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REPRODUCTIVE SUCCESS OF *FUCUS VESICULOSUS* (PHAEOPHYCEAE) IN THE BALTIC SEA[1]

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Marine organisms colonizing brackish habitats such as the Baltic Sea must cope with the negative effects of low salinities on reproductive success because these may reduce gamete viability and/or increase polyspermy. Reproductive characteristics of the marine seaweed *Fucus vesiculosus* L. were studied in several brackish habitats, particularly in the northern Baltic Sea, to understand its ability to reproduce where few other marine species survive. Polyspermy and fertilization success were variable at the boundary of the continuous distribution of *F. vesiculosus* in the Baltic Sea, and polyspermy was high (10%–30%) when fertilization was successful. A strong female bias (80%–86%, ca. 5.5:1) was found at the northernmost limit of Baltic *F. vesiculosus*. Electrophysiological studies showed that many eggs have a high input resistance (519 ± 150 MΩ [mean ± SE, n = 14] at Drivan, 1995), which may be helpful in preventing polyspermy in this brackish habitat. The polyspermy block remains sodium-dependent in the northern Baltic. Sperm bound quickly to northern Baltic eggs in natural water, but fertilization was delayed compared to marine *F. vesiculosus*. A subset of northern Baltic eggs studied during an optimal reproductive period (7–11 July 1995) had a membrane potential (E_m) of ca. −100 mV and an effective fertilization potential (FP) of ca. 2 min with a plateau of −25 mV, but repolarized too rapidly for the FP to be protective. Pronuclear migration and cell wall secretion occurred more slowly in Baltic than in marine zygotes. The reproductive success of these boundary populations may be dependent upon windows of opportunity when there are favorable combinations of the levels of salinity, water motion, population density, and sex ratio. These factors and the short duration of the reproductive season in the northern Baltic Sea may result in reproductive failure in some years.

Key index words: **Baltic Sea; estuaries; fertilization success; fucoids; *Fucus*; polyspermy; reproductive ecology**

Abbreviations: **AW, artificial water; E_m, membrane potential; $E_{m\text{-}bf}$, membrane potential before fertilization; FP, fertilization potential; psu, practical salinity unit**

Marine organisms colonizing brackish habitats (e.g. estuaries, the Baltic Sea) must be able to cope with the negative effects of low salinities on reproductive success. Low salinities may decrease the success of fertilization by reducing the motility and longevity of gametes (e.g. Serrão et al. 1996a). Increased polyspermy is another effect that low salinities might have on the reproductive success of marine organisms (Brawley 1992). Polyspermy is lethal for most species (Jaffe and Gould 1985); therefore, it is not only essential for an egg to be fertilized, but also to avoid being fertilized by more than one sperm. Many organisms have several types of polyspermy blocks to prevent the entry of additional sperm after the first sperm-egg fusion, and these include the fast block (i.e. an electrical block) and a slow block (e.g. secretion of a fertilization envelope or cell wall) (see review by Jaffe and Gould 1985). Fast blocks against polyspermy act on scales of milliseconds after the first sperm entry and are usually

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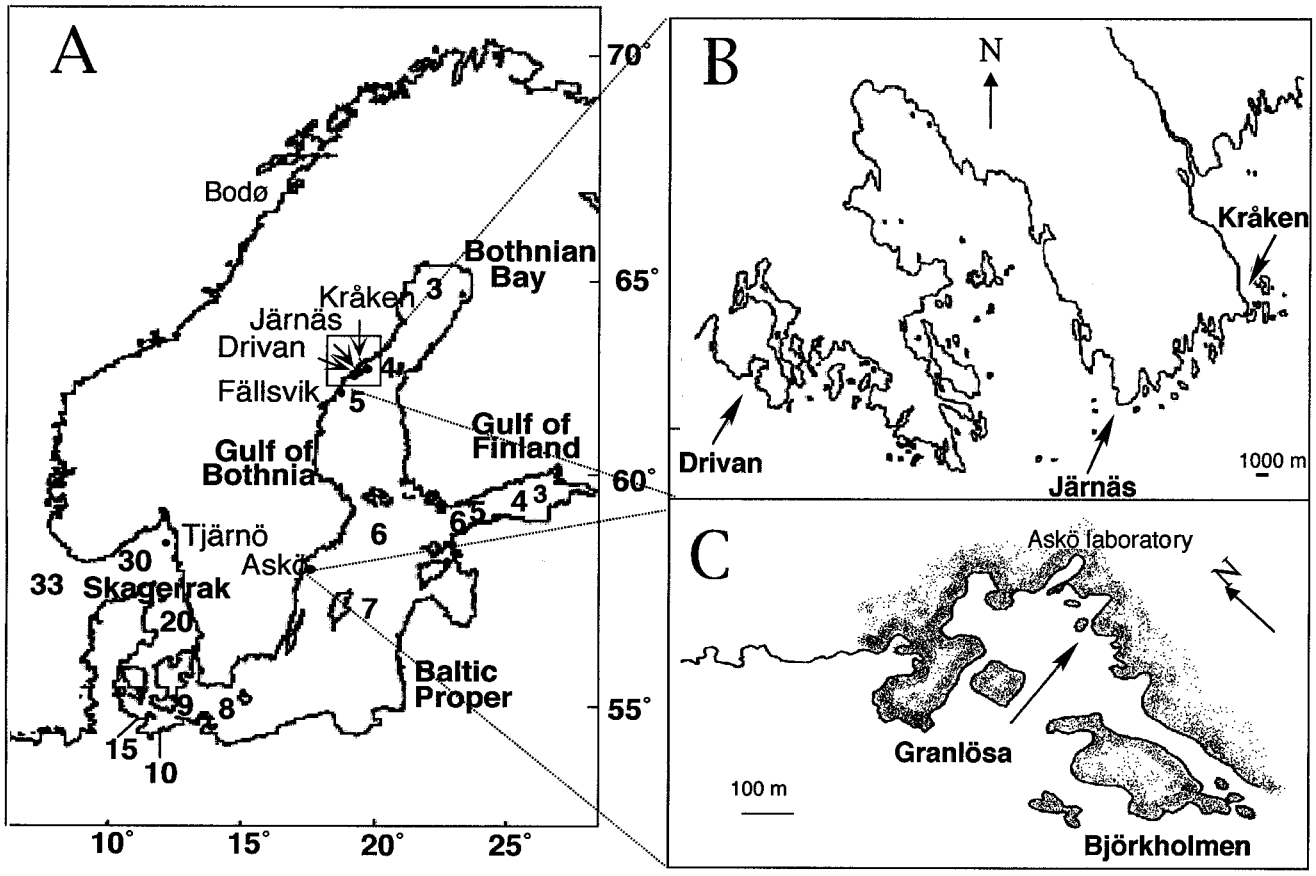

FIG. 1. (A) Map showing the location of the Baltic Sea, of the sites where field work took place, and/or where receptacles were collected. The approximate levels of salinity (in psu) are indicated. (B) Map of the study sites located near the northernmost distribution of continuous populations of *Fucus vesiculosus* in the Baltic Sea. (C) Approximate location of the two sites in the central Baltic at Askö.

based on a rapid depolarization of the egg membrane that reduces the probability of fertilization by further sperm, although eggs can still become polyspermic if the sperm:egg ratio is high (Jaffe and Gould 1985). An electrical polyspermy block is present in eggs from a wide range of organisms, including echinoderms (Jaffe 1976, Miyazaki and Hirai 1979), echiuroid worms (Gould-Somero et al. 1979), amphibians (Cross and Elinson 1980, Grey et al. 1982, Iwao 1989), nemerteans (Kline et al. 1985), crabs (Goudeau and Goudeau 1989), fucoid algae (Brawley 1991, Taylor and Brownlee 1993), lamprey (Kobayashi et al. 1994), ascidians (Goudeau et al. 1994), and mussels (Togo et al. 1995). Influx of Na^+ at fertilization causes membrane depolarization in most marine eggs (e.g. Jaffe 1976, Brawley 1987, 1991), whereas in freshwater eggs, depolarization occurs due to Cl^- efflux (e.g. Grey et al. 1982, Kobayashi et al. 1994). The external concentration of Na^+ may thus become a limiting factor when marine organisms attempt to colonize habitats with lower salinity, because the typical fast block against polyspermy will be less effective, and potential recruits may be lost to polyspermy (Brawley 1992).

The predictable alternation between lower and higher salinities in estuaries at low and high tide, respectively, provides windows of opportunity for organisms to release their gametes under conditions in which the fast block against polyspermy will be most efficient, and this occurs in the estuarine alga *Fucus ceranoides* (Brawley 1992). Here, high reproductive success occurs because adults release gametes at the time of the tidal cycle when salinity is highest, resulting in most eggs being fertilized near slack high tide (Brawley 1992). However, the largest brackish sea on Earth, the Baltic Sea (Fig. 1A), lacks tidal fluctuations. A stable gradient of salinity occurs across the Baltic, from its opening to the North Sea (ca. 10 practical salinity units [psu]) to the Bay of Bothnia (0.1 psu). It has been undergoing a large-scale decrease in salinity since the end of the ''*Littorina* Sea'' period (ca. 7500–3000 BP). At that time, the connection to the North Sea was wider and the Baltic was colonized by marine species that have since disappeared (Ignatius et al. 1981, Russell 1985). Among the numerous fucoid species present in the North Sea, *Fucus vesiculosus* is the only one that presently occurs throughout most of the Baltic, where it forms continuous stands from the entrance to the Baltic to regions of approximately 3.5–4 psu

in the Gulf of Bothnia and Gulf of Finland (Waern 1952, Kautsky et al. 1992, Serrão et al. 1996a). *Fucus serratus* is also present in the Baltic Sea but only occurs in the more saline southern areas, reaching a limit at ≥7 psu, and *Fucus evanescens* has been reported from a southerly location (Kiel) of higher salinity (Schueller and Peters 1994). *Fucus ceranoides* is absent from the Baltic Sea, despite being able to grow at low salinities (Bäck et al. 1992), possibly because adults release few gametes under brackish conditions (Brawley 1992).

The absence of other fucoids from most of the Baltic, despite the close relationship among all *Fucus* species (Serrão 1996, Leclerc et al. 1998, Serrão et al. 1999), suggests that *F. vesiculosus* has developed unique features that allow it to reproduce successfully in low salinities. These might include characteristics that prevent polyspermy, such as a change in the ionic basis of the fast polyspermy block and/or mechanisms for maintaining a low sperm:egg ratio at fertilization. Alternatively, high levels of polyspermy might occur, but enough recruits survive to allow persistence of these populations.

