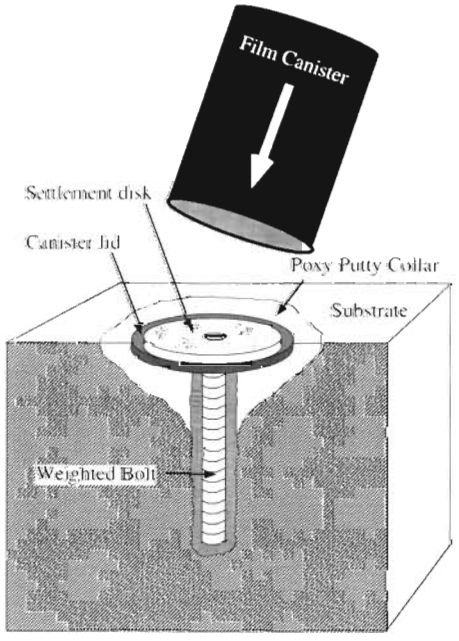


Fig. 2. Settlement disk positioned in the substrate. The disk is held in place in a canister lid that fits flush to a collar of Poxy Putty, both flush with the substrate. The weighted bolt protrudes into the central hole to anchor the assembly. The assembly is removed by snapping the canister onto the lid to retain the sample, and lifting it from the pool

Maps of each pool were printed on paper, cut out, and weighed to allow the surface area to be estimated from the weight of paper of known area. Percentage cover of *F. distichus* over the entire area of each pool was estimated using a 50 × 50 cm quadrat subdivided into 10 × 10 cm squares.

During low tide the pools were drained, and sites for settlement disks were selected haphazardly within patches of plants (n = 3 per pool). Settlement disk assemblies consisted of a film canister lid (diameter ca 38 mm) into which Sea Goin' Poxy Putty™ (Permalite Plastics, Newport Beach, CA) disks (diameter 28 mm) were fitted; these were held together with a bolt (Fig. 2). Holes were drilled into the rock with a gas-powered drill (Ryobi Ltd, Hiroshima, Japan): a shallow hole at the surface that was large enough for the settlement disk (4 to 5 cm diameter), and a smaller, deeper hole in its center (ca 1 cm diameter) into which the weighted bolt protruded (Fig. 2). The shallow hole was filled with Poxy Putty™, with care taken to avoid filling the deeper hole. A film canister lid was covered with a thin film of silicone grease and pushed into the Poxy Putty™ until flush with the surrounding rock surface. After the Poxy Putty™ dried, the lid was removed to leave a depression into which disk assemblies could be fitted. This design allowed sample retrieval underwater with a minimum of disturbance, by snapping a film canister carefully onto the lid to seal the sample before lifting the assembly from the pool. The simplicity and rapidity of this system were advantages when working at low temperature, as samples were often collected from under thick ice cover.

**Sampling and field conditions.** Daily sampling of eggs and zygotes was performed prior to immersion of the pools by a daytime or evening high tide (i.e. following a daytime low tide). The disk assemblies were replaced with fresh ones on each occasion. Unlike many invertebrates, there is no larval stage in the life history of fucoid algae, and therefore settlement of eggs and zygotes into the shallow water of these isolated tide pools during the period of low tide directly reflects gamete release. Breaks in the daily sampling regime in 1995 occurred between January 31 and February 10 and between February 17 and 21. In addition, ice cover prevented sampling on several occasions when either (1) algae overlying settlement disks were embedded in the ice, preventing their removal to reach samples beneath, or (2) settlement disks were themselves embedded in ice. On returning to the laboratory, both the settlement disk and the seawater in the canister were examined with a dissecting microscope, and the number of eggs and zygotes was recorded (during peaks of settlement, subsamples were counted to provide estimates of settlement per disk). At the same time that disks were retrieved, air and pool water temperatures were recorded, and samples of water from the top and bottom of each pool were removed with a 1 ml syringe and transferred to microcentrifuge tubes for measurement of osmolality. Osmolality of water samples was determined in the laboratory using a Wescor 5500 vapor pressure osmometer (Logan, UT). Irradiance measurements in the field or at the Darling Marine Center, ME (6 miles from field sites) were recorded between 12:00 and 14:30 h EST (Eastern Standard Time; hereafter all times given in EST) using a Li-Cor LI 188B quantum meter with spherical sensor.

To resolve settlement patterns on a finer temporal scale, settlement disks (on lids) were placed haphazardly among algae in Pools A2, A4, B3, and B4 during single low tides on 2 occasions (January 26, low tide = 12:29 h; and January 30, low tide = 16:25 h). Following isolation of the pools by the receding high tide, samples were collected at 90 min intervals until the following high tide. Water motion was essentially absent during the sampling period, so disks were not anchored to the substrate, but sample collection was otherwise as described above.

Gamete release and settlement during daytime low tides (DLT) and nighttime low tides (NLT) were determined in March 1996 in 2 pools (A2 and B4) by placing settlement disks haphazardly among algae with mature receptacles, as described above. These data

were log-transformed and analysed by repeated-measures ANOVA (Systat Inc. version 5.2.1) and means were compared using Tukey's test.

Release of eggs in Pool B4 was compared at low and high tide by placing fertile branches (n = 6 to 8 receptacles) in 50 ml polypropylene centrifuge tubes with the side walls replaced by 40 µm nylon mesh to retain fucoid eggs (ca 70 µm diameter). During neap tides on March 25 and 26 pools did not receive tidal input, whereas on April 1 and 2 the pools were flushed at high tide. Tubes containing fertile branches were tethered to bolts in pools for the sampling period, after which the receptacles were removed for fresh weight determination, and the tubes resealed and transported in seawater back to the laboratory to count eggs.

