The Role of Water Motion in Algal Reproduction

Richard Gordon

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THE ROLE OF WATER MOTION IN
ALGAL REPRODUCTION

By

Richard Gordon

B.S. University of Washington, 1997

A THESIS
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
(in Marine Biology)

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THE ROLE OF WATER MOTION IN
ALGAL REPRODUCTION

By Richard Gordon

Thesis Advisor: Dr. Susan Brawley

An Abstract of the Thesis Presented
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Environmental conditions, such as water motion, can influence fertilization success and spore dispersal in marine algae. Previous studies on fucoid algae showed that gamete release is restricted to, or enhanced by, periods of low water motion. Few other algal taxa have been investigated, however, including species with an alternation of generations. I investigated gamete and spore release in the macroalgae *Alaria esculenta* and *Ulva lactuca*, as well as in the diatom *Pseudo-nitzschia multiseries* to determine if water motion is inhibitory or stimulates propagule production and release. I used orbital shakers to simulate water motion; these were interspersed with stationary platforms within a walk-in culture chamber. Gamete and zoospore release (*A. esculenta* and *U. lactuca*) or gamete and zygote production (*P. multiseries*) were determined daily for several days. *Alaria esculenta* released a significantly higher number of zoospores under turbulent conditions (P = 0.0001, 2-way ANOVA) and a higher number of sperm under calm conditions (P = 0.0052, 2-way ANOVA). Juvenile *A. esculenta* sporophytes were present in significantly higher numbers in calm treatments (P = 0.001 2-way ANOVA; contact time, male + female gametophytes = 150 min). Turbulent conditions resulted in a significantly higher release of both gametes (P = 0.0021, 2-way ANOVA) and zoospores...
(P = 0.0028, 2-way ANOVA) in Ulva lactuca. Pseudo-nitzschia multiseries had significantly higher gametogenesis under calm conditions in one mating cross (P = 0.0055) but not in the other cross. A significantly higher number of zygotes were produced under calm conditions (P = 0.0001, 2-way ANOVA) in all crossed strains of P. multiseries. These data demonstrate that different algal taxa have varying reproductive responses to water motion. The results are of interest with respect to life-history adaptation and fertilization success.
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Chapter 1

Introduction

In intertidal and subtidal communities where organisms using external fertilization are present, hydrodynamic forces can play an important role. Gametes of benthic invertebrates who broadcast spawn in this environment were quickly diluted as distance between individuals and water velocity increased when adults were spawned artificially (Pennington 1985, Levitan et al. 1992). Whether such effects are observed naturally is unknown because few studies of broadcast spawning invertebrates have examined fertilization success in the context of natural gamete release. Several algal species achieve high levels of fertilization success by synchronizing gamete release and restricting it to periods of low water motion despite living in habitats frequently characterized by high water motion. This phenomenon maximizes fertilization success by causing the release of gametes under conditions where they will not be diluted; this has been demonstrated in estuarine habitats using Fucus ceranoides Linnaeus (Brawley 1992) and in brackish and marine habitats with Fucus vesiculosus Linnaeus (Serrão et al. 1996, Berndt et al. 2002). The tidepool alga Fucus distichus achieves high levels of fertilization in the field by releasing gametes synchronously at low tide, a time when the pools are isolated from waves and therefore experience very low water motion (Pearson and Brawley 1996). In these studies, fertilization often reached levels as high as 100%, indicating that timing reproduction to calmer periods prevents recruitment failures in fucoids relative to gamete release and fertilization. In the laboratory, with water motion simulated by orbital shakers, gamete release was inhibited in Ascophyllum nodosum,

Fucoids achieve these high levels of fertilization through a chemical signal. Under calm conditions, carbon becomes limiting in the boundary layer surrounding the thallus and results in an interruption of photosynthesis (Pearson et al. 1998). In the only non-fucoid alga tested to date, Bryopsis plumosa (Hudson) C. Agardh, water motion did not inhibit gamete release. Calm conditions, however, increased gamete release (Speransky et al. 2000).

Although much attention has been paid to the role that light, nutrients, and temperature play in gamete release and fertilization in algae (reviewed in Brawley and Johnson 1992), little is known about the role water motion plays in the release of propagules outside of the fucoids and Bryopsis. To consider light, nutrients, and temperature without the physical and chemical role of water motion creates a fundamental gap in the understanding of algal reproduction. Reproduction in algae is an interesting problem because many algae have complex life histories that may use different stimuli to trigger propagule release at each life history stage. Species with a biphasic life history release spores into the water column from one phase; these do not have to fuse with any other propagule. At a different time in the life history, gametes are released; a sperm and an egg must fuse for fertilization to succeed. Both of these processes take place in a hydrodynamically variable environment with currents and waves. In addition, pheromones are present in many algal taxa. These chemicals may be less effective in attracting gametes when water motion is high because of dilution.
Some level of water motion may be required for sperm and egg contact, as well as reproduction in planktonic species. Small-scale turbulence can increase the contact rates of plankton (Rothschild and Osborn 1988), facilitate the formation of marine snow (Kiorboe 1997), and increase the encounter rates between predators and prey (Dower et al. 1997, Sundby, 1997). In these situations, water motion increases contact because the particles, organisms, or cells in the water column are already at a low concentration relative to each other and water motion increases the encounter rate.

The complex life histories exhibited by many algae may affect how each phase responds to water motion. To date, there is no agreement as to the role sporophytes and gametophytes play in the recruitment and dispersal of algae with biphasic life histories. No attempts have been made to experimentally investigate the roles of reproduction in haplo-diploid life histories. Although there have been many ideas to explain the evolution of both isomorphic and heteromorphic life histories, including as a response to grazing pressure (Slocum 1980, Lubchenco and Cubit 1980, Dethier 1981), physiological adaptations (Conway et al. 1976, Zupan and West 1990), and gametophytes as a dormant stage (Hall and Murray 1998, Edwards 2000), no studies have examined experimentally the reproductive response to the physical environment.

Theoretical attempts to explain the evolution of algal life histories have focused on the physical environment as a selective agent. Neushul (1972) proposed that kelps might be adapted to different turbulence regimes. That is, the macroscopic phase had adapted to the surf zone, and the microscopic phase had adapted to the boundary layer at the substratum. He further proposed that propagule release at each stage reflected this adaptation. A similar argument was made by Bell (1997), who provided a testable
hypothesis. He proposed that a haploid macrothallus became specialized as a
gametophyte over evolutionary time by shedding chemotactic gametes in a low velocity
environment. According to Bell, the fertilized zygote only disperses locally over short
distances and gives rise to a diploid macrothallus. This stage, which is specialized as a
sporophyte, can shed spores above the substrate in a high velocity environment where
they can disperse over wide distances. Another theoretical proposal is the hypothesis that
heteromorphic life histories are under bimodal selection pressures, towards opposite ends
of the r and K selection spectrum (Vadas 1979). Under this hypothesis, one morph is
slow-growing and long-lived, whereas the other morph is a short-lived, weedy, rapid
colonizer, quickly reaching reproductive maturity.

Both Smith (1938) and Fritsch (1942) believed that haploids were the ancestral
condition, giving rise to an isomorphic alternation of generations that then gave rise to
the heteromorphic alternation of generations. This process, according to Fritsch,
originated with asexual and sexual haploid individuals. The transfer of meiosis to the
asexual phase gave rise to isomorphic haplo-diploids, which then underwent divergent
development of the two phases to give rise to heteromorphic haplo-diploids.

If isomorphy evolved first and then gave rise to heteromorphy, the question
remains, why have isomorphic haplo-diploids persisted? Valero et al. (1992) argued that
isomorphic haplo-diploids are maintained because of genetic or morphological
constraints or that they are an intermediate step in evolution towards either a
heteromorphic life history or a strict haploid or diploid life history. A strict haploid or
diploid life history would occur as soon as selective advantage favored one of these
phases over an isomorphic haplo-diploid. Also it is possible that haplo-diploidy is an adaptive trait by itself, or that it is neutral to selection (Klinger 1993).

Isomorphic species may actually be ecologically different despite the similar appearance of the two phases. Differences may be at the level of propagule release, settlement, physiological adaptation, or resistance to herbivory. Undetectable differences may exist between some species, or in the ancestral alga that gave rise to an isomorphic alternation of generations. Hughes and Otto (1999) point out that differences, even if only slight, can lead to the evolution of a biphasic life cycle, under a broad range of conditions. In their model, they assert that no matter what the differing trait is, if the two ploidy phases differ enough to exploit an environment more efficiently together than they would alone, then a biphasic life history will be favored. In addition, if haploid or diploid phases have identical fecundities, and carrying capacities, haplo-diploidy will be favored as long as inter-ploidy competition is less intense than intra-ploidy competition. Another hypothesis suggested by Hughes and Otto (1999) is that the differences in competitive ability by one phase can overcome any advantage of higher survival by the other phase. For example, diploid spores could be poor competitors for haploid resources or haploids could be better overall competitors.

There is some experimental work that supports the Hughes and Otto (1999) model. Destombe et al. (1993) found that in *Gracilaria verrucosa* (Hudson) Papenfuss haploids grew better than diploids in low-nutrient seawater, and that this effect was reversed when the water was enriched with nutrients. This suggests that haploids are better adapted for exploiting low-resource environments than diploids. In addition, holdfasts of haploid juveniles grew better than diploid holdfasts under nutrient-limited
conditions; diploids had a higher tolerance to Pb than haploids; and diploids grew better than haploids under UV radiation (Destombe et al. 1993). In an earlier study on *Gracilaria verrucosa*, Destombe et al. (1989) found additional differences between haploids and diploids. Diploids had a higher propagule release as well as a higher juvenile and adult survival. These findings, if ecologically relevant, may mean that *Gracilaria* is better able to exploit resources with a biphasic life history.

Physiological and ecological differences, although existing between stages in some species, do not always exist between stages in other algal species. Littler et al. (1987) investigated this problem in the red alga *Polycavernosa debilis* (Forsskål) Fredericq et J. Norris by looking at primary production, calorific values, percent organic contents, and susceptibility to predation by herbivorous fish. They found no significant differences between gametophytes and sporophytes in any of these categories, thereby falsifying the hypothesis (for the variables studied) that ploidy level alone leads to ecological and physiological differences between the phases. As an alternate hypothesis they proposed that genetic or reproductive constraints may be the dominant factors that maintain isomorphic diplo-haplontic algae and that this life history type can be maintained if there is some adaptive value to having both sporic and gametic types of reproduction.

