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RESEARCH ARTICLE

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Effects of water motion on propagule release from algae with complex life histories

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Abstract Reproductive marine algae with complex life histories may respond differently to water motion depending upon whether the spore-producing or gamete-producing phase is considered. Two such species, the kelp *Alaria esculenta* (L.) Greville (Phaeophyceae) and *Ulva lactuca* L. (Chlorophyta), were examined experimentally in the laboratory. The kelp was collected in April–June 2000 and *U. lactuca* was collected in July–August 2000, from four intertidal habitats at Schoodic Point, Maine, USA. Orbital shakers were used to generate water motion. Sporophylls of *A. esculenta* released more zoospores under shaken versus calm conditions, whereas fewer antheridia on the microscopic male gametophytes released sperm under shaken versus calm conditions when male and female gametophytes were placed together. However, antheridial sperm release was equivalent when male gametophytes were exposed to undiluted media from dense cultures of mature female gametophytes under shaken versus calm conditions. These data suggest that water motion inhibited sperm release by diluting the sperm-releasing pheromone produced by ripe eggs below a threshold required to cause sperm release. Water motion stimulated both gamete and zoospore release from *U. lactuca*. This is the first report in an alga of stimulation of gamete release by increased water motion, but it is notable that parthenogenesis occurs in *Ulva* spp.; thus, gametes may develop into gametophytes (1 N) or parthenosporophytes (2 N). This study demonstrates that water motion has strong effects upon algal reproduction, but that the effects may vary between species, possibly due to their different life histories.

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Introduction

Recent studies of fertilization success in a variety of marine taxa fail to support predictions (e.g. Pennington 1985; Denny and Shibata 1989; Levitan et al. 1992) of widespread sperm limitation and reproductive failure in marine organisms. High fertilization success has been found in many different species in studies of natural populations and processes (e.g. Petersen 1991; Babcock and Mundy 1992; Babcock et al. 1992; Brawley 1992; Petersen et al. 1992; Sewell and Levitan 1992; Serrão et al. 1996; Kaczmarska and Dowe 1997; Engel et al. 1999). Meanwhile, experimental studies have focused on factors such as gamete longevity and mode of release (e.g. Serrão et al. 1996; Pearson and Brawley 1996, 1998; Pearson et al. 1998; Meidel and Yund 2001; Yund and Meidel 2003), and have used better model parameters (e.g. Denny et al. 2002; Farley 2002) to understand fertilization success. Only further studies of natural fertilization success can resolve the continuing debate (e.g. Levitan 2002; Wahle and Gilbert 2002; Yund and Meidel 2003) on levels of fertilization success in species such as sea urchins, but sperm competition appears common in nature in marine organisms (review by Yund 2000; Berndt et al. 2002; Franke et al. 2002). It is, however, likely that many species are potentially affected by both sperm competition and sperm limitation in some ecological situations and certainly in their evolutionary histories. Thus, it is of particular interest to continue mechanistic explorations of traits that increase fertilization success. One trait known to be particularly important in some marine algae is acute sensitivity to higher levels of water motion, where increased motion would dilute gametes and decrease fertilization success. Fucoid algae release gametes only under calm or nearly

calm conditions (e.g. Serrão et al. 1996; Berndt et al. 2002) due to detection of water motion via chemical cues in the boundary layer (Pearson and Brawley 1996, 1998; Pearson et al. 1998). However, many marine algae have complex life histories, and these typically include more than one type of reproductive propagule. Here, we examine the effect of water motion on gamete release in such algae.

Life history could have an important effect upon reproductive responses to water motion in algae. For example, Bell (1997) hypothesized that haploid macrothalli become specialized as gametophytes over evolutionary time by releasing chemotactic gametes into low-velocity environments. The resulting zygotes would disperse short distances and germinate into diploid macrothalli, which release spores into high-velocity environments to achieve dispersal. This hypothesis can be tested in kelps, which have a heteromorphic life history consisting of a large sporophyte and microscopic male and female gametophytes. A pheromone produced by mature eggs regulates sperm release and guidance to the egg (Lüning and Müller 1978), which is retained on the female gametophyte. Here we test the responses of kelp sporophytes and gametophytes to water motion using the perennial kelp *Alaria esculenta* (Sears 2002), in which sporangia develop on multiple pairs of sporophylls at the base of the large blade rather than on the blade proper. This provided us with a manageable experimental unit for laboratory studies.

