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Chemical Competition between Microscopic Stages of Macrocystis pyrifera and Five Native Kelp Species: Does Giant Kelp Always Lose?

Maria Suzanne Christensen
San Jose State University

CHEMICAL COMPETITION BETWEEN MICROSCOPIC STAGES OF *MACROCYSTIS PYRIFERA* AND FIVE NATIVE KELP SPECIES: DOES GIANT KELP ALWAYS LOSE?

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SAN JOSÉ STATE UNIVERSITY

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Michael Graham, Ph.D.	Moss Landing Marine Laboratories
Scott Hamilton, Ph.D.	Moss Landing Marine Laboratories
Chris Lane, Ph.D.	University of Rhode Island

ABSTRACT

CHEMICAL COMPETITION BETWEEN MICROSCOPICAL STAGES OF *MACROCYSTIS PYRIFERA* AND FIVE NATIVE KELP SPECIES: DOES GIANT KELP ALWAYS LOSE?

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The giant kelp *Macrocystis pyrifera* is often considered competitively dominant to other kelp species due to its high productivity. However, on the microscopic level, previous studies found that *Macrocystis* can be inferior to other kelp species through microscopic interspecies chemical competition. Recruitment failure can be caused by neighboring kelps because there is no species specificity in the stereochemistry of the signaling chemical used during reproduction to initiate spermatozoid release; therefore, *Macrocystis* spermatozoid release is pre-empted by that of its competitors. To date, this interaction has been tested between *Macrocystis* and only one other kelp taxon, *Pterygophora*. To test whether *Macrocystis* is always chemically outcompeted microscopically, I investigated the competitive outcome, by tracking sporophyte production, between *Macrocystis* and five native kelps using laboratory studies. Tests with *Pterygophora californica* and *Ecklonia arborea* showed asymmetric results indicating that *Macrocystis* was the inferior kelp. Studies using *Alaria marginata* and *Egregia menziesii* found symmetric results where both competing species did poorly in the presence of *Macrocystis*. Lastly, when *Macrocystis* was settled with *Postelsia palmaeformis*, there was no significant difference in sporophyte production between polycultures and monocultures for either species. These results indicate that the competitively superior species will vary depending on the specific species interaction.

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INTRODUCTION

Foundation species have disproportionate positive effects on the structure and function of marine ecosystems – through the provision of habitat complexity and energy – playing a central role in sustaining ecosystem services (Dayton 1972, Bruno and Bertness 2001). Numerous foundation species have been described worldwide, including canopy-forming trees, salt marshes and mangroves, hermatypic corals, seagrasses, and kelps (Ellison et al. 2005, Reed and Hovel 2006, Graham et al. 2007, Angelini et al. 2011, Osland et al. 2013). Foundation species regulate population and community dynamics in many ecosystems by creating the vital biogenic structure of the community that not only stabilizes the local conditions but also the ecosystem processes within the system, such as productivity, competition, and water flow (Reed and Foster 1984, Ellison et al. 2005, Angelini et al. 2011, Falkenberg et al. 2012, Graham et al. 2016). Understanding the ecology of these foundation species is of great importance since their existence is a necessity to the success of the ecosystem they inhabit (Bruno and Bertness 2001, Graham 2004, Ellison et al. 2005, Gedan and Bertness 2010, Angelini et al. 2011, Graham et al. 2016, Teagle et al. 2017).

Kelps, brown marine macroalgae in the Order Laminariales, are often referred to as foundation species since they are important species in the communities they inhabit, by providing complex habitat, food and provisions to other species (Mann 1982, Dayton 1985, Foster and Schiel 1985, Holbrook et al. 1990, Stachowicz 2001, Graham 2004, Graham et al. 2007, Graham et al. 2016, Teagle et al. 2017). These seaweeds contribute to their community through their high productivity, high diversity and their complex

biological structure (Dayton 1985, Graham et al. 2007, Schiel and Foster 2015, Graham et al. 2016, Teagle et al. 2017); however, kelps are very diverse and are characterized by distinct physical and biological attributes. Kelps can vary tremendously in morphology, size, life span, phenology, fecundity, growth rates, environmental tolerance, habitat, and degree of chemical defense. These Laminarian species can be annuals (e.g., *Nereocystis luetkeana* (Mert.) Postels and Ruprecht; Amsler and Neushul 1989) or long-lived perennials (e.g., *Macrocystis pyrifera* (L.) C. Agardh; Papenfuss 1942, North 1971). Different kelps also occupy different niches constrained by the differential response of individual kelps or kelp populations to available resources, such as light (quantity and/or spectral quality), nutrients, and space, which can be modified by competitors and physical disturbance (Lüning and Neushul 1978, Dayton 1985, Graham et al. 1997, Reed et al. 1996, Schiel and Foster 2015, Graham et al. 2016).

Kelp forest community development and zonation is usually controlled by several interacting processes, including recruitment, growth, and competition for resources (Mann 1973, Dayton 1985, Carpenter 1990, Graham et al. 1997, Steneck et al. 2002, Arkema 2009, Schiel and Foster 2015). Kelps are influenced by, and affect, physical factors such as light, water motion, nutrients and available substrate for settlement and growth (Dayton 1985, Eckman et al. 1989, Schiel and Foster 2006, Christie et al. 2007 Muth 2012, Teagle et al. 2017). Kelp populations can fluctuate in size and distribution over time and space due to predictable events such as seasonal changes, or unpredictable events such as intensity of winter storms (Dayton 1985, Reed et al. 2006, Schiel and Foster 2015).

Shallow subtidal rocky-bottom areas of temperate regions of the Eastern Pacific are dominated by kelp forests (Dayton 1985, Steneck et al. 2002, Reed et al. 2004, Teagle et al. 2017) and the giant kelp *Macrocystis* is considered the dominant canopy-forming species in both hemispheres (Buschmann et al. 2006, Graham et al. 2007, Schiel and Foster 2015, Graham et al. 2016). *Macrocystis* functions as a "foundation" species in these habitats by modifying the local environment for other organisms (Schiel and Foster 2006, Graham et al. 2007, Schiel and Foster 2015, Graham et al. 2016), by altering light (Reed and Foster 1984, Dayton et al. 1999, Clark et al. 2004), physical disturbance (Jackson and Winant 1983, Jackson 1983, Rosman et al. 2007) and sedimentation (North 1971, Muth 2012). *Macrocystis* is also a foundation species by supporting high levels of biodiversity and biomass of other species (Dayton 1985, Steneck et al. 2002, Graham 2004) by providing complex habitats (Quast 1971, Foster and Schiel 1985, Holbrook et al. 1990, Carr 1994) and through its high productivity (Parker 1963, Gerard 1976), both through drift production and direct grazing opportunities. Earlier studies investigating *Macrocystis* found that this foundation species supports from 40 to over 275 common species by providing energy and habitat (Graham 2004, Graham et al. 2007) and is of great ecological and economical importance worldwide (Graham et al. 2007) by being the pillar for one of the world's most productive ecosystems which supports many human uses and activities (Schiel and Foster 2015).

Giant kelp forests exist along the California coast where the coastal climate is highly seasonal (Foster 1982, Graham et al. 2007, Schiel and Foster 2015). Winter storms create large swells and upwelling is most prominent in the spring (Huyer 1983, Foster 1982,

Graham 1997, Biller et al. 2013). Tidal flushing from nearshore submarine canyons (Breaker and Broenkow 1994) and strong upwelling (Huyer 1983, Traganza et al. 1981) result in year round presence of cold nutrient-rich water, which promotes the presence of thriving kelp forests (Graham et al. 1997). Oceanographic variability at the scale of seasons (e.g., winter storms), years (e.g., El Niño Southern Oscillation) or decades (e.g., Pacific Decadal Oscillation) drive subsequent variability of species composition, abundance, and distribution in kelp forests (Cowen et al. 1982, Foster 1982, Dayton et al. 1984, Dayton and Tegner 1984, Foster and Schiel 1985, Dayton et al. 1992, Graham et al. 1997, Dayton et al. 1999). Large swells during the winter remove many of the larger dominant canopy-forming kelps, such as *Macrocystis*, thinning the canopy cover of the kelp forest and subsequently preventing competitive exclusion of many understory species, thereby increasing overall biodiversity (Gerard 1976, Dayton 1985, Dayton et al. 1992, Graham et al. 1997, Dayton et al. 1999, Clark et al. 2004). Depending on the kelp species, recruitment can occur continuously (e.g., *Macrocystis*), or during specific temporal recruitment windows when environmental conditions are favorable (e.g., *Pterygophora californica* Rupr.). Various factors can affect the magnitude of kelp recruitment, such as light and nutrient concentrations (Lüning and Neushul 1978, Deysher and Dean 1986a, Deysher and Dean 1986b, Kinlan et al. 2003), zoospore settlement densities (Reed 1990) and aggregations (Foster 1975a), available substrate (Muth 2012), and levels of competitors and grazers (Reed ad Foster 1984, Ebeling et al. 1985, Harrold and Reed 1985, Reed 1990).