**Fertilization success, time of fertilization and polyspermy analysis.** Levels of natural fertilization success and polyspermy (i.e. the fertilization of an egg by more than one sperm; a lethal condition) in the field were essentially determined as described by Brawley (1992). Sea Goin' Poxy Putty™ plates (28.3 $cm^2$) were placed in upturned lids of polypropylene containers of an appropriate size. These were placed haphazardly among algae in the pools during periods of gamete release, following which the container was carefully placed over the lid. After being removed from the pool, the sample was quickly filtered through 40 µm nylon mesh to retain eggs, and the filter plus a minimal volume of seawater (to prevent bursting of unfertilized eggs) was transferred to acetic acid:ethanol (1:3) for fixation in the field. This procedure took less than 5 min per replicate. A different method was used on March 10, 11 and 26: Samples were aspirated directly from the pools with a Pasteur pipette and placed in fixative in small vials. In the laboratory, fixed eggs and zygotes were filtered through 125 µm nylon mesh to remove large detritus, stained with Wittman's aceto-iron hematoxylin (Wittman 1965) and examined under the microscope to determine the presence or absence of sperm pronuclei (Brawley 1987; N.B. eggs containing more than one sperm pronucleus are polyspermic). When the sperm pronucleus was in the same plane of focus as the egg nucleus, the distance of both from the egg surface was recorded using a calibrated ocular micrometer. From these data the time of fertilization, relative to the time of fixation, was determined from a calibration curve in the laboratory (see below: 75 zygotes were counted per sample; n = 3 or 5 samples per pool).

The rate of sperm pronuclear migration towards the egg pronucleus and the stage of karyogamy were determined in the laboratory at both 3°C and 8°C. Receptacles in seawater were incubated in the light in a walk-in culture chamber and were removed within 30 min of the onset of gamete release (the time of removal was designated $t = 30$), by which time a sufficient quantity of gametes for analysis had been released. Subsamples of eggs and zygotes were fixed in acetic acid:ethanol (1:3) at regular time intervals, stained and examined as described above (n = 25 eggs). These data were used to construct calibration curves, from which the timing of fertilization in the field was estimated as described by Brawley (1992). Stages of karyogamy were assigned a stage (A to E) after Brawley (1992).

**Laboratory culture of *Fucus distichus* zygotes.** Receptacles of *Fucus distichus* released gametes into filtered seawater after 3 to 4 h of illumination. The zygotes were passed through 125 µm nitex to remove oogonia, washed 3 times in filtered seawater, allowed to settle on replicate glass cover slips in 60 mm Petri dishes, and cultured at 0 or 5°C with 80 µmol photons $m^{-2}$ $s^{-1}$ irradiance and a 12 h light:12 h dark photoperiod. Development and adhesion of zygotes and embryos on replicate cover slips were monitored at regular intervals with a stereozoom microscope. Analyses are based on counts of 30 to 40 zygotes per replicate coverslip.

## RESULTS

### Patterns of gamete release and settlement in the field

Antheridia containing sperm and oogonia containing eggs were shed onto the surface of receptacles and then settled directly below the point of release, because there was little (if any) water motion in the pools; oogonia, eggs, zygotes and antheridia are negatively buoyant, and sperm are negatively phototactic. During spring tides (i.e. high-amplitude tides occurring in association with full and new moons), the pools at both sites were flushed by wave action around high tide, and during such times populations of *Fucus distichus* were subjected to highly turbulent flow regimes. Pools at Site A were generally flushed ca 30 min earlier on the rising tide than those at Site B. However, during neap tides, pools at both sites were isolated for periods up to 3 d. Settlement varied between pools over ca 2 orders of magnitude (Fig. 3; cf. Pools A2, B1 and B4). The areas of pools were between 0.36 (B3) and 4.81 $m^2$ (B4) and percentage cover varied from 8.7 (A4) to 43.4% (A2), but additional data are required to assess the effects of pool size or cover on the magnitude or timing of gamete release. The position of the settlement disks relative to the largest reproductive adults in a patch was an important source of variation since eggs and zygotes settling during low tide fell vertically below the point of release. The distribution of *F. distichus* within each pool was highly contagious, as