It is possible that the ecological similarity or differences between the phases in an isomorphic haplo-diploid should reflect the ratio of haploids to diploids in the field. There is a similar ratio of haploids to diploids in some species, but not all. Destombe et al. (1989) found no difference between haploid and diploid individuals of *Gracilaria verrucosa* at three sites in northern France. However, differences in the haplo-diploid
ratio have been found for *Gracilaria* in other locations. Haploid gametophytes dominate in Manila Bay (Trono and Azanza-Corrales 1981), and diploid sporophytes dominate in British Columbia (Whyte et al. 1981). In a three year study, May (1986) found that Puget Sound populations of *Iridaea cordata* (Setchel and Gardner) Abbot were dominated by haploid gametophytes. These data contrast with observed diploid dominance at sites in Monterey Bay, California (Hansen and Doyle 1976) and Oregon coastal populations (Dyck et al. 1985). Diploid sporophytes most often dominate isomorphic algal populations. An expanded series of reasons for this were advanced by De Wreede and Klinger (1988), which include greater fitness of diploid sporophytes over haploid gametophytes, production of adventitious thalli by sporophytes, and apomeiosis or recycling of the sporophyte stage. Not included in this line of reasoning are gamete release or fertilization success. Naturally high or low levels of fertilization success, or spore release can drive the ratio of diploid sporophytes to haploid gametophytes, by producing bottlenecks at the different stages.

If gametes are diluted, then fertilization will not take place, and the gametes will be wasted. Gametophytes, therefore, should be selected to release gametes during conditions optimal to maximize their fertilization success, unless they are able to develop parthenogenetically. Spores, on the other hand, are suitable for dispersal because of their ability to develop without fusion. Kelp spores are thought to disperse only short distances (Dayton 1972, Reed et al. 1988, Reed et al. 1997). Kelp and other species, however, can travel long distances due to passive transport by currents, or increased wave action from storms (Amsler and Searles 1980, Reed et al. 1988, Reed et al. 1997).
Spore dispersal and settlement can strongly affect fertilization success in some species. Reed (1991) found that successful recruitment of new sporophytes in the kelps, *Pterygophora californica* Reprecht and *Macrocystis pyrifera* (Linnaeus) C. Agardh only occurred naturally when gametophyte densities were between 1 - 10 / mm². In addition, high densities can negatively affect kelp recruitment through competition among gametophytes (Reed 1990). These results suggest that a balance is needed in the kelp life history. If spores disperse too far, the gametophyte density will be too low to successfully recruit the next sporophyte generation; if they don't disperse far enough, then densities will be too high to maximize recruitment.

There is a wide range in the genetic diversity among populations of different seaweed species (Pearson and Murray 1997), which can be influenced by dispersal distances. Limited dispersal results in less similarity among *Silvetia compressa* (as *Pelvetia fastigiata* (J. Agardh) De Toni) populations (Williams and Di Fiori 1996). In contrast, *Halidrys dioica* Gardner populations were more similar because of the release of reproductive fronds that are carried by currents to new locations (Lu and Williams 1994). Kelp spores are known to remain viable in the water column for several days and to be able to germinate without settlement (Reed et al. 1992). Recently, Kusumo and Druehl (2000) found that there was greater genetic similarity between patches of *Alaria marginata* Postels and Ruprecht at wave-sheltered sites compared with wave-exposed sites. These data, may be the result of inefficient fertilization or a limited ability of spores to disperse at wave-sheltered locations.

The purpose of this research is to determine if a reproductive response to water motion can be detected in algae other than fucoids (Phaeophyceae) and *Bryopsis*
(Chlorophyceae) and, if the response differs within different life history stages of the same species. Results of this work may lead to a better understanding of genetic variability within these species, reproductive success, dispersal ability, life history adaptations, and the roles that heteromorphic and isomorphic species play in reproductive response to environmental conditions. I hypothesize that gamete and spore release will show different responses to water motion; gamete release will be higher under calm conditions and spore release will be higher under turbulent conditions. To test these hypotheses, I investigated three species of algae, *Alaria esculenta* (Linnaeus) Greville (Phaeophyceae, Laminariales), *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle (Bacillariophyceae, Pennales), and *Ulva lactuca* Linnaeus (Chlorophyta, Ulvales). These species were selected because much is known about their life histories and conditions required for their culture in the laboratory. They also represent a diverse set of algae relative to life histories and taxonomic affinities.
Chapter 2

Alaria esculenta

Introduction

Kelps (Laminariales) occupy two different hydrodynamic regimes because of their heteromorphic alternation of generations. The macroscopic sporophytes rise into the water column whereas the microscopic gametophytes grow within the boundary layer of the substratum. Gamete and zoospore release may reflect these morphological differences, and the morphology may be a functional adaptation to maximize both dispersal and fertilization (Neushul 1972, Bell 1997). To test the hypothesis that gamete and zoospore release differ in their response to water motion, I investigated the kelp *Alaria esculenta*. This species was chosen because the sporophytes are abundant and easily collected on the coast of Maine. Furthermore, male and female gametophytes can be isolated and cultured in the laboratory for long periods of time.

*Alaria esculenta* (Linnaeus) Greville (Phaeophyceae, Laminariales) has a typical laminarian life history (Sauvageau 1915). This heteromorphic alga alternates between a macroscopic (diploid) sporophytic stage (Figure 1) and a microscopic (haploid) gametophytic stage that is dioecious. Reproductive maturity for *A. esculenta* sporophytes in Maine begins in the spring and continues through late summer in individuals growing at greater depth (personal observation). The zoospores develop within sporangia on sporophylls attached to the stipe at the base of the blade. Zoospores can develop within a sorus on the vegetative blade if the sporophylls are removed experimentally (Pfister
1991, Pfister 1992). When released, the zoospores settle and develop into male and female gametophytes. Reproduction is oogamous. Once extruded from the oogonium of the female gametophyte, eggs stimulate the release of sperm and attract them using the pheromone lamoxirene. Sperm release is stimulated when concentrations greater than or equal to 10 pM are detected at the antheridium (Müller et al. 1985, Lünning and Müller 1978, Maier and Müller 1987, Maier and Müller 1986). Once fertilized, new sporophytes germinate directly on the female gametophyte. Some members of this genus are reported to be capable of parthenogenetic development of haploid and diploid sporophytes from unfertilized eggs, but these sporophytes are often abnormal, and have not been observed to grow to reproductive maturity (Nakahara and Nakamura 1973).
Figure 1. *Alaria esculenta* sporophyte.

Bar = 30 cm.
Arrow indicates sporophylls
Materials and Methods

Sporophyte Experiments

Zoospore Release

To determine the timing of zoospore release in _Alaria esculenta_, five sporophylls were collected haphazardly from five individuals in tidepools at low tide from Schoodic Point, Maine, on March 20, 2000. At the end of the photoperiod, each sporophyll was placed in 900 mL seawater within a 1L Erlenmeyer flask in a walk-in culture chamber (9 °C, 35-45 μmol photon • m⁻² • sec⁻¹, 12:12 L:D). The sporophylls were monitored for signs of zoospore release 1 h before the start of the light period, through 1 h after the end of the light period for two days. The seawater was changed daily.

Hydrodynamic Experiments

To test the effect of water motion on zoospore release, six trials were conducted. Sporophylls were collected from Schoodic Point, Maine, on April 16 (trial 1), May 7 (trial 2), June 2 (trial 3), June 14 (trial 4), June 23 (trial 5), and July 7, 2000 (trial 6). Collections made on April 16 and May 7 were from tidepools in the mid-intertidal zone on spring tides; collections on June 2, June 14, and June 23 were made from the shallow subtidal zone on spring tides. The July 7 collection was made from the shallow subtidal zone on a neap tide. Pairs (i.e. 2 sporophylls opposing each other on the stipe) of mature sporophylls of approximately the same age were collected from six haphazardly selected individuals on each experiment date (N=6). Sporophylls were cut from the stipe with a knife, placed into a plastic bag, put on ice, and transported back to the laboratory. Each collection was made during the afternoon or evening low tide to minimize disruption of
the photoperiod. In the lab, each sporophyll was attached by the base with a stainless steel binder clip to a string and suspended into a 1L Erlenmeyer flask containing 900 mL seawater. One sporophyll from each pair was assigned to the shaken treatment on an orbital shaker (VWR) set for 170 rpm and the other was assigned to the calm treatment on an adjacent stationary platform of the same dimensions and height. All treatments took place within a walk-in culture chamber (9 °C, 35-45 µmol photons • m\(^{-2}•\) sec\(^{-1}\), 12:12 L:D). Seawater was changed daily 30 min after the end of the light period.

For three consecutive days at 1 h after the start of the light period, the sporophylls were temporarily removed while the flasks were swirled and 1 mL water samples were taken. The water samples were diluted 1:1 in 1 mL deionized water to immobilize the zoospores. The number of zoospores was then counted with a hemacytometer and the number of cells • mL\(^{-1}\) was determined with the following equation: cells • mL\(^{-1}\) = average of 10 counts • dilution factor • 10\(^4\). The sorus area on each sporophyll was determined by tracing each sorus onto transparency film, scanning the image, and using NIH Image (http://rsb.info.nih.gov/nih-image/) to calculate the area. The total number of zoospores released over the three days was divided by the sorus area and log transformed. The experiments were analyzed using a 2-factor ANOVA (treatment and trial) and a Fisher's Protected LSD was performed using SAS statistical software (version 6.07).

**Gametophyte Experiments**

**Cultures of Female and Male Gametophytes**

Six sporophylls were collected haphazardly from Schoodic Point, Maine, on April 16, May 7, June 2, June 14, June 23, July 7, and August 24, 2000. At each collection, the sporophylls were detached at their bases, placed in a plastic bag, put on ice, and
transported back to the laboratory. The sporophylls were wiped with a paper towel to remove epiphytes and then placed in a plastic bag overnight at 9°C. The sporophylls were then placed into 1L sterile seawater and allowed to release zoospores for approximately 1 h at 9°C. Zoospore concentrations were determined by counting with a hemacytometer, and the zoospores were plated onto 2 x 2 cm glass chips within petri dishes at a concentration of 1-10 spores • mm⁻² in sterile seawater containing 1 mg • L⁻¹ germanium dioxide (Sigma). After two weeks, the 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 after a minimum of two weeks, they were separated using a micropipette. The male and female gametophytes were cultured separately in replicate deep 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 • sec⁻¹ (Lüning and Neushul 1978) (Figure 2).
Figure 2. *Alaria esculenta* gametophytic vegetative growth.