Competition, whether it is between species or between individuals within a species, plays an important role in the structuring of seaweed populations and communities (Reed and Foster 1984, Santelices and Ojeda 1984, Dayton 1985, Olson and Lubchenco 1990, Graham 1997, Arenas and Fernandez 2000). In a population, determinants of population growth, such as size and age structure, may be affected by competition (Olson and Lubchenco 1990). On the community-level, competition may influence patterns such as species diversity and succession (Lubchenco and Gaines 1981). At least one shared resource must be in limited supply for competition to occur and spatial and temporal variability in resource supply will determine the intensity and nature of a competitive interaction (Carpenter 1990). Interspecific competition is the competitive interaction between species, which results from one species using an available limited resource, such as space or light, at the expense of the other (Connell 1961, Connell 1983a, Schoener 1983). Intraspecific competition arises when individuals from the same species compete for a limited resource. It is common for an organism to overlap in resource utilization, not only with individuals from the same species, but also among several other species; therefore, an organism can be engaged in multiple intra-and interspecific interactions simultaneously (Diamond 1978).

Macrocystis is considered to have great ecological success around the world and is often named the competitive dominant kelp on the macroscopic scale because of its high plasticity in form and function (Dean et al. 1989, Graham et al. 2007, Schiel and Foster 2015). Unlike other kelps and macroalgae, *Macrocystis* displays an extreme adaptability to variable environmental conditions (Santelices 1990, Graham et al. 2007) by changing

its growth, productivity, or reproductive pattern. Due to the heteromorphic life-history of *Macrocystis* and all other kelps, competition for resources (such as substratum and light) can occur at both the macroscopic and microscopic level (Graham et al. 2007). Both intra-and interspecific competition can occur on the microscopic level, potentially affecting the successful recruitment of new individuals that is crucial for the replenishment and ultimate persistence of kelp populations (Graham et al. 1997). Recruitment of these sessile organisms is a multifaceted process including dispersal, settlement, gametogenesis, fertilization and survival to a macroscopic stage (Reed 1990, Graham et al. 2007). Kelp microscopic stages must, therefore, withstand many physical and biological stressors to produce viable macroscopic sporophytes, including sedimentation, water flow, light and nutrient quality, grazing, and intra and/or interspecific competition for resources such as space and light (Devinny and Volse 1978, Lüning and Neushul 1978, Dayton et al. 1984, Deysher and Dean 1986, Dean et al. 1989, Leonard 1994, Sala and Graham 2002, Schiel and Foster 2006, Graham et al. 2007, Muth 2012).

One potential mechanism for microscopic interspecific competition in kelps, that could affect the successful recruitment of new individuals within a population, is the idea of interference competition (sensu Park 1962) through "chemical warfare" (Reed 1990). Species using chemical compounds to their advantage when competing has been explored in both terrestrial (Vivanco et al. 2004) and marine systems (Jackson and Buss 1975, Sheppard 1979). Terrestrial plant species and seaweeds have been found to produce allelochemicals hindering growth and reproduction in their competitors (Whittaker and

Feeny 1971, Harlin and Rice 1987, Denboh et al. 1997, Callaway 2002, Karban 2007, Rasher and Hay 2014).

In order to understand how this chemical microscopic competition may occur between kelps, one must be familiar with how the kelp lifecycle functions (Fig. 1). Kelps exhibit two morphological phases in their lifecycle: the sexual microscopic haploid gametophyte stage and the asexual macroscopic diploid sporophyte stage (Sauvageau 1915, reviewed by Kain 1979). Haploid spores are produced and released from the macroscopic diploid sporophyte to settle on the ocean substratum. These spores germinate into haploid gametophytes, and when sexually mature, they produce oogonia (eggs) or antherozoids (sperm), a process known as gametogenesis. To increase fertilization success, the egg releases the pheromone lamoxirene that induces sperm release from the male gametophyte (Lüning and Muller 1978, Maier and Muller 1986, Maier 1987, Maier 1995, Maier et al. 2001). This signaling chemical creates a chemotactic orientation that guides the sperm toward the egg over distances of 1mm. Lamoxirene is the only known pheromone for the order Laminariales and there is no species' specificity in the stereochemistry of the signaling chemical (Maier et al. 2001). If the sperm reaches the egg, syngamy occurs, which results in the diploid embryonic sporophyte that develops into the macroscopic alga (Fig. 1 #10). Based on differences in phenology, different species of kelp use varying abiotic cues to signal the proper timing of gametogenesis, such as temperature or day length; therefore, one kelp species may recruit earlier or later than another depending on environmental conditions (Lüning and

Neushul 1978, Lüning and Dring 1979, Reed 1990, Graham et al. 2007, Schiel and Foster 2015).

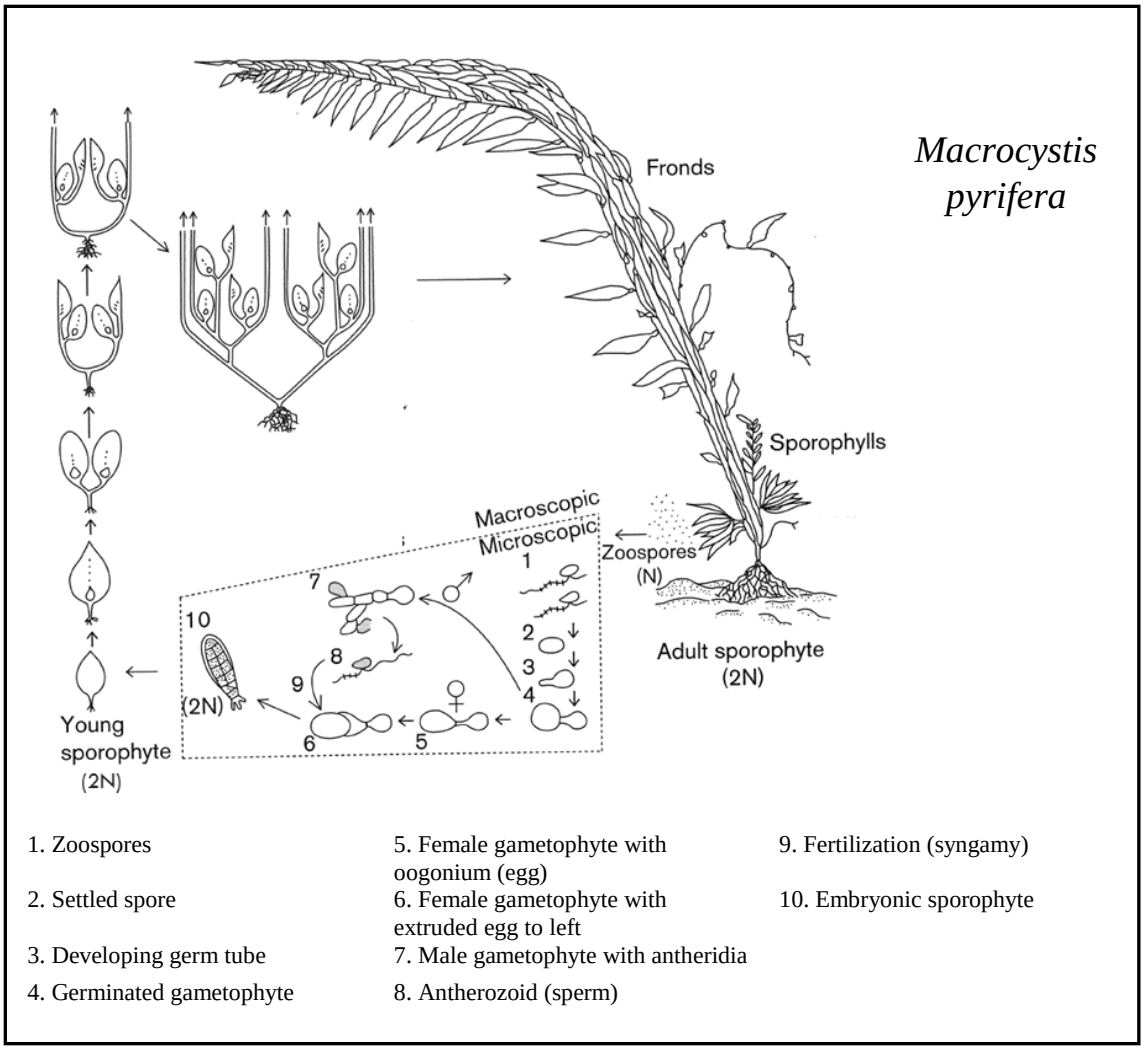

Figure 1. The life history of *Macrocystis*, representing the biphasic lifecycle of all kelps (Schiel and Foster 2015).