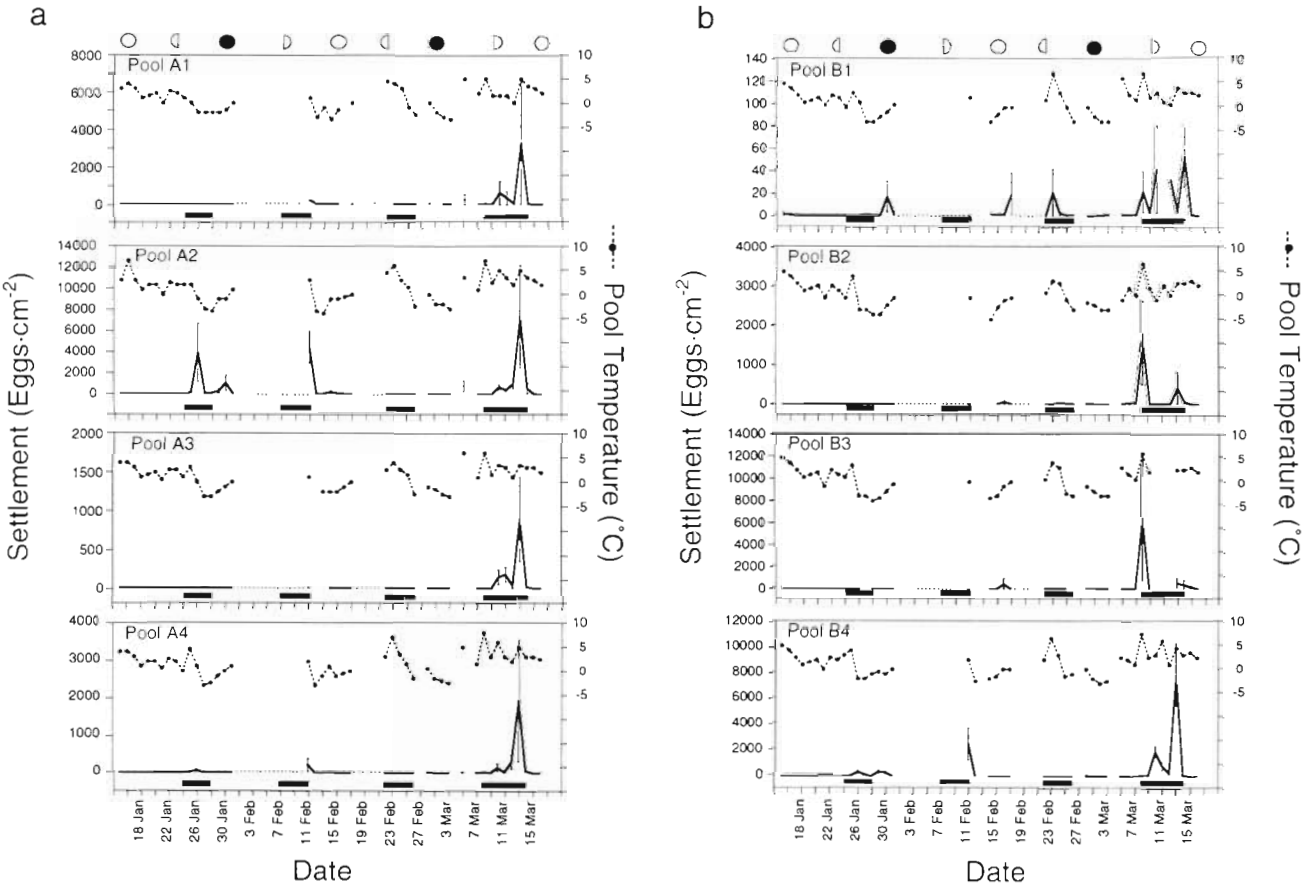


Fig. 3. Settlement of eggs and zygotes (——) of *Fucus distichus* between January 15 and March 16, 1995 (n = 3 settlement disks, except Pool A1, where n = 2; mean ± SE), and the temperature of water in the pools at the time of sampling (--●--) for (a) Site A and (b) Site B. Lunar phase is shown along the top; blocks on the *x*-axis indicate days on which the low tide fell between 10:00 and 14:00 h, and on which most settlement events were recorded. For the settlement data, the thin dotted line represents deliberate gaps in sampling; other missing data resulted because of excessive ice cover on pools

shown by values of the variance to mean ratio that exceeded 20 for each pool [data not presented; a value of 1 indicates a random distribution, and increasingly higher values indicate more contagious distributions (Pielou 1977)].

Settlement was generally synchronous in populations both within and across several pools, suggesting that common proximal factors influenced release of gametes at these sites (Fig. 3). For example, on January 26 (A2, A4 and B4) and 30 (A2, B1 and B4), on February 11 (A1, A2, A4 and B4), and particularly on March 13 (A1 to 4 and B2 to 4), pools shared synchronous gamete release and settlement events. DLT is a good indicator of gamete release (as determined from settlement), since 28 of the 37 settlement events recorded for all of the pools during 1995 occurred when the DLT fell between 10:00 and 14:00 h, and a further 4 occurred within 2 d of this 'reproductive window'. Major peaks in gamete release and zygote settlement were not correlated with lunar phase, although minor episodes occurred around the time of full and new moon (e.g. January 30 in Pools A2, B1 and B4; February 14 in Pool A2; February 16 in Pools B2 and B3; February 17 in Pool B1).

The major period of gamete release observed during 1995 occurred in March (Fig. 3). Gametes continued to be released into early April (data not shown), followed by rapid necrosis and loss of receptacles during mid to late April, prior to a resumption of vegetative growth. It is likely that the increasing level of gamete release from January to March was a result of receptacle maturation, although gametangia appeared to be mature throughout this period.

There was a large temporal and spatial variation in the osmolality of the pools relative to seawater (ca 1000 mM kg$^{-1}$) during the sampling period, due to both climatic conditions and tidal periodicity (Fig. 4). Vertical salinity gradients were formed in 2 ways: as water froze at the surface, salts were concentrated in bottom water relative to the surface; secondly, input of fresh water by precipitation (rain or fog) caused the salinity of the water near the surface to decrease, but had only