Bar = 10 μm
Gametophyte Experiment 1

To test the effect of water motion on sperm release in *Alaria esculenta*, vegetative masses of male and female gametophytes were fragmented separately to a 2-8 cell size (Figure 3) for 2 minutes in 200 mL PES inside a Waring blender (Vadas 1972), and then plated out at a concentration of 5 gametophytes • mm$^{-2}$ in 50 mL PES into 6 (male), or 9 (female) replicate petri dishes, each containing 25, 1.0 μm Cyclopre clear polycarbonate filter membranes (Whatman) cut into 2 x 40 mm strips. These strips allowed male and female gametophytes to be manipulated and placed together for a fixed amount of time under experimental conditions. The polycarbonate membranes were attached to the bottom of the petri dish using silicone vacuum grease (Dow Corning). Half of the male and female replicates were grown for two weeks under shaken conditions on two orbital shakers (VWR, model 98001) set for 50 rpm, while the other half was grown for two weeks under calm conditions on two adjacent stationary platforms inside a walk-in culture chamber (9°C,12:12 L: D, 4 μmol photons • m$^{-2}$ • sec$^{-1}$). After two weeks when mature oogonia and antheridia were visible, the polycarbonate strips with male and female gametophytes were placed in contact with each other under experimental conditions; four male strips and four females strips were positioned alternately with vacuum grease into 25 mm deep petri dishes and filled with 50 mL PES. The dishes were then assigned to the following treatments: grown calm - treated calm, grown calm - treated shaken, grown shaken - treated calm, and grown shaken - treated calm. Shaken treatments were placed on three orbital shakers set at 100 rpm and calm treatments were on three interspersed stationary platforms. There were nine replicates of each treatment, three on each platform or shaker. Nine replicate plates, grown calm, were
also made containing four female strips placed 2 mm apart to control for the possibility of parthenogenetic development of unfertilized eggs. It took 30 minutes to position all the strips in all the treatments, after which time the dishes were placed into treatment conditions. All treatments took place inside a walk-in culture chamber (9°C, 12:12 L:D, 4 \mu\text{mol} \text{ photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}).

After 2.5 h, the four male membranes were removed from each dish and fixed in 1.5% glutaraldehyde in seawater. Ten haphazardly selected fields were used to count total antheridia and empty antheridia on the four membranes (400x, field diameter = 0.89 mm) using phase contrast optics (Figure 4). Percent release was calculated as release (%) = empty antheridia / total antheridia × 100. These values were arcsin transformed for statistical analysis using a 2-factor ANOVA (culture condition and treatment condition). Female gametophytes on membranes were allowed to develop for two weeks under the experimental conditions and then the total number of juvenile sporophytes in each replicate was counted (Figure 5).
Figure 3. Fragmented male (M) and female (F) gametophytes of *Alaria esculenta*.

Bar = 10 μm
Figure 4. Male gametophyte of *Alaria esculenta*.

Bar = 10 μm

a. Empty antheridium
b. Mature antheridium
Figure 5. Juvenile sporophytes of *Alaria esculenta*.

Bar = 10 μm
Gametophyte Experiment 2

Male gametophytes were plated into twelve, 25 mm deep petri dishes each containing two 25 mm diameter, 1µm pore size Cyclopore clear polycarbonate filter membranes (Whatman) with 50 mL PES. The gametophytes were grown under calm conditions for ten days (9°C, 12:12 L:D, 4 µ mol photons • m⁻² • sec⁻¹). After mature antheridia were observed, 6 of the plates were assigned haphazardly to shaken treatments on orbital shakers set for 100 rpm (two dishes per shaker, three shakers) and 6 were haphazardly assigned to calm treatments on adjacent stationary platforms (two dishes per platform, three platforms) inside a walk-in culture chamber (9°C, 12:12 L:D, 4 µ mol photons • m⁻² • sec⁻¹). The seawater medium was removed and 20 mL of the PES in which female gametophytes had been growing was added (Maier 1995). After 2.5 h, the membranes were removed and fixed in 1.5% glutaraldehyde in seawater. Counts of individual male gametophytes (five from each membrane) for total antheridia and empty antheridia were made from each replicate in ten fields (400x magnification, field diameter = 0.89 mm) using phase contrast optics. Percent release was calculated as release (%) = empty antheridia / total antheridia x 100 and arcsin transformed for statistical analysis using a single – factor ANOVA.

Gametophyte Experiment 3

Six sporophylls were haphazardly collected from separate sporophytes at Schoodic Point, Maine, on May 1, 2001, from the shallow subtidal zone and transported, on ice, back to the laboratory. Zoospores were released into sterile seawater containing 1 mg • L⁻¹ germanium dioxide and plated into twelve, 25 mm deep petri dishes, each
containing 2, 25mm diameter 1μm pore size Cyclopore clear polycarbonate filter membranes (Whatman) attached with silicone vacuum grease (Dow Corning). After one week, when the zoospores had settled and germinated, the membranes were transferred to new 25 mm deep petri dishes containing 50 mL PES. Six of the plates were haphazardly assigned to shaken treatments on orbital shakers set for 100 rpm (two dishes per shaker) or adjacent stationary platforms (two dishes per platform) for an additional three weeks inside a walk-in culture chamber (9°C, 12:12 L:D, 4 μ mol photons • m⁻² • sec⁻¹). The medium was changed daily.

The membranes were removed from the dishes and fixed in 1.5% glutaraldehyde in seawater after the first observation of empty antheridia (t = 3 weeks). Ten fields were used to count individual male gametophytes (five from each membrane) for total antheridia and empty antheridia in each replicate, as above. Percent release was calculated as release (%) = empty antheridia / total antheridia x 100 and was arcsin transformed for statistical analysis using a single – factor ANOVA.

**Gametophyte Experiment 4**

To reduce the amount of manipulation time experienced in setting up the strips (Experiment 1) the same experiment was conducted with a reduction in replicates and only two treatments (shaken and calm). Vegetative masses of male and female gametophytes were fragmented separately to a 2-8 cell size in a Waring blender (Vadas 1972), and then plated out at a concentration of 5 gametophytes • mm⁻² onto 25 2x40 mm, 1 μm Cyclopore clear polycarbonate filter membranes (Whatman) inside petri dishes containing 50 mL PES. The polycarbonate membranes were attached to the petri dish
using silicone vacuum grease (Dow Corning). In total, 4 replicate male and 4 replicate female plates were made. All replicates were grown under calm conditions in 50 mL PES (9°C, 12:12 L:D, 4 μ mol photons • m⁻² • sec⁻¹).

The male and female gametophytes were placed in contact with each other under experimental conditions after two weeks when mature oogonia and antheridia were visible. Four male strips and four female strips were attached alternately with silicone vacuum grease (Dow Corning) into 25 mm deep petri dishes and filled with 50 mL PES. The dishes were then assigned to calm or shaken treatments. Shaken treatments were on three orbital shakers set for 100 rpm and calm treatments were on three interspersed stationary platforms. There were three replicates of each treatment. All treatments took place inside a walk-in culture chamber (9°C, 12:12 L:D, 4 μ mol photons • m⁻² • sec⁻¹).

After 2.5 h, the four male membranes were removed from each dish and fixed in 1.5% glutaraldehyde in seawater. Ten haphazardly positioned fields of total antheridia and empty antheridia were counted on the four membranes (400x, field diameter = 0.89 mm) with phase contrast optics. Percent release was calculated as release (%) = empty antheridia / total antheridia and was arcsin transformed for statistical analysis using a single-factor ANOVA. The female gametophytes on membranes were cultured for an additional week under the experimental conditions and then the total number of juvenile sporophytes in each replicate was counted.

**Characterization of Water Velocity**

Water velocity was determined by the same method as 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 a 45° angle to the direction of
the water flow. Ground black pepper was used as a particle to reflect the acoustic signal. Output was recorded at 5 mm • sec⁻¹ on a high speed chart recorder (Gould, model 220). Water flow was generated by an orbital shaker (VWR) set at 170 rpm (sporophyte experiments) or 100 rpm (gametophyte experiments) within a 1 L Erlenmeyer flask with 900 mL seawater (sporophyte experiments) or a 25 mm deep petri dish with 50 mL seawater (gametophyte experiments).
Results

Sporophyte Experiments

Zoospore Release

All sporophylls released zoospores within two days of culture and within 3.5 h from the start of the light period. Zoospore release was observed when the seawater was changed during the light period, but release was not observed when the seawater was changed 30 minutes after the end of the light period (Table 1). This release pattern determined sampling and seawater change times for other experiments.

Table 1. Hours after the onset of the light period before the first observed zoospore release.

<table>
<thead>
<tr>
<th>Sporophyll Number</th>
<th>3/21/00</th>
<th>3/22/00</th>
<th>3/23/00</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3.5</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>No release</td>
<td>0.16</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.16</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>No release</td>
<td>0.16</td>
<td>No release</td>
</tr>
<tr>
<td>5</td>
<td>No release</td>
<td>3.5</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Hydrodynamic Experiments

A significantly higher number of zoospores was released under shaken than calm treatments. There was also a significant difference in release between trials; however, there was no significant treatment x trial interaction. In addition, there was no significant difference among individuals within trials (Table 2; Figure 6).

A Fisher's protected LSD found significant differences ($\alpha = 0.05$) between trials. Experiments 1, 3, and 5 were significantly different from experiments 2, 4, and 6. In
addition, experiments 1, 4, and 5 were different from experiments 2 and 6 (Table 3).

These differences, however, are not meaningful in terms of collection date, location (tide pool or shallow subtidal), and tide series (spring or neap). The differences only reflect the relative magnitude of release during the experiments.

Table 2. Two – way ANOVA table for _Alaria esculenta_ zoospore release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2.189</td>
<td>2.189</td>
<td>22.54</td>
<td>0.0001</td>
</tr>
<tr>
<td>Trial</td>
<td>5</td>
<td>3.232</td>
<td>0.646</td>
<td>6.66</td>
<td>0.0003</td>
</tr>
<tr>
<td>Trial x Treatment</td>
<td>5</td>
<td>0.324</td>
<td>0.065</td>
<td>0.67</td>
<td>0.6510</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individuals (Trials)</td>
<td>30</td>
<td>4.627</td>
<td>0.154</td>
<td>1.59</td>
<td>0.1055</td>
</tr>
<tr>
<td>Residual</td>
<td>30</td>
<td>2.913</td>
<td>0.097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>10.372</td>
<td>0.253</td>
<td>2.61</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

Table 3. Fisher's Protected LSD for _Alaria esculenta_ zoospore release.