Reed (1990) and Howard (2014) found that, although *Macrocystis* outcompetes its co-occurring species *Pterygophora* at a macroscopic scale, *Pterygophora* can outcompete *Macrocystis* at a microscopic scale when zoospores of both species settle in the same place at the same time. Reed (1990) suggested that *Pterygophora*'s competitive

advantage over *Macrocystis* was due to *Pterygophora* reaching sexual maturity approximately four days earlier than *Macrocystis* (Reed et al. 1991, Howard 2014). By maturing earlier, *Pterygophora* female gametophytes can emit lamoxirene into the benthic boundary layer prior to *Macrocystis* females becoming gametogenic, and thus trigger the release of *Macrocystis* sperm before its' eggs are ready for fertilization. This effectively purges *Macrocystis*'s recruitment potential, providing *Pterygophora* with a competitive advantage at the microscopic scale. Reed (1990) suggested that such chemical warfare amongst gametophytes may exist between all kelp species that overlap geographically and in their reproductive periods. Gametophytic interspecific competition in kelps could play an important role in their recruitment and consequently the structuring of kelp communities (Reed 1990, Howard 2014), but it is currently unknown whether chemical competition occurs between species other than *Macrocystis* and *Pterygophora* (Graham et al. 2007).

This study focused on interspecific microscopic competition between the giant kelp *Macrocystis* and five California native kelp species. The goal of this study was to investigate if microscopic competition is common between *Macrocystis* and other kelp species, and if competition does occur, is *Macrocystis* always competitively inferior to other co-occurring species? The current paradigm is that all kelps use lamoxirene; hence, if settled together, all kelp should compete chemically on the microscopic level if the timing of sexual maturity varies among species. This paradigm has only been tested using *Macrocystis*, *Pterygophora* and *Nereocystis* (Reed 1990, Howard 2014). I solely focused on microscopic competition between *Macrocystis* and other native species as competition

among kelps on the macroscopic level has been well studied (Pearse and Hines 1979, Foster 1982, Dayton et al. 1984, Reed and Foster 1984, Dayton et al. 1992, Clark et al. 2004, Edwards and Hernandez-Carmona 2005, review in Schiel and Foster 2015).

METHODS

Competitive Dynamics

The occurrence and outcome of microscopic competition was experimentally tested using one trial each between *Macrocystis* and five central California kelps: *Pterygophora* (perennial, subtidal), *Egregia menziesii* (Turner) Areschoug (perennial, intertidal/subtidal), *Ecklonia arborea* Areschoug (formerly *Eisenia arborea* Areschoug; perennial/subtidal), *Alaria marginata* Postels and Ruprecht (annual, intertidal) and *Postelsia palmaeformis* Ruprecht (annual, intertidal). Additional experiments were conducted testing interspecific competition between *Macrocystis* and *Nereocystis luetkeana*, *Laminaria farlowii* Setchell, and *Lessoniopsis littoralis* Farlow and Setchell ex Tilden, but these experiments failed due to low spore release or low spore settlement from the three potential competitors. Competitors from the successful experiments therefore represented three of the four kelp families (Lane et al. 2006). Reproductive tissue from each species was collected from either Stillwater Cove (36°33'55.30"N, 121°56'36.05"W) or Soberanes Point (36°26'50.94"N, 121°55'39.72"W), located approximately 14 kilometers apart along the central California coast south of Monterey. The timing of fertile tissue collection was dependent on the reproductive window for each species (Fig. 2). The first experiment began at the end of September 2014 and the last experiment ended in the middle of December 2015. Fertile tissue for each species

was collected from a minimum of five individuals, but if possible, the preferred number of collected individuals was ten, separated by as much distance as possible within each site (Reed 1990). The reproductive tissue was kept separate by species and brought back to the laboratory for spore release and culturing using a cooler.

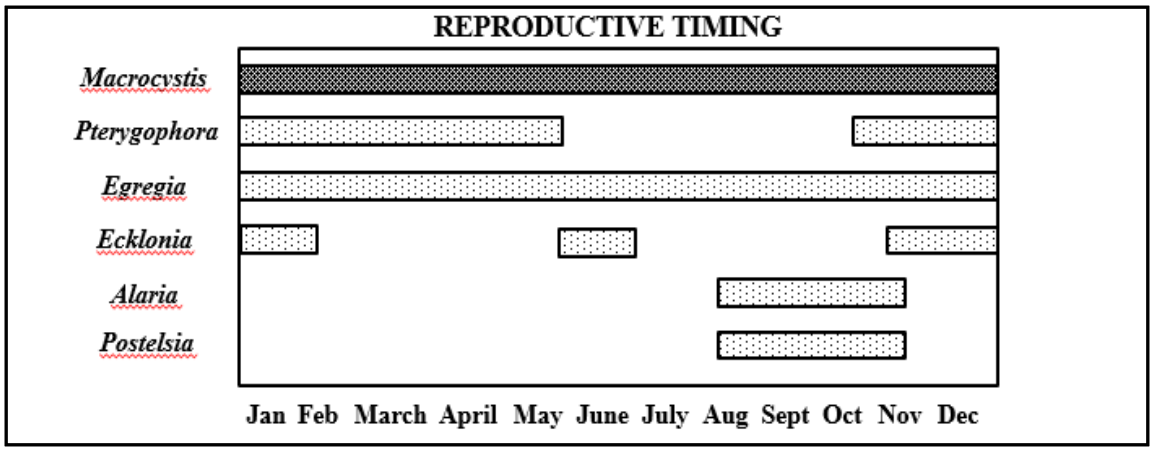

Figure 2. Reproductive timing for the six kelp species used for this study (modified from Abbott and Hollenberg 1976, Blanchette 1996, Reed et al. 1996, McConnico and Foster 2005, Graham et al. 2007).

Keeping species separate, all tissue was cleaned in the laboratory using a 3-step method to control diatom growth. The reproductive material was placed in a 1% iodine solution for 30 seconds, transferred to sterile water for 20 s, and soaked in artificial seawater (Instant Ocean Spectrum Brands, 3001 Commerce St. Blacksburg, VA 24060-6671) for 1 minute.

After the tissue was cleaned, it was layered between moist paper towels and placed in the dark for a minimum of 3 hours at 10 ^{o}C. After dehydrating in the dark, spore release was initiated by immersing the tissue in artificial seawater at 18 ^{o}C for 1 h, stirring frequently to encourage spores to stay in suspension (Reed 1990, Muth 2012). The resulting spore density was determined using a hemocytometer at 400x magnification and

diluted with artificial seawater (if necessary) to reach a desired concentration of approximately 20 spores/mm^2 per species (Reed 1990, Reed et al. 1991). Initial spore settlement density of *Macrocystis* and its competitor was determined microscopically for all treatments after approximately 24 hours using 15 haphazardly chosen fields of view (FOV) under 200x - 400x magnification, to ensure that a similar density was obtained for both species before any further data were recorded for each experiment.

