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## Effects of Temperature on Sexual Competition in Kelps: Implications for Range Shifts in Foundation Species

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EFFECTS OF TEMPERATURE ON SEXUAL COMPETITION IN KELPS:  
IMPLICATIONS FOR RANGE SHIFTS IN FOUNDATION SPECIES

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

San José State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Alexis Cynthia Howard

May 2014

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The Designated Thesis Committee Approves the Thesis Titled  
EFFECTS OF TEMPERATURE ON SEXUAL COMPETITION IN KELPS:  
IMPLICATIONS FOR RANGE SHIFTS IN FOUNDATION SPECIES

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May 2014

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## ABSTRACT

### EFFECTS OF TEMPERATURE ON SEXUAL COMPETITION IN KELPS: IMPLICATIONS FOR RANGE SHIFTS IN FOUNDATION SPECIES

By Alexis Cynthia Howard

Kelp populations inhabit some of the most dynamic environments on the planet and often exist close to the limits of their temperature tolerances. Temperature cues reproductive processes in many kelps and fluctuating temperatures can affect kelp recruitment and population persistence. Some kelps compete sexually through their microscopic life history stages by releasing a pheromone that triggers the premature release of spermatozooids of neighboring species, leading to recruitment failure. It is unknown, however, whether changing temperature modifies competitive hierarchies among kelp species. To address this issue, I investigated how temperature affects sexual competition between microscopic stages of three co-existing and possibly competitive kelps in central California. Laboratory studies were conducted to test the effects of temperature on germination, gametogenesis, fertilization, and recruitment. At 4°C, 8°C, and 12°C, *Macrocystis pyrifera* outcompeted *Nereocystis luetkeana*, but was outcompeted by *Pterygophora californica*. At 16°C, *Nereocystis* did not survive and *Pterygophora* sporophyte recruitment decreased relative to that of *Macrocystis*. All three of these kelps showed increased time to fertility of female gametophytes with decreasing temperatures. This demonstrated that temperature could alter the competitive hierarchies among these three species and suggests that increasing ocean temperatures due to climate change will favor *Macrocystis* over *Nereocystis* and *Pterygophora*, increasing *Macrocystis*' dominance along the central California coast.

## ACKNOWLEDGEMENTS

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## INTRODUCTION

Foundation species play essential roles in the stability and structure of ecological communities through the creation of biogenic habitat (Dayton 1972, Bruno et al. 2003, Rohr et al. 2009) and the provisioning of energy and nutrients to food webs. The presence of foundation species has been shown to positively affect biodiversity and even alter hydrology (Ellison et al. 2005). Foundation species include corals, grasses, oysters, many canopy-forming trees, and kelps (Harvell et al. 1999, Ellison et al. 2005, Altieri and Witman 2006). It is important to understand the role that each of these foundation species plays in the ecosystem to predict how they, and the ecosystem they inhabit, may be affected by perturbations (Ebenman and Jonsson 2005).

Many species respond negatively to anthropogenic stresses (Ellison et al. 2005). For example, tree species can decline in their survival due to a variety of factors, including over-harvesting and high-intensity forestry, deliberate removal of certain forest species, native pests, and introductions and outbreaks of nonindigenous pests and pathogens (Ellison et al. 2005). There is currently no sign that these anthropogenic stressors will be diminishing any time soon. Many tree taxa have exhibited range shifts, either in latitude or elevation, in response to climate change, and paleoecology evidence indicates that range shifts occurred rapidly at the end of the last glacial interval (Davis and Shaw 2001). With increasing temperatures predicted to accelerate over the next century, the distributions of many species have begun shifting pole-ward or are hypothesized to begin shifting soon (Walther et al. 2002). All foundation species are limited by additional factors other than temperature. Reef building corals may not be

able to shift their range due to light limitations at extreme latitudes (Walther et al. 2002). This would negatively impact all of the species that depend on coral reefs for food, refuge, or habitat, and allow for less complex turf algae to take its place. This multifactor effect may affect many foundation species and with increasing threats, conservation actions may be needed to help preserve these species that establish and maintain habitats that support other species (Rohr et al. 2009).

Kelp forests are some of the most diverse and productive ecosystems in the marine environment (reviewed by Dayton 1985, Steneck et al. 2002, Graham et al. 2007). Kelps are large conspicuous brown algae (order Laminariales) that are found on every continent around the world, except Antarctica. Forest-forming kelps are considered to be foundation species because they provide food and habitat for marine flora and fauna (North 1971). The complex morphology and high biodiversity associated with kelps make them essential to community structure because they dampen water motion, shade the benthos, scrub nutrients, alter sediment transport, and provide a fairly stable physical structure for organisms (North 1971, Reed and Foster 1984, Dayton 1985, Clark et al. 2004, Graham et al. 2007). Kelps not only change the abiotic environment, but they also provide energy and habitat through kelp forests that support 40 to over 275 common species (Graham 2004, Graham et al. 2007).