Means with the same letter are not statistically different (α = 0.05).

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean (log total release)</th>
<th>N</th>
<th>Exp.</th>
<th>Date (2000)</th>
<th>Collection Location and Tide</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.6357</td>
<td>12</td>
<td>3</td>
<td>June 2</td>
<td>Subtidal collection, Spring</td>
</tr>
<tr>
<td>A</td>
<td>6.5809</td>
<td>12</td>
<td>5</td>
<td>June 23</td>
<td>Subtidal collection, Spring</td>
</tr>
<tr>
<td>B A</td>
<td>6.5647</td>
<td>12</td>
<td>1</td>
<td>April 16</td>
<td>Tidepool collection, Spring</td>
</tr>
<tr>
<td>B A</td>
<td>6.3506</td>
<td>12</td>
<td>4</td>
<td>June 14</td>
<td>Subtidal collection, Spring</td>
</tr>
<tr>
<td>B C</td>
<td>6.2051</td>
<td>12</td>
<td>6</td>
<td>July 7</td>
<td>Subtidal collection, Neap</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tidepool collection, Spring</td>
</tr>
<tr>
<td>D C</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td>Subtidal collection, Spring</td>
</tr>
<tr>
<td>D</td>
<td>6.0631</td>
<td>12</td>
<td>2</td>
<td>May 7</td>
<td>Tidepool collection, Spring</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Subtidal collection, Spring</td>
</tr>
</tbody>
</table>
Figure 6. *Alaria esculenta* zoospore release.

Mean ± SE.
Gametophyte Experiments

Gametophyte Experiment 1

There was a significant difference in the number of empty antheridia between calm and shaken treatments (Table 4). Higher release was observed in calm treatments, which had a mean antheridial release of 66.3%. Shaken treatments had a mean antheridial release of 24.4%. Conditions during culture (calm or shaken) had no significant effect on the number of antheridia released. There was a trend, however, for increased release when the gametophytes were cultured under calm conditions (Figure 7). There was a significant culture condition x treatment interaction. The treatment effect did not change, however, with respect to developmental conditions, only the magnitude of the response. In both cases, a higher percent of antheridia was released under calm compared to shaken conditions.

Table 4. Gametophyte Experiment 1: Two-way ANOVA table for *Alaria esculenta* sperm release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.143</td>
<td>0.143</td>
<td>9.05</td>
<td>0.0052</td>
</tr>
<tr>
<td>Culture</td>
<td>1</td>
<td>0.048</td>
<td>0.048</td>
<td>3.06</td>
<td>0.0900</td>
</tr>
<tr>
<td>Treatment x Culture</td>
<td>1</td>
<td>1.521</td>
<td>1.521</td>
<td>96.48</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.489</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>1.708</td>
<td>0.569</td>
<td>36.12</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Calm treatments had a significantly higher number of developing juvenile sporophytes than did shaken treatments. Culture conditions did not have an effect on the total number of juvenile sporophytes, and there was no significant culture condition x treatment interaction (Table 5; Figure 8). No juvenile sporophytes were found in the control dishes containing only female gametophytes (i.e. parthenogenesis did not occur).

Figure 7. Gametophyte Experiment 1: *Alaria esculenta* antheridial release.  

Mean ± SE.
Table 5. Gametophyte Experiment 1. Two-way ANOVA table for *Alaria esculenta* juvenile sporophytes.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>86493.940</td>
<td>86493.940</td>
<td>45.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>Culture</td>
<td>1</td>
<td>158.219</td>
<td>158.219</td>
<td>0.08</td>
<td>0.7760</td>
</tr>
<tr>
<td>Treatment x Culture</td>
<td>1</td>
<td>1753.233</td>
<td>1753.233</td>
<td>0.91</td>
<td>0.3468</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>59546.208</td>
<td>1920.845</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>88405.392</td>
<td>29468.464</td>
<td>15.34</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Figure 8. Gametophyte Experiment 1: *Alaria esculenta* juvenile sporophytes.

Mean ± SE.
Gametophyte Experiment 2

There was no significant difference in antheridial release between calm and shaken treatments when media in which female gametophytes were growing was added to cultures of male gametophytes (Table 6). The mean released antheridial number (%) was nearly the same in both treatments; calm treatments released a mean antheridia number of 28.6% while shaken treatments released a mean of 26.8%. (Figure 9).

Table 6. Gametophyte Experiment 2: ANOVA table for *Alaria esculenta* sperm release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.002</td>
<td>0.002</td>
<td>0.33</td>
<td>0.5791</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.054</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>0.002</td>
<td>0.002</td>
<td>0.33</td>
<td>0.5791</td>
</tr>
</tbody>
</table>
Figure 9. Gametophyte Experiment 2: *Alaria esculenta* antheridal release.
Mean ± SE.
Gametophyte Experiment 3

Calm treatments had a significantly higher antheridal release (%) compared to shaken treatments (Table 7; Figure 10). All female gametophytes with mature oogonia, either straight or branched, were filaments consisting of ≤3 cells. Other female gametophytes, from 4 to 10 cells long were present, but not reproductive. In addition, not all of the male gametophytes had mature antheridia. There were no discernable morphological differences, however, in the size of reproductive and unreproductive males.

Table 7. Gametophyte Experiment 3: ANOVA table for *Alaria esculenta* sperm release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.182</td>
<td>0.182</td>
<td>6.93</td>
<td>0.0251</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.263</td>
<td>0.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>0.182</td>
<td>0.182</td>
<td>6.93</td>
<td>0.0251</td>
</tr>
</tbody>
</table>
Figure 10. Gametophyte Experiment 3: *Alaria esculenta* antheridial release. Mean ± SE.
Gametophyte Experiment 4

Calm treatments had significantly higher antheridal release (%) compared to shaken treatments (Table 8; Figure 11). Although there was a trend (Figure 12) for a higher number of juvenile sporophytes, this trend was not significant (Table 9).

Table 8. Gametophyte Experiment 4: ANOVA table for Alaria esculenta sperm release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.346</td>
<td>0.346</td>
<td>260.27</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.005</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>0.346</td>
<td>0.346</td>
<td>260.27</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 9. Gametophyte Experiment 4: ANOVA table for Alaria esculenta juvenile sporophytes.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>29680.667</td>
<td>29680.667</td>
<td>4.66</td>
<td>0.0970</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>25462.667</td>
<td>6365.667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>29680.667</td>
<td>29680.667</td>
<td>4.66</td>
<td>0.0970</td>
</tr>
</tbody>
</table>
Figure 11. Gametophyte Experiment 4: *Alaria esculenta* antheridal release. 
Mean ± SE.
Figure 12. Gametophyte Experiment 4: *Alaria esculenta* juvenile sporophytes.

Mean ± SE.
Figure 13. Flow characteristics for conditions used in adverse eculizumab experiments.

Time (s)

Flow Velocity (cm/s)
Discussion

Water motion affected zoospore and gamete release in *Alaria esculenta*.

Zoospore release occurred under calm conditions and was greatly enhanced by shaken conditions. In contrast, sperm release, although not inhibited by water motion was increased under calm conditions.

The release of sperm under calm conditions enhanced the production of the diploid sporophytic phase, as found in Experiments 1 and 4, although the results were only statistically significant in Experiment 1. This difference may be due, in part, to the smaller sample size in Experiment 4 compared to Experiment 1. A decrease in sample size between the two experiments was necessary because of the time it took to manipulate membranes in Experiment 1. If the experiment had not been repeated on a smaller and more rapidly manipulated scale, then it would be uncertain whether the effects of the treatment in Experiment 1 on sperm release were real or an artifact of the 30 min set-up period (i.e. the period during which all gametophytes experienced calm conditions).

The fact that sperm release was greater under calm conditions in Gametophyte Experiments 1 and 4, but not in Experiment 2, suggests that the relevant effect of water motion is dilution of the pheromone rather than an effect on the antheridia. Lamoxirene, the sperm attractant produced by the egg (Müller et. al. 1985), should be diluted rapidly when water motion is high. In contrast, under calm conditions, the pheromone can concentrate at the substrate close to the male gametophytes, triggering sperm release. When both treatments are exposed experimentally to pheromone at the same high concentration, as I infer was the case in Experiment 2, pheromone dilution would not occur in the shaken treatment.
Results from the zoospore release experiments agree with findings in other kelps. Kusumo and Druehl (2000) found increased genetic variability among patches of *Alaria marginata* (Postels et Ruprecht) on the coast of British Columbia, Canada, at wave-exposed sites compared to wave-sheltered sites. The authors noted that once released, seaweed propagules may have little control over their destiny but, "the plants may have strategies to optimize their dispersal" (p. 407). The intertidal kelp *Postelsia palmaeformis* (Ruprecht) releases zoospores at low tide, resulting in short dispersal distances (Dayton 1973) and a low degree of genetic variability (Kusumo et al. 2001). In contrast, *Alaria esculenta* zoospores are released in higher numbers in response to shaken conditions, demonstrating that this species has the ability to maximize dispersal by increasing zoospore release under conditions of high water motion.

Reed et al. (1988) observed an increase in sporophyte recruitment in the kelps *Macrocystis pyrifera* (Linnaeus) C. Agardh and *Pterygophora californica* (Ruprecht) following winter storms in California. These winter storms also increased the dispersal distance for both species. Although zoospore concentrations were not measured directly in Reed et al. (1988)'s study, the increased recruitment after winter storms is consistent with my laboratory findings of increased zoospore release during periods of high water motion. As a result, kelp species may be able to rapidly colonize habitats cleared by disturbance and recruit into areas where the gametophytes would otherwise be competitively inferior.

The difference in the response to water motion by sporophytes and gametophytes in *Alaria esculenta* may 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) or within the boundary layer (microscopic) may reflect the relative functions of the propagules. In the case of A. *esculenta*, the relative role of the macroscopic sporophytic phase is one of dispersal. Zoospores do not need to fuse with another cell in order to germinate into gametophytes. In contrast, the relative role of the microscopic phase is one of genetic recombination. The gametophytes maximize sperm release under calm conditions because of the nature of the pheromone, which must be perceived by the male gametophyte. These results, therefore, support Neushul’s hypothesis of the functional adaptation of seaweed morphology.