Five replicate mixed-species cultures (polycultures) were cultured in Petri dishes per interspecies experiment, aiming for a spore density ratio of ~20/20 spores/mm^2, where one of the species in each experimental trial was chosen to be settled first for 24 hours. After 24 hours, the spore solution of the first settled species was poured out, leaving only the settled spores and any potential spores sitting in the boundary layer, then new artificial seawater was added. This water was vigorously swirled around, poured off, and finally each dish was submerged upside down in new artificial seawater. The methods described here were experimentally tested before this study began and they were used to make sure that the boundary layer in the Petri dishes was broken to flush out any remaining spores that had yet to attach to the dishes. The study testing the effectiveness of breaking the boundary layer also ensured that the already settled spores weren't damaged and remained viable. Next, the second species in the experimental trial was added to the Petri dishes for another 24 hours before rinsing the dishes once again using the methods described above in order to break the boundary layer. Once washed out, the dishes were then filled with Provasoli (PES) (1968) enriched seawater. To determine if any interspecific competition occurred, 18 fields of view (FOV) per polyculture dish

(n=90 per experiment) were tracked over time using photos in order to distinguish microscopic *Macrocystis* from its competitor species. Earlier studies by Reed (1990) and Howard (2014) used chemical methods to distinguish each species that could not be implemented for this study (see end of Methods section). A mark was randomly made on the bottom of each polyculture Petri dish to locate the general vicinity of the FOV in repeated observations. Then the FOV was marked inside the dish using very fine tweezers and pictures were taken using Spot Insight QE Model # 4.2 (Fig. 3).

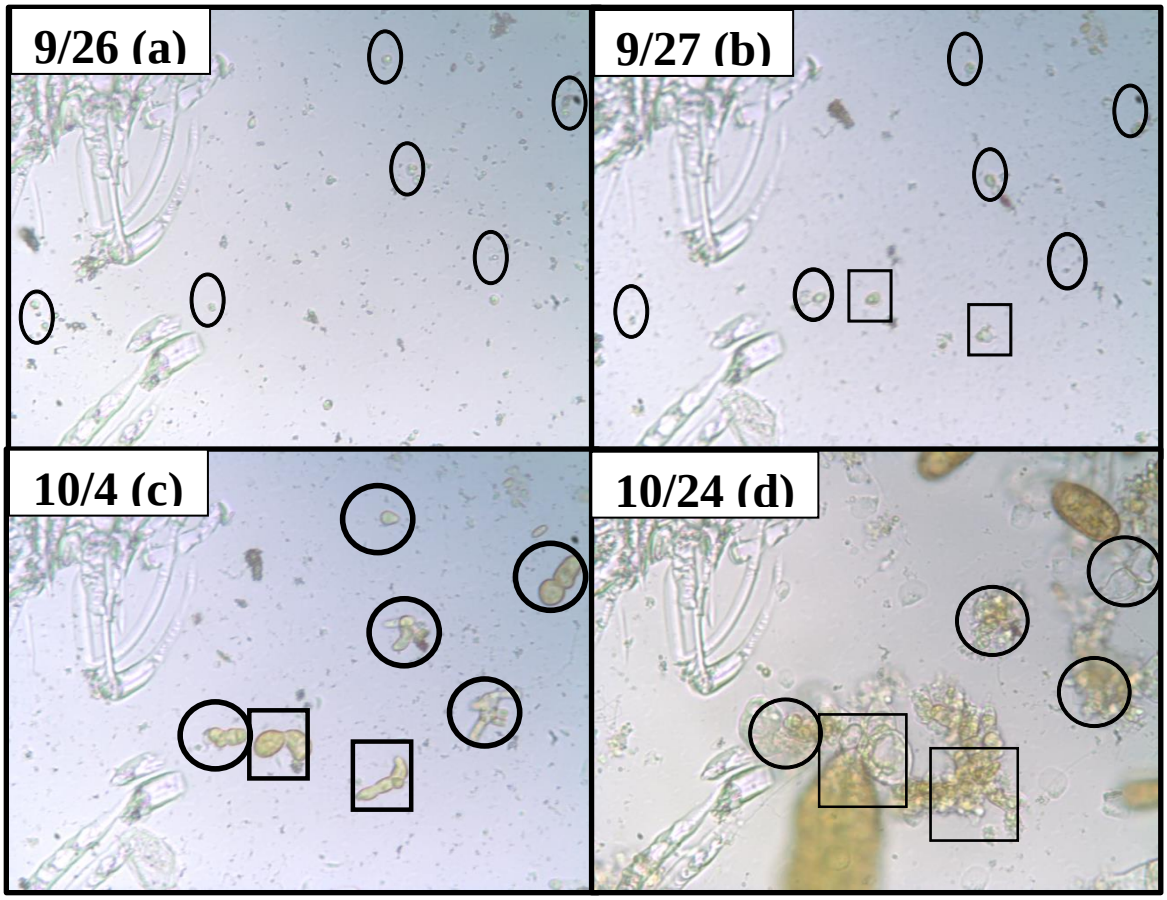

Figure 3. Time-lapse from 9/26/14 to 10/24/14 of *Macrocystis* and *Pterygophora* development from spores (a - *Macrocystis* only and b - *Macrocystis* and *Pterygophora*), to gametophytes (c), to eventually sporophytes (d). *Macrocystis* is marked with circles and *Pterygophora* with squares (400x magnification). *Macrocystis* was settled first in this experiment.

The first photo of each FOV (18/dish/date) was taken after the first species had settled but before the second species was added. The second photo was taken 24 hours later once the second species had settled. By using this method, the same FOV could be located repeatedly over time, settled spores could be distinguished between the two species, and fertilization success resulting in sporophytes, could be tracked. For each FOV, only spores that developed into female gametophytes were tracked over time for each species (Fig. 3) and if fertilization was successful, the number of resulting sporophytes was recorded weekly.

In order to compare the number of sporophytes produced by females in the polycultures, an additional ten dishes for each species per experiment were settled with monocultures, five of which contained a density of approximately 20 spores/mm^2 (similar density as seeded in the polyculture experiments per species), and five contained a density of twice the initial monoculture density to mimic the total spore density in the polycultures. All of the monoculture experiments utilized the same methods as the polyculture dishes, in terms of stimulating settlement and breaking the boundary layer as described earlier.

The monocultures containing~20 spores/mm^2 were used as positive controls; hence, to determine the sporophyte recruitment success of each species when settled without a competitor. The same total number of female gametophytes that were tracked in the polycultures were randomly chosen in the~20 spores/mm^2 monoculture treatments and all female gametophytes were surveyed for production of sporophytes. The monocultures with twice the original monoculture density were used to control for any density-

dependent (intraspecific) effect that could arise in the ~20/20 spores/mm^2 (polyculture) treatments. These monocultures were seeded to mimic the same density as that of the combined densities of the two kelp species in the mixed-species treatment (Underwood 1986) and to ensure that any potential negative effect on sporophyte production found in the polycultures was not due to "overcrowding". Sporophyte production in both monoculture treatments was surveyed to establish any potential intraspecific competition at the end of each experiment by randomly choosing 15 FOV per Petri dish (n=75/experiment). The area of the FOV was estimated to the nearest mm^2 and counts are presented as sporophyte density (i.e., the number of sporophytes per mm^2). If the monocultures with twice the spore density had significantly fewer sporophytes than the ~ 20 spores/mm^2 monoculture treatments, then intraspecific competition would be assumed to be occurring.

A total of 25 Petri dishes were used for each experimental trial (5 polycultures, 5~20 spores/mm^2 monoculture treatments for *Macrocystis* and 5~20 spores/mm^2 monoculture treatments for competitor, 5 2X monoculture density for *Macrocystis* and 5 dishes with 2X monoculture density for the competitor). All replicates were cultured at 12 ^{o}C, an irradiance of 40 µmol $\cdot m^{-2} \cdot s^{-1}$, and a 14:10 light/dark photoperiod, with PES replaced weekly (Lüning and Neushul 1978, Reed et al. 1996). Sporophytes were only counted when both lateral and vertical cell divisions were clearly visible. In order to obtain the maximum number of sporophytes present for both species, counts of sporophytes (after the presence of lateral and vertical divisions) were made weekly in the polycultures and in the ~20 spores/mm^2 monocultures, until numbers began to decline or the FOV became

too overgrown to distinguish individual gametophytes. Once a decline was observed, the experiments were ended.