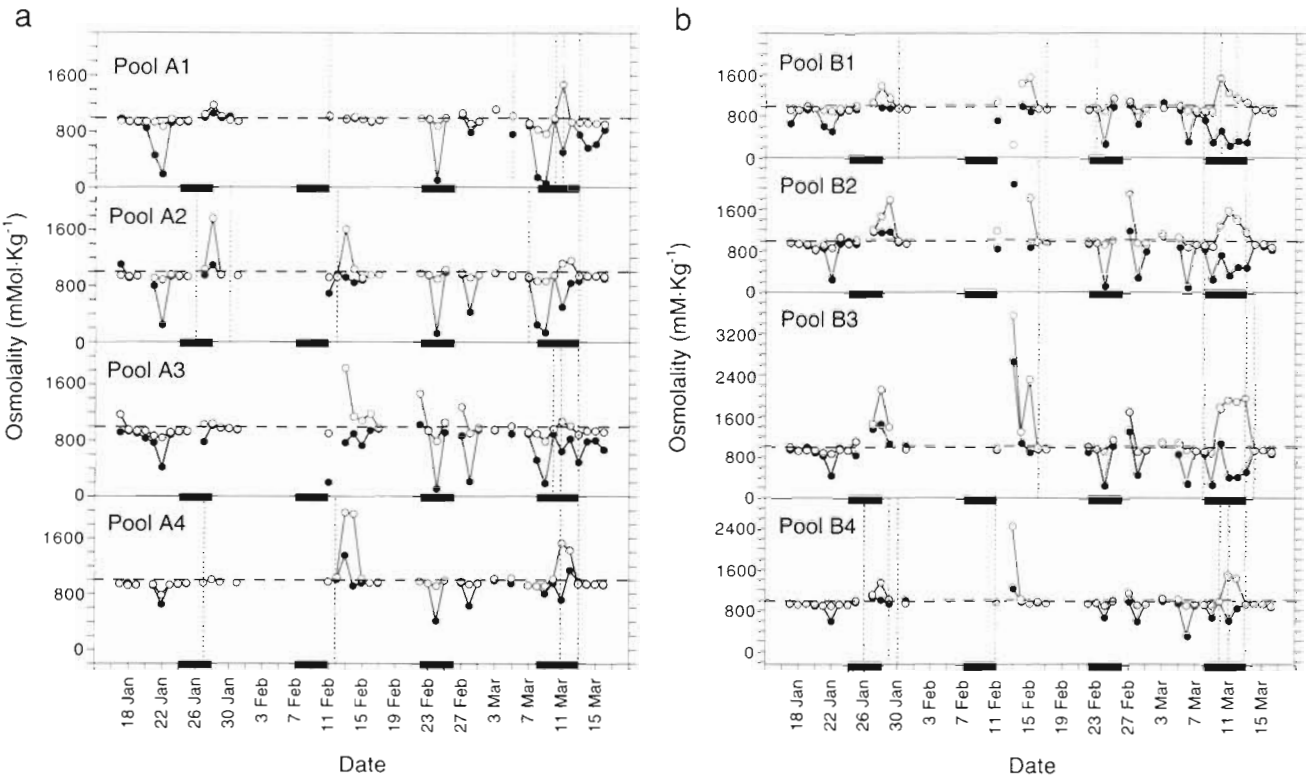


Fig. 4. Osmolality of water in tide pools at (a) Site A and (b) Site B, between January 15 and March 16, 1995. Samples of surface (●) and bottom water (○) were taken near the end of a low tide period. The horizontal broken line represents the osmolality of normal seawater (ca 1000 mM $kg^{-1}$); vertical broken lines show dates on which gamete release was recorded for the particular pool. Blocks on the *x*-axis show days on which the low tide fell between 10:00 and 14:00 h

a minor effect on salinity near the bottom. The maximum recorded osmolality at the bottom of the pools was 3540 mM $kg^{-1}$ (Pool B3, March 13) and exceeded 1500 mM $kg^{-1}$ in all of the pools periodically. The minimum recorded osmolality of water at the pool surface was 63.5 mM $kg^{-1}$ (Pool A1, March 9), and frequently dropped below 500 mM $kg^{-1}$ in all of the pools (Fig. 4). During neap tide periods that coincided with DLT, pools often did not receive tidal input for several days, suggesting that osmotic stimuli might also influence gamete release. For example, none of the pools was flushed during high tide between March 10 and 12 and a modest amount of settlement was recorded in most of the pools. Then, on March 13, the morning high tide flushed all the pools in Site A as well as Pool B4 (the latter receiving only limited wash by larger waves), and in each case, high settlement was recorded. It should be noted, however, that significant numbers of eggs and zygotes were released in pools B2 and B3 on these days in mid March, and these pools were not washed by waves on March 13. Other than the possible coincidence (in some pools) of gamete release with large osmotic changes on several days in mid March, however, gamete release was not related to osmotic changes.

Pool temperature during isolation of the pools at low tide varied between −5°C (under thick ice cover on February 14) and +8°C (March 8), while the mean sea surface temperature during the sampling period was 5.1°C in January, reaching a low of 3.2°C in February before rising slightly to 3.4°C in March. High levels of gamete release were recorded at temperatures between −1 and +7°C, and 29 of the 38 events occurred at temperatures above freezing. The temperature of the pools may, therefore, be correlated with settlement, although at temperatures significantly below zero the formation of ice causes: (1) increased salinity and (2) trapping of receptacles (precluding gamete release); without experimental manipulations, the effects of these factors cannot be resolved from the effects of temperature per se. Temperature has a marked effect on the adhesion of zygotes to the substrate; following culture for 30 h at 5°C in the laboratory 69.3 ± 2.9% (SE) of zygotes had attached, whereas this figure was reduced to 20.9 ± 2.7% at 0°C (n = 14 coverslips).

No relationship between the level of irradiance and patterns of gamete release was evident. Although some release events occurred on sunny days, when irradiance was typically ca 2500 µmol photons $m^{-2}$ $s^{-1}$ (e.g. January 26 and 30), others occurred at intermedi-

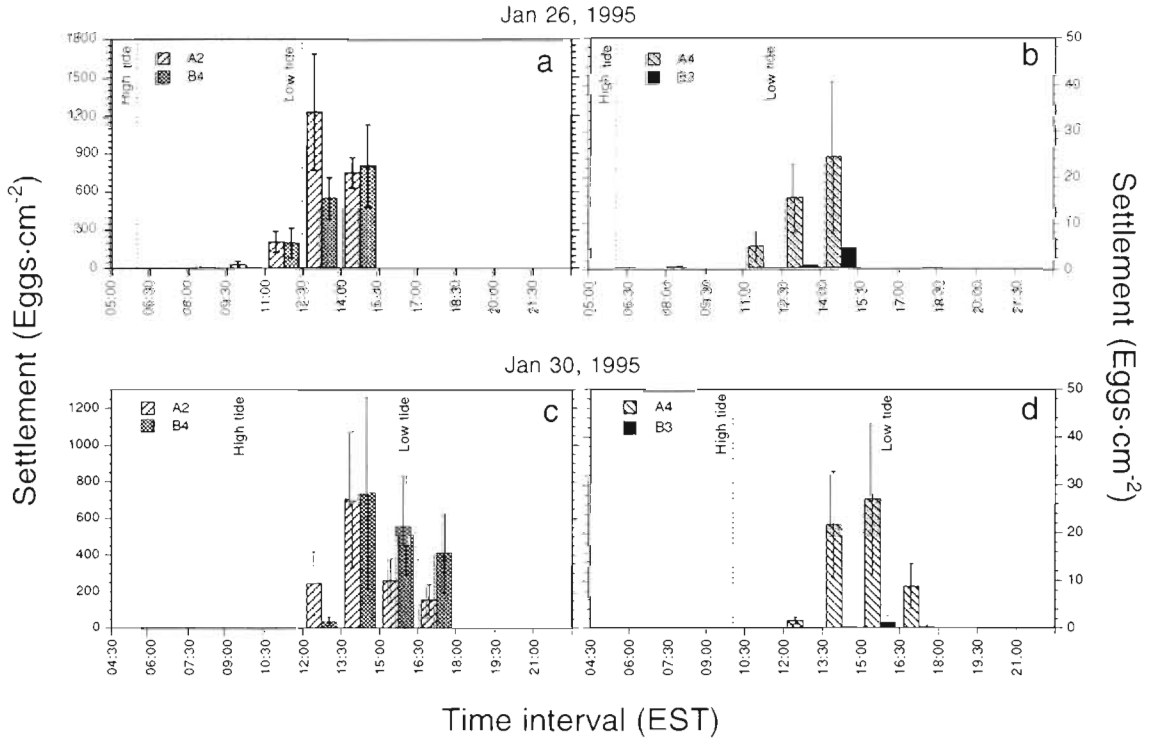


Fig. 5. Settlement of eggs and zygotes of *Fucus distichus* in Pools A2 and B4 on January 26 (a and c), and A4 and B3 on January 30, 1995 (b and d) during single DLTs (n = 5 settlement disks; mean ± SE). Samples were collected every 1.5 h after pools were isolated by the receding high tide

ate (March 8, 997 µmol photons $m^{-2}$ $s^{-1}$) or low irradiance (March 13, 173 µmol photons $m^{-2}$ $s^{-1}$).