Kelp populations inhabit some of the most dynamic environments on the planet (Graham et al. 2007) and changing environmental conditions, such as temperature, salinity, light, nutrients, sedimentation, or wave action, can heavily influence the condition and health of kelps (Dayton 1985, Springer et al. 2006). Environmental change

may also influence the diversity of organisms that depend on kelp forest systems, especially along changing coastlines (North 1971). Healthy kelp populations can be either annual or perennial. However, environmental fluctuations can have an impact on the annual stability of the population and the successful recruitment of subsequent cohorts (Graham et al. 2007).

Climate change may have dramatic effects on kelp populations through factors other than temperature (Dayton et al. 1998, Graham et al. 2007). Not only is temperature expected to increase with climate change, but salinity could change due to increased storms or droughts, nutrients could be depleted from decreased upwelling, changes in pH due to CO<sub>2</sub> and sedimentation, and wave action could increase from increasing numbers and severity of storms (Briggs 1995, Harley et al. 2006). All of these factors could affect the successful recruitment of kelps as well as their ability to mature (Dayton et al. 1998).

Successful recruitment of kelps is essential to the persistence of kelp forest populations. Variability in the abiotic system (e.g., changes in temperature, light, salinity, etc.) can be important to each life history stage by affecting survival, maturity, and reproduction (Luning and Neushul 1978). Kelps have a biphasic life history that includes macroscopic and microscopic stages. The large conspicuous individuals that make up a kelp forest are comprised of the 2N sporophyte stage (Fig. 1). This form becomes reproductive when conditions allow (high nutrients, low temperature, enough light) and produces specialized blades called sporophylls that have sporangia aggregated in sori (Neushul 1963). These sporangia contain microscopic biflagellate zoospores created through meiosis followed by mitosis (Fritsch 1945, Graham et al. 2007). Once

released, 1N zoospores disperse and settle, germinate, and become microscopic male and female 1N gametophytes. After maturation, females produce oogonia (eggs) that release the pheromone lamoxirene (the pheromone released by all kelp taxa at this stage of reproduction), which triggers male gametophytes to release their spermatozooids (Maier et al. 1987, 2001) that then track the pheromone back to the egg for fertilization (Maier et al. 1987, 2001). Spore settlement densities must be greater than 1 per mm<sup>2</sup> to ensure successful fertilization, because male spermatozooids have limited dispersal abilities and male gametophytes must be close enough to females to detect the pheromone cue (Reed