The sodium-dependent fast block against polyspermy is characterized in marine *F. vesiculosus* by a fertilization potential that consists of a depolarization from about −65 mV to −25 mV for 6 min (Brawley 1991). The rate and duration of the depolarizations are slower in artificial seawater in which Na^+ has been replaced by the impermeant cation *N*-methyl glucamine, and eggs are more susceptible to polyspermy in such media (Brawley 1991). Cell wall formation in marine zygotes of this species is first observed about 10 min postfertilization (Brawley et al. 1976, Brawley 1991); this constitutes a permanent slow block against polyspermy. In addition, there is an intermediate block of unknown mechanism, which causes additional sperm to detach from the egg soon after fertilization (Brawley 1991).

Polyspermy is directly dependent on the sperm:egg ratio at fertilization (Jaffe and Gould 1985, Brawley 1987), and in marine *F. vesiculosus,* artificial seawaters with [Na^+] similar to those that occur in the central Baltic Sea (ca. 80 mM) increase polyspermy appreciably only if the sperm:egg ratio is high (Brawley 1990a, 1991). However, in artificial seawaters with [Na^+] similar to those that occur at the limit of *F. vesiculosus* in the Baltic Sea (ca. 45 mM), polyspermy in marine eggs is high even at low sperm:egg ratios (Brawley 1990a, 1991). Laboratory studies with marine *F. vesiculosus* (Brawley 1990a, 1991) show that 10% of the zygotes would become polyspermic at a sperm:egg ratio of 25:1, 20% at a sperm:egg ratio of 250:1, and 40% at 2000:1 or 4300:1 in 50 mM Na^+, which corresponds to approximately 4 psu, the salinity at the limit of *F. vesiculosus* in the Baltic Sea.

The major objective of this study was to use *F. vesiculosus* from the Baltic Sea, and in some comparative studies also from Atlantic estuaries, as models to study reproductive adaptations of marine organisms colonizing low-salinity habitats. In particular, the specific questions addressed in this study for these brackish populations were: 1) what are the natural levels of fertilization success and polyspermy, 2) what are their sex ratios, 3) are polyspermy levels higher at lower external [Na^+], 4) what are the electrophysiological characteristics of eggs from brackish habitats, and 5) how does salinity affect the duration of gamete viability, the release of eggs from oogonia, and the timing of pronuclear migration and the intermediate and slow polyspermy blocks? These questions were addressed to provide insight into the ability of *F. vesiculosus* to reproduce under conditions where few other marine species are found.

MATERIALS AND METHODS

The model system. The reproductive season of *F. vesiculosus* in the Baltic Sea is restricted largely to May–July in the northern Baltic (Kautsky et al. 1992), whereas a longer reproductive period with two major peaks during spring and fall occurs in southern areas (Carlson 1991). *Fucus vesiculosus* is dioecious and settlement is directly related to gamete release because oogonia, antheridia, and eggs sink, and the sperm are negatively phototactic. The zygotes attach to the bottom approximately a day after fertilization at the temperatures (ca. 8–14° C) commonly found during most of its reproductive season in the Baltic at 6.5 psu (pers. observ.).

Egg settlement. Patterns of natural egg settlement were monitored daily during the reproductive seasons of 1993 and 1994 (central Baltic Sea) and in 1995 and 1996 (northern Baltic Sea), to estimate the days when enough eggs could be sampled for determinations of natural fertilization success and polyspermy. The populations living at the lowest salinities in the Baltic were found by examining geological maps for rocky areas at ≥3 m depth and surveying these areas by diving. There were well-developed beds of *F. vesiculosus* at Drivan (Fig. 1A, B), but at Kråken (Fig. 1A, B) no continuous stands were found (at ca. 2.5 m), although well-developed individuals were found in small patches or as isolated individuals. The regions where natural egg settlement was studied were located near the northern limit of the distribution of *F. vesiculosus* in the Baltic Sea at Drivan (Fig. 1A, B) and in the central Baltic at Askö (Fig. 1A, C). The study area at Askö is located at 0.3–1 m depth and is exposed to westerly winds; the salinity is approximately 6.5 psu. At Drivan, *Fucus* occurs at approximately 2.5–6 m depth and is frequently exposed to turbulent conditions. During the 1995 and 1996 reproductive seasons, the salinities at the Drivan site varied between approximately 3.5 and 4.5 psu, and the temperatures varied between approximately 5° and 14° C.

During the 1993 reproductive season in the Baltic Sea at Askö (Granlösa), settlement of eggs/zygotes from *F. vesiculosus* was monitored on epoxy disks of 1.8 cm diameter (prepared as in Brawley and Johnson 1991). Each disk was held on the bottom over a peg on a brick. Ten females of *F. vesiculosus* were chosen randomly, and three disks were placed haphazardly around each. The same locations were sampled every day; disks were collected underwater and replaced by new ones at 2030–2300 (i.e. after the natural period of maximal release each day [Serrão et al. 1996b]) after being covered with 40-μm nylon mesh to prevent loss of eggs during retrieval from the water. The number of eggs per disk was counted under a dissecting microscope. Water motion was described qualitatively as calm or rough by visual observation near the anticipated period of gamete release (early evening). Egg settlement and water motion in 1994 are described in Serrão et al. (1996b).

Settlement of eggs in the northern Baltic (ca. 3–5 m depth) was monitored during a 9-day period in 1995 and a 26-day period

in 1996, coinciding with the main reproductive season in the northern Baltic. Eggs and zygotes were collected on epoxy plates as described in Serrão et al. (1996b). At each site, two plates (1995) or one plate (1996) was placed on inverted plastic lids in each of 10 (1995) or 20 (1996) haphazardly chosen locations with *Fucus*. Samples were retrieved daily between 0130 and 0430 (in 1995) and between 2030 and 2230 (in 1996) by capping the plastic box underwater to prevent loss of sample. After each plate was collected, a receptacle was collected from all of the individuals within 1 m^2 of the plates in order to determine the sex ratio on a local scale using a water-filled loop of garden hose to mark the sampling area (see below). New plates were then assigned to new locations with *Fucus*.

Since gamete release in *Fucus* is dependent on photosynthesis and requires calm conditions in light (Serrão et al. 1996b, Pearson et al. 1998), current velocities and light levels were determined. Peak current velocities were recorded daily during the studies in the northern Baltic using a Marsh-McBirney 511 (Frederick, Maryland) current meter using a time constant of 0.2 s and with the probe positioned at the level of the receptacles (approximately 0.2 m above the bottom). The natural light levels during a sunny day in the northern Baltic at Drivan were determined with an Underwater Quantum Sensor (LI-COR, Lincoln, Nebraska) suspended from a boat every 3 h from 0900 to 1800 at 0, 2, and 4 m depth (the latter represents the natural depth of most of the *Fucus* stands) and above the sea surface. Natural light levels in the central Baltic at Askö were measured with an Underwater Quantum Sensor placed at the natural level of the receptacles (0.2–1.5 m depth).

Polyspermy levels in natural populations. The proportion of polyspermic eggs formed during natural fertilization events was determined in the continuous stand of *F. vesiculosus* in the northern Baltic at Drivan (2.5–5 m depth; 1995, 1996, Fig.1A, B) and in two stands in the central Baltic at Askö (1 m depth; Granlösa [1993, 1994] and Björkholmen [1994], described in Serrão et al. [1996b], Fig. 1A, C). Eggs and zygotes were collected on epoxy plates of 100 cm^2 as described in Serrão et al. (1996b). These plates were placed on inverted plastic lids under randomly (Askö) or haphazardly (Drivan) chosen individuals. For each site and date, 10–20 plates were placed in the field; of those, settlement occurred on a subset and only these were analyzed (6–12 plates per date and ca. 50 eggs per plate analyzed for the central Baltic; 1–10 plates per date and ca. 30 eggs per plate analyzed for the northern Baltic). Haphazard selection of sites was used at Drivan due to the time constraints involved in daily relocation of sampling areas after collecting the samples by SCUBA diving. After the natural period of gamete release (see Serrão et al. 1996b), each sample was retrieved by capping the plastic box underwater and was immediately fixed in 1:3 acetic acid:ethanol. A subsample was stained with aceto-iron hematoxylin (Wittmann 1965) to determine the number of sperm pronuclei present inside the eggs. Fertilization success was calculated as the proportion of eggs that had at least one sperm pronucleus inside. Polyspermy was calculated as the proportion of fertilized eggs that had more than one sperm pronucleus inside. In the northern Baltic (Drivan), water samples were taken daily from the surface and at the bottom from the deepest (5 m) and the shallowest (2.5 m) locations within the sampled area, and their salinities were determined with a WTW Microprocessor Conductivity Meter (LF 196, Christian Berner PB, Germany).

Sex ratios. The sex ratio was determined in several stands of *F. vesiculosus*: in the central Baltic (Askö, Fig. 1A, C), in the northern Baltic (Fällsvik, Fig.1A, B), at the northernmost limit of *F. vesiculosus* in the Baltic (Drivan and Kråken, Fig. 1A, B), in the Skagerrak (Tjärnö, near the connection of the Baltic with the North Sea, Fig. 1A), and in Maine (U.S.A.), both in estuaries (i.e. at the uppermost limit of *F. vesiculosus* in the Narraguagus River, the Union River, and the Penobscot River) and on the outer coast (at Schoodic Point and Pemaquid Point). At each site, locations (n = 15–82 locations) were randomly (Maine) or haphazardly (northern Baltic and Skagerrak) chosen, and 1 m^2 areas were delimited at each location with a reconnected garden hose filled with water. One receptacle was picked from each individual growing within the 1 m^2 to determine its sex (total sampling = 1961 individuals at Schoodic, 1459 at Pemaquid, 2016 at Tjärnö, 82 at the Narraguagus River, 199 at the Penobscot River, 506 at the Union River, 129 at Fällsvik, 928 at Drivan site 1). At Askö, the sex ratio was estimated by determining the sex of a receptacle from every reproductive individual found within an area of 31 m^2 at Granlösa (n = 297) and 35 m^2 at Björkholmen (n = 120). The sex ratio at a second site in Drivan was determined from haphazard collections of individuals throughout the algal bed (n = 127), and at Kråken, where the population density was very low, the sex ratio was determined from receptacles from every individual found during a dive (n = 22). Collections from Drivan and Askö were made on multiple days throughout the reproductive season; other data were collected on single days during the middle of the reproductive season. Receptacles were sectioned in the laboratory and observed with a microscope to determine the sex of each individual. A test for proportions was used to test the null hypothesis of unskewed sex ratios (i.e. H_0: proportion of females (or males) = 0.5).