Temporal patterns of egg and zygote settlement during a single DLT on January 26 and 30 are shown in Fig. 5. On January 26, sampling began at 08:00 h, after the pools were isolated on the falling tide following the 06:00 h high tide. Settlement was nearly absent until the 11:00 to 12:30 h sampling period, but high levels of settlement occurred after the 12:30 h low tide, between 12:30 and 15:30 h (Fig. 5a, b). This pattern was found in each of the 4 pools studied. On January 30, settlement occurred over the same time period as on January 26; settlement began during the first sampling period after the 10:00 h high tide, between 12:00 and 13:30 h, and reached a peak between 13:30 and 16:30 h (Fig. 5c, d). On both days the range of pool temperatures recorded was similar (−3 to +1°C on January 26 and −2 to 0°C on January 30), and although no irradiance data for these 2 d are available, the weather was sunny and clear. These data suggest that settlement (and therefore gamete release) coincides with DLT, not with a particular phase of the tidal cycle.

During peaks of release associated with DLT on March 12 (09:49 h) and 13 (10:55 h) 1996, gamete release during NLT was very low (Fig. 6). Repeated-measures ANOVA indicated significant differences between release during DLT and NLT for Pools A2 and B4 ($F_{1,10}$ = 39.67; $p < 0.001$). Comparisons of means showed that within a pool release during NLT on March 11/12 and 12/13 was lower than DLT on March 12 and 13 except in Pool B4, where release during NLT of March 12/13 was not significantly lower than release during DLT on March 13 (following the peak of release on March 12).

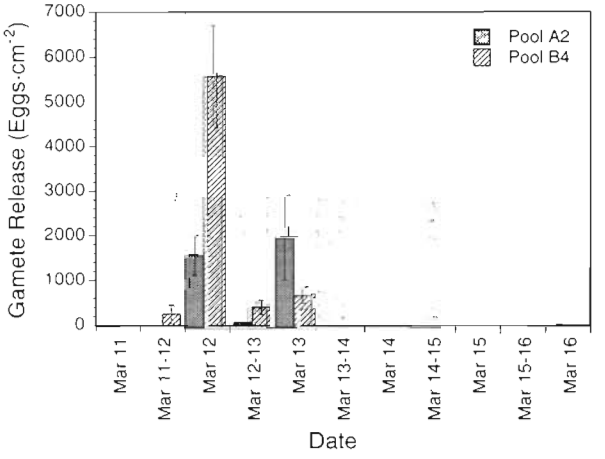


Fig. 6. Egg release by *Fucus distichus* during DLT and NLT in Pools A2 and B4 between March 11 and 16, 1996. Sampling intervals were the periods between immersion by the high tide. Alternating light and dark blocks represent day and night. Values are means ± SE (n = 6 settlement disks)

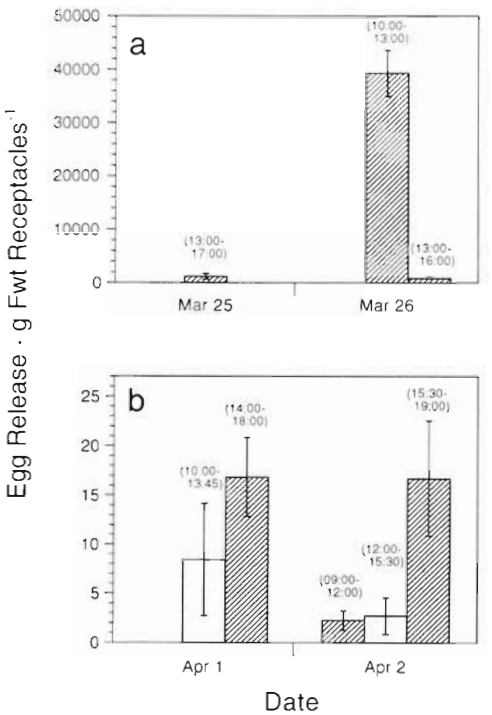


Fig. 7. Egg release by *Fucus distichus* during (a) DLT (March 25 and 26, 1995) and (b) DHT (April 1 and 2, 1995). Hatched bars are samples taken during isolation of the pools from wave action (low tide), and shaded bars are samples taken when pools are subjected to wave action (high tide). Values are means ± SE (n = 6 nitex bags)

Settlement might underestimate gamete release during turbulent periods of wave exposure at high tide, because of the potential for sample loss due to water motion. Therefore, gamete (egg) release was measured directly during times when low tide versus high tide occurred in the middle of the day in order to compare gamete release in calm and turbulent conditions during this period. Egg release was measured in Pool B4 on days coinciding with DLT (March 25 and 26) and daytime high tides (DHT; April 1 and 2), as shown in Fig. 7. On March 26, egg release was high between 10:00 (when release of gametangia onto the surface of receptacles was quite apparent) and 13:00 h, prior to the low tide at 13:00 h. Between 13:00 and 16:00 h, egg release was more than 2 orders of magnitude lower (Fig. 7a). On March 25, egg release was only determined between 13:00 and 17:00 h and therefore it is uncertain if higher release occurred earlier in the day, although little gametangial release from individuals in the pool was observed visually, suggesting that this was not the case. March 25 and 26 fell during a neap tide series, and the pools at Site B were not washed by the high tide on either day; therefore, there were no periods of turbulence during the tidal cycle. Egg release during DHT on April 1 and 2 was more than 3 orders of magnitude lower than during DLT on March 26, and average egg release was less than 20 eggs $g^{-1}$ fresh wt during calm periods following the high tide on both days (Fig. 7b). These data: (1) support the hypothesis that gamete release occurs primarily during DLT periods; (2) show that gamete release is very low when the pools are being flushed by the high tide during this interval; and (3) are in agreement with data from laboratory experiments showing a strong inhibition of gamete release by water motion in *Fucus distichus* (Serrão et al. 1996).