Previous methods used by Reed (1990) and Howard (2014) to distinguish kelp species from one another when settled together were not logistically feasible for this study, which is why the tracking method using photos, as described earlier, was utilized instead. Reed's antibody-staining method was not feasible because currently only antibodies for *Macrocystis* and *Pterygophora* have been produced (Hempel et al. 1989) and it was too labor intensive and expensive to try to create antibodies for the other species that were used for this research. Howard's (2014) method of staining one competitor with calcofluor was originally utilized for this study; however, perhaps due to manufacturing errors or changes, the 0.01% calcofluor white stain "Fungi-fluor™", I experienced complete mortality of all kelp spores exposed to the stain, resulting in zero recruitment in multiple trials. Additionally, another dye "Solophenyl Flavine 7GFE 500", also known as "Direct Yellow 96", was tested at various concentrations and staining times and was excellent in staining kelp tissue; however, it occasionally dissipated or potentially leaked out of the tissue within days and could therefore not be utilized in this study given the elapsed times of the experiments (i.e., multiple weeks). Direct Yellow 96 is known for staining plant cell walls and fungal cell walls and septa in a similar way as calcofluor white (Hoch et al. 2005, Anderson et al. 2010, Knight and Sutherland 2011) by being selective for beta-linked polysaccharides, but has never been utilized to stain kelp tissue.

Data Analysis of Competition Experiments

To determine if sporophytes occurred less frequently in polycultures than monocultures (indicative of interspecific competition) the observed frequency of sporophytes in either treatment (polyculture and monoculture) was compared to the expected frequency of sporophytes. Expected frequency of sporophytes was calculated by adding the observed number of sporophytes in the polycultures and monocultures (~20 spores/mm^2) and dividing by two. Expected frequency of sporophytes was tested for any significant difference relative to the observed frequency of sporophytes using the chi-square goodness of fit test with Yates correction for each species in each experiment.

Univariate two-way fixed-factor analysis of variance (ANOVA) was conducted for each experiment to analyze if there was an effect on sporophyte recruitment in the monocultures due to the greater densities of kelp spores settled initially (~20 spores/mm^2 versus ~40 spores/mm^2), where the interaction between species (n=2) and the settlement density (n=2) was the output of interest.

Timing of Egg Production

To get a better understanding of female gametophyte development, egg production was studied for all species (except *Ecklonia*) by surveying female gametophytes in the monocultures used as positive controls (n=5/species). Fifty randomly-chosen females were sampled every one to four days to calculate the ratio of females with eggs to females without eggs for each date. Unfortunately, the initiation of egg production for *Postelsia*, *Alaria* and *Pterygophora* was not documented due to their unexpected rapid initial rate of production as the first observations were made after 13 days. Egg

production was observed for the five species either until they reached eighty percent of the individuals, or a decline in eggs was observed, or the dishes were too overgrown that the eggs could not be distinguished any longer. Since each experiment was only run once it was not possible to test any differences in the rate of egg production so these rates are mere observations that can possibly help in understanding the polyculture results in this study.

RESULTS

Competitive Dynamics

Out of the five polyculture experiments performed, the study with both *Pterygophora* and *Ecklonia*, when settled with Macrocystis, found that *Macrocystis* showed a significant decrease in sporophyte recruitment while the competitors did not, indicating interspecific chemical competition with *Macrocystis* being inferior to the competitor (Table 1a-b; Fig. 4-1ab, 2ab). When *Macrocystis* was settled with *Postelsia*, the experiment found no significant difference in sporophyte recruitment between both species' polycultures and monocultures suggesting a lack of competition and potential coexistence (Table 1c; Fig. 4-3ab). Lastly, both *Alaria* and *Egregia*, when settled with *Macrocystis*, found a significant decrease in sporophyte recruitment for both *Macrocystis* and the competitor in the polycultures compared to monocultures (Table 1 d-e; Fig. 4-4ab, 5a-b), potentially indicating some sort of competition other than chemical competition.

Table. 1. Total number of female gametophytes tracked over time using photos in polycultures and their sporophyte production number for each experiment. The same number of females were randomly surveyed for sporophytes in the monocultures for each experiment. Number of sporophytes were documented and compared to the number of expected # of sporophytes for both treatments for each species.

a. *Pterygophora and Macrocystis*

Pterygophora	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	67	67	134
Total # sporophytes	50	46	96
Total *Expected* # sporophytes	48	48	96
Macrocystis	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	54	54	108
Total # sporophytes	41	13	54
Total *Expected* # sporophytes	27	27	54

b. *Ecklonia and Macrocystis*

Ecklonia	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	253	253	506
Total # sporophytes	11	5	16
Total *Expected* # sporophytes	8	8	16
Macrocystis	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	161	161	322
Total # sporophytes	7	1	8
Total *Expected* # sporophytes	4	4	8

c. *Postelsia and Macrocystis*

Postelsia	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	76	76	152
Total # sporophytes	46	46	92
Total *Expected* # sporophytes	46	46	92
Macrocystis	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	117	117	322
Total # sporophytes	17	10	27
Total *Expected* # sporophytes	13.5	13.5	27

d. *Alaria and Macrocystis*

Alaria	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	74	74	148
Total # sporophytes	108	44	152
Total *Expected* # sporophytes	76	76	152
Macrocystis	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	86	86	172
Total # sporophytes	6	0	6
Total *Expected* # sporophytes	3	3	6

e. *Egregia and Macrocystis*

Egregia	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	54	54	506
Total # sporophytes	27	11	38
Total *Expected* # sporophytes	19	19	38
Macrocystis	*Monoculture*	*Polyculture*	*Total*
Total ♀ gametophytes surveyed/tracked	82	82	322
Total # sporophytes	24	7	31
Total *Expected* # sporophytes	15.5	15.5	31

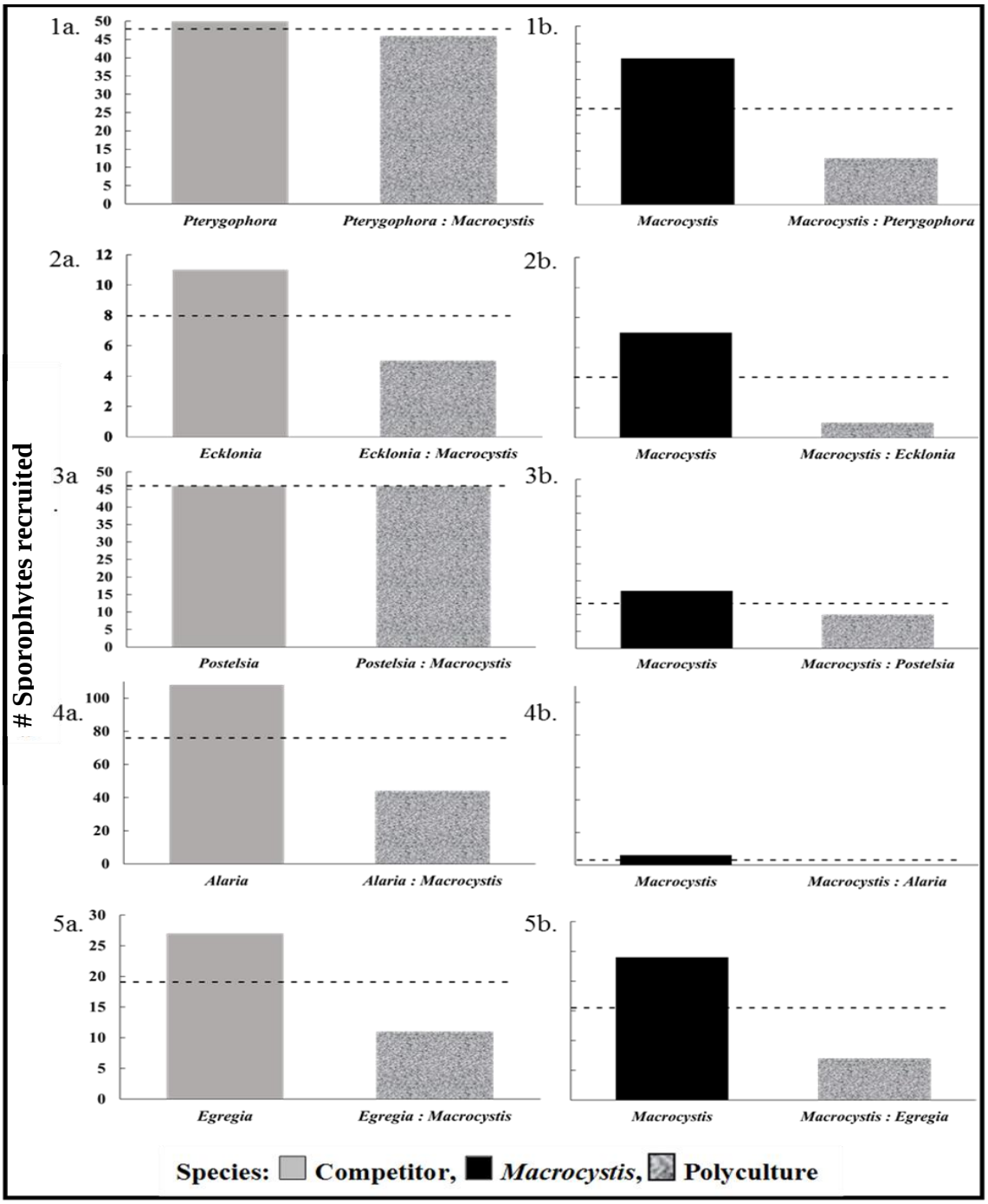

Figure 4. Sporophyte recruitment densities of (1a) *Pterygophora* and (1b) *Macrocystis*, (2a) *Ecklonia* and (2b) *Macrocystis*, (3a) *Postelsia* and (3b) *Macrocystis*, (4a) *Alaria* and (4b) *Macrocystis*, and (5a) *Egregia* and (5b) *Macrocystis* using the same number of female gametophytes in mixed-species treatments and monocultures. The dotted line represents the expected sporophyte recruitment number for each treatment.