Effect of sodium on polyspermy levels. Gametes from the Baltic at Fällsvik were used to test the effects on polyspermy of manipulating the external $[Na^+]$ in artificial water (AW). The $[Na^+]$ tested at the osmolality of northern Baltic water (125 $mmol \cdot kg^{-1}$) was 1.1 mM and 54 mM (as in northern Baltic water) and a control in natural Baltic water. To compare polyspermy at 450 mM Na^+ (as in marine water) with that at 54 mM (as in northern Baltic water), a treatment with the ionic composition of Baltic water but the osmolality of marine water was also used to separate effects of $[Na^+]$ and osmolality. Eggs were released from the receptacles in artificial Baltic water with an ionic composition similar to that at the northern Baltic for the treatments at 125 $mmol \cdot kg^{-1}$ osmolality or in the same water with the impermeant cation *N*-methyl-glucamine used as a Na^+ replacement to an osmolality of 980 $mmol \cdot kg^{-1}$ (as in marine water). Northern Baltic (i.e. Fällsvik/Drivan) artificial water was 53 mM NaCl, 3.3 mM $MgCl_2$, 3 mM $MgSO_4$, 1.5 mM $CaCl_2$, 1.4 mM KCl, and 1.1 mM $NaHCO_3$ (Serrão et al. 1996a). Sperm were released in water with the same ionic composition but with all NaCl replaced by *N*-methyl-glucamine chloride (such that only 1.1 mM Na^+ remained from the $NaHCO_3$) for the treatments at 125 $mmol \cdot kg^{-1}$ osmolality or in the same water with *N*-methyl-glucamine to an osmolality of 980 $mmol \cdot kg^{-1}$. Eggs were separated from intact oogonia by filtering with 105-μm nylon mesh and were inseminated 1.5 h after the oogonia and antheridia were released. Fertilizations were performed at a 1000:1 sperm:egg ratio in glass dishes (n = 2) containing 5 mL solution at 10° C. The eggs from each treatment were fixed in 1:3 acetic acid:ethanol after 9 h (treatments at 980 $mmol \cdot kg^{-1}$) or after 12 h (treatments at 180 $mmol \cdot kg^{-1}$). A subsample of 50 eggs from each replicate was stained with aceto-iron hematoxylin (Wittmann 1965) to determine the number of sperm pronuclei inside each egg.

Electrophysiology. Receptacles were stored damp at 4° C in the dark until use, and in all experiments they were used within 4 days of collection. Sperm and eggs were obtained for experiments by placing numerous receptacles into glass dishes half-covered by medium. The medium was Baltic water collected from the bottom of natural beds at Drivan (3.9–4.0 psu at times of collections for these experiments) or Fällsvik (4.3–4.6 psu) or a 63 mM NaCl-artificial northern Baltic (i.e. Fällsvik/Drivan) water (4.6 psu) for Baltic eggs, seawater from Bodø, or step-dilutions of seawater (30–32 psu) from the Darling Marine Center (University of Maine) made with deionized water (18 MΩ) from a Barnstead system. Eggs and sperm were used within 2 h of release of the gametangia from receptacles in most experiments.

Electrophysiological studies of eggs from 1) Fällsvik (1994–1995) and Drivan (1995–1996) on the Swedish coast in the Baltic Sea, 2) the Penobscot River estuary (1995, 2 miles north of Bucksport, Maine, U.S.A.), and 3) Bodø, Norway (1995), were conducted by techniques similar to those described in Brawley (1991). The recording chamber (see Brawley 1991: fig. 1) was placed in a water-cooled cold stage (AQLM-Video, Ltd., Woods Hole, Massachusetts) on a Zeiss IM-35 inverted microscope, and a Lauda circulating water bath kept the seawater in the chamber

at 10° C (Baltic and Norwegian measurements) or 14° C (Penobscot). A few drops of an aqueous solution of poly-D-lysine hydrobromide (150–300 kDa) were placed on a fragment of clean coverslip for a few seconds. The coverslip was rinsed with distilled water and placed at the bottom of the water-filled chamber. Immediately, eggs were pipetted into the chamber and stuck lightly to the coverslip upon contact. The amount of poly-D-lysine that could be used varied, and this was a critical parameter for successful recording. An aqueous solution of 2 mg·mL^{-1} was used for marine eggs, 0.5–1 mg·mL^{-1} for estuarine eggs, and 2–20 μg·mL^{-1} for Baltic eggs. Eggs were impaled with glass microelectrodes (1.2 mm o.d.; WPI, Inc., New Haven, Connecticut, or Radnoti, Inc., Monrovia, California) pulled a few hours before use with a horizontal electrode puller (ISA, Inc., Flushing, New York). When back-filled with 1.5 M potassium acetate (pH 7.0 with HCl), 80–120 MΩ (Baltic eggs) and 60–90 MΩ (Norway, Maine eggs) microelectrodes were used. The bath was grounded with a bridge of 2% agar in seawater or Baltic water.

A Biodyne (Santa Monica, California) AM-2 voltage follower, Nicolet (Madison, Wisconsin) 310 oscilloscope, Narashige micromanipulators, Gould 220 chart recorder (Cleveland, Ohio), and homemade stimulator (designed by Ray Kado) were used to record membrane potential. Baltic and Norwegian eggs were recorded at 50 Hz, so duration data were multiplied by 1.2 to standardize all data to a 60 Hz recording speed. Data were not used if the tip potential changed by more than 3 mV during an experiment. The high-resistance microelectrodes required with Baltic eggs led to about one in five records being unusable with this criterion. The bridge balance was retested during the experiment and readjusted as necessary. The microelectrode was inserted into the egg by a transient increase in the ''negative capacitance'' adjustment of the voltage follower. Recovery to a resting potential of −50 mV (or more negative potential) and an input resistance of 90 MΩ or higher generally occurred within 15 min of impalement. After a steady resting potential was reached, data on membrane input resistance were taken, followed by the addition of sperm to the bath.

Duration of gamete viability. The duration of gamete viability for fertilization in *Fucus* from the Northern Baltic at Fällsvik was evaluated in natural water from the Baltic by releasing gametes from males (n = 3) and females (n = 3) and using these in fertilization assays (5° C, 5 mL, sperm:egg ratio = 100) at different times (0, 2, 4, and 6 h after gamete release) with newly released gametes of the opposite sex. The aging gamete suspensions were kept in 500-mL beakers at 5° C. Another female and male were used in identical assays but in artificial water with marine salinity. Fertilization success was evaluated by staining the eggs with calcofluor white approximately 24 h after the initial gamete release.

Salinity and light effects on egg release from the oogonia. To determine the effects of salinity and light on loosening of the oogonial sheath and release of eggs, oogonia were released from three females from Drivan and transferred to experimental dishes (5 mL) at the salinity and light conditions of each treatment. The media were: 1) artificial water with the same ionic composition as Drivan water (same as Fällsvik), 2) artificial water with the same ionic composition as Drivan water but at 63 mM NaCl, and 3) natural Baltic water from Drivan. The photon flux densities were: 1) 300 μmol photons·m^{-2}·s^{-1} and 2) 120 μmol photons·m^{-2}·s^{-1}. The experiment was fully factorial; all six combinations of these levels of the two factors were used as treatments (three replicates per treatment). Released oogonia were concentrated by allowing them to settle in glass beakers and removing most of the water by aspiration. Approximately 100 μL of concentrated oogonia were transferred to glass dishes under the treatment conditions. The eggs and oogonia were fixed in 70% ethanol 5 h after the treatments and the numbers of oogonia that had or had not released their eight eggs were determined by counting under a dissecting microscope.

Timing of pronuclear migration and the intermediate and slow polyspermy blocks. In order to determine the rate of pronuclear migration, gametes from the Baltic at Askö were released in their natural water (6.5 psu), and the intact oogonia were removed from the eggs with 105-μm nylon mesh. Eggs were fertilized at a sperm:egg ratio of 1000:1, in a 100-mL beaker, at 12° C. Immediately after adding sperm and every 30 min for the following 6 h, an aliquot of eggs was fixed in 1:3 acetic acid:ethanol. For each fixation time, 100 eggs were stained with aceto-iron hematoxylin (Wittmann 1965), and the position of the sperm pronucleus or stage of karyogamy was recorded.

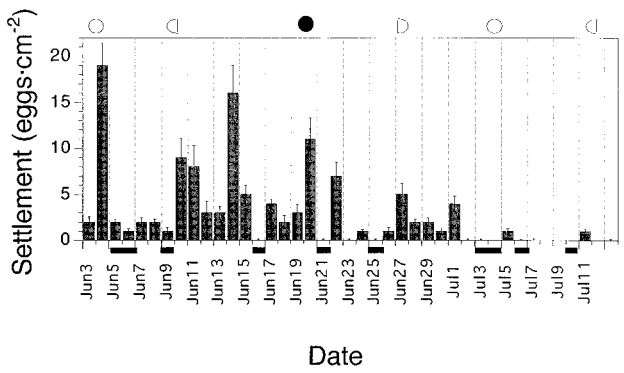

FIG. 2. Daily egg settlement (mean ± SE) for *Fucus vesiculosus* from the Baltic Sea during the reproductive season of 1993 at Askö, Granlösa, showing that high release and settlement of eggs occurred only on calm days. Phases of the moon are shown above the graph. Black bars on the *x*-axis represent days when currents caused movement of receptacles during the natural time of gamete release in early evening.

The timing of the intermediate polyspermy block was determined in gametes from Askö fertilized in petri dishes (n = 3) with 5 mL of natural Baltic water (5° C in a cold room) from Askö, at a sperm:egg ratio of 1000:1. A sample was taken from the petri dish every minute and observed at the microscope until the sperm swam away from the eggs (i.e. the intermediate polyspermy block) and the eggs stopped spinning. For determining this timing with Drivan gametes, eggs in natural water (10° C on a cold stage) were observed at an IM-35 (Zeiss) microscope from the time when sperm was added until the sperm swam away. Individual eggs or three to four eggs per observation were observed in 1995 and 1996 (n = 19 eggs).

The timing of development of the cell wall (i.e. the slow polyspermy block) after insemination of Baltic eggs was determined in eggs from Fällsvik (n = 3 assays) and from Askö (n = 2 assays). Eggs and sperm were released from receptacles from Fällsvik (at 10° C) or Askö (at 12° C) in their natural waters, and fertilizations were performed at sperm:egg ratios of 1000:1 in 100 mL of natural Baltic water from the respective areas. Immediately after adding sperm to the egg suspensions, samples of eggs were pipetted at frequent intervals and stained with one drop of 0.0001% (v/v) calcofluor white, which stains β-linked cell wall polysaccharides such as cellulose and alginate (Callow et al. 1978, Brawley and Bell 1987). The numbers of eggs with and without any calcofluor fluorescence were counted using a Zeiss IM-35 or an Olympus BH-2 fluorescent microscope with UV excitation.