### Fertilization success and polyspermy

Fertilization success and levels of polyspermy in replicate samples of 50 to 100 eggs and zygotes were determined on 4 dates: January 30, March 10, 11 and 26 (Table 1). On each date, samples were collected 3 to 4 h after low tide, several hours after receptacles were observed to be releasing oogonia and antheridia. An additional sample was collected on March 26, between 12:00 and 12:40 h (prior to low tide), to investigate the changes in fertilization level over the settlement period. Because gamete release was high in most of the pools on March 26, eggs and zygotes could be pipetted directly from the area of Poxy Putty™ surrounding the settlement disks, obviating the need to place fertilization plates in the pools on this date. However, since low settlement was observed in certain pools, only 1 replicate was obtained from Pools B1 and B2 at both sampling times, and from Pool B3 at 12:20 h (Table 1). Fertilization success in samples taken 3 to 4 h after low tide was 96.8 to 100%, except on March 10 and 11 when fertilization success in Pool B4 was between 78 and 79%. The reason for these slightly lower values is not clear; however, the pool temperature on March 10 and 11 was lower than that on March 26 (3, 6 and 10°C respectively), which may have affected the rate of gamete release from oogonia and antheridia (Quatrano 1980). Thus, it is possible that eggs were continuing to be fertilized at the time the samples were taken on March 10 and 11. On January 30, the maximum temperatures of Pools A2 and B4 were low (2.5 and 3.0°C, respectively), but samples were taken later in the evening, since the high tide was later. On March 26, fertilization levels of 66.3 to 100% were found in samples taken prior to lowest low tide, and these values increased in the same pools to between 99.6 and 100% when samples were collected 4 h later (Table 1).

The estimated timing of fertilization in the field revealed differences between the samples from January 30 and March 26. On January 30, fertilization in Pools A2 and B4 occurred in the late afternoon or early

Table 1. Fertilization success and polyspermy levels in samples of eggs and zygotes of *Fucus distichus* collected from tide pools at Chamberlain, Maine, between January and March 1995. For each sample (pool), n replicates of between 50 and 100 eggs per zygote were counted. The site (A or B), pool and date are shown, together with the time of collection (EST) in relation to the daytime low tide (LT). The estimated range of times of fertilization in field samples was obtained from laboratory calibrations of sperm pronuclear migration rate and stage of karyogamy. nd: not determined

| Pool | Date | LT | Fixation time | n | % Fertilized mean (SE) | Estimated time of fertilization | % Polyspermic mean (SE) |
|---|---|---|---|---|---|---|---|
| A2 | Jan 30 | 16:25 h | 18:30 h | 5 | 96.8 (0.9) | 16:30–17:30 | 1.3 (0.7) |
| B4 | Jan 30 | 16:25 h | 19:00 h | 5 | 96.8 (1.0) | 18:00 h | 2.9 (0.8) |
| B4 | Mar 10 | 11:35 h | 15:30 h | 3 | 78.3 (2.9) | nd | 2.5 (0.3) |
| B4 | Mar 11 | 12:35 h | 16:30 h | 3 | 78.8 (4.9) | nd | 4.9 (0.6) |
| A2 | Mar 26 | 13:11 h | 12:30 h | 3 | 97.5 (0.7) | 08:30–11:30 h | 1.3 (0.0) |
| A2 | Mar 26 | 13:11 h | 16:40 h | 3 | 100.0 (0.0) | Before 12:30 h | nd |
| A3 | Mar 26 | 13:11 h | 12:40 h | 3 | 100.0 (0.0) | 08:30–10:00 h | 1.7 (1.1) |
| A3 | Mar 26 | 13:11 h | 16:50 h | 3 | 100.0 (0.0) | Before 12:30 h | nd |
| B1 | Mar 26 | 13:11 h | 12:15 h | 1 | 77.5 (–) | After 11:15 h | 2.5 (–) |
| B1 | Mar 26 | 13:11 h | 16:00 h | 1 | 100.0 (–) | Before 12:00 h | nd |
| B2 | Mar 26 | 13:11 h | 12:00 h | 1 | 83.8 (–) | 11:00 h | 1.3 (–) |
| B2 | Mar 26 | 13:11 h | 16:00 h | 1 | 100.0 (–) | Before 12:00 h | nd |
| B3 | Mar 26 | 13:11 h | 12:20 h | 1 | 66.3 (–) | 11:30 h | 1.3 (–) |
| B3 | Mar 26 | 13:11 h | 16:20 h | 2 | 100.0 (0.0) | 12:30–13:30 h | nd |
| B4 | Mar 26 | 13:11 h | 12:30 h | 3 | 76.6 (2.9) | 11:30–12:00 h | 1.5 (0.8) |
| B4 | Mar 26 | 13:11 h | 16:30 h | 3 | 99.6 (0.4) | 11:30–13:30 h | nd |

evening 1 to 2 h prior to sample collection, whereas the majority of eggs were fertilized before noon on March 26, and fertilization was complete before 12:30 h in 4 of the 6 pools sampled (A2, A3, B1 and B2), and by 13:30 h in the remaining 2 (B3 and B4). The rate of sperm pronuclear migration and stage of karyogamy were temperature-insensitive in laboratory calibrations at 3°C (Fig. 8) and 8°C (data not shown), representing temperatures encountered in the field when collecting samples for analysis of fertilization success. Therefore, any variation in the timing of fertilization between samples in the field reflects variation in timing of: (1) gamete release from individual adults, (2) breakdown of oogonia and antheridia, and/or (3) fertilization processes such as sperm motility and sperm dispersal (availability); it is not an artifact due to variation in sperm pronuclear migration rate. The timing of fertilization was reconstructed in samples of eggs and zygotes fixed at a discrete point in time during the low tide (18:30 and 19:00 h on January 30; 12:30 h on March 26). The time at which fertilization occurred in each zygote sample was calculated from the calibration curve (Fig. 8) of sperm pronuclear migration. On both days, fertilization in Pool A2 (Fig. 9a, c) began earlier and occurred over a broader time interval than in Pool B4 (Fig. 9b, d). The data from Pool A2 show considerable variability both within and between replicate sampling sites, e.g. 60, 9 and 5% of zygotes collected on March 26 at the 3 sampling sites had been fertilized for >4 h prior to fixation. This interesting variability between replicates could result from: (1) a difference in the time of fertilization of eggs which are available to be fertilized (e.g. due to variation in sperm concentration), or (2) variation in the time of release of gametes by individuals across Pool A2. Sampling of gametes (eggs and sperm) and zygotes at intervals throughout the period of gamete release and fertilization is required to distinguish between these 2 possibilities.