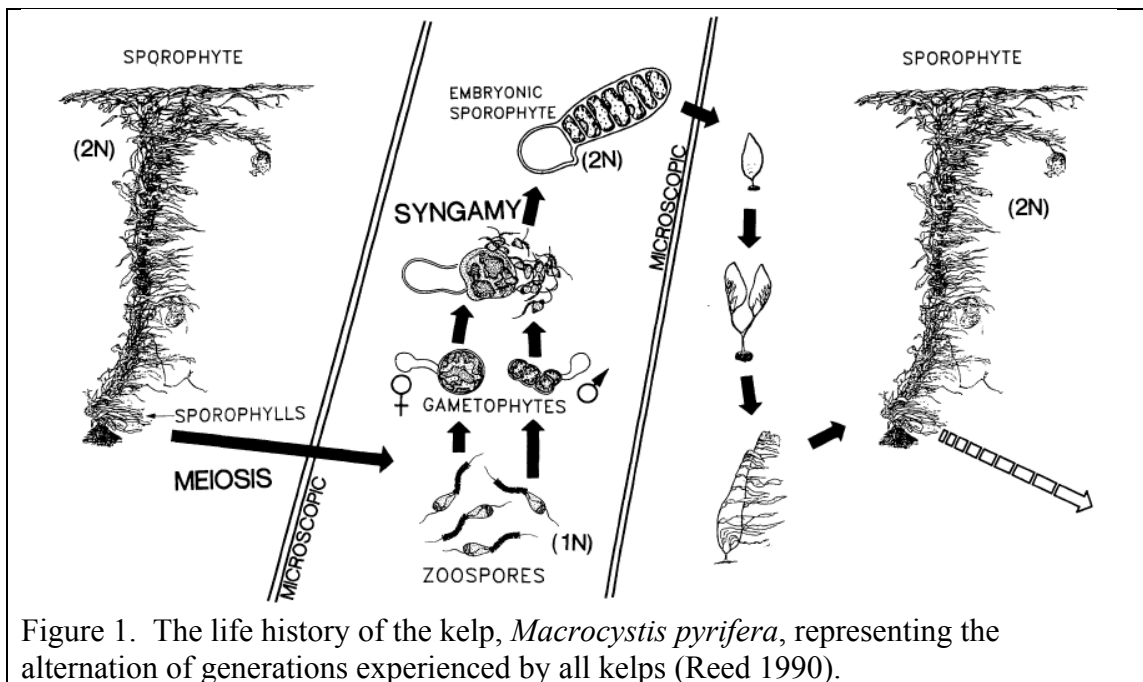


Figure 1. The life history of the kelp, *Macrocystis pyrifera*, representing the alternation of generations experienced by all kelps (Reed 1990).

1990). Conditions, such as light ( $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and temperature ( $2^{\circ}\text{C}$  to  $18^{\circ}\text{C}$  depending on the species), must be right for germination, gametogenesis, fertilization, and the eventual production of a microscopic sporophyte. Once the egg is fertilized

through syngamy, a 2N embryonic sporophyte develops, which eventually will become a macroscopic sporophyte and complete the life cycle. Kelps have shown plasticity in reproductive timing by responding to favorable environmental conditions, making them more successful than some other algae despite their fairly short life span (Reed et al. 1996).

Differential timing of gametogenesis among kelp taxa can lead to competitive interactions between microscopic stages that can influence recruitment patterns (Reed 1990). Chemical competition experiments among microscopic stages of *Pterygophora californica* Rupr. and *Macrocystis pyrifera* (L.) C. Agardh showed that inhibition of *Macrocystis* recruitment by *Pterygophora* was asymmetrical (Reed 1990); *Macrocystis* never inhibited *Pterygophora*, but *Pterygophora* inhibited *Macrocystis*. *Pterygophora*'s competitive advantage was likely caused by *Macrocystis* male gametophytes sensing the pheromone released by *Pterygophora* female gametophytes before *Macrocystis* female gametophytes were mature (Maier et al. 2001). The use of this pheromone for the premature release of spermatozoids by *Macrocystis* could cause it to miss the fertilization of an entire cohort. This reproductive inhibition has been demonstrated only once before between these two species and has yet to be explored between any other kelp taxa or at temperatures above or below ambient, and it remains unclear how environmental factors might influence this interaction (Reed 1990).

Variability in the abiotic system (i.e., temperature, light, salinity, etc.) can affect the survival, maturity, and reproduction of each life history stage (Luning and Neushul 1978). Temperature is one of the environmental cues that trigger reproduction, and



variation in temperature can strongly influence the physical maturation and life history of kelps. In addition to abiotic environmental variables (Reed 1990), competition can be important in determining kelp recruitment. The chemical competition among microscopic stages of *Pterygophora californica* and *Macrocystis pyrifera* was mediated by the release of a pheromone (lamoxirene) by female gametophytes of both species. The species that matures and releases the pheromone first may cause premature release of male spermatozooids of the other species, thus resulting in a subsequent recruitment failure (Reed 1990, Maier et al. 2001). Such competition can be critical to kelp recruitment success and be heavily influenced by environmental factors, especially temperature.

The frequency of warm sea surface temperature events have increased since 1977, and is predicted to increase further, but how this affects marine populations and communities is not well understood (Briggs 1995, McGowan et al. 1998). There is additional evidence to show that ocean temperatures in upwelling zones may decrease, or have longer cool periods due to intensification of wind-driven ocean upwelling from greenhouse related thermal low-pressure cells (Bakun 1990, Snyder et al. 2003, Bakun et al. 2010). However, a pole-ward shift/expansion of the warm-temperate regions along the coast of western North America is expected at the expense of the cold-temperate regions (Bartsch et al. 2012). Many kelps live near their temperature tolerances especially when close to their lower latitude range (Harley et al. 2006); therefore, any change in temperature could greatly affect the performance and survival of a population. Due to pending climate change, scientists have warned that negative effects on kelp

populations may have lasting impacts on the entire ecosystem (Dayton et al. 1998, Winder and Schindler 2004, Sexton et al. 2009). Thus, it is important to study the effects of temperature (decreases and increases) on the different microscopic and macroscopic kelp life stages, and how kelp populations will react to these changes.

All kelp life stages respond physiologically to changes in temperature above and below their optimal temperature which varies among locations, with decreases in growth or survival (