RESULTS

Natural settlement. In the central Baltic (i.e. at Askö), the settlement periodicity did not follow a clear semilunar cycle; instead, high egg and zygote settlement occurred at several times during the reproductive season, exclusively under calm conditions (Fig. 2; see also Serrão et al. [1996b] for gamete release and settlement in the central Baltic in 1994). In the northern Baltic only one single peak of settlement was detected each year during the reproductive period studied at Drivan, and this yearly peak occurred when current velocities were ≤0.03

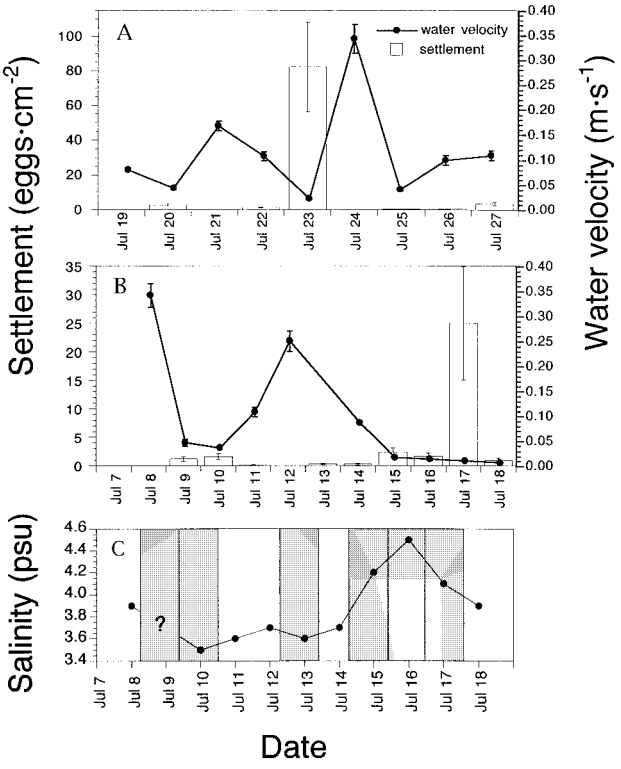

FIG. 3. Daily egg settlement (mean ± SE) for *Fucus vesiculosus* from the northern Baltic Sea (Drivan) and peak current velocities (mean ± SE) quantified during periods of the reproductive season of 1995 (A) and 1996 (B). Settlement between 23 June and 7 July 1996 was zero or close to zero every day (data not shown). (C) Salinities at ca. 4 m depth in the northern Baltic at Drivan during the period of the reproductive season when settlement was observed in 1996. The days when enough eggs were collected for polyspermy analyses are shaded. The question mark indicates that water samples were not collected on 9 July.

m·s^{-1} (Fig. 3A, B). The periods with high water motion at Askö correspond to peak currents of approximately 0.2–0.4 m·s^{-1}, and during calm periods these currents were 0.04–0.17 m·s^{-1} (Serrão et al. 1996b). At Drivan, peak currents varied from 0.02 to 0.34 m·s^{-1} during the reproductive season of 1995 and from 0.01 to 0.35 m·s^{-1} in 1996 (Fig. 3A, B).

The levels of salinity in the *Fucus* stand at Drivan during the 1996 reproductive season were always low, but varied from day to day between 3.5 and 4.5 psu (Fig. 3C). The period with the highest salinities coincided with the first major gamete release of the reproductive season. Release of gametes had been low during the previous 3 weeks when the weather was stormy every afternoon, as detected both by 1) collection of the settlement data—settlement between June 23 and July 7 (data not shown) was zero or close to zero every day—and 2) daily observation of receptacles as information on sex ratios was collected—the conceptacles contained many oogonia and these had cleaved into eight eggs.

Fucus vesiculosus typically grows between 2 and 5 m at Drivan, but most of the bed is at a depth of ca. 4 m. The photon flux density during a calm, sunny

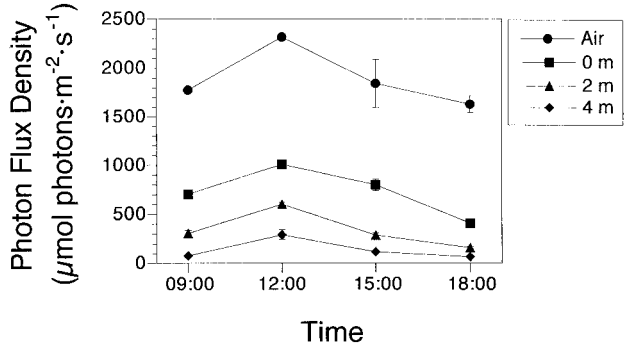

FIG. 4. Changes in light intensity experienced by *Fucus vesiculosus* during a sunny day (18 July 1996) in the Baltic Sea at Drivan at approximately 4 m depth. Light levels at the surface and at 0 m and 2 m are also shown for comparison.

day (18 July 1996) in this bed varied between 70 and 300 μmol photons·m^{-2}·s^{-1} at 4 m and between 160 and 600 μmol photons·m^{-2}·s^{-1} at 2 m (Fig. 4).

Polyspermy levels in natural populations. Natural levels of polyspermy were low (ca. 1%–6%) in the central Baltic (Fig. 5) at times when fertilization was nearly 100% (data on fertilization success for these days appear in Serrão et al. [1996b]). In the northern Baltic, however, approximately 10%–30% of the zygotes were polyspermic (Fig. 6). The natural success of fertilization was variable; the proportion of eggs fertilized varied from 5% to 100% on different days (Fig. 6). Polyspermy was absent when fewer than 10%–15% of the eggs were fertilized, in contrast to the high levels of polyspermy associated in most cases with high levels of fertilization (Fig. 6). The zygotes collected in the field on days with high fertilization success often had extra sperm attached, apparently trapped in the cell wall material. Many fertilized eggs were still weakly attached to other

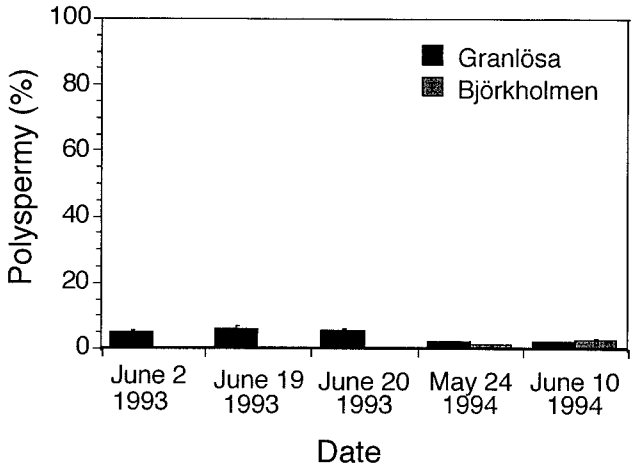

FIG. 5. Natural polyspermy levels (mean percentage of eggs with >1 sperm pronucleus ± SE) of *Fucus vesiculosus* in the central Baltic in two sites at Askö (Granlösa and Björkholmen). Fertilization success in the same days and at the same sites was always nearly 100% (Serrão et al. 1996b).

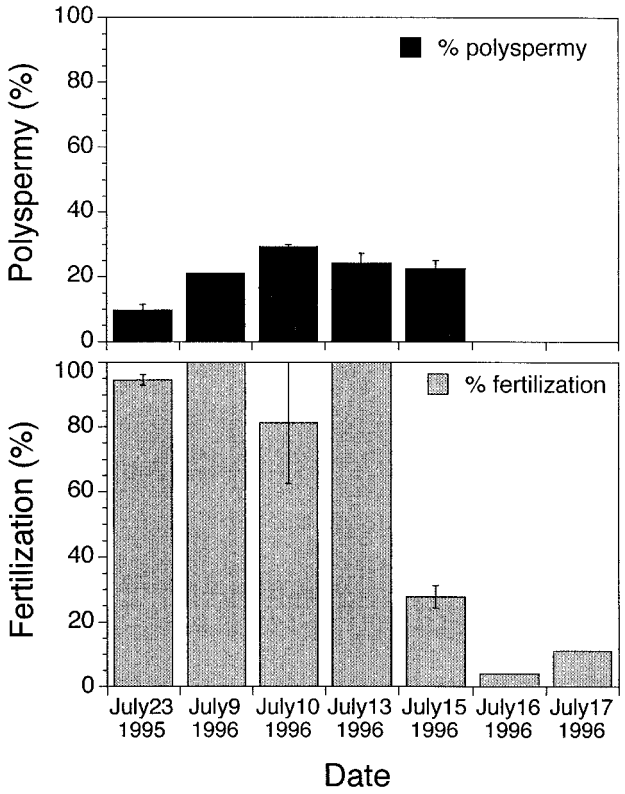

FIG. 6. Natural polyspermy levels (mean percentage of eggs with >1 sperm pronucleus ± SE) and fertilization success (mean percentage of eggs with ≥1 sperm pronucleus ± SE) of *Fucus vesiculosus* in a natural stand in the northern Baltic at Drivan.

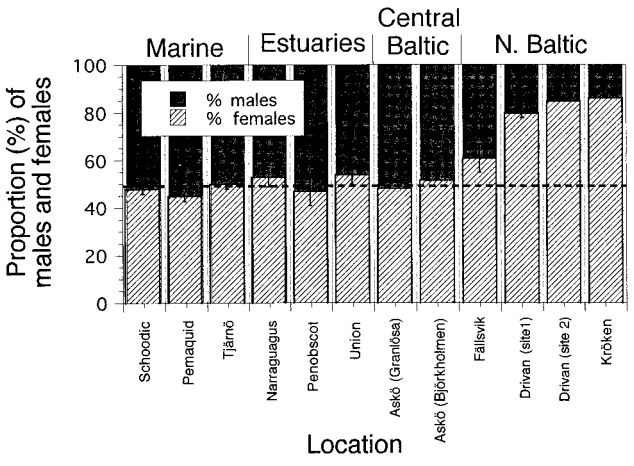

FIG. 7. Sex ratio (mean percentage of females ± SE) in populations of *Fucus vesiculosus* from two marine sites in Maine (Pemaquid and Schoodic), the Skagerrak (Tjärnö, at the boundary between the Baltic and the North Sea), the uppermost limit of *F. vesiculosus* in rivers (Union, Narraguagus, and Penobscot) in Maine (U.S.A.), the central Baltic (Askö), and the northern Baltic (Fällsvik, and at the northernmost limit of *F. vesiculosus* in the Baltic: Drivan and Kråken). The broken line represents 50% of each sex.

eggs from the same oogonium due to incomplete dissolution of the oogonial sheath.

Sex ratios. The sex ratio in natural populations of *F. vesiculosus* (Fig. 7) was skewed toward females in the northern Baltic Sea. There was a trend toward a female bias (61 ± 6% females, mean ± SE, n = 30 sites) at Fällsvik, but it was not significant ($P > 0.05$) due to a higher spatial variability in the sex ratios. The sex ratio was significantly ($P < 0.05$) skewed at the northernmost limit of *F. vesiculosus* in the Baltic, at Drivan (80 ± 2% females, n = 82 sites) and Kråken (86% females). The proportion of females was not significantly ($P > 0.05$) different from 0.5 in the central Baltic (Askö), at the uppermost limit of *F. vesiculosus* in rivers (Union, Narraguagus, and Penobscot), at the connection between the Baltic and the North Sea (Tjärnö), and in one of the exposed marine populations from Maine (Schoodic). At Pemaquid, an exposed marine site in Maine, the sex ratio (45% females) was significantly male biased ($P < 0.05$).