Bell (1997) proposed that this specialization and functional adaptation was the driving force 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. This happens, because living within the boundary layer, gametes can be released into the environment at a time of little water movement. Gametes within the boundary layer will disperse locally over very short (mm) distances. Therefore, the diploid macrothallus would become specialized as a sporophyte and release spores into a high velocity environment where they can disperse widely. These experiments provide a direct test of Bell’s hypothesis, and fully support it.

There are several possible mechanisms for increased zoospore release under turbulent conditions. One possibility is that water motion is directly responsible for mechanical rupture of the sporangium. If this was true, it would not explain why zoospores were released in the calm treatments, where shear forces were absent. Moreover, zoospores in *A. esculenta* are released only at the beginning of the light cycle,
which implies that a light-driven pathway may be responsible for triggering zoospore release. Zoospore release may be optimized under turbulent conditions if a photosynthetic pathway is involved because the sporophyll is continually washed with nutrients and dissolved organic carbon. Under calm conditions, nutrients may become limiting and reduce photosynthesis, inhibiting zoospore release. The production and secretion of mucilage during photosynthesis could cause sporangia to swell and rupture (Toth 1976). Further experiments are needed to determine the mechanism of zoospore release.
Chapter 3

*Ulva lactuca*

Introduction

The effects of water motion on zoospore versus gamete release can be tested best using species with an isomorphic alternation of generations. Even though the adults look the same and live in the same place, the two phases of an isomorphic species can vary in ecologically important ways (Destombe et al. 1989, Destombe et al. 1993). If differences exist between the two generations in the relative timing of gamete or zoospore release, then conditions for fertilization, dispersal, and recruitment may be optimized over the two life history phases. In addition, differences in the timing of gamete or zoospore release may be critical in the species' ability to colonize new habitats, especially if the species is an opportunist.

I investigated the effects of water motion on the release of gametes and zoospores in the green seaweed *Ulva lactuca* Linnaeus (Chlorophyta, Ulvales) (Figure 14) to test if an alga with an isomorphic alternation of generations responds to water motion at the level of propagule release. This species was selected because it is easily collected and abundant on the coast of Maine.

*Ulva lactuca*, an isomorphic diplohaplontic alga, inhabits many of the world's coasts. The haploid gametophytes release positively phototactic, biflagellate gametes (Figure 15) that fuse to form zygotes that germinate into diploid sporophytes. The sporophytes meiotically form negatively phototactic, quadriflagellate zoospores (Figure
that grow into haploid gametophytes when released (Föyn 1934, Föyn 1962, Bilding 1968, Koeman and van den Hoek 1981). Parthenogenesis occurs in at least two species of Ulva, including Ulva lactuca, and the rarity of sporophytes has been used to suggest that fertilization is rare (Lövlie and Bryhni 1978). Parthenogenesis also results from sloughed vegetative cells of the gametophyte that settle and germinate (Bonneau 1978). However, periodic gamete release that is synchronous with spring and neap tides has been observed in Ulva lactuca and several other species of Ulvales (Smith 1947, Christie and Evans 1962, Sawada, 1972, Okuda 1984, Okuda and Yamasaki 1987). Although there can be variability in the timing of gamete release between localities, synchrony with respect to individual locations has been observed within 1-7 days (Smith 1947, Sawada 1972, Okuda 1984, Okuda and Yamasaki 1987). The synchronous gamete release observed by these authors suggests that there might be a mechanism to detect conditions that maximize fertilization.

Whether or not parthenogenesis or fertilization is the predominant process in the life history of Ulva remains unresolved. These questions, however, cannot be resolved without a complete understanding of propagule release. To better understand the reproductive processes in Ulva, I tested the hypothesis that gamete and zoospore release differ with respect to the level of environmental water motion. Gametes may be released during periods of low water motion in order to maximize fertilization success and provide genetic recombination. Zoospores are predicted to be the main dispersal stage and to be released during periods of high water motion to maximize dispersal.
Figure 14. *Ulva lactuca*.

Bar = 5 cm
Figure 15. *Ulva lactuca* gametes.

Bar = 10 μm
Figure 16. *Ulva lactuca* zoospores.

Bar = 10 μm
Materials and Methods

Sporophyte and Gametophyte Experiments

*Ulva lactuca* was collected randomly on spring tides at Schoodic Point, Maine, on August 12 (trial 1), August 24 (trial 2), September 10 (trial 3), and September 26, 2000 (trial 4). Each collection consisted of 18 individual thalli from exposed shores in the lower intertidal zone; no individuals were collected from tidepools. In each case, the entire thallus was removed from the substrate at the holdfast, placed in a plastic bag, and transported back to the lab on ice. All collections were made during the afternoon low tide to minimize disruption of the photoperiod.

In the lab, epiphytes were removed from the thalli, and individuals were spun in a salad spinner to remove excess water and weighed before being placed in 250 mL Erlenmeyer flasks containing 200 mL sterile seawater. Nine flasks were assigned haphazardly to shaken treatments on orbital shakers (VWR) at 170 rpm and nine flasks were assigned to calm treatments on interspersed stationary platforms of the same height in a walk-in culture chamber (13°C, 70-80 μmol photons • m² • sec⁻¹, 16:8 L:D).

Each day for four consecutive days, the flasks were observed for the presence of green clouds of cells within the flask. Notes were made on the distribution of these clouds (surface of water or bottom of flask, towards the light source, or evenly distributed in the flask). Six hours after the start of the light period, thalli were removed temporarily from the flask, while the flask was swirled and a 1 mL water sample was removed and diluted in 1 mL deionized water. Adult thalli were then placed in flasks with fresh medium under the previous treatment conditions. Zoospores or gametes in each sample
were counted with a hemacytometer and the number of cells • mL\(^{-1}\) was calculated from the following equation: cells • mL\(^{-1}\) = average of 10 counts • dilution factor • 10\(^4\).

Gametes and zoospores were determined by counting the number of flagella (gametes = 2 flagella, zoospores = 4 flagella) using 400x magnification and phase contrast optics. This determination was also verified by observing the behavior and distribution of the cells in the flasks (gametes = positively phototactic, zoospores = negatively phototactic).

The total number of cells released (gametes or zoospores) was divided by the wet weight of the reproductive thalli and log-transformed. This number was used for analysis in a 2-factor ANOVA (treatment and trial) with SAS statistical software (version 6.07).

**Gametophyte : Sporophyte Ratios**

Gametophyte : sporophyte ratios were determined using the release data from the hydrodynamic experiments. Additional collections were made on June 3, 2001, from Great Head, Maine, and on June 4, 2001, from Schoodic Point, Maine. Both collections consisted of 20 individuals, randomly collected from exposed shores along a transect line of 20 random numbers laid haphazardly in the *Ulva* patch. No individuals were collected from tidepools. Each thallus was placed in a 250 mL Erlenmeyer flask containing 200 mL seawater and cultured. (12°C, 80 \(\mu\)mol photons • m\(^{-2}\) • sec\(^{-1}\), 14:10 L:D). Once propagule release was observed in a flask, a sample was taken and the number of flagella was counted (gametes = 2 flagella, zoospores = 4 flagella) as above.
Neap Tide Release

Visual observations were made of clouds of cells (swarmers) being released from *Ulva lactuca* in tidepools on a neap tide at Blueberry Hill, Maine (Schoodic), on May 1 and June 12, 2001, and at Great Wass Island on May 12, 2001. A water sample was removed from a tidepool at Blueberry Hill on May 1 and transported back to the lab to determine if the cells being released were gametes or zoospores. Twenty individuals were collected randomly from Blueberry Hill on May 1 and brought back to the lab, placed in a 250 mL Erlenmeyer flask containing 200 mL seawater, and cultured (12°C, 80 μmol photons • m⁻²• sec⁻¹, 14:10 L:D) for two days. No water samples or individuals were collected from Great Wass Island.

Characterization of Water Velocity

Water velocity was determined by the method of 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 a 45° angle to the direction of the water flow. Ground black pepper was used as a particle to reflect the acoustic signal in a 250 mL Erlenmeyer flask with 200 mL seawater.


## Results

### Spore Release

*Ulva lactuca* released a significantly higher number of zoospores under shaken treatments compared to calm treatments (Figure 17). There was no significant difference between trials and there was no trial x treatment interaction (Table 10).

**Table 10. Two-way ANOVA table for *Ulva lactuca* zoospore release.**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>5.751</td>
<td>5.751</td>
<td>11.77</td>
<td>0.0034</td>
</tr>
<tr>
<td>Trial</td>
<td>3</td>
<td>0.792</td>
<td>0.264</td>
<td>0.54</td>
<td>0.6613</td>
</tr>
<tr>
<td>Trial x Treatment</td>
<td>3</td>
<td>0.999</td>
<td>0.500</td>
<td>1.020</td>
<td>0.382</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>7.816</td>
<td>0.488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>9.106</td>
<td>1.518</td>
<td>3.11</td>
<td>0.033</td>
</tr>
</tbody>
</table>
Gamete Release

*Ulva lactuca* released a significantly higher number of gametes under shaken treatments compared to calm treatments (Figure 18). There was no significant difference between trials, and the trial x treatment interaction was not significant (Table 11).
Table 11. Two-way ANOVA table for *Ulva lactuca* gamete release.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>7.323</td>
<td>7.323</td>
<td>16.22</td>
<td>0.0002</td>
</tr>
<tr>
<td>Trial</td>
<td>3</td>
<td>3.66</td>
<td>1.223</td>
<td>2.71</td>
<td>0.0579</td>
</tr>
<tr>
<td>Trial x Treatment</td>
<td>3</td>
<td>0.975</td>
<td>0.325</td>
<td>0.72</td>
<td>0.5462</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>18.060</td>
<td>0.452</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>10.592</td>
<td>1.513</td>
<td>3.35</td>
<td>0.0066</td>
</tr>
</tbody>
</table>
Figure 18. *Ulva lactuca* gamete release.

Mean ± SE

**Gametophyte : Sporophyte Ratios**

Gametophyte : sporophyte ratios changed from August to September 2000 at Schoodic Point. There was a trend for more gametophytes to be present as the season progressed. A survey during following June (2001) showed the same ratio of gametophytes to sporophytes as the previous September. During June 2001, the
gametophyte: sporophyte ratio appeared lower at Great Head than at Schoodic Point (Table 12).