Macrocystis had significantly lower recruitment densities ($\chi 2$ yates =14.54, df =1, p=0.000137) when settled with *Pterygophora* versus when settled alone (Fig.4-1b), while *Pterygophora* did not show a significant decrease when settled in the polycultures ($\chi 2$ yates =0.177, df =1, p=0.67396) (Fig.4-1a) indicating *Pterygophora* was the dominant kelp in the experiment. *Macrocystis* was settled first in this experiment.

The same pattern was seen in the experiment with *Ecklonia*, where *Macrocystis* females producing sporophytes occurred significantly more in monocultures than polycultures ($\chi 2$ yates =4.625, df =1, p=0.031509) (Fig.4-2b) but *Ecklonia* showed no significant difference in sporophyte recruitment between monoculture and polyculture ($\chi 2$ yates =2.3125, df =1, p=0.12833) (Fig.4-2a) indicating *Ecklonia* was superior to *Macrocystis*. *Ecklonia* was settled first in this experiment.

When *Macrocystis* was settled with *Postelsia*, neither species had a significantly lower sporophyte recruitment in the polycultures versus the monocultures ($\chi 2$ yates 1.852, df =1, p=0.173551) (Fig.4-3b) and ($\chi 2$ yates =0, df =1, p=1) (Fig.4-3a) respectively. *Postelsia* was settled first in this experiment.

Macrocystis had significantly lower sporophyte recruitment densities in the polycultures versus monocultures in both the experiment with *Alaria* ($\chi 2$ yates = 4.4163, df =1, p=0.035597) (Fig.4-4b) and with *Egregia* ($\chi 2$ yates =9.354, df =1, p=0.002225) (Fig.4-5b). Similarly, both *Alaria* and *Egregia* had significantly fewer females producing sporophytes in their polycultures than their monocultures ($\chi 2$ yates = 26.953, df = 1, p<0.00001) (Fig.4-4a) and ($\chi 2$ yates = 6.762, df = 1, p=0.009312) (Fig.4-5a) respectively.

Alaria was settled first in the experiment with *Macrocystis* while in the experiment with *Egregia*, *Macrocystis* was settled first.

The study found no significant density-dependent effect in the monocultures that could have indicated that any intraspecific competition occurred for any of the five polyculture experiments (Table 2, Fig.5a-e).

Table 2. Two-way analysis of variance for (a) *Pterygophora* and *Macrocystis*, (b) *Ecklonia* and *Macrocystis*, (c) *Postelsia* and *Macrocystis*, (d) *Alaria* and *Macrocystis*, and (e) *Egregia* and *Macrocystis*, showing sporophyte recruitment numbers at different settlement densities.

a. *Pterygophora and Macrocystis*

Source	*df*	*MS*	*F value*	*P*
Settlement Density	1	0.8914	0.0295	0.8657
Species	1	1529.3	50.672	<.0001*
Species x Settlement Density	1	0.8000	0.0265	0.8727
Error	16	30.181		

b. *Ecklonia and Macrocystis*

Source	*df*	*MS*	*F value*	*P*
Settlement Density	1	12.623	10.123	0.0058*
Species	1	10.272	8.2381	0.0111*
Species x Settlement Density	1	9.0377	7.2480	0.0160*
Error	16	1.2469		

c. *Postelsia and Macrocystis*

Source	*df*	*MS*	*F value*	*P*
Settlement Density	1	12.978	2.9216	0.1067
Species	1	70.939	15.969	0.0010*
Species x Settlement Density	1	12.978	2.9216	0.1067
Error	16	4.4423		

d. *Alaria and Macrocystis*

Source	*df*	*MS*	*F value*	*P*
Settlement Density	1	0.20343	2.8698	0.1096
Species	1	2.8822	40.661	<.0001*

Species x Settlement Density	1	0.01157	0.1632	0.6915
Error	16	0.07089		

e. *Egregia and Macrocystis*

Source	*df*	*MS*	*F value*	*P*
Settlement Density	1	0.047704	0.3848	0.5438
Species	1	0.71965	5.8052	0.0284*
Species x Settlement Density	1	0.10727	0.8653	0.3661
Error	16	0.12397		

When analyzing the sporophyte density in both monoculture treatments, the~40 spores/mm^2 treatment never had significantly fewer sporophytes than the~20 spores/mm^2 treatment for each species, showing that the six species in these experiments do not experience any significant intraspecific competition when seeded at approximately~40 spores/mm^2.