Effect of sodium on polyspermy levels. The incidence of polyspermy in Baltic eggs was affected by the external sodium concentration (Fig. 8). At the natural osmolality (125 mmol·kg^{-1}) and [Cl$^-$] of northern Baltic water and a sperm:egg ratio of 1000:1, more eggs became polyspermic when the concentration of sodium in the water was 1.1 mM than when it was 54 mM (Fig. 8). At an osmolality equivalent to seawater (980 mmol·kg^{-1}), approximately 10% of the eggs became polyspermic at 450 mM Na^+, and this value increased to nearly 20% at 54 mM Na^+. The variable levels of polyspermy at 54 mM Na^+, 125 mmol·kg^{-1} osmolality, were caused by variable fertilization success, probably as a consequence of damage induced when handling the gametes in one replicate, as fewer eggs were fertilized and only 7% of

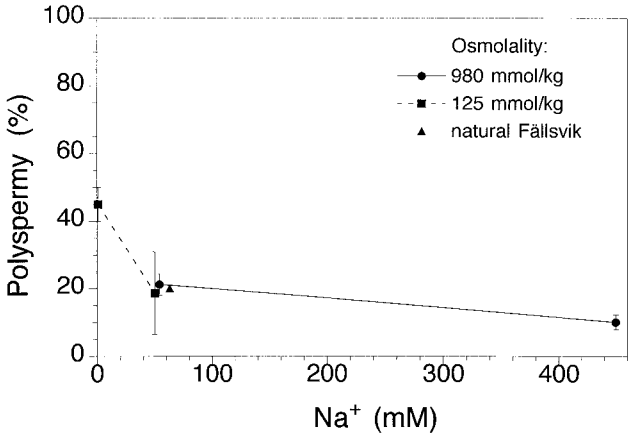

FIG. 8. Polyspermy (mean percentage of eggs with >1 sperm pronucleus ± SE) in *Fucus vesiculosus* from the northern Baltic at Fällsvik increases as the external concentration of sodium decreases (sperm:egg ratio = 1000:1, n = 2). The variable levels of polyspermy at 54 mM Na^+, 125 mmol·kg^{-1} osmolality, coincide with variable fertilization success. (In a replicate where fewer eggs were fertilized, only 7% were polyspermic versus 30% in the other replicate with higher fertilization success.)

those were polyspermic (versus 30% in the other replicate).

Electrophysiology of brackish eggs: Baltic Sea. Data on basic electrophysiological characteristics of Baltic eggs (Table 1) are presented only for Drivan because the osmolalities used in most studies of Fällsvik eggs were higher than natural. There were notable differences between the input resistances of eggs before fertilization in the 2 years in which studies were made, with the 1995 eggs having especially high resistances (Table 1). A range of 70–1870 MΩ (n = 14) was observed in 1995, but the 1996 eggs were less resistive and a range of 110–1010 MΩ (n = 32) was observed. Recording was discontinued in many eggs in both years due to shallow membrane potentials that did not recover following impalement or that depolarized from shallow initial values. Resting potentials measured in the unfertilized egg (Table 1) tended to be more negative than those of marine eggs, both in previous recordings of marine *F. vesiculosus* from Massachusetts (Brawley 1991) and in recordings of marine eggs from the Norwegian coast in this study.

Sperm attached rapidly (less than 1 min after addition to bath) to all eggs from Fällsvik and Drivan. Following attachment, however, a long period typically ensued during which attached sperm failed to elicit an electrical response (Fig. 9). Eggs remained excitable at the end of such periods in response to the passage of small square pulses of current (Fig. 9). In the small number (i.e. four eggs in 1995, one egg in 1996) of eggs in which a fertilization potential (FP) was observed in natural water, a range of times between sperm adhesion and depolarization was observed (0.7–55 min, Table 1). The mean plateau of the FP in those eggs shown in Figure 10A and B is similar to that of marine eggs (ca. −25 mV, Fig. 10C), but the mean for all observations was more negative (Table 1). Eggs in which FPs were observed tended to be exceptionally excitable; 0.03 nA of current elicited a response from one egg (FP of this egg shown in Fig. 10A, excitability not shown). Current was not passed through the egg from which the other complete FP in Drivan natural water (Fig. 10B) was observed, due to its rapid attainment of a steady resting potential at −100 mV. Sperm were added immediately to this egg because we had found that passage of current in such cases (membrane potential [E_m] = −90 to −110 mV) appeared to cause the microelectrode to pop out of the egg. Thus, the slower depolarization in this egg may reflect an artefact due to incomplete recovery from impalement, a possibility that data on resistance would have evaluated. These two eggs were still excitable after repolarization of the FP and still had high membrane input resistance (mean of 2095 MΩ, Table 1). All other eggs showing FP-like depolarizations were ones in which the microelectrode popped out before full repolarization was observed; thus, smaller sample sizes are available for some

TABLE 1. *Electrophysiological characteristics of eggs from two brackish populations and one marine population of Fucus vesiculosus.* $E_{m\text{-}bf}$, membrane potential before fertilization; n.d., not determined.[a]

	Drivan 95	Drivan 96	Bodø 95	Penobscot 95		
				(7–8 psu)	(10–12 psu)	(24–32 psu)
Resistance (MΩ)						
Before fertilization	519 ± 150 (14)	248 ± 31 (32)	141 ± 21 (8)	190 ± 31 (7)	146 ± 9 (7)	252 ± 55 (4)
After fertilization	2095 ± 29 (2)	—	50 ± 8 (4)	76 ± 14 (3)	78 ± 14 (3)	87 ± 12 (2)
Membrane potential (mV)						
E_m before fertilization	−68 ± 4.0 (17)	−58 ± 2.0 (32)	−63 ± 3.0 (8)	−65 ± 3.0 (7)	−62 ± 3.0 (7)	−61 ± 3.0 (4)
E_m after fertilization	−97 ± 5.0 (3)	—	−78 ± 1.0 (3)	−81 ± 9.4 (3)	−79 ± 0.9 (5)	−72 ± 2.0 (2)
E_m plateau of FP	−32 ± 8.0 (3)	−32 (1)	−22 ± 1.4 (4)	−33 ± 1.0 (4)	−24 ± 2.0 (5)	−24 ± 0.4 (4)
Timing (min)						
Time FP more positive than $E_{m\text{-}bf}$	4.6 ± 1.4 (2)	1.0 (1)	6.9 ± 2.2 (4)	8.5 ± 0.2 (3)	14.3 ± 2.2 (5)	5.0 ± 0.3 (3)
Time FP more positive than −45 mV	2.3 ± 0.1 (3)	0.1 (1)	3.8 ± 0.3 (4)	2.1 ± 0.3 (3)	4.6 ± 0.5 (5)	4.4 ± 0.3 (4)
Sperm adhesion to FP	18 ± 11.0 (4)	0.7 (1)	1.3 ± 0.4 (4)	2.5 ± 2.0 (4)	3.4 ± 0.8 (6)	0.2 ± 0.1 (4)
FP to sperm detachment	5.1 ± 1.9 (2)	—	n.d.	2.6 ± 0.3 (4)	5.3 ± 3.3 (5)	1.8 ± 0.1 (4)
Aborted recordings (E_m remaining depolarized following impalement)	28	45	0	4	3	0
Excitable	yes	yes	yes	yes	yes	yes

[a] Values are means ± SE (n).

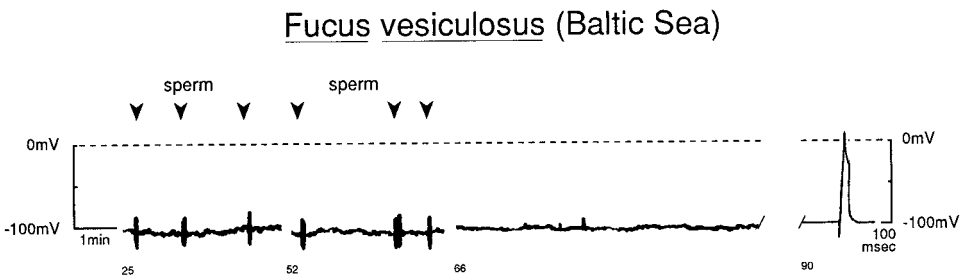

FIG. 9. Fällsvik egg recorded in Fällsvik natural water. Sperm (in natural water) were added at several points (arrows), but depolarizations did not occur and the egg remained excitable when 0.2 nA was passed (right panel).

measurements (Table 1). Membrane resistances at the steady resting potential (MΩ, mV) before fertilization of those eggs from which a partial or full FP was observed were (MΩ, mV): a) n.d., −90; b) 1870, −110; c) 680, −60; d) 627, −85; e) 193, −90. The resting potential became more negative (mean of −97 mV, Table 1) following repolarization of the FP; the calculated equilibrium potentials for K^+ and Cl^- at Drivan based upon chemical analysis of natural water, the intracellular ion concentrations of marine fucoids determined by Allen et al. (1972), and a temperature of 10° C are E_{K^+} = −130 mV and E_{Cl^-} = +20 mV. The rapid repolarization of the Baltic eggs shown in Figure 10 is notable and has not been observed in any previous electrophysiological work on marine *Fucus* (Brawley 1991) or here.

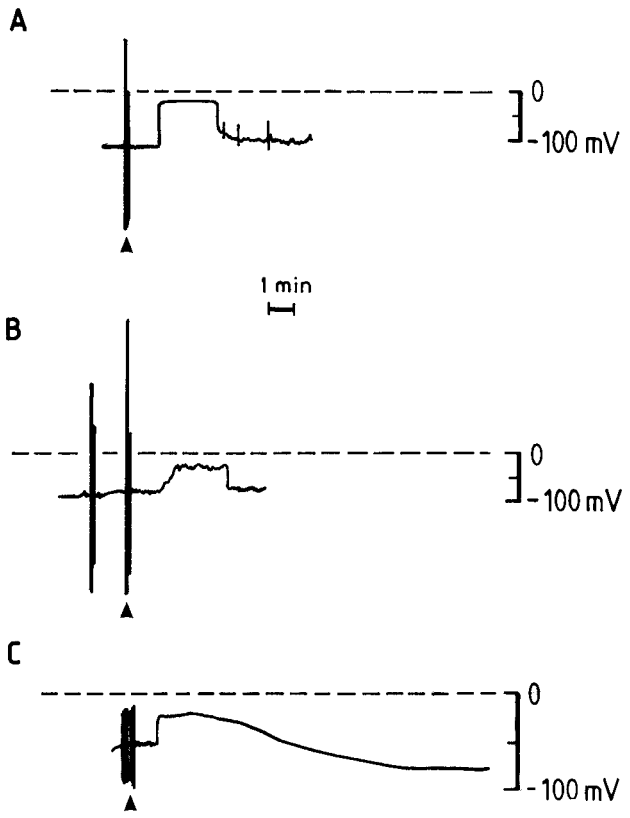

FIG. 10. Fertilization potentials of *Fucus vesiculosus* eggs from the northern Baltic at Drivan (A, B) recorded in Drivan natural water, on 8 and 10 July 1995, respectively, and from Bodø, Norway (C) recorded in seawater. Sperm were added to the bath at the points marked with arrowheads. Note the rapid repolarization of the membrane potential at the end of the fertilization potential in Baltic eggs.