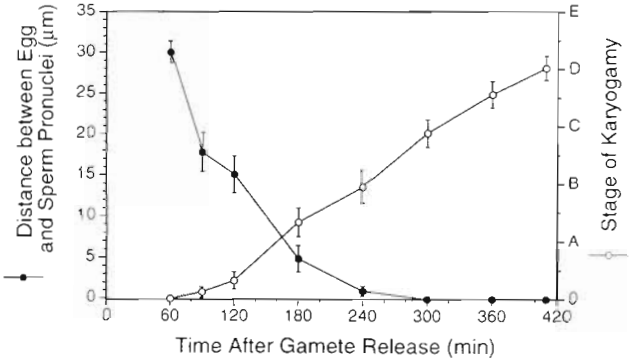


Fig. 8. Laboratory calibration of sperm pronuclear migration and stage of karyogamy with time at 3°C in *Fucus distichus*. Receptacles were placed into seawater at time = 0 min, and removed following gamete (eggs and sperm) release 30 min later. Aliquots were fixed at 30 to 60 min intervals for 7 h. Zygotes were stained with aceto-iron haematoxylin to determine the positions of the sperm and egg pronuclei and stage of karyogamy. Values are means ± SE (n = 25)

Polyspermy was low in all samples, typically 1 to 3% except in Pool B4 on March 11, where it was 4.9%. Settlement on January 30, and on March 10 and 11 was not high (Fig. 3), and it could be argued that polyspermy was low on these occasions because the sperm

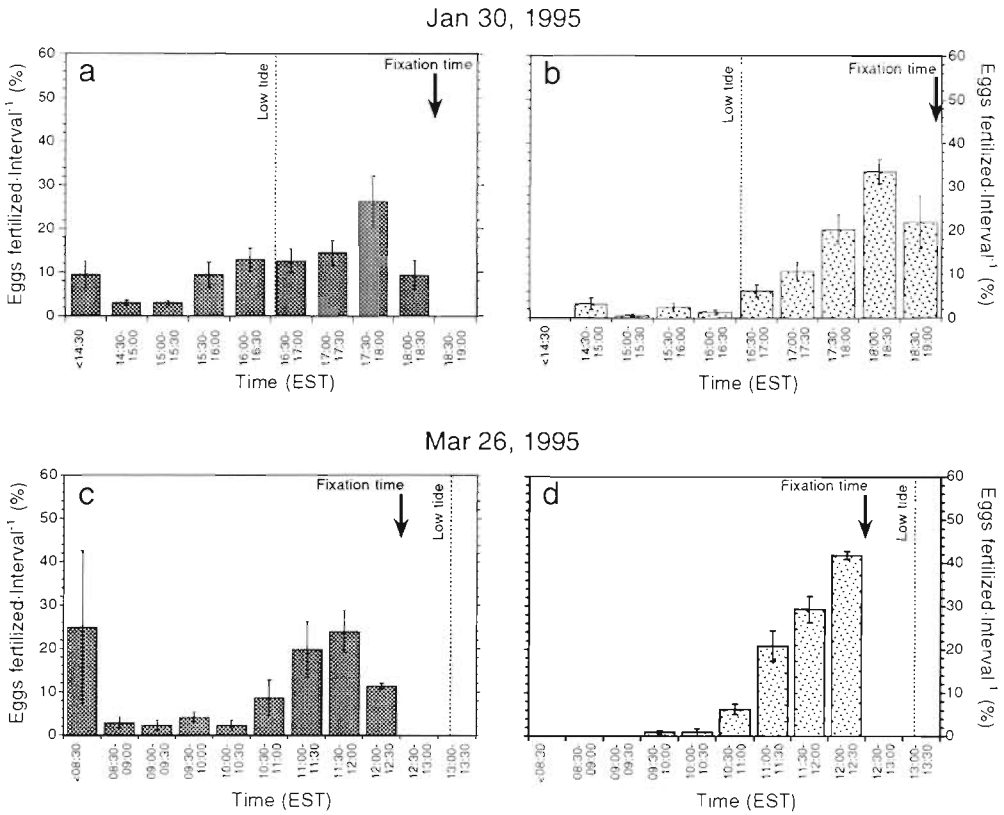


Fig. 9. Time during emersion of tide pools A2 (a and c) and B4 (b and d) when eggs of *Fucus distichus* become fertilized. Data are for January 30 (a and b) and March 26, 1995 (c and d). Values are means ± SE of n = 5 (January 30) or n = 3 (March 26) samples (75 eggs per sample)

concentration in the pools was relatively low. However, on March 26 a visual assessment suggested that both gamete release and settlement on disks was very high (Fig. 7), while polyspermy was low (Table 1). Therefore, the available data suggest a low incidence of polyspermy in *Fucus distichus*, even when gametes are released into relatively low volumes of calm water in the pools.

## DISCUSSION

The data presented in this paper show that the fucoid seaweed *Fucus distichus* L. is able to achieve high levels of fertilization success in the intertidal zone (78 to 100%) by releasing gametes synchronously during periods of very low water motion in tide pools isolated at low tide. Major periods of gamete release were not associated with the lunar or tidal phase, in contrast to several other seaweeds (Williams 1905, Hoyt 1927, Christie & Evans 1962, Fletcher 1980, Norton 1981, Brawley 1992); instead, they occurred when the DLT was between 10:00 and 14:00 h. Following release and fertilization during low tide, zygotes remain unattached and potentially subject to dispersal for a period of at least 1 tidal cycle at the low temperatures recorded during the reproductive season. The magnitude of gamete release was greatest in March, and fertilization occurred more rapidly during the low tide period in March samples than samples taken in January. These observations suggest either that receptacle maturity increases through the reproductive season, or that environmental factors such as pool temperature are more favorable for gamete release and fertilization at this time.