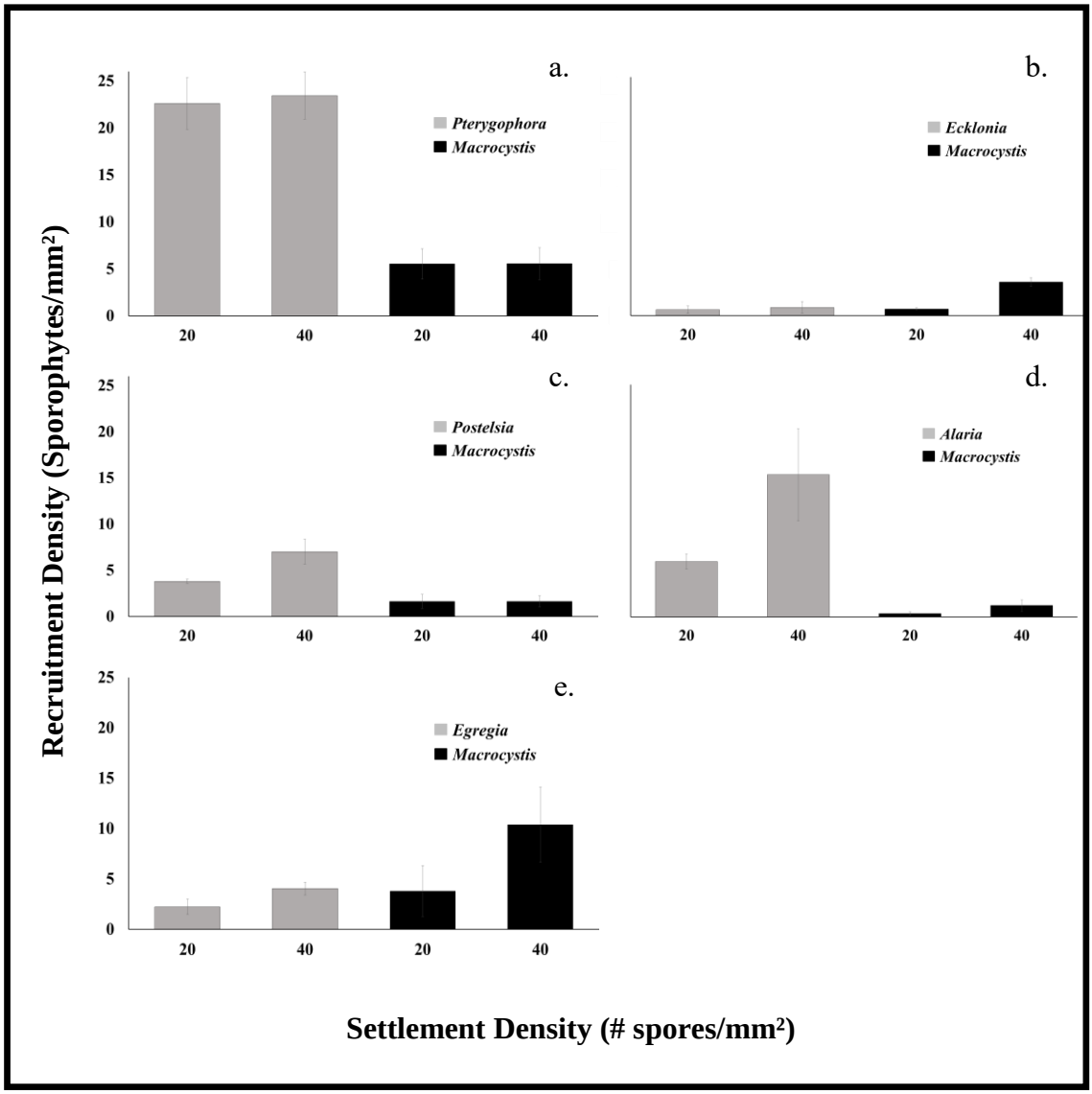

Figure 5. Sporophyte recruitment density in monocultures for *Macrocystis* and (a) *Pterygophora*, (b) *Ecklonia*, (c) *Postelsia*, (d) *Alaria*, and (e) *Egregia*. Spores were settled at 20 or 40 spores/mm². Each Species * Settlement Density combination was replicated 5 times; Data are means ± 1 SE.

Timing of Egg Production

Based on the observations of egg production in five out of the six species (no observation for *Ecklonia* was made), the species seemed divided into two groups. *Postelsia*, *Alaria*, and *Pterygophora* all had a fast initial rate of egg production observed from day thirteen. *Postelsia* reached 80% egg production already by day fifteen while *Alaria* and *Pterygophora* took seventeen and nineteen days, respectively (Fig. 6). The slower group included *Egregia* and *Macrocystis* who both had a much slower production rate, not even reaching 80% egg production before the end of the observations at day twenty-seven. At this time, *Egregia* female gametophytes started to die, hence the decline seen in egg production, and *Macrocystis* gametophytes were so overgrown at day twenty-six that it was impossible to distinguish any eggs (Fig. 6). Overall, *Macrocystis* seemed to have the slowest egg production rate based on my observations.

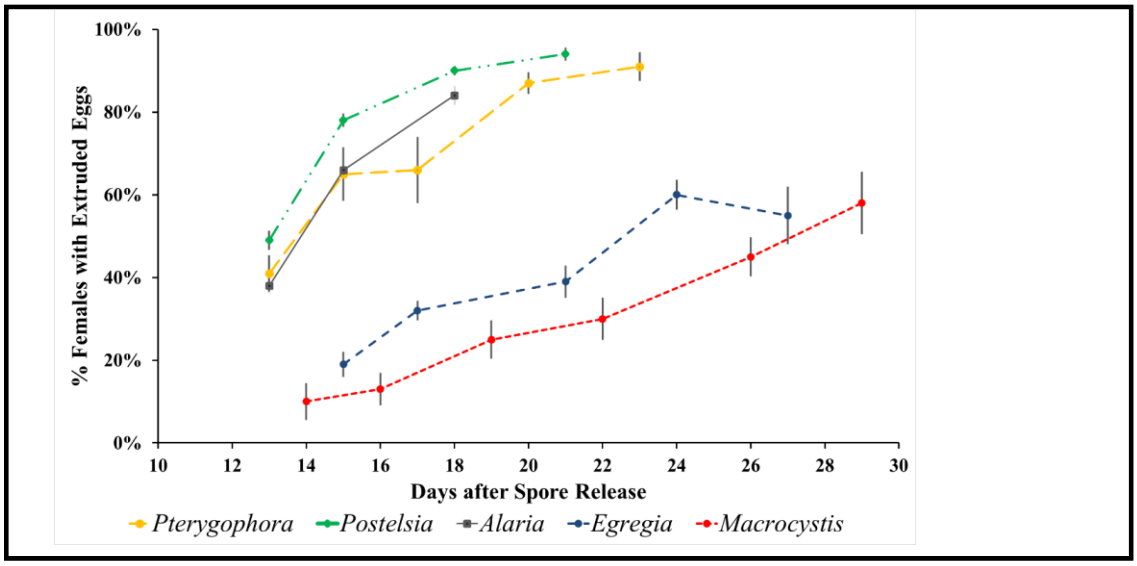

Figure 6. Egg production observations made every 1 to 4 days for *Macrocystis* and four other kelp species used in this study. Shown are the mean (± 1 SE) percent of females with extruded eggs as a function of the time since spore release.

DISCUSSION

The recruitment of *Macrocystis* and all other kelp species can be affected by several different abiotic and biotic factors, such as temperature, salinity, light and nutrient availability, spore settlement densities (Lüning and Neushul 1978) and potentially by chemical competition between microscopic stages (Reed 1990, Howard 2014). This study aimed to test whether interspecific competition always occurs between microscopic stages of *Macrocystis* and those of other kelps. Furthermore, if competition occurs, is it chemical competition and who is the winner? During the recruitment phase of the kelp biphasic lifecycle, mature female gametophytes emit the pheromone lamoxirene to release and attract the antherozoids from its male gametophytes (Maier 1987, Maier et al. 2001). This chemical is species-independent; hence, if two species have settled close enough to each other, then one species could mature earlier and trigger a premature release of antherozoids from the other species, which could potentially create a loss of an entire cohort (Reed et al. 1991). This chemical warfare is described as chemical or sexual competition in the literature and is hypothesized to have the potential to always occur between kelp species (Reed 1990). Previous studies observed chemical competition between microscopic stages of *Macrocystis* and two other species, *Pterygophora* (Reed 1990, Howard 2014) and *Nereocystis* (Howard 2014). Despite using very different methods, this study had similar results to those of Reed (1990) and Howard (2014) when experimenting with *Macrocystis* and *Pterygophora*. Here, I validated that when *Macrocystis* and *Pterygophora* are settled together, the results are asymmetrical, with *Pterygophora* being the competitive dominant. *Pterygophora*'s sporophyte recruitment

does not seem affected by *Macrocystis* presence, but *Macrocystis*'s sporophyte recruitment is negatively affected by *Pterygophora*'s presence indicating that competition is occurring, possibly due to the pheromone interactions previously described. Both Reed (1990) and Howard (2014) hypothesized that *Pterygophora*'s advantage is due to its earlier maturation of eggs, and faster release of lamoxirene. The egg production observations in this study (Fig. 6) indicate that *Pterygophora* has a faster production rate than *Macrocystis*. Validating *Pterygophora*'s dominance over *Macrocystis* on the microscopic level using new methods helps solidify the paradigm that *Pterygophora* has an early ecological advantage over *Macrocystis* due to the timing of gametogenesis.

This study also found asymmetrical results when *Macrocystis* and *Ecklonia* were settled together and *Ecklonia* outcompeted *Macrocystis*. *Ecklonia* may have produced eggs faster than *Macrocystis*; however, there was no observation made to support this claim in this study and the timing of egg production for *Ecklonia* has not been previously studied. There was a potential artifact of the experiment. What if every winner of each experiment in this study was simply due to who was settled first? It is difficult to answer this question unless the experiments had been run multiple times, each time changing the species that was settled first. That was not feasible for this study due to multiple reasons, but mainly due to the difficulty of getting each experiment up and running and the constraint of time. However, out of the five experiments in this study, *Macrocystis* was settled first twice but it didn't outcompete the other kelp species in either case. In the three experiments where *Macrocystis* was settled second, only once did the competitor do better, while in the other two experiments both *Macrocystis* and the competitor did

poorly. The experiment on *Ecklonia* and *Macrocystis* was the only one where *Ecklonia* could have benefitted from being settled first and that could have been the reason that it outcompeted *Macrocystis*; therefore, further studies are needed to investigate this potential competitive interaction.

Postelsia and *Macrocystis* experienced no significant competition when settled together. However, *Macrocystis* experienced a non-significant reduction in sporophyte production when settled together with *Postelsia* compared to when settled alone. Based on the observations of egg production for these two species, this result could be because *Postelsia* matures much faster than *Macrocystis*. Therefore, even if *Postelsia* emitted lamoxirene, *Macrocystis*'s male gametophytes would not be mature enough to release its sperm. Hence, *Postelsia* is already growing its sporophytes when *Macrocystis* is beginning to produce eggs. The two species can emit lamoxirene at two different times and not interfere with each other's sperm release and subsequent sporophyte production. The observations of *Postelsia*'s egg production in this study suggest that about 80% of females produce eggs at day 15, which is similar to an earlier study that found 100% of females fertile at day 15 (Lewis 1995).