Microelectrodes were withdrawn from the eggs shown in Figure 10 soon after repolarization in order to avoid the potential loss of records due to clogged microelectrodes, which is known to occur in many fucoid eggs during prolonged recording after fertilization. In two eggs from Fällsvik, however, the possibility of further fertilizations is suggested by the occurrence of second (or more) stages of depolarization following repolarization of the first FP (Fig. 11).

Electrophysiology of brackish eggs: estuarine eggs. Fucus vesiculosus is also found at the brackish extremes of estuaries in the western Atlantic, and the electrophysiological properties of *Fucus* eggs from a Maine estuary were examined as a comparison to Baltic eggs (Figs. 12, 13). Eggs did not recover from impalement (i.e. resting potentials remained at about −10 mV) in diluted seawaters ≤7.8 psu (n = 7) with one exception. Although all eggs were excitable, they tended to be less excitable than marine or Baltic eggs and to depolarize more slowly (Figs. 9, 13); a second step of depolarization was sometimes observed (Fig. 12B). The plateau of the fertilization potential in eggs fertilized in 10–12 psu or in 24–32 psu seawaters was similar to that of marine eggs fertilized in seawater, but the fertilization potential in 7.8–8 psu had a more negative plateau and was of shorter duration above the critical value (Brawley 1991) of −45 mV (Table 1). Eggs studied at 7–12 psu fertilized more slowly after sperm attachment than in seawater or as compared to marine eggs (Table 1).

Duration of gamete viability for fertilization. Northern Baltic gametes lost fertilization capability faster in their natural brackish water than in marine water (i.e., artificial seawater) (Fig. 14). The performance of gametes from different individuals, however, was variable; the gametes from some individuals (e.g. female 1, Fig. 14A) were completely inviable as soon as 2 h after release (Fig. 14).

Salinity and light effects on loosening of oogonial sheath and egg release from ooogonia. Salinity influenced the dissolution of the oogonial sheath and consequent release of eggs (Fig. 15). At the salinities found near the northernmost limit of distribution of *F. vesiculosus* in the Baltic (53 mM NaCl), approximately

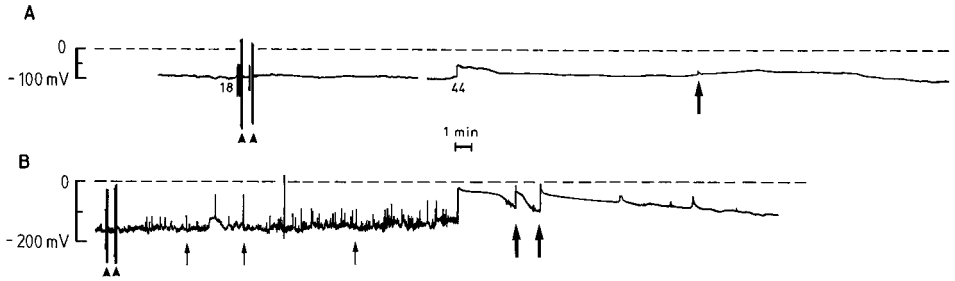

FIG. 11. Fertilization potentials in two Baltic (Fällsvik) *Fucus vesiculosus* eggs. Note the long time following addition of sperm (arrowheads) before a fertilization potential occurs. The egg in (A) lost excitability soon after fertilization, but a second depolarization occurred (arrow), and this suggests the occurrence of polyspermy. Note that the FP occurred 44 min following initial impalement of the egg. The egg in (B) had an unusually negative resting potential, and multiple fertilizations after the first fertilization potential are suggested by the rapid FP-like depolarizations (arrows) following the first. Sharp spikes in the record (small arrows mark examples) were stimulated by passing current through the microelectrode. These were not removed from the V record because of the absence of a corresponding I record (one of the recorder's two channels was damaged during transport to Sweden in 1994). Recordings were made in Fällsvik water at ca. 5% higher than ambient osmolality (supplied with *N*-methyl glucamine chloride).

90% of the oogonia did not release their eggs (Fig. 15). Photon flux density had no effect on the proportion of oogonia releasing eggs at 53 mM NaCl, but at the salinities found more commonly at Fällsvik and on some days at Drivan (≈63 mM NaCl), more oogonia released their eggs at the higher photon flux density (Fig. 15).

Timing of pronuclear migration and the intermediate and slow polyspermy blocks. Pronuclear migration was lengthy (Fig. 16). This corresponds both to a delay in the beginning of migration of the sperm pronucleus and to a slower rate of migration through the egg cytoplasm compared to estuarine *F. ceranoides* (Brawley 1992) and marine *F. vesiculosus* (Brawley and Quatrano 1979). Migration did not begin until 2 h postfertilization; prior to this, the sperm pronucleus was found in the cortex (i.e. at ca. 6 μm below the egg surface; the eggs are ca. 60–90 μm in diameter).

In the central Baltic Sea, the intermediate block against polyspermy (i.e. the time after fertilization when the extra sperm swim away from the eggs) was evident by 7.3 ± 0.3 min (n = 3 batches of eggs from replicated fertilization assays) after the sperm were added. This corresponds to the time when most sperm were observed to swim away from the eggs, although a few remained attached.

In various laboratory assays of the slow block (i.e. cell wall formation), staining of eggs with calcofluor white was detected over a variable time period: 9–30 min in Askö eggs, 10–30 min in Fällsvik eggs (Fig. 17). The proportion of Fällsvik eggs that were stained increased with time for 1 h in one replicate assay and for approximately 3 h in the other before

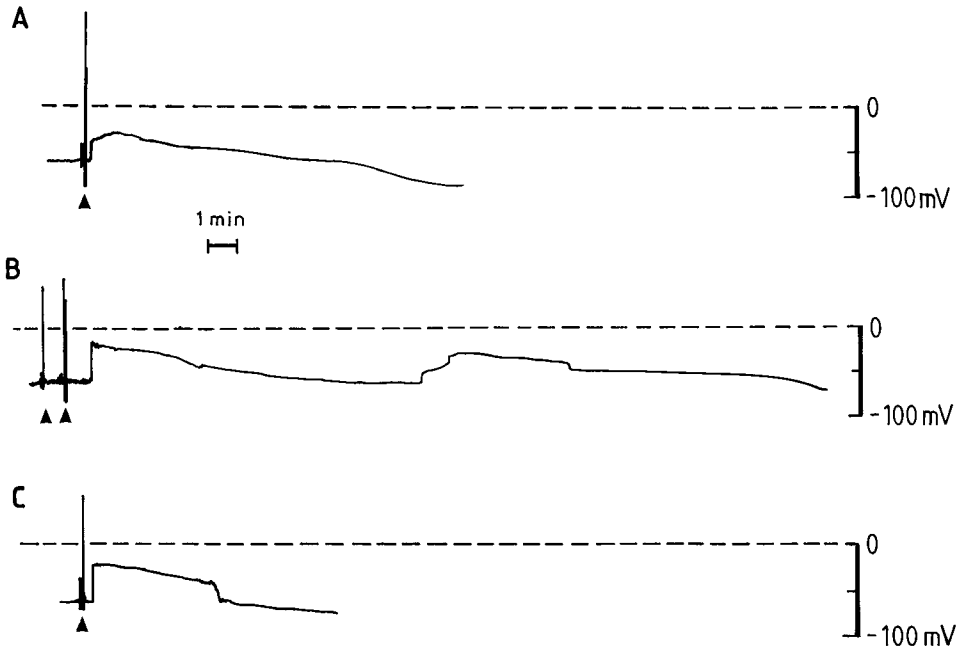

FIG. 12. Fertilization potentials of estuarine eggs (Penobscot River, Maine) of *Fucus vesiculosus* in 8 psu (A), 11 psu (B), and 32 psu (C) seawater (or dilutions of seawater). The fertilization potentials of (B) and (C) are similar to those of marine eggs, although a second depolarization occurred in (B), suggesting polyspermy. In (A), the depolarization is less rapid and the first portion of the FP is about 10 mV more negative than the plateau, reached about 1 min after rise of the FP.

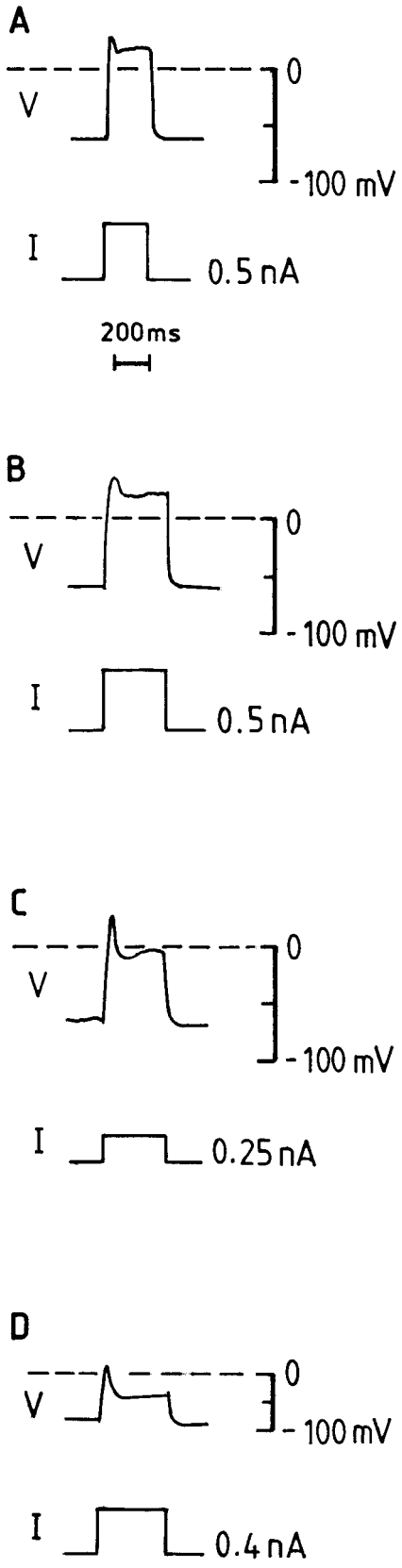

FIG. 13. Estuarine *Fucus vesiculosus* eggs (A–C) are excitable (V) in response to passage of square pulses of current (I), as are Baltic eggs (D). The (A–C) panels were taken during the recording of the eggs shown in Figure 12 (A–C, respectively). Excitability is reduced at lower salinities and typically required pulses of 0.5 nA to demonstrate (compare A and B with C). (D) Drivan egg in Drivan water (4 psu).

reaching a plateau with 30%–40% of the eggs fertilized, suggesting high variability in the time of fertilization or rate of cell wall formation. The final proportion of eggs with cell wall, measured 7–10 h after addition of sperm (not shown in Fig. 17), was 55% in the replicate with highest values and 33% in the other. Additionally, the intensity of calcofluor white staining took nearly 24 h to become as bright as is common in marine eggs within ca. 15 min after fertilization (Brawley 1991). Organelles were still visible through the lightly stained cell surface both in the central (Askö) and northern (Fällsvik) Baltic during the first 24 h after fertilization.