The good agreement found between periods of gamete release and days on which low tide fell between 10:00 and 14:00 h suggests that an absence of water motion and a period of irradiance are both necessary for release. This is further supported by the lack of gamete release during NLT. Both endogenous rhythms (e.g. circadian ones) and proximal environmental factors could contribute to the timing of gamete release. Almost no shedding occurred on days coinciding with daytime high tides, particularly during the period when the pools were being washed by waves. Endogenous rhythms of algal gamete release have been demonstrated previously in a few species (Müller 1962, Phillips 1988), although in other species gametes are released only in response to environmental cues associated with tidal periodicity (Ohno 1972; further references in Brawley & Johnson 1992). We have recently shown that receptacles of *Fucus distichus* collected 2 d prior to the onset of DLTs, and placed in culture under constantly calm or simulated tidal conditions, released gametes only with the onset of DLTs

and gamete release in the field (Serrão et al. 1996). However, gamete release was suppressed during this period if cultures were subjected to high water motion, and photosynthetic competence under calm conditions was required for release (Serrão et al. 1996).

Other than water motion and light, none of the environmental variables measured in this study is a good predictor of gamete release. However, several of the larger release events in March occurred coincidently with above average daily pool temperature (measured near the end of low tide). Higher temperatures would increase the rate of gamete maturation and any physiological processes involved in gamete release, although gametes were also released at temperatures as low as −1°C, well below the ambient sea surface temperature. In addition, gamete release may be indirectly affected by low temperatures, which cause ice to cover the pools during their isolation at low tide, and therefore reduce irradiance and increase salinity.

Gamete release was generally low when large salinity gradients were present in the pools (but see data for Pool B4 on March 11; Figs. 3b & 4b). Similar variations in salinity are experienced by *Fucus distichus* populations in Nova Scotia, Canada (Edelstein & McLachlan 1975), although the maximum values found by these authors were exceeded in this study. Both hyper- and hypoosmotic conditions can potentially affect gamete release and fertilization, since receptacles from a single alga may be near both the top and bottom of a pool's water column. After settlement, potential hyperosmotic stress is more important, because zygotes remain near the bottom of the pools: it is known that osmolalities within the range of those measured in the tide pools inhibit the expression of polarity in *Fucus vesiculosus* for up to 14 d (Torrey & Galun 1970). In their experiments, Torrey & Galun used sucrose as osmoticum, but zygotes in seawater of similar osmolality will presumably be exposed to the added stress of potentially cytotoxic intracellular concentrations of ions. Acclimation of adult *F. distichus* to hyperosmotic conditions has been shown to contribute to increased freezing tolerance in laboratory studies (Pearson & Davison 1994). Zygotes can be maintained at 0 and 5°C with limited development for weeks, or even months (McLachlan 1974), but the physiological and developmental consequences of combined low temperature and high salinity have not been investigated for early developmental stages.

The ability of *Fucus distichus* to synchronize gamete release during periods of low (near zero) water motion is an extremely valuable adaptation for an organism inhabiting the intertidal zone. Both modeling approaches and experimental field studies of induced spawning have shown that fertilization success declines rapidly with increasing water motion (Pennington 1985, Denny & Shibata 1989, Levitan et al. 1992). A small amount of water motion should be beneficial for fertilization, because it ensures adequate mixing of gametes (Denny & Shibata 1989). However, it may be less important for *F. distichus*, which is a monoecious seaweed capable of self-fertilization in which sperm are actively attracted to eggs by a pheromone (Maier & Müller 1986). Following the release of gametangia from the conceptacles, there is a short period during which gametes remain associated with the parent receptacles, since a quantity of mucilage is released together with the gametangia. Presumably, during this period eggs are closely associated with sperm from the same parent, as well as sperm from receptacles of neighbors when densities of individuals are high. Eggs are fertilized over a period of >4 h in some pools, providing some support for our prediction that gamete release in monoecious, self-fertilizing species might be less synchronous between individuals than for dioecious species. Fucoid algae should prove to be excellent model organisms in which to test such hypotheses in future studies. The high levels of fertilization success found in this study (78 to 100%) are similar to those found in dioecious species of fucoid algae from estuaries (Brawley 1992) and the Baltic Sea (Serrão et al. 1996).

The results of this study raise 2 intriguing questions concerning gene flow in *Fucus distichus*. The first concerns the extent to which self-fertilization occurs, and the second concerns the ability of zygotes to disperse between tide pools. Because eggs and sperm are released simultaneously by *F. distichus* into a restricted volume of very calm water, sperm limitation is unlikely to occur. However, sperm competition is likely to strongly favor self-fertilization because eggs would be closer to their sibling sperm than to more distant potential competitors (Grosberg 1991, Petersen 1991, Yund & McCartney 1994, Levitan & Petersen 1995, Yund 1995). Further studies to look at gene flow within pool populations, with the aid of molecular genetic techniques (allozyme or nucleic acid markers), will be necessary to determine the relative proportion of self-fertilization versus out-crossing. Whether or not self-fertilization limits gene flow between individuals, the restriction of gamete release to low tide periods means that individuals within a tide pool form an isolated breeding population, and gene flow between pools is largely dependent on the dispersal of zygotes. The low temperatures encountered by reproductive *F. distichus* result in slow rates of adhesive production by zygotes, potentially allowing 1 to several high tide periods during which dispersal to neighboring tide pools could occur. The success of dispersal and the scale over which it may effectively occur are not known, however, and must await future investigation.

The first event in the life history of *Fucus distichus* is the production of zygotes by external fertilization. Successful external fertilization is achieved by synchronizing gamete release with periods of very low or zero water motion in tide pools isolated by the low tide, and does not occur during periods of high water motion at high tide. These data also suggest that *F. distichus* has an endogenous rhythm that permits gamete release to occur under the most favorable conditions; calm conditions alone do not stimulate gamete release in this species (Serrão et al. 1996; these results). Moreover, calm conditions during DLTs are required. Finally, these observations suggest that models predicting low external fertilization success in intertidal organisms do not apply to tide pools and possibly other situations (e.g. slack high tide) where water motion in the environment varies predictably in time.

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