Postelsia and *Macrocystis* are also the two closest genetically related species in this study (Lane et al. 2006) and perhaps this lack of (or lower level of) competition is because they are closely related as hypothesized by some early ecologists (Lack 1954, MacArthur 1958). It is possible that these two closely related species have evolved to have very different timing to gametogenesis so as to not overlap in production of eggs and the release of lamoxirene. However, many community ecologists hypothesize the

opposite: that more closely related species will experience greater competition with each other since they are more ecologically similar than distantly related species (Elton 1946, Park 1948, Maherali and Klironomos 2007, Jiang et al. 2010, Violle et al. 2011). Close relatives are thought to be more similar in "habits and constitution" (Darwin 1859), hence overlap in niche utilization, which results in competitive exclusion (Gause 1934, Hardin 1960). The results found by Howard (2014) between *Macrocystis* and *Nereocystis* resonate with the idea that competition increases as genetic relatedness increases. This experiment with *Macrocystis* and *Postelsia* suggests the opposite. The paradigm instilled by Darwin suggests that interspecific competition shall increase as the genetic distance between species decreases. Recent studies that investigated this paradigm have studied different organisms and arrived at contradicting conclusions. Cahill et al. (2008) termed the paradigm the "competition-relatedness hypothesis" and used a meta-analysis of several plant competition experiments. The study found no significant relationship between interspecific competition and genetic distance, also known as phylogenetic relatedness (Cahill et al. 2008). Contrarily, a study by Violle et al. (2011) investigated this paradigm using bacterivorous protist species and found supporting evidence for the hypothesis. Using a multigenerational experiment, the study found that increasing phylogenetic relatedness resulted in an increased frequency and tempo of competitive exclusion of the inferior competitor (Violle et al. 2011). It is possible that *Postelsia* and *Macrocystis* simply coexist when settled together on the microscopic level because on the macroscopic level they share very opposite habitats and they would rarely, if ever, have their spores settle together. Like Gause (1934) and Hardin (1960) suggested, species that

are less ecologically similar are more likely to occupy different niches and are therefore able to coexist. This explanation resonates with the results I found with *Macrocystis* and *Postelsia*. Even though they are genetically very close (Lane et al. 2006), in nature they share very different habitats. Also supporting this argument is that the strongest competition in this study was observed between *Macrocystis* and *Pterygophora* and *Macrocystis* and *Ecklonia* and these species are not closely related (Lane et al. 2006) but they are the most similar ecologically in this study. This idea is also supported by Howard's (2014) results where *Macrocystis* outcompeted *Nereocystis*. These two kelps are genetically close but they are also ecologically similar, so competition occurs. Based on this research and Howard's study it's fair to suggest that ecological similarity may be what drives competition more than phylogenetic relatedness in kelps; however, this idea should be investigated further.

Macrocystis experienced symmetric competition with both *Alaria* and *Egregia* so the mechanism for the competition could be something different than chemical competition. This is because not only did *Macrocystis*'s sporophyte recruitment decrease in the polycultures, but *Alaria*'s and *Egregia*'s recruitment also decreased. There was a negative effect on recruitment for all species in each polyculture experiment which could have indicated density-dependent effects; however, the results of the two-way analysis of variance for each experiment were not significant. The results from the experiments with *Alaria* and *Macrocystis* are difficult to explain. Overall, *Macrocystis* had poor sporophyte recruitment. In the monoculture experiment, with 86 female gametophytes being surveyed, only six sporophytes were produced versus zero in the polycultures for the

same amount of females. However, based on the monocultures this observed low recruitment was not due to competition for space since there was no significant intraspecific competition occurring. In comparison, *Alaria* produced about 110 sporophytes by the 74 female gametophytes that were surveyed in the monocultures. Overall, the results for *Macrocystis* when settled with *Alaria* could be due to low recruitment for a reason not known to this study, and not due to the presence of *Alaria* gametophytes. However, the sporophyte production for *Alaria* was decreased by more than half when settled with *Macrocystis* and it was settled first. There is a possibility that with *Alaria* producing multiple eggs per female that these eggs are ready for fertilization at different times and *Macrocystis* caused a premature sperm release that hindered fertilization for some of *Alaria*'s eggs. Hence, this interspecies interaction could be asymmetric with *Macrocystis* being the competitive dominant even though the results of this study does not support that.

Furthermore, *Egregia* only sometimes occupies similar habitats as *Macrocystis*, especially in shallow waters. This could explain the result found for these two species; there isn't a strong competitive dominant relationship between the two species like the study found with *Pterygophora* and *Ecklonia*; hence, when both species are settled together they both do poorly due to some sort of competition other than chemical competition.

Overall, this study did not find any significant negative density dependence for any of the six species when they were settled at ~20 spores/mm^2 and ~40 spores/mm^2 in their monocultures. These results supported that there was no significant intraspecific density

effect in any of the polyculture experiments and that any negative sporophyte density results were from interspecies competition. The fact that there was no density decrease for any of the six species even at ~40 spores/mm^2 supports Reed et al. (1991) results where their study found that sporophyte production in non-aerated cultures was the greatest at about 50 spores/mm^2. However, since all of the experiments in this study were grown as non-aerated cultures it is worth mentioning that this could have caused nutrient limitation (Reed et al. 1991); which, potentially could affect each species differently in terms of growth and reproduction. For future experiments studying microscopic competition, aerated cultures are strongly suggested.

Reed (1990) and Howard (2014) both suggested that chemical competition occurs between *Pterygophora* and *Macrocystis*, since *Pterygophora*'s females mature about four to six days earlier than *Macrocystis*. This study found the same asymmetrical result as those two studies for both *Pterygophora* and *Ecklonia*. Egg production for *Pterygophora* was recorded once during this study but unfortunately no egg production for *Ecklonia* was obtained. Based on the egg production recorded, *Pterygophora* had a 12 day advantage over *Macrocystis* to reach 40% of females with eggs (Fig. 6). Howard (2014), who collected kelp from similar locations as this study, found that *Pterygophora* had about a five to six day advantage over *Macrocystis* to reach 40% of females with eggs. However, the kelp tissue for each study was collected during different years, and potentially during different times of the year, which could contribute to the difference seen in egg production for *Pterygophora* and *Macrocystis*.

Overall, *Pterygophora*, *Postelsia* and *Alaria* all have similar egg production rates, while *Egregia* and *Macrocystis* are slower to reach the same level of fertility. However, these data were only observed once and replicate experiments should be run in the future to detect any variation within species, and over time, in order to detect annual variation in egg production.

Many factors can affect kelp recruitment such as: zoospore settlement densities, temperature, and distance to other zoospores within a species and to neighboring kelps. It is important to understand how all of these factors affect each kelp species' recruitment since the structure and foundation of the kelp forests depend on the persistence of kelp populations (Graham et al. 1997). This study suggests that *Macrocystis* competes both asymmetrically and symmetrically with other kelp species in addition to exhibiting no competition with the closest related species. These results indicate that there are other mechanisms for competition than only chemical competition. The competitive interaction between species needs to be better understood since microscopic interspecies competition may have a large effect on both kelp populations and kelp community dynamics.

CONCLUSIONS

This study provides insight into microscopic interspecies competition between *Macrocystis* and five native species. Chemical warfare with *Macrocystis* was observed for two species in this study, supporting earlier studies suggesting that species compete chemically using the pheromone lamoxirene. However, for three other species chemical competition was not observed. Interestingly, the two species that *Macrocystis* chemically competed with are the two species in this study that utilize a habitat similar to

Macrocystis's. Studying how *Macrocystis* competes with species microscopically is essential to understanding its recruitment and subsequent population structure which provides the biogenic habitat in the dynamic kelp forest. Furthermore, as a foundation species, *Macrocystis* is often the dominant kelp macroscopically so it is important to learn if other kelp species uses chemical competition to dominate *Macrocystis* on the microscopic level to maintain their populations. Overall, microscopic chemical competition between *Macrocystis* and other kelp species needs to be better understood since it may be more important in regulating species and community dynamics than previously thought.

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