DISCUSSION

The variable levels of polyspermy and fertilization success in natural stands in the northern Baltic indicate that successful fertilization may depend more on stochastic effects, related to prevailing oceanographic and meteorological conditions (e.g. salinity, water motion), than in more saline populations. Polyspermy can cause the loss of approximately a quarter of the zygotes in natural stands of *F. vesiculosus* in the northern Baltic Sea (at ca. 3.5–4.5 psu). Few (ca. 1%–6%) eggs became polyspermic in the central Baltic Sea (6.5 psu). Thus, the loss of potential recruits to polyspermy occurs mostly at the salinities experienced near the limit of the continuous populations of *F. vesiculosus* in the Baltic.

The populations of *F. vesiculosus* in the northern Baltic at Drivan live at the limits of their gametes' salinity tolerance (Serrão et al. 1996a); therefore, it is to be expected that even the small variations in salinity that occur in the northern Baltic at Drivan (i.e. 3.5–4.5 psu) would affect fertilization success by reducing it when salinity was lowest. However, the results from the 1996 reproductive season contradict these predictions: natural fertilization in these populations was successful at salinities as low as 3.5 psu, although polyspermy was high, and the lowest fertilization success was observed when salinity was maximal within this range. Therefore, small-scale changes in salinity within the natural range of 3.5–4.5 psu are not enough to explain why fertilization in these populations nearly failed on some days. A possible cause for these patterns is reduced gamete viability due to a too-long period of storage in the receptacles while the environmental conditions prevented release. Gamete release in *Fucus* requires calm conditions in the light (Pearson and Brawley 1996, 1998, Serrão et al. 1996b, Pearson et al. 1998). During the 1996 reproductive season when most of the samples were taken, however, there was an ex-

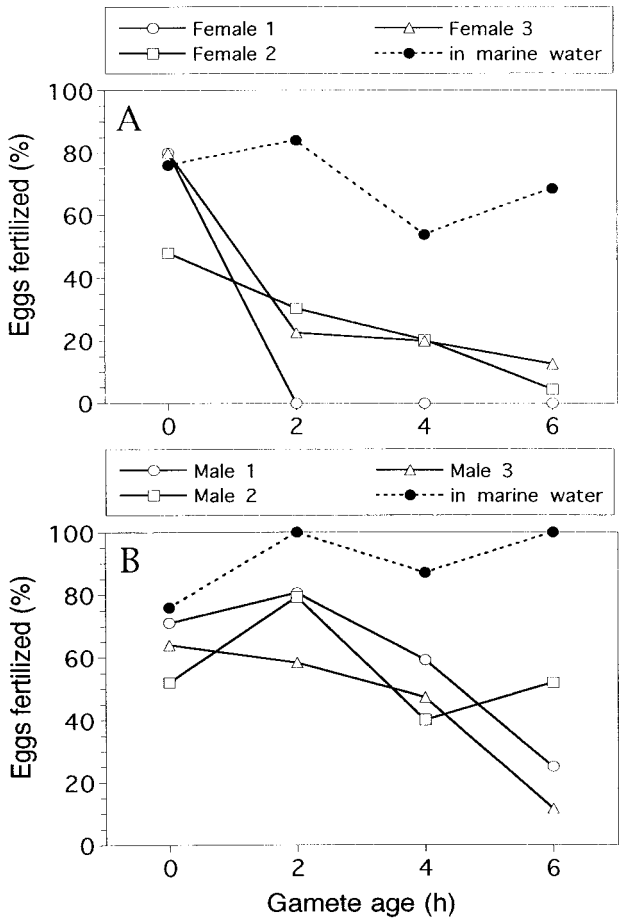

FIG. 14. Duration of gamete viability for fertilization in *Fucus vesiculosus* from the northern Baltic at Fällsvik, evaluated as the proportion of eggs fertilized when the eggs (A) or sperm (B) were kept in their natural water at 5° C for 0–6 h following their release. Each line represents an independent assay. Gametes from one female (A) and one male (B) were allowed to age in marine artificial water for comparison.

tended period of stormy and/or turbulent conditions for approximately 3 weeks. The few days that were calm were also overcast, and gamete release was low. During these days, the few eggs collected in the field had high fertilization success, but many were polyspermic. When the first day with favorable conditions for gamete release (i.e. a calm, sunny day) occurred, most gametes had been stored in the receptacles for a long time (ca. 3 weeks). The characteristics of fertilization are altered in gametes that have been aged; for example, barriers to cross-fertilization between different *Fucus* species seem to be eliminated as gametes age (Bolwell et al. 1977), and in starfish (Miyazaki and Hirai 1979) and mussels (Togo et al. 1995), eggs aged after release are more susceptible to polyspermy. Thus, low fertilization success after lengthy bad weather that inhibited gamete release may have been caused by over-mature gametes. This hypothesis is supported by the fact that few eggs could be fertilized in simultaneous laboratory experiments.

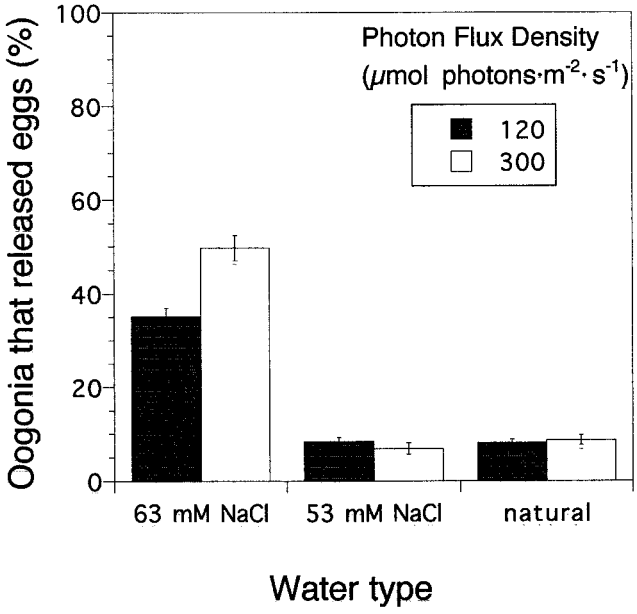

FIG. 15. Effect of [NaCl] and light intensity (120 or 300 μmol photons·m^{-2}·s^{-1}) on the proportion of eggs that are released from Drivan oogonia. The media were artificial northern Baltic water (see Methods) with 53 or 63 mM NaCl and natural water collected from the northern Baltic, which contained approximately 53 mM NaCl on this collection date.

High fertilization success and polyspermy on days when few gametes were released suggest that high sperm:egg ratios at fertilization can be achieved even outside of the main peaks of gamete release. This is somewhat surprising given that the female-biased sex ratios in the northern Baltic are likely to contribute to a lower sperm:egg ratio that, in addition to reducing polyspermy, would also reduce fertilization success. In the northern Baltic, fertilization may still be facilitated by the natural low salinities, which affect the ability of the oogonia to release eggs (Serrão et al. 1996a). Oogonia from Drivan

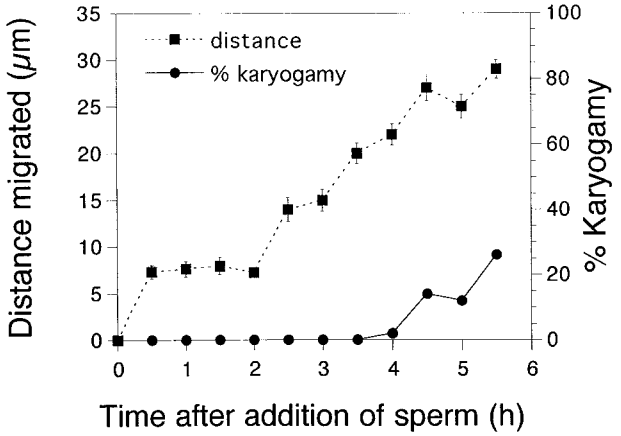

FIG. 16. Rate of migration of the sperm pronucleus and proportion of eggs that had started karyogamy at 0–6 h following mixing of sperm and eggs from *F. vesiculosus* from the Baltic Sea at Askö.

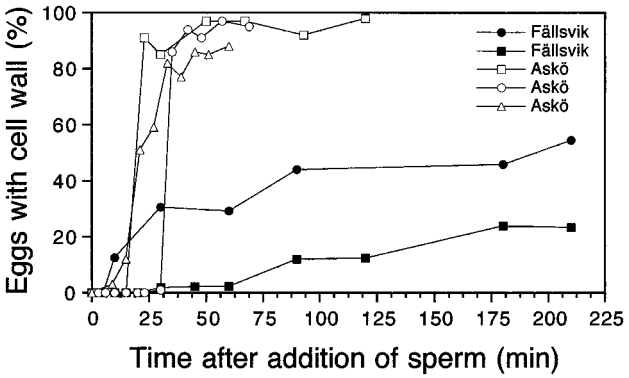

FIG. 17. Proportion of eggs from the central Baltic (Askö) and the northern Baltic (Fällsvik) that had started secreting cell wall material (i.e. the slow block against polyspermy) at various times after adding sperm to the eggs in their respective natural Baltic waters (ca. 4 psu for Fällsvik, ca. 6.5 psu for Askö). Each line represents an independent assay.

take a longer time to release their eggs at 4.0 psu than at 4.6 psu, and the sperm are attracted preferentially to oogonia compared to eggs, probably because the concentration of pheromone is higher around the oogonia. Thus, when each oogonium finally releases its eight eggs *in situ,* many sperm may already have concentrated near them. The observation that zygotes were often attached to each other and/or had oogonial sheaths suggests that fertilization might occur while the eggs are still inside the inner layer of the oogonium; this has been observed in other fucoid algae (Müller and Gassmann 1985, Brawley 1990b): as soon as a small tear forms in the oogonial sheath, sperm may enter the oogonia. Eggs lyse soon after being released at these low osmolalities (Serrão et al. 1996a), so fertilization of eggs still partially within the oogonium would be protective, aside from the possibly negative effect on the sperm: egg ratio leading to an increase in polyspermy. This hypothesis is supported by the fact that during the days when fertilization success was high, many zygotes collected in the field had an irregular shape (i.e. as when they are inside the oogonium), were still attached to each other, and/or had parts of the oogonial sheath still around them.