Table 12. Gametophyte: sporophyte ratios

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Ratio (Gametophyte:Sporophyte)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/16/00</td>
<td>Schoodic</td>
<td>0.88</td>
</tr>
<tr>
<td>8/24/00</td>
<td>Schoodic</td>
<td>2</td>
</tr>
<tr>
<td>9/1/00</td>
<td>Schoodic</td>
<td>1.83</td>
</tr>
<tr>
<td>9/30/00</td>
<td>Schoodic</td>
<td>8</td>
</tr>
<tr>
<td>6/4/01</td>
<td>Schoodic</td>
<td>8</td>
</tr>
<tr>
<td>6/3/01</td>
<td>Great Head</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Neap Tide Release

Samples of swarmers collected from tidepools at Blueberry Hill on May 1, 2001, consisted exclusively of gametes. All cells in the water sample were biflagellate, and positively phototactic; both in the lab and in the tidepool they aggregated at the surface of the water. Of the twenty individuals collected on this date, eight released propagules, which consisted entirely of gametes. No release was observed in any tidepools at Blueberry Hill on June 12, 2001.

All of the swarmers from Great Waas Island on May 12, 2001, aggregated at the surface layer. I assume, therefore, that they were gametes.
Figure 19. Flow characteristics for containers used in *Ulva lactuca* experiments.

A 250 mL flask with 200 mL seawater, orbital shaker, 170 rpm.
Discussion

Water motion affected gamete and zoospore release in *Ulva lactuca*. The release of both gametes and zoospores was enhanced when water motion was high. Furthermore, calm conditions did not inhibit the release of either gametes or zoospores. This has several implications for the population biology of the species.

The increased release of zoospores under shaken conditions agrees with the notion that zoospores act as dispersal agents, and are released during conditions that optimize dispersal. This strategy may contribute to the species' success as an opportunistic, rapid colonizer.

Releasing gametes during periods of high water motion can dilute gametes and decrease fertilization success. Since *Ulva lactuca* releases more gametes under shaken conditions, gametes may become diluted and fertilization success may be low. However, *Ulva lactuca* is known to release gametes synchronously on the coast of California (Smith 1947), which indicates that there may be other mechanisms besides sensitivity to water motion to maximize fertilization. *Ulva* gametes are positively phototactic and swarm at the surface of the water. Swarming at the surface of the water may facilitate fertilization. By aggregating in two dimensions, instead of three, the gametes can increase their probability of encounter, as do gametes of many corals (Babcock 1995).

Stratmann et al. (1996) found that the presence of a swarming inhibitor, a chemical secreted from the gametophyte, regulated gamete release in *Ulva mutabilis* Føyn. The authors found that gamete release was inhibited when this chemical accumulated in culture media. The results from Stratmann et al. (1996) may explain the results seen in the gametophyte experiments. A chemical released from the gametophyte
could build up in high concentrations close to the thallus in the calm treatments and inhibit gamete release. In the shaken treatments, the swarming inhibitor would be diluted by the agitation and have less of an effect on gamete release. It is not clear how this chemical affects natural gamete release, but it may coordinate the process in dense patches of *Ulva*.

*Ulva* is also known to regenerate the gametophytic stage through parthenogenesis of unfused gametes (Lövlie and Bryhni 1978), providing an auxiliary mechanism for population success. The presence of parthenogenesis has been argued to be the reason for high gametophyte : sporophyte ratios (Lövlie and Bryhni 1978). Gametophytes dominated the population at Schoodic Point except for the August 16, 2000, collection. The same result was found for Great Head. These results suggest that fertilization is low among *Ulva* at those two locations. Predicting an optimal gametophyte : sporophyte ratio is difficult because factors such as fertilization success, recruitment success, and survivorship are not known for this species. If those factors are optimized for both gametophytes and sporophytes, a ratio of 2:1 gametophytes : sporophytes should result. Although it is tempting to make these kinds of assumptions with an isomorphic species, such assumptions should be avoided because isomorphic species can vary considerably with respect to the ecology and physiology of different life history stages (Destombe et al. 1989, Destombe et al. 1993). Nonetheless, data from this study demonstrate the likelihood that more gametes are released during periods that are not optimal for fertilization success. Therefore it is likely that parthenogenesis is common among *Ulva* on these shores, and that fertilization success is highly variable. Further study, however, is needed to determine if this is indeed the case, and if *Ulva lactuca* can achieve high
fertilization success under conditions of high water motion due to the aggregation of gametes at the water’s surface.
Chapter 4

Pseudo-nitzschia multiseriess

Introduction

The reproductive ecology and environmental conditions that stimulate or inhibit sexual reproduction in Pseudo-nitzschia multiseriess (Hasle) Hasle (Bacillariophyceae) are not fully understood. This species is responsible for amnesic shellfish poisoning through the production of domoic acid, a neurotoxin (Bates et al. 1989). To date, the only environmental variable tested for its effect on reproduction has been light (Hiltz et al. 2000). Due to the common worldwide occurrence of domoic acid-producing species (Bates 2000, Bates et al. 1998), it is important to understand the conditions under which toxic blooms occur. Therefore, other important environmental conditions such as water motion need to be examined for possible effects on sexual reproduction and other bloom dynamics.

Asexual reproduction in diatoms is accomplished through vegetative cell division. This process results in a decrease in the overall cell size in the population with each cycle of division. Sexual reproduction is the only way to restore the maximum cell size and produce genetic recombinants (Geitler 1932; also see Drebes 1977, Round et al. 1990, Edlund and Stoermer 1997); and therefore is a necessary part of diatom life-history. In addition, sexual reproduction produces empty frustules that sink and is regarded as important to the biogeochemical cycling of silica (Crawford 1995).
In order for sexual reproduction to be successful, gametes must fuse to form a zygote. In pennate diatoms, the gametes are amoeboid rather than flagellated. The parent cells, therefore, rely on close proximity and contact to initiate gametogenesis (van den Hoek et al. 1995). Pairing of parent cells may be disrupted by high water motion, and may cause gametogenesis to be inhibited or decrease fertilization success.

Harmful algal blooms are often associated with calm water conditions (Wyatt and Horwood 1973, Thomas and Gibson 1990) because of the negative effect water motion has on the growth of phytoplankton. (Savidge 1981, Thomas and Gibson 1990, Berdalet 1992, Juhl et al. 2000, Juhl et al. 2001. Estrada et al. 1987, Estrada et al. 1988, Zirbel et al. 2000). Much of the current knowledge of the effects of water motion on phytoplankton is based on the studies of dinoflagellates but cells of similar size and shape will experience similar hydrodynamic parameters. In summary, water motion may affect growth, gametogenesis, or gamete fusion in diatoms.

To determine whether water motion has an effect on diatom reproduction, I utilized the pennate diatom *Pseudo-nitzschia multiseries*. In pennate diatom, contact between parent cells is necessary for gametogenesis and successful reproduction (Davidovich and Bates 1998), therefore, I hypothesized that water motion would inhibit gametogenesis and decrease fertilization success due to an inability of the parent cells to achieve contact under conditions of high water velocity. I chose this species because its sexual reproduction had been described in detail (Davidovich and Bates 1998) and sexually mature clones are easily obtained.

*Pseudo-nitzschia multiseries* undergoes vegetative binary fission under normal culture conditions, reducing its cell size each time. When the cell length reaches < 80 μm,
it undergoes sexual reproduction to restore large, new cells. Two cells, "female" and "male", align themselves valve-to-valve (Davidovich and Bates 1998, Kaczmarska et al. 2000). Meiosis results in two gametes per cell and loss of the parent vegetative cell. Gametes are either active "male" or passive "female". The frustules of the parent cells open, and the unflagellated active gametes move towards the passive gametes by amoeboid action. The zygotes that result from this fusion grow into long auxospores to produce the initial cell and restore the maximal cell length (Davidovich and Bates 1998). As *P. multiseries* decreases in cell size through normal vegetative growth, its domoic acid production also decreases. The domoic acid concentration increases after the cells undergo sexual reproduction and restore maximal cell size (Bates et al. 1999).

Sexual reproduction is most successful under 10:14 L:D growth conditions (Hiltz et al. 2000) This light regime corresponds with the shorter light period in the fall, when monospecific blooms of *P. multiseries* have been documented off Prince Edward Island (Bates et al. 1989).
Materials and Methods

Effect of Water Motion

*Pseudo-nitzschia multiseries* clones CLN-8, CLN-14, and CLN-15 were obtained from Dr. Stephen Bates at Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, New Brunswick. Clones used in this study were the sexual offspring of *P. multiseries* clones that had been isolated previously by Claude Léger (Fisheries and Oceans Canada, Moncton) from water samples taken in New London Bay, Prince Edward Island, Canada, in November 1996 (Table 13). All stock cultures were maintained by C. Léger in f/2 media within a culture chamber (20°C, 100 μm photons•m⁻²•sec⁻¹, 10:14 L:D). I conducted all experiments at Fisheries and Oceans Canada in Moncton, New Brunswick and analyzed the results at the University of Maine.

Table 13. Origin and apical cell length of *Pseudo-nitzschia multiseries* clones used in the experiment.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Parental Clones</th>
<th>Sex</th>
<th>Date Isolated</th>
<th>Apical Cell Length (μm; mean ± SD; n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLN-8</td>
<td>CL-45 x CL-47</td>
<td>&quot;female&quot;</td>
<td>October, 1997</td>
<td>47.9 ± 2.2</td>
</tr>
<tr>
<td>CLN-14</td>
<td>CL-46 x CL-48</td>
<td>&quot;male&quot;</td>
<td>October, 1997</td>
<td>49.6 ± 2.0</td>
</tr>
<tr>
<td>CLN-15</td>
<td>CL-46 x CL-48</td>
<td>&quot;female&quot;</td>
<td>October 1997</td>
<td>39.0 ± 2.5</td>
</tr>
</tbody>
</table>

Exponentially growing cells were used for mating crosses (Davidovich and Bates 1998). *Pseudo-nitzschia multiseries* clone CLN-14 was crossed with clones CLN-8 and CLN-15 in 6-well culture plates. Twelve, 6-well plates were used for both mating
crosses. Four drops (0.16 mL) of each starting culture at a concentration of $2.56 \times 10^5$ cells $\cdot$ mL$^{-1}$ (CLN-8), $1.53 \times 10^5$ cells $\cdot$ mL$^{-1}$ (CLN-14), or $2.14 \times 10^5$ cells $\cdot$ mL$^{-1}$ (CLN-15) were placed in each of 72 wells (6 wells per plate, 12 plates per mating cross) containing 5 mL of 0.4 $\mu$m filtered f/2 media. Six plates of each mating cross were assigned haphazardly to the calm treatment and six plates were assigned haphazardly to the shaken treatment on interspersed orbital shakers (VWR model 98001, 170 rpm) and stationary platforms in a walk-in culture chamber (20° C, 10:14 L:D, 79 – 111 $\mu$mol photons $\cdot$ m$^{-2}$ $\cdot$ sec$^{-1}$). Each day for four consecutive days the entire 5 mL mating cross was removed with a pipette from one pre-assigned well on each replicate plate in each treatment and fixed with 2 drops of Lugol’s iodine solution.