We also tested reproductive responses in the green alga *Ulva lactuca* (''sea lettuce'', an annual), which has an isomorphic alternation of generations. *U. lactuca* gametes are positively phototactic, whereas zygotes and zoospores are negatively phototactic (e.g. Smith 1947; Hiraoka et al. 2003). Parthenogenesis occurs in at least two species of *Ulva*, including *U. lactuca*, and the rarity of sporophytes suggested to Løvlie and Bryhni (1978) that fertilization is rare in *Ulva* spp. ''Parthenogenesis'' also results from sloughed vegetative cells of the gametophyte that settle and germinate (Bonneau 1978). Periodic gamete release that is synchronous with spring and neap tides has been observed in several Ulvales, including *U. lactuca*; although the timing of gamete release varies between localities with respect to the tidal cycle, synchrony over 1–7 days within individual locations is common (Smith 1947; Sawada 1972; Okuda 1984; Okuda and Yamasaki 1987). This suggests that the algae may possess a mechanism to detect environmental variability that influences fertilization success. Despite being isomorphic, *U. lactuca* sporophytes versus gametophytes might demonstrate different responses to water motion during propagule release. Other studies of isomorphic species of algae have demonstrated different responses to environmental factors in some cases (e.g. Destombe et al. 1993), but not in others (e.g. Littler et al. 1987). Recent theoretical work (Hughes and Otto 1999) supports the possibility that even slight differences in isomorphic individuals can support continued selection of a biphasic life history. Whether isomorphic or heteromorphic, algal gametes must fuse to produce a zygote, and hence dilution of gametes by high water motion would theoretically lead to low fertilization success without respect to life-history differences.

Here, we determine whether water motion is inhibitory or stimulatory to gamete and zoospore release in *A. esculenta* and *U. lactuca*. Using *A. esculenta* as a model system, we tested Bell's (1997) hypothesis that heteromorphic alternation of generations is a strategy that specializes the sporophyte as a dispersal agent and the gametophyte as the agent of genetic recombination. Under this scenario, we predicted that water motion would stimulate or increase zoospore release while inhibiting or decreasing gamete release. We made the same prediction for *U. lactuca*. Although it is isomorphic, the fact that it exhibits synchronous gamete release around spring and neap tides (Smith 1947; Sawada 1972; Okuda 1984; Okuda and Yamasaki 1987) led us to hypothesize that gametophytes might react to water motion as fucoid algae do (e.g. Serrão et al. 1996; Berndt et al. 2002), whereas the sporophytic phase would be adapted for dispersal.

Materials and methods

Source of materials

Pairs of mature sporophylls (one pair per individual) were collected from six haphazardly selected individuals of *Alaria esculenta* (L.) Greville at Schoodic Point, Maine, USA, on 16 April (tidepool), 7 May (tidepool), 2 June (subtidal), 14 June (subtidal), 23 June (subtidal), and 7 July (subtidal) 2000. Sporophylls were cut from the stipe with a knife and transported to the laboratory in plastic bags on ice. Materials were collected near the end of the day, and experiments (see below) were set up immediately to minimize disruption of the natural photoperiod.

Most experiments using gametophytes of *A. esculenta* were set up from established cultures of gametophytes that were obtained initially by releasing zoospores from mature sporophylls of six individuals collected haphazardly at Schoodic Point. The zoospore concentration was determined with a hemocytometer, and zoospores were then plated onto 2×2 cm pieces of glass slides within Petri dishes containing sterile seawater and 1 mg l^{-1} GeO_2 (Sigma Chemical, St. Louis) at a concentration that provided 1–10 spores mm^{-2} and grown at 9°C. After 2 weeks, cultures were placed in Provasoli's enriched seawater (PES, Provasoli 1968), which was changed every 2–3 weeks. When male and female gametophytes could be distinguished (i.e. after about 2 weeks), they were separated with a micropipette and cultured separately in deep dish culture dishes containing 250 ml PES for 5 months to promote vegetative growth at 9°C under constant light at 2 μmol photons m^{-2} s^{-1} (Lüning and Neushul 1978). When gametophytes were needed for experiments under different treatment conditions (e.g. calm versus shaken), vegetative masses of male and female gametophytes were fragmented separately to filaments, two to eight cells long, in 200 ml PES for 2 min using a Waring blender (Vadas 1972), and then plated onto Cyclopore (1.0 μm, clear) polycarbonate filter membranes (Whatman Int., Maidstone, UK, Cat. 7091–2510; Graham 1999) at a final concentration of 5 fragments mm^{-2} in 50 ml PES per Petri dish. In a few gametophyte experiments, zoospores were plated directly onto Cyclopore membranes and placed under experimental conditions (see below) without being maintained for long intervals in the laboratory in vegetative condition.

Ulva lactuca was collected randomly using a transect line and a random numbers table on spring tides at Schoodic Point, Maine, USA, on 12 August, 24 August, 10 September, and 26 September 2000 for four separate experiments. A total of 18 thalli from exposed shores in the lower intertidal zone were collected each time, removing the entire thallus from the substrate at the holdfast and transporting algae back to the laboratory in plastic bags in a cooler. All collections were made during afternoon low tides, and experiments were started immediately to minimize disruption of the photoperiod. Epiphytes were removed from the thalli. Individuals were spun in a salad spinner to remove excess water and weighed before being placed in 250-ml flasks containing 200 ml sterile seawater. Experiments were conducted in a walk-in culture chamber at 13°C and 70–80 μmol photons m^{-2} s^{-1} (16 h light:8 h dark cycle).

Characterization of water motion

Water motion was determined by the method described in Pearson et al. (1998). Measurements were made with a pulsed doppler flowmeter (Crystal Biotech CBI 8000, model PD-10). A 2-mm crystal transducer was placed at 45° to the direction of the water flow. Ground black pepper was used as a particle to reflect the acoustic signal in a 1-l Erlenmeyer flask containing 900 ml seawater on an orbital shaker (VWR) at 170 rpm (*A. esculenta* sporophyte experiments), in a 25-mm deep Petri dish containing 50 ml seawater on an orbital shaker at 100 rpm (*A. esculenta* gametophyte experiments), or in a 250-ml Erlenmeyer flask containing 200 ml seawater on an orbital shaker (VWR) at 170 rpm (*U. lactuca* experiments). Output was recorded on a high-speed chart recorder (Gould, model 200). In all cases, the flow was oscillatory, consisting of 3 cycles s^{-1} at −1 to 20 cm s^{-1} (*A. esculenta* sporophyte experiments), 2 cycles s^{-1} at −1.2 to 14.0 cm s^{-1} (*A. esculenta* gametophyte experiments), and 3 cycles s^{-1} at 1.7–14.1 cm s^{-1} (*U. lactuca* experiments). Such oscillatory flows produced by motion on orbital shakers (see Fig. 3a, b in Pearson et al. 1998) are similar to the water motion experienced by these organisms in nature (e.g. Fig. 2a, b in Koehl and Alberte 1988).

Experimental treatments: kelp sporophytes

The effect of water motion on zoospore release from sporophylls of *A. esculenta* was examined on six separate occasions by collecting pairs of sporophylls (i.e. two sporophylls that opposed each other on a stipe) of similar maturity from six haphazardly selected individuals. One sporophyll of each pair was assigned to a calm treatment, and the other sporophyll, to a shaken treatment (VWR orbital shaker, 170 rpm). Each sporophyll was suspended in a 1-l Erlenmeyer flask containing 900 ml seawater by a stainless steel binder clip attached to a string. Flasks were placed on interspersed orbital shakers (3) and stationary platforms (3) at the same height in a walk-in culture chamber adjusted to 9°C and 35–45 μmol photons m^{-2} s^{-1} (12 h light:12 h dark cycle). Preliminary experiments with hourly sampling of zoospore release from sporophylls demonstrated that most zoospores were released within the first hour after lights-on and not during the dark period. In addition, preliminary experiments demonstrated that the number of zoospores released day-to-day was variable and unpredictable; an individual sporophyll can release millions of zoospores on 1 day, zero the following day, and then release millions of zoospores again on subsequent days. Thus, to examine the effect of water motion, sporophylls were temporarily removed from flasks at 1 h after lights-on on three successive days, and a 1-ml water sample was collected from each flask after swirling the seawater. Zoospores were immobilized by diluting the sample 1:1 with deionized water, counted with a hemocytometer, and the number of cells per milliliter determined. The sorus area on each sporophyll was determined using NIH Image (http://rsb.info.nih.gov/nih-image) to analyze the scanned image. The total number of zoospores released over the 3 days was divided by the sorus area and log transformed. Data were analyzed using a two-factor ANOVA (treatment and trial) and a Fisher's Protected LSD with SAS statistical software (v.6.07).

Experimental treatments and rationale: kelp gametophytes

Three different types of experiments were performed to examine the effect of water motion on the release of sperm from antheridia. Two experiments examined sperm release and fertilization by placing (time-controlled encounter; TCE) or growing (simulated natural encounter; SNE) male and female gametophytes together, whereas another experiment (high pheromone; HP) determined the effect of water motion on male gametophytes exposed to media from female cultures. TCE had the advantage of putting strips of ripe females and ripe males together for a precisely defined interval and, importantly, making it possible to culture the female gametophytes in subsequent isolation from any males to determine zygote production during the experimental treatment by quantifying blade development (juvenile sporophytes, Fig. 1). The disadvantage of TCE was that the genetic composition of the gametophytes on the strips was undefined, and some gametophytes could have been clonal cultures derived from the same fragmented gametophyte (N.B. Six adults were used to produce zoospores for gametophyte cultures, gametophytes were grown vegetatively for months under conditions inhibitory to gametogenesis, and vegetative masses were blended into fragments for seeding onto strips, as described below). To eliminate this problem and also to avoid potential artifacts from use of gametophytes that had been cultured vegetatively for months before the water-motion experiments, zoospores were allowed to settle directly onto the membranes in the SNE experiment to produce gametophytes. This meant, however, that many male and female gametophytes were growing together when they became ripe, and it was impossible to isolate female gametophytes (and developing sporophytes) after the experimental treatments for subsequent study. When an inhibitory effect of water motion on sperm release was observed in TCE and SNE (see below), we conducted HP to see whether the effect of water motion disappeared when both calm and shaken treatments were done in media presumed to contain high concentrations of pheromone because of the length of time (4 days) that ripe female gametophytes had been cultured in those media. Our hypothesis was that pheromone was diluted by water motion in TCE and SNE to levels below the threshold required for sperm release by male gametophytes, but that dilution below that threshold would be impossible in HP.

In TCE, male gametophytes and female gametophytes were established on separate strips (2×40 mm) of Cyclopore membranes from separate male and female vegetative cultures. This was done by fragmenting vegetative masses of gametophytes into two to eight cell pieces in a Waring blender (2 min, in 200 ml PES) according to Vadas (1972), and plating these onto membranes at 5 gametophytic fragments mm^{-2} in replicate male and female Petri dishes containing 50 ml PES. The strips were affixed to the Petri dish bottoms with silicone vacuum grease (Dow Corning). Half of the male and female replicates were grown for 2 weeks under shaken conditions on two orbital shakers (VWR, model 98001, 50 rpm), while the other half of the replicates was grown for 2 weeks under calm conditions on two interspersed stationary platforms; all were grown inside a walk-in culture chamber (9°C, 4 μmol photons m^{-2} s^{-1}, 12 h light:12 h dark cycle). When antheridia and oogonia matured after 2 weeks of culture, four female and four male strips were repositioned in an alternating array, held down by vacuum grease, in each of 36 replicate, 25-mm-deep Petri dishes containing 50 ml PES. Dishes were then assigned to one of four treatments: grown calm/calm treatment, grown calm/shaken treatment, grown shaken/calm treatment, and grown shaken/shaken treatment. Nine replicates of each treatment were used. Shaken treatments were placed on three orbital shakers (three dishes per shaker per treatment) set at 100 rpm, and calm treatments (three dishes per shaker per treatment) were placed on three interspersed stationary platforms. A control for parthenogenetic development consisted of nine replicate female plates ($n = 4$ female strips $dish^{-1}$; $n = 0$ male

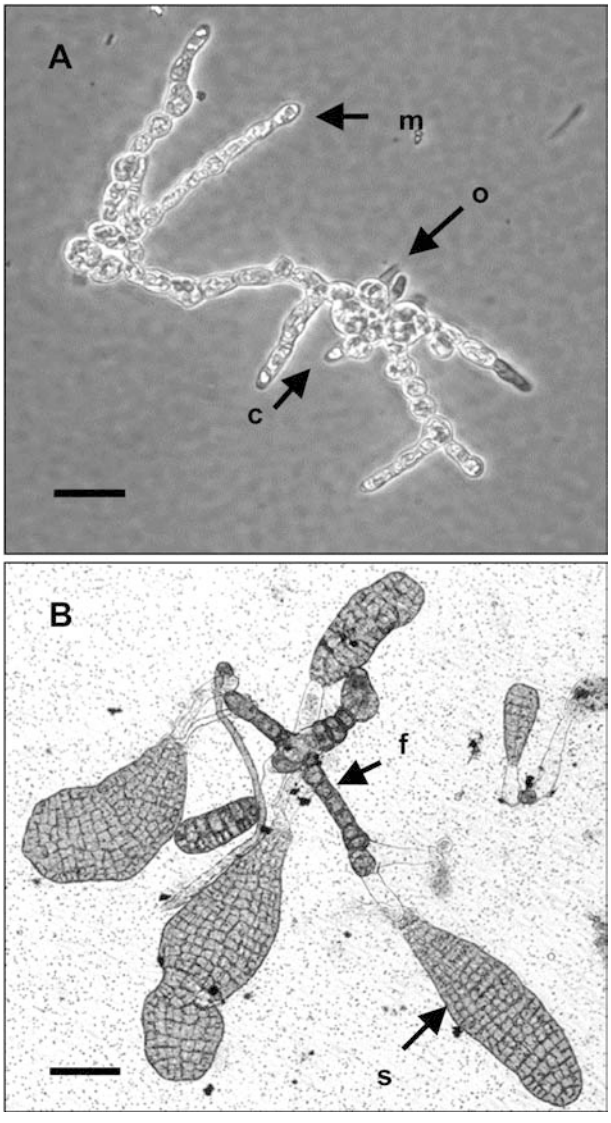

Fig. 1A, B *Alaria esculenta*. **A** Filamentous male gametophyte (*m*) with open (*o*) and unopened (*c*) antheridia, which contain sperm. **B** Young sporophytes (*s*) germinate and develop as blades on the filamentous female gametophyte (*f*). Kelps are oogamous, and the egg is retained on the gametophyte at fertilization; therefore, each blade represents an independent fertilization event. *Scale bar*: 10 μm

strips dish^{-1}) on the three stationary platforms. The experiment was conducted in the walk-in culture chamber (same conditions as above). After 2.5 h, the four membranes with males attached were removed and fixed in 1.5% glutaraldehyde in seawater. Total antheridia and empty (discharged) antheridia were counted over ten haphazardly chosen fields in the phase microscope (×400, field diameter = 0.89 mm) for each of the four male gametophyte membranes per replicate dish. Percent release was calculated as: release (%) = empty antheridia/total antheridia×100. Values were arcsine transformed prior to analysis with a two-factor ANOVA [culture condition (calm or shaken), experimental condition (calm or shaken)]. Female gametophytes were cultured for another 2 weeks under the experimental conditions, and then the total number of juvenile sporophytes on the four female gametophyte strips per replicate (nine per treatment) was determined as a proxy for fertilization; the fertilized zygote is retained on the female gametophyte and develops into a sporophyte (Fig. 1).

To control for the potential loss of natural responses to water motion during long-term culture, we also grew gametophytes directly from zoospores in SNE. Zoospores were obtained from six sporophylls collected from six different individuals. The zoospores were released into sterile seawater containing 1 mg l^{-1} GeO_2 and plated into 12 Petri dishes (25 mm deep), each containing two Cyclopore clear polycarbonate filters (25 mm diameter, 1 μm pore size). These were attached to the bottom of the dish with vacuum grease. The zoospores were allowed to germinate in calm culture for 1 week. Following germination, membranes were transferred to new 25-mm-deep Petri dishes containing 50 ml PES, and six dishes were haphazardly assigned to shaken treatments (100 rpm, orbital shakers, two dishes per shaker), while six plates were placed on interspersed stationary platforms (two dishes per platform), all inside a walk-in culture chamber (9°C, 4 μmol photons m^{-2} s^{-1}, 12 h light:12 h dark cycle). Media were changed and dishes were observed daily using an inverted microscope in the culture chamber to determine whether gametangia had developed, because in this experiment male and female gametophytes always grew in close proximity. Following the first observation of empty antheridia ($t = 3$ weeks), membranes were removed from the dishes and fixed in 1.5% glutaraldehyde in seawater. Total antheridia and empty (discharged) antheridia were counted in ten haphazardly chosen fields in the phase microscope (×400, field diameter = 0.89 mm) for each membrane of male gametophytes. Percent release was calculated as: empty antheridia/total antheridia×100, and was arcsine transformed for statistical analysis using a single-factor ANOVA.

The final experiment (HP) examined the effects of water motion on gametophytes to determine whether release would be equivalent from male gametophytes under calm and shaken conditions if "egg water" (Maier 1995) from female cultures was added to replicates of both treatments. Female gametophytes and male gametophytes were grown separately on Cyclopore membranes (25 mm diameter) from fragmented vegetative masses (see experiment TCE above) of male and female gametophytes. A total of 12 dishes (two membranes per dish, 50 ml PES) of gametophytes were cultured under calm, standard conditions (see above) for 10 days. After mature antheridia and oogonia were observed, six dishes of male gametophytes were haphazardly assigned to the calm treatments, and six dishes, to the shaken treatment (100 rpm, interspersed treatments, as above). Then, 20 ml of the PES in the male dishes was replaced with 20 ml of PES in which female gametophytes were growing. After 2.5 h, membranes were transferred to 1.5% glutaraldehyde in seawater and percent release of antheridia was determined as above, with single-factor ANOVA after arcsine transformation.

U. lactuca experiments

U. lactuca was randomly collected on four dates (12 August, 24 August, 10 September, and 26 September 2000) from the lower intertidal zone, avoiding tidepools. Entire thalli of 18 individuals were collected for each of the four experiments in late afternoon, transported to the laboratory in bags in a cooler, spun individually in a salad spinner to remove excess water, weighed, and placed in 250-ml Erlenmeyer flasks containing 200 ml sterile seawater. Nine flasks were assigned haphazardly to shaken treatments (three VWR orbital shakers, 170 rpm), and nine flasks were assigned to interspersed, calm treatments on three stationary platforms of the same height, all placed in a walk-in culture chamber (13°C, 70–80 μmol photons m^{-2} s^{-1}, 16 h light:8 h dark cycle).

Daily for four consecutive days, each thallus was transferred to another flask with fresh medium 6 h after the beginning of the light period. Each flask was initially examined for the presence of a cloud of green cells (swarmers), and their position relative to the light source in the culture chamber was noted to determine whether they were positively or negatively phototactic [gametes = positively phototactic; zoospores (and zygotes) = negatively phototactic]. The medium in each old flask was then swirled briefly, and a 1-ml water sample removed and diluted 1:1 with deionized water to immobilize gametes or zoospores, which were quantified with a hemocytometer.

Whether the propagules were gametes (i.e. released from gametophytes) or zoospores (i.e. released from sporophytes) was determined by the number of flagella (zoospores = 4; gametes = 2; exceptions are known) and the phototaxis of the cells; the number of flagella is not sufficient by itself (Tanner 1981). The total number of cells released (gametes or zoospores) was divided by the fresh weight of the reproductive portion of each thallus, to standardize results between replicates, and log transformed before statistical analysis. Two-factor ANOVAs (treatment, experiment date) were used for analysis (SAS statistical software, v.6.07).

Results

Alaria esculenta reproduction

Zoospores were released from sporophylls within 4 h of the beginning of the light period; frequently, release occurred within the first 30 min of lights-on. More zoospores were released from sporophylls under shaken [$5.25\times10^6 \pm 4.51\times10^5$ (SE) zoospores] than calm [$2.43\times10^6 \pm 3.66\times10^5$ (SE) zoospores] conditions. There was a significant effect of experimental date, but the treatment×experiment date interaction was not significant (Table 1). The differences among experimental dates were related only to the magnitude of zoospore release, and not to collection date, location of individuals (tidal pool versus subtidal), or tidal cycle (i.e. spring versus neap).

Significantly more antheridia released sperm in the calm versus shaken treatments in the TCE experiment (i.e. when male and female gametophytes were placed together): a mean of 66.3% compared to 24.4% (Table 2; Fig. 2). There was a significant interaction between culture condition and treatment (Table 2), but this was due to differences in magnitude of release in calm treatments because of culture history (i.e. whether gametophytes were cultured under calm or shaken conditions); there was a trend for more release by male gametophytes cultured under calm conditions (Fig. 2). Subsequent culture of female gametophytes resulted in a significantly higher number of juvenile sporophytes in calm treatments versus shaken treatments (Table 3; Fig. 3). Culture conditions had no effect on the total number of juvenile sporophytes (Table 3). Control dishes containing female gametophytes that had been cultured by themselves as controls had no sporophytes at the end of the experiment although there were many mature eggs (i.e. parthenogenesis did not occur).

In the SNE experiment (i.e. male and female gametophytes grown together from zoospore settlement and germination), calm treatments resulted in a significantly higher release of sperm from antheridia compared to shaken treatments, as evaluated by the number of empty antheridia [calm treatments: $48.0 \pm 11.1\%$ (SE) empty antheridia; shaken treatments: $26.9 \pm 9.2\%$ (SE) empty antheridia; $P = 0.0251$]. All female gametophytes with mature oogonia in this experiment were filaments consisting of only three cells. Other female gametophytes consisting of four- to ten-celled filaments were present, but they did not become reproductive within the

Table 1 *Alaria esculenta*. Two-way ANOVA results for zoospore release from sporophylls under calm versus shaken treatments

Source	*df*	MS	*F*	*P*
Treatment	1	2.189	22.54	0.0001
Experiment date	5	0.646	6.66	0.0003
Treatment×Date	5	0.065	0.67	0.6510
Error				
Sporophyll(Flask)	30	0.154	1.59	0.1055
Residual	30	2.913	0.097	

Table 2 *Alaria esculenta*. Two-way ANOVA results for number of empty antheridia (sperm release) under shaken versus calm treatments after calm or shaken culture (TCE experiment)

Source	*df*	MS	*F*	*P*
Treatment	1	0.143	9.05	0.0052
Culture	1	0.048	3.06	0.0900
Treatment×Culture	1	1.521	96.48	0.0001
Error	32	0.016		

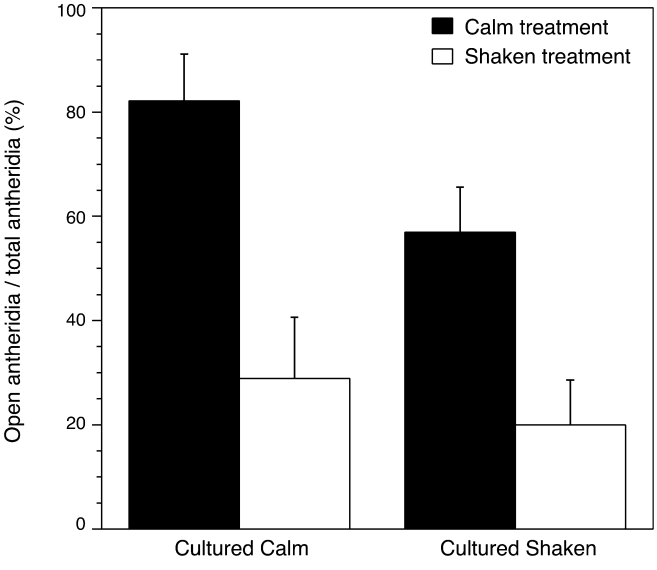

Fig. 2 *Alaria esculenta*. More antheridia (%, mean ± SE) released sperm when ripe male and female gametophytes were placed together under calm versus shaken treatments (experiment TCE, see "Results"), regardless of whether prior culture of gametophytes was under calm or shaken conditions

Table 3 *Alaria esculenta*. Two-way ANOVA analysis of numbers of juvenile sporophytes (germinated zygotes) developing in cultures of gametophytes (TCE experiment) under shaken versus calm treatments after calm or shaken culture

Source	*df*	MS	*F*	*P*
Treatment	1	86,493.940	45.03	0.0001
Culture	1	158.219	0.08	0.7760
Treatment×Culture	1	1,753.233	0.91	0.3468
Error	32	1,920.845		

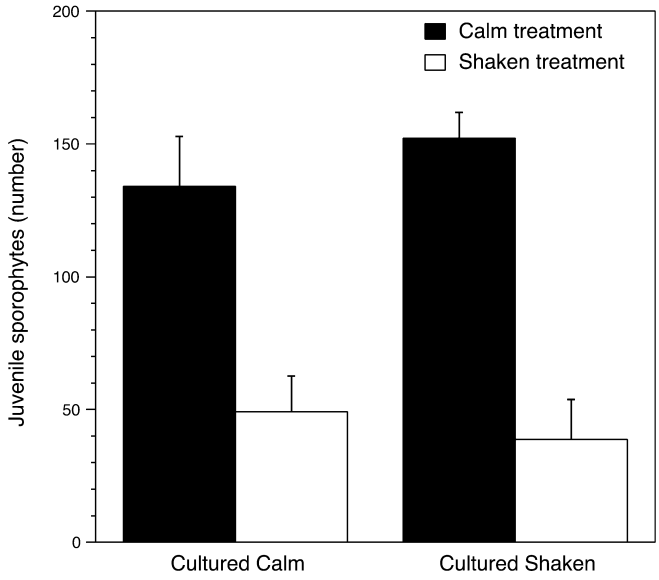

Fig. 3 *Alaria esculenta*. Number of juvenile sporophytes (mean ± SE) developing from female gametophytes in experiment TCE (see Fig. 2). Sperm release under calm experimental conditions produced more juvenile sporophytes (i.e. germinated zygotes)

period of this experiment. Additionally, not all male gametophytes had mature antheridia, but unlike female gametophytes, the reproductive and non-reproductive male gametophytes were morphologically similar.

In the HP experiment (i.e. "egg water" from female cultures added to male gametophytes), antheridial release was not significantly different between calm and shaken treatments when media from female gametophytes bearing ripe eggs were added to male gametophytes under these two treatment conditions ($P = 0.5497$). Calm treatments had $28.6 \pm 12.2\%$ (SE) empty antheridia compared to $26.8 \pm 13.0\%$ (SE) in shaken treatments.

Ulva lactuca experiments

Significantly more zoospores were released in shaken compared to calm treatments [shaken: $2.17\times10^8 \pm 1.16\times10^8$ (SE) zoospores; calm: $1.58\times10^7 \pm 4.41\times10^6$ (SE) zoospores; Table 4]. Also, significantly more gametes were released under shaken conditions [shaken: $1.09\times10^9 \pm 1.62\times10^8$ (SE) gametes; calm: $4.69\times10^8 \pm 1.26\times10^8$ (SE) gametes; Table 5].

Table 4 *Ulva lactuca*. Two-way ANOVA results for zoospore release under calm versus shaken treatments

Source	*df*	MS	*F*	*P*
Treatment	1	5.751	11.77	0.0034
Experiment date	3	0.792	0.54	0.6613
Treatment×Date	3	0.999	1.020	0.382
Error	15	0.488		

Table 5 *Ulva lactuca*. Two-way ANOVA results for gamete release under calm versus shaken treatments

Source	*df*	MS	*F*	*P*
Treatment	1	7.323	16.22	0.0002
Experiment date	3	1.223	2.71	0.0579
Treatment×Date	3	0.325	0.72	0.5462
Error	40	0.452		

Discussion

These laboratory studies show that water motion affects reproduction in both phases of the life histories of the two algal species examined. In the green alga *Ulva lactuca*, water motion produced by orbital shakers increased both gamete and zoospore release when compared with calm conditions. Similar studies in the green alga *Bryopsis plumosa* found that gamete release was enhanced by calm conditions, but also occurred at a high level in replicates on shakers (Speransky et al. 2000). Thus, studies to date of "weedy" green algae demonstrate a neutral to positive effect of water motion on propagule release. On the other hand, our data on the effects of water motion on sperm release by the brown macrophyte *Alaria esculenta* show an inhibition by water motion, similar to our field and laboratory work on another group of brown macrophytes, the fucoids (Serrão et al. 1996; Pearson and Brawley 1996, 1998; Pearson et al. 1998; Berndt et al. 2002). In contrast, we find that zoospore discharge from *A. esculenta* in the laboratory is enhanced by water motion. Clearly, water motion can affect reproduction of different species in different ways, and can even affect propagule release from different life-history phases of a single species (e.g. *A. esculenta*) differently.

Calm conditions likely favored sperm release by *A. esculenta* (TCE and SNE experiments) in our studies due to the inhibitory effect of water motion on pheromone gradients rather than to a direct (e.g. mechanical) effect on the antheridium of pheromone production by the ripe egg. Lamoxirene, the sperm-release and attractant pheromone produced by the ripe egg (Müller et al. 1985), should be diluted rapidly when water motion is high, such as under the temporally and/or spatially limited contact between ripe male and female gametophytes in TCE and SNE. In these experiments, sperm release was greatest in calm treatments, where steep and sufficient gradients of pheromone from ripe eggs to adjacent antheridia would occur, in contrast to shaken replicates where the pheromone putatively produced from ripe eggs would have been diluted below the threshold required for causing release of sperm. Had the experiment continued for many days without exchange of seawater, no treatment effect would be expected because lamoxirene would build up in the shaken flasks to a point above threshold. Experiment HP tested this

possibility by transferring media in which ripe female gametophytes had been growing to replicate sets of male gametophytes in calm and shaken treatments; this produced an equivalent level of antheridial release.