Gametes of *F. vesiculosus* from the lowest salinities in the Baltic (ca. 4 psu) where extensive beds of fucoids are found are more tolerant of brackish conditions than eggs from the Penobscot River estuary, where fertilization appears to cease at salinities below 8 psu. Baltic eggs are more excitable and some depolarize rapidly when the fertilization potential is triggered. Another notable characteristic of eggs from the northern Baltic is their tendency to have high input resistances. Eggs in both locations, however, appear to be susceptible to polyspermy because they repolarize substantially before sperm detachment has occurred, unlike marine eggs (Brawley 1991, these Results). It is unknown, however, whether estuarine eggs are released during the beginning and end of the high tide, which is the only time during which salinity is this low. Salinities are 15 psu or higher (data not shown) during most of the tidal cycle at this site in the estuary, and at 10–12 psu, the time the egg is depolarized to potentials more positive than −45 mV is greater than the time from rise of the FP to sperm detachment. At salinities of 10 psu and above, the plateau of the fertilization potential is also similar to that of marine eggs, whereas it is more negative at ca. 8 psu.

An important question is why a fertilization potential could be observed in so few Baltic eggs. Although Baltic sperm at these low salinities swim more slowly (and sometimes erratically) than marine sperm (Serrão et al. 1996a), Baltic sperm found eggs in the recording dish quickly and attached to them. This suggests that pheromone is secreted as in marine eggs (Maier and Müller 1986) to form a steep gradient from the egg that directs local sperm taxis. Most eggs at ca. 4 psu, however, do not fertilize but remain excitable after having sperm bound for nearly an hour. These eggs could be 1) old, 2) lack complete sperm receptors, 3) be under osmotic stress such that the arrangement of receptors in the membrane is inhibitory, or 4) be damaged by impalement. The eggs certainly appear to be fragile, requiring use of smaller microelectrode tips, and at the low ionic strength of Baltic water, a sparser coating of poly-D-lysine to immobilize eggs. Northern Baltic (i.e. from Fällsvik) eggs and sperm were much more sensitive to aging in their natural water than in water with an osmolality equivalent to that of seawater, with eggs becoming much less fertilizable after 0–2 h of aging and sperm after 4–6 h of aging. Fertilization potentials in these eggs were observed more commonly when gametes were released and studied in waters with higher than natural osmolalities. Eggs aged in their natural water also begin to lyse (Serrão et al. 1996a). Release of eggs from oogonia also proceeds more slowly at a salinity of 4.0 psu compared to 4.6 psu, which means that there are likely to be important stochastic environmental effects upon fertilization in the northern Baltic at Drivan, given that salinity in the bed was observed to fluctuate between 3.6 and 4.5 psu within 1 week during the reproductive season. Sperm continue to move at their site of attachment as if trying to achieve better binding; this suggests the possibility that in Baltic eggs, which have a larger diameter (Serrão et al. 1996a), receptor orientation in the egg membranes might be abnormal, making full binding (and activation) unlikely. Osmotic stress could cause this to be a major problem in eggs as they age. Thus, several lines of evidence suggest that fertilization of Baltic eggs must occur soon after their release from oogonia to be successful. Furthermore, electrophysiological recordings, in which −90 to −110 mV resting potentials were reached soon after impalement and FPs were observed in natural water, were obtained from Drivan eggs over

only one period: 7–11 July 1995, although recording with freshly collected and released materials spanned mid-June through late July in 1995 and 1996. This suggests a seasonally-narrow reproductive window.

The potassium conductance of the marine fucoid zygote is higher than that of the egg (Taylor and Brownlee 1993). This also appears to be true for Baltic and estuarine eggs, which approach their Nernst E_K as zygotes. Interestingly, the reduced excitability of estuarine eggs is similar to the effect of low external Ca (1 mM) on marine eggs in seawater observed by Taylor and Brownlee (1993). Influx of Ca is actually increased at 1 mM Ca_{ext} (Robinson 1977), and under such conditions, excitability should be diminished. This will lead to a slower rise of the fertilization potential, a characteristic of estuarine but not most Baltic eggs. Most Baltic eggs are excitable and depolarization was usually rapid when a FP occurred; these should be more protective against polyspermy than the slower depolarizations observed at 7.8–8.0 psu in estuarine eggs. Measurement of the intracellular ion concentrations of northern Baltic eggs are required to understand if these have phenotypically or genotypically diverged to values that are lower than those of marine fucoids. We were unable to determine the intracellular ion concentrations of Baltic eggs due to the eggs being so fragile that some always ruptured, releasing their ions into the wash water, but this is a possibility that should be further investigated in future studies.

How do Baltic eggs achieve rapid depolarization in brackish water? One possibility is the unusually high membrane resistance of many Baltic eggs at 4 psu, as in the case of Miyazaki's (1979) findings for starfish eggs. Starfish eggs become much more resistive when mature than when either immature or over-mature. Consequently, the increase in Na^+ influx caused by the fertilizing sperm causes depolarization to occur rapidly, which prevents polyspermy in mature eggs, whereas immature and over-mature eggs suffer polyspermy associated with a slower rate of depolarization (Miyazaki 1979, Miyazaki and Hirai 1979). Even in the one Drivan egg in which rapid depolarization to −25 mV was observed, the rapid and very steep repolarization after ca. 2 minutes will leave the egg subject to polyspermy, due to the slow appearance of the intermediate and slow polyspermy blocks. Thus, in the absence of a sustained depolarization, mediated by an ion other than Na^+, monospermy might depend upon the skewed sex ratio of Baltic populations at their salinity extremes. Determinations of sperm:egg concentrations in the water column are needed to confirm the anticipated effect of the skewed sex ratio.

In the Baltic Sea, the slow block against polyspermy was first detected many minutes (9–30 min) after the eggs were inseminated. This might be caused by a delay in sperm:egg fusion, but the weak cell wall staining (see Results) demonstrates that the egg's capacity to secrete a cell wall quickly after fertilization also seems to be impaired. This may not result in higher polyspermy levels if the intermediate block against polyspermy is effective. However, the intermediate block also appeared slow (≤7 min) compared to marine eggs (Brawley 1991). This timing is important because the egg membrane would have repolarized to levels permissible for fertilization before 7 min passed. In marine *F. vesiculosus,* sperm swim away from the eggs 1–3 min after fertilization (Brawley 1991).

Other cellular processes also take longer in Baltic *F. vesiculosus.* Migration of the sperm pronucleus takes several hours longer than in marine *F. vesiculosus,* in which karyogamy starts 2 h after fertilization (Brawley and Quatrano 1979). In Baltic *F. vesiculosus* it takes at least 2 h for the sperm pronuclei to begin to migrate; an additional ≥3 h is required for karyogamy. The delay in initiation of pronuclear migration may be caused by slower polymerization of microtubules required for pronuclear migration (Brawley and Quatrano 1979, Swope and Kropf 1993). The rate of many processes may be limited by [ATP] due to the increased demands of osmoregulation under brackish conditions; respiratory oxygen consumption by these receptacles is unusually high (Samuelsson, pers. observ.).

It is difficult to interpret the female-skewed sex ratios in the northern Baltic populations because the basis for sex determination in fucoids is unknown. The female bias may occur due to 1) production of gametes that have a female-biased sex determinant, 2) lower fertilization of putative male-determining gametes, or 3) reduced survival of males at prereproductive stages. The last phenomenon appears to explain a bias in the sex ratio across depth in surfgrass (Williams 1995). In other organisms, both unexplained populational variation in the sex ratio within a species (e.g. review by Pearse and Cameron 1991) and environmental effects on sex determination and sex ratio (e.g. Crawford and Balfour 1983 and reviews by Lloyd 1974, Ghiselin 1987, Pearse and Cameron 1991) are known. Sex ratios in some organisms vary during the reproductive season (e.g. Barrett and Helenurm 1981), but the consistency in the female bias throughout the reproductive season in the northern Baltic was notable. The female bias in the northern Baltic was not a discrete effect; that is, present or not. Instead, fewer males were found progressively toward the north. Serrão et al. (1996a) suggested that the northern limit of *F. vesiculosus* in the Baltic might be determined by the negative effects of low osmolality on fertilization, because fucoid eggs do not develop parthenogenetically. The present results raise other possibilities: 1) a salinity level at which the sex ratio would eventually approach 100% females, an unsustainable population as parthenogenesis does not occur, 2) susceptibility of gametes to over-maturation, and 3) higher levels of polyspermy.

Organisms that live at the boundaries of their species' distribution can be affected more severely by external constraints. The northern Baltic is one such area, where there is a fragile environment for the reproduction of *F. vesiculosus*. This work demonstrates some adaptations to brackish conditions (e.g. electrophysiological characteristics of Baltic versus estuarine eggs), but low salinity impairs the gametes' ability for fertilization in many ways (see Serrão et al. 1996a, these Results). Sensitivity to water motion and the light regime leads to synchronous gamete release but, during prolonged stormy periods, may cause the gametes to become over-mature prior to release and reduce their capacity to be fertilized. When high fertilization occurs, many zygotes may die from polyspermy (e.g. caused by the low $[Na^+]$). Thus, there may be years when few germlings are produced in the northern Baltic, and these may be consumed by the abundant populations of grazing amphipods and snails present in the area that usually feed on adult *Fucus* and filamentous algae (Kangas et al. 1982). In marine environments, high grazing pressures may be outweighed by the large number of embryos.

In conclusion, this study has contributed to our knowledge of processes that may influence the reproductive success of species with external fertilization. Predictions that polyspermy would rise at the lowest salinities have been confirmed in this study. However, these apparent effects of salinity on polyspermy are confounded with the effects of salinity and other factors on the success of fertilization, because any factor that decreases the probability of an egg being fertilized at all (fertilization success) also decreases the probability of it being fertilized by more than one sperm (polyspermy). Information on the natural concentrations of sperm surrounding an egg at the moment when fertilization takes place under a variety of environmental conditions would help to interpret variations in polyspermy levels in nature. Future work should test these hypotheses: 1) that the reproductive success of these boundary populations depends on windows of opportunity when there are favorable combinations of several interacting factors and 2) that natural polyspermy levels in these boundary areas depend not only on effects of low sodium on the electrical polyspermy block, but also on effects of the same factors that cause daily fertilization levels in *Fucus* stands to vary from nearly 0% to 100%. Such key interacting processes determining fertilization success and polyspermy levels in this area may be 1) effects of salinity, population density and sex ratio, synchrony of release, and water motion on the concentration of viable sperm surrounding the eggs during the periods when fertilization takes place and 2) the effects of low salinities on the ability of gametes to fuse and on the fast block against polyspermy. The reproductive success of these boundary populations appears to be dependent on a delicate juxtaposition of physical and chemical conditions that may produce large annual variations in fertilization success.

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