All cells were allowed to settle in a petri dish (diameter = 5.0 cm). Five positions on the dish were selected haphazardly and the total number of vegetative cells (Figure 20), gametes (Figure 21) (free or within frustules, 5 $\mu$m cells), zygotes (Figure 22) (free or attached to frustules, 10 $\mu$m cells), and auxospores were counted on an inverted microscope at 100x magnification (viewing diameter = 2.0 mm) or 200x magnification (viewing diameter = 0.89 mm). The counts were corrected for the different viewing areas by multiplying by the ratio of the two areas. Percent gametogenesis, percent zygotes, and fertilization success were calculated for each day as:

- **Gametogenesis (%)** = $\frac{(G/2)}{(V + Z + (G/2))} \times 100$
- **Zygotes (%)** = $\frac{Z}{(V + Z + (G/2))} \times 100$
- **Fertilization success (%)** = $\frac{Z}{(Z + (G/2))} \times 100$

where $G$ = the mean number of gametes, $V$ = the mean number of vegetative cells, and $Z$ = the mean number of zygotes. This method differs from previous calculations of
reproductive output (Hiltz et al. 2000) but provides a better estimate of the total number of cells present by taking into account the number of vegetative cells lost to reproduction during each 24-hour sampling period. The data were arcsin transformed and a 2-factor ANOVA (treatment and date) was conducted for each mating cross with the a priori assumption that the two mating crosses would behave different (SAS mainframe statistical software, version 6.07).

**Characterization of Water Velocity**

Water velocity was determined by the method of 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 a 45° angle to the direction of the water flow. Ground black pepper was used as a particle to reflect the acoustic signal. Output was recorded at 5 mm • sec\(^{-1}\) on a high speed chart recorder (Gould, model 220). Water flow was generated by an orbital shaker (VWR model 98001). The shaker was set at 170 rpm with 5 mL seawater in 1 well of a 6-well plate.
Figure 20. *Pseudo-nitzschia multiseries* vegetative cells.

Bar = 25 μm
Figure 21. *Pseudo-nitzschia multiseries* gametes. Arrows indicate gametogenesis within a vegetative cell.

Bar = 25μm
Figure 22. *Pseudo-nitzschia multiseries* zygote. Arrow indicates zygote attached to parent frustule.

Bar = 25 μm
Results

No gametes or zygotes were present in either the calm or shaken treatments in samples harvested on the first day of the experiment. Thus, this day was excluded from further analysis of gamete and zygote production. In addition, no auxospores were found in any replicates in either treatment on any day.

CLN-14 x CLN-8 Crosses

Populations in the calm and shaken treatments had the same growth rate from day 1 to day 2. On the third day there was a slight difference in growth rate with fewer cells in the calm treatment. The number of vegetative cells in both calm and shaken treatments was equivalent by the fourth day of sampling (Figure 23).

![Graph showing vegetative growth](image)

**Figure 23.** Vegetative growth for *Pseudo-nitzschia multiseries* CLN-14 x CLN-8 mating cross.

*Mean ± SE.*
*Points are the mean of 6 replicates. Error bars are too small to be seen.*
There was no significant difference in gametogenesis between the calm and shaken treatments. There was a significant effect of the sampling date, reflecting the relative amount of gametes present. There was also a significant date by treatment interaction due to the change from higher gametogenesis (%) in the calm treatment on days 2 and 3 to higher gametogenesis (%) in the shaken treatment on day 4 (Table 14; Figure 24).

**Table 14. Pseudo-nitzschia multiseries CLN-14 x CLN-8 mating cross: Two-way ANOVA for gametogenesis.**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0000331</td>
<td>0.0000331</td>
<td>0.43</td>
<td>0.5157</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>0.0020765</td>
<td>0.0010382</td>
<td>13.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment X Date</td>
<td>2</td>
<td>0.0006070</td>
<td>0.0003035</td>
<td>3.97</td>
<td>0.0295</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.0022923</td>
<td>0.0000764</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.0027166</td>
<td>0.0005433</td>
<td>7.11</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Calm treatments had a significantly higher number of zygotes compared to shaken treatments. There was a significant effect of date, reflecting the relative number of zygotes present on each day, but no significant date by treatment interaction was found (Table 15; Figure 25). Calm treatments also had higher fertilization success compared to shaken treatments on each of the days in which gametes were produced. (Table 16).

**Figure 24. Gametogenesis for Pseudo-nitzschia multiseries**
CLN-14 x CLN-8 mating cross.

**Mean ± SE**
Table 15. *Pseudo-nitzschia multiseries* CLN-14 x CLN-8 mating cross:
Two-way ANOVA for zygote production.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0000568</td>
<td>0.0000568</td>
<td>147.29</td>
<td>0.0007</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>0.0001432</td>
<td>0.0000716</td>
<td>18.01</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment X Date</td>
<td>2</td>
<td>0.0000245</td>
<td>0.0000122</td>
<td>3.07</td>
<td>0.0610</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.0001193</td>
<td>0.0000040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.0002244</td>
<td>0.0000449</td>
<td>11.29</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Figure 25. Zygote production for *Pseudo-nitzschia multiseries* CLN-14 x CLN-8 mating cross.

Mean ± SE

Note: No zygotes were produced on day 2 in the shaken treatment.
Table 16. Fertilization success for *Pseudo-nitzschia multiseries* CLN-14 x CLN-8 mating cross.

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Treatment</th>
<th>Fertilization Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Calm</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Shaken</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Calm</td>
<td>33.33 ± 6.44</td>
</tr>
<tr>
<td>3</td>
<td>Shaken</td>
<td>4.12 ± 0.78</td>
</tr>
<tr>
<td>4</td>
<td>Calm</td>
<td>41.45 ± 7.73</td>
</tr>
<tr>
<td>4</td>
<td>Shaken</td>
<td>18.64 ± 3.51</td>
</tr>
</tbody>
</table>

**CLN-14 X CLN-15 Crosses**

Vegetative cells had the same division rate from day 1 to day 2. On the third day, there were slightly fewer cells in the calm treatment and the growth rate diverged. The number of vegetative cells in both calm and shaken treatments was equivalent by the fourth day of sampling (Figure 26).

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There was significantly more gametogenesis (%) in the calm treatment than in the shaken treatment. There was a significant effect of date, reflecting the relative number of gametes on each sampling date; however, there was no significant date by treatment interaction (Table 17; Figure 27).
Table 17. *Pseudo-nitzschia multiseries* CLN-14 x CLN-15 mating cross: Two-way ANOVA for gametogenesis.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0002904</td>
<td>0.0002904</td>
<td>8.95</td>
<td>0.0055</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>0.0002976</td>
<td>0.0001488</td>
<td>4.58</td>
<td>0.0183</td>
</tr>
<tr>
<td>Treatment X Date</td>
<td>2</td>
<td>0.0000782</td>
<td>0.0000391</td>
<td>1.20</td>
<td>0.3139</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.0009737</td>
<td>0.0000325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.0006662</td>
<td>0.0001332</td>
<td>4.10</td>
<td>0.0059</td>
</tr>
</tbody>
</table>
A significantly higher number of zygotes was present in the calm treatment. There was a significant effect of sampling date as well as a significant date by treatment interaction (Table 18; Figure 28). Calm treatments also had higher fertilization success compared to shaken treatments on each of the sample dates in which gametes were produced (Table 19).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.0000870</td>
<td>0.0000870</td>
<td>6923</td>
<td>0.0001</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>0.0000611</td>
<td>0.0000306</td>
<td>24.34</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment X Date</td>
<td>2</td>
<td>0.0000196</td>
<td>0.0000098</td>
<td>7.81</td>
<td>0.0019</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.0000337</td>
<td>0.0000013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.0001677</td>
<td>0.0000335</td>
<td>26.70</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Figure 28. Zygote production for *Pseudo-nitzschia multiseries* CLN-14 x CLN-15 mating cross.

Mean ± SE

Note: No zygotes were produced on days 2 and 3 in the shaken treatment.
Table 19. Fertilization success for *Pseudo-nitzschia multiseries* CLN-14 x CLN-15 mating cross.