The difference in the response to water motion by sporophytes and gametophytes in *A. esculenta* appears to be an adaptation to different hydrodynamic regimes by the two life-history phases. Neushul (1972) proposed that differences in the release of propagules by phases living in the surge zone (macroscopic sporophyte) or within the boundary layer (microscopic gametophyte) should reflect the relative functions of the propagules (i.e. zoospores, dispersal; gametes, genetic recombination). Bell (1997) also proposed that reproductive specialization and functional adaptation were the driving forces in the evolution of the heteromorphic life history in the Phaeophyceae. He proposed that heteromorphy evolves when the haploid microthallus is specialized as a gametophyte living within the boundary layer, where gametes could be released into the environment at a time of little water movement. Gametes within the boundary layer would disperse locally over very short (mm) distances, but fertilization would be efficient and provide genetic recombination. The diploid macrothallus would become specialized as a sporophyte to release spores into a high-velocity environment, where they would be dispersed widely. Our experiments provide a direct test of Neushul's and Bell's hypotheses, and fully support them.

There are several possible mechanisms to account for increased zoospore release under turbulent conditions. One possibility is that water motion is directly responsible for mechanical rupture of the sporangium. If this were true, it would not explain why zoospores were released in the calm treatments, where shear forces were absent. Moreover, zoospores were released only at the beginning of the light cycle, which implies that a light-driven pathway may be responsible for triggering zoospore release. The production and secretion of mucilage during photosynthesis could cause a sporangium to swell and rupture (Toth 1976); further work is required to elucidate the mechanism of zoospore release.

We are just beginning to understand the genetic structure of kelp populations (e.g. Coyer et al. 1997; Kusumo and Druehl 2000), and the results of the present study are relevant to their interpretation. Our laboratory studies suggest that *A. esculenta* sporophytes release more zoospores in higher flows, which would favor wider dispersal and recruitment of gametophytes and less genetic structure than if release were restricted to calm conditions. Reed et al. (1988) observed an increase in sporophyte recruitment in the kelps *Macrocystis pyrifera* and *Pterygophora californica* following winter storms in California. Reed et al. (1988) also observed that the winter storms increased the dispersal distance for both species. Although zoospore concentrations were not measured directly by Reed et al. (1988), the increased recruitment after winter storms is consistent with our laboratory studies.

U. lactuca differs from other algal species studied by our laboratory in that gamete release was clearly stimulated by higher levels of water motion. *U. lactuca* is a rapid colonizer that usually occurs in dense stands in nature. Gamete release may actually be inhibited in such stands under calm(er) conditions, because Stratmann et al. (1996) found that gametophytes in culture release a swarming inhibitor that prevents gamete release. This finding could explain the lower gamete release in our calm cultures than in the shaken cultures; however, our culture media were changed daily. Gamete release when water motion is high will aid dispersal if viable offspring can still result, and this may be the crux of the response: *Ulva* spp. can develop parthenogenetically from unfertilized gametes (Føyn 1958; Løvlie and Bryhni 1978). The individual that results from parthenogenesis in *U. mutabilis* can either be a functional gametophyte (1 N) or, through a diploidization process, become a functional parthenosporophyte (2 N). *Ulva* spp. gametes are positively phototactic, and green slicks have been observed on the sea surface following gamete release (e.g. Smith 1947; Gordon 2001). Phototaxis appears to be a strategy to concentrate gametes in a plane, not unlike reproductive strategies of some invertebrates, such as corals, with buoyant gametes (e.g. Babcock et al. 1986). Gametes of species such as *U. lactuca* may still be able to concentrate at the surface and achieve successful fertilization under moderately energetic conditions, or, when the sea settles after moderate disturbances. Alternatively, zygote formation may occur only when gametes are released under calm(er) conditions, but dispersal of unfertilized gametes under higher velocity conditions may lead to ecologically favorable dispersal and establishment of genetically identical adults via parthenogenesis. Such adults would be ready to engage in sexual reproduction under favorable environmental conditions.

The differences in gamete release by green and brown algae in calm and turbulent conditions may have a taxonomic basis, but a functional group basis seems equally likely. There are a number of small, weedy brown algae (e.g. *Ectocarpus* spp. and similar species) that offer an appropriate functional group comparison to macrophytic brown algae, such as kelps and fucoids; parthenogenesis occurs in such algae, as in many of the weedy green algae. Finding a macrophytic comparison in the marine green algae for species such as *U. lactuca* is difficult; brackish/freshwater green macrophytes such as *Chara* spp. and *Nitella* spp., which have oogamous reproduction (as do fucoids and kelps), might be suitable. The present studies and parallel studies of other organisms demonstrate a diversity of selected traits that ensure successful reproduction in many marine organisms.

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