**Mean ± SE**

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Treatment</th>
<th>Fertilization Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Calm</td>
<td>10.34 ± 1.14</td>
</tr>
<tr>
<td>2</td>
<td>Shaken</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Calm</td>
<td>43.39 ± 5.81</td>
</tr>
<tr>
<td>3</td>
<td>Shaken</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>4</td>
<td>Calm</td>
<td>42.53 ± 5.01</td>
</tr>
<tr>
<td>4</td>
<td>Shaken</td>
<td>31.68 ± 3.58</td>
</tr>
</tbody>
</table>

The total number of cells (vegetative, gametes, and zygotes) on the fourth day was extrapolated to determine how many of each type of cell was present at the end of the experiment. This was done by multiplying the ratios of the areas of the counting field of view by the ratio of the entire petri dish used for the counts. The results of this analysis are presented in Table 20.
Table 20. Day 4 vegetative cells, gametes, and zygotes extrapolated to the entire 5 mL sample.

a. CLN-14 x CLN-8 mating cross.
b. CLN-14 x CLN-15 mating cross.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vegetative Cells Mean ± SE</th>
<th>Gametes Mean ± SE</th>
<th>Zygotes Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calm</td>
<td>5.66 x 10⁶ ± 2.71 x 10⁵</td>
<td>1.05 x 10³ ± 1.27 x 10⁴</td>
<td>3.75 x 10⁴ ± 7.94 x 10³</td>
</tr>
<tr>
<td>Shaken</td>
<td>5.31 x 10⁶ ± 2.91 x 10⁵</td>
<td>1.54 x 10⁵ ± 6.29 x 10⁴</td>
<td>1.80 x 10⁴ ± 3.86 x 10³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vegetative Cells Mean ± SE</th>
<th>Gametes Mean ± SE</th>
<th>Zygotes Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calm</td>
<td>5.86 x 10⁶ ± 8.38 x 10⁵</td>
<td>9.37 x 10⁴ ± 7.36 x 10³</td>
<td>1.94 x 10⁴ ± 1.87 x 10³</td>
</tr>
<tr>
<td>Shaken</td>
<td>5.05 x 10⁶ ± 1.61 x 10⁵</td>
<td>3.23 x 10⁴ ± 1.01 x 10³</td>
<td>7.4 x 10³ ± 4.09 x 10³</td>
</tr>
</tbody>
</table>
Figure 29. Flow characteristics for containers used in *Pseudo-nitzschia multiseries* experiments. A 6-well plate (10 mL capacity) with 5 mL seawater, orbital shaker set at 170 rpm.
Discussion

Results from these experiments demonstrate that water motion has an effect on sexual reproduction in *Pseudo-nitzschia multiseries*. This effect was mainly at the level of fertilization success and zygote production. Both clones had a significantly higher number of zygotes produced in the calm treatment compared to the shaken treatment. Although there was a significantly higher number of gametes produced in the calm treatment for the CLN-14 X CLN-15 crosses, this effect was not observed in the CLN-14 X CLN-8 crosses. There was, however a trend during sample days 2 and 4 for increased gamete production in the calm treatment. This trend reversed itself in the final day of sampling, possibly due to the increase in cell density on the final day. This, as well as the relative difference in the number of gametes produced each day between mating crosses, suggests that there is some degree of clonal variation.

In both mating crosses, zygote production increased with time in both calm and shaken treatments. Gamete production did not follow this pattern. Although it is not known how long *P. multiseries* gametes are viable, this study suggests that gametes from the first day are not being fertilized within the 24-hour period between samples and that gametes from one day are being carried over and fertilized on subsequent days.

In this study the calm and shaken treatments produced a large difference in fertilization success. Fertilization success in the shaken treatment lagged far behind that in the calm treatment and did not start to increase until the fourth day. This may have been caused by the increase in density of the vegetative cells and the gametes after four days. Under a high enough density, the vegetative cells may begin to cluster, facilitating zygote production even under hydrodynamically unfavorable conditions. Alternatively,
water, motion may facilitate cell-to-cell contact at high densities (Rothschild and Osborn 1988).

Water motion is known to affect cell division in marine phytoplankton (Tuttle and Loeblich 1975, Gallerton 1976, Pollingher and Zemel 1981, Berdalet 1992). Under high shear, the total amount of DNA within the cells of the dinoflagellate Gymnodinium nelsonii Martin increases without cell division; the hydrodynamic effect of turbulence is thought to be a physical disturbance of the spindle, preventing separation of the chromosomes (Berdalet 1992). Comparisons between my experiment and those done with dinoflagellates should be treated with caution, however, because diatoms have shown greater variability in their growth response to high water velocity and are considered less sensitive than dinoflagellates to water motion (Thomas and Gibson 1990). The response of diatoms to varying hydrodynamic conditions can be nutrient-specific (Savidge 1981), indicating the possibility for chemical perception of the level of water motion. In my experiments with P. multiseries, cells in the calm and shaken treatments had similar division rates (Figure 23; Figure 26), therefore, the treatment effect was not caused by a disturbance of the cell cycle.

It is likely that the cause of reduced gamete and zygote production is a decrease in the cell-to-cell contact caused by shear. Two parent cells must be in contact in order to reproduce successfully, and free-floating gametes do not fertilize due to the lack of substrate required for amoeboid movement (Davidovich and Bates 1998). Paired cells are easily disturbed, breaking the weak bond that holds them together (personal observation). Bates et al. (1998) postulated that under bloom conditions, sexual reproduction would be favored because the high density would increase the chance of
encounter between cells. Although this study cannot support or refute that idea, these data indicate that calm water is the environmental condition most favorable for reproduction and suggest that when water motion is present, fertilization is only successful when the population reaches very high densities. Although *P. multiseries* reproduces when it is under log-growth conditions, in the ocean, water motion can dilute the cells, and thus decrease the chance of two cells encountering each other. During a bloom, cells may reach high enough densities for gametogenesis to occur but successful fertilization will only happen if cell-to-cell contact is not disturbed by water motion.

In this experiment, the cells were likely not being diluted by turbulence after the third day. This is due, in part, to the starting concentration of cells, as well as the size of the container used. The starting concentration used was needed to assure that reproduction would take place, because of the need for cells to encounter each other. The size of the container used was necessary to conduct the experiment with the number of replicates used. This experiment can now be repeated over a longer time period and using larger containers to better test the dilution effect of water motion on the cells.
Chapter 5

Concluding Remarks

This study of these algae demonstrates that reproductive responses to water motion differ among species as well as between life-history phases within the same species. These differences reflect the overall biology, life histories, and ecology of the species studied. In addition, these experiments open up new questions related to the timing and frequency of reproduction, dispersal ability, and levels of genetic variability within populations of *Alaria esculenta*, *Ulva lactuca*, and *Pseudo-nitzschia multiseries*.

The different responses to water motion between the sporophytic and gametophytic stages of *Alaria esculenta* suggest that the life history of this species is an adaptation to maximize dispersal and fertilization. This agrees with the hypotheses of Neushul (1972) and Bell (1997). However, to test if increased zoospore release in response to turbulence maximizes dispersal, measurements of zoospore release under natural conditions are needed. These data need to be collected at different localities and compared to the genetic variability within and between populations. Kusumo and Druehl (2000) published population genetic data for *Alaria marginata* and correlated it to wave exposure but, to date, we lack any data on natural zoospore or gamete (sperm) release in *Alaria*.

In my experiments, I demonstrated that sperm release and fertilization success in *A. esculenta* are increased under calm conditions. I believe that this effect was due to a dilution of the sperm releasing and attracting pheromone under increased levels of water motion, however, other factors cannot be ruled out by my experiments. It is possible that
female gametophytes do not produce as much pheromone under turbulent conditions, or that there is mechanical damage to the antheridia in turbulent conditions that inhibits sperm release. In order to test my hypothesis, further experiments are needed. In Gametophyte Experiment 2, I added female-conditioned media (i.e., medium in which female gametophytes had grown) directly to male gametophytes under calm and shaken conditions. This resulted in the only case where there was no significant treatment effect on sperm release. Pheromone was assumed to be present in the media because sperm were released Maier (1995). Since the pheromone concentration is unknown, similar experiments should be repeated with known concentrations of the pheromone. These experiments would give a better understanding of the role the pheromone has under calm and shaken conditions.

The results from my experiments with *Ulva lactuca* have implications for dispersal, fertilization success, and genetic variability within *Ulva* populations. I demonstrated that both gamete and zoospore release increase when levels of water motion increase. This has the potential to maximize dispersal. *Ulva* is capable of parthenogenesis through the development of unfertilized gametes (e.g. Lövlie and Bryhni 1978). Gametes released under calm conditions may produce zygotes to provide genetic recombination whereas gametes released on days with high water motion may disperse and develop parthenogenetically. Since individual *Ulva lactuca* individuals in Maine release gametes within a window of 1-3 days around spring and neap tides (personal observation), any individual may experience several different hydrodynamic conditions within that period. This would optimize both dispersal and fertilization.
To better understand the reproductive biology of *Ulva*, the release of gametes and zoospores should be quantified in the field along with the hydrodynamic conditions at the time of release. Population genetic studies should also be done to determine the amount of genetic variability within and between populations. In addition, long-term monitoring of gametophyte : sporophyte ratios should be done at a variety of locations. This would help in understanding the relative roles of fertilization and parthenogenesis within populations.

Since differences in the response of gametogenesis to water motion were found between different clonal crosses, my experiments with *Pseudo-nitzschia multiseries* should be repeated with the same clones, as well as additional clones. Other experiments should include a cessation of shaking after several days to determine if these cells can reach fertilization levels comparable to those in the calm treatment. These experiments should also be repeated using larger containers. The small volume used in these experiments may have concentrated the cells when cell density increased. Larger containers, as well as containers of different shape should allow the cells to be diluted enough to better simulate the effects of wave dilution.

From my experiments on *P. multiseries*, I cannot determine if the contact rates between cells are increased or decreased due to turbulence, cell density, or both (Bates 1988, Rothschild and Oborn 1988). My data on fertilization success suggest that if cells in hydrodynamically energetic habitats come in contact long enough to undergo gametogenesis, they do not stay in contact with each other long enough for successful fertilization. It would be of interest to design experiments to determine what effect water
motion has on contact rates in *P. multiseries* because this information is critical to understanding the conditions under which sexual reproduction may occur in nature.

These experiments demonstrate the importance of examining the biology of gametes, spores, zygotes, and vegetative cells in relation to environmental variables. Further work in this area will help to better understand the life histories, bloom dynamics, and ecology of other algal species.
Bibliography


BIOGRAPHY OF THE AUTHOR

Richard Gordon was born in Portland, Oregon, on October 28, 1974. He was raised in Woodinville, Washington, and graduated from Woodinville High School in 1992. He attended The University of Washington and graduated in 1997 with a Bachelor’s degree in Biology: Ecology, Evolution, and Conservation. After spending two years in Chicago, Illinois, teaching science for the Chicago Academy of Sciences, he moved to Maine and entered the Marine Biology graduate program at The University of Maine in the fall of 1999.

Richard received a National Science Foundation GK-12 Teaching Fellowship in his second year at The University of Maine. This gave him the opportunity to teach science in fourth and fifth grade classrooms at Lewis Libby Elementary School in Milford, Maine. He is a member of the Northeast Algal Society and the Phycological Society of America (student member of the education committee). His Master’s research is published as an abstract from a presentation at the annual meeting of the Phycological Society of America in Estes Park, Colorado, in June 2001 (Gordon, R. J. and Brawley, S. H. Hydrodynamics and algal reproduction J. Phycol. 37 (3) suppl.: 19). After receiving his degree, he will be moving to Seattle, Washington, with his wife, Laura Pisconski. Richard is a candidate for the Master of Science degree in Marine Biology from The University of Maine in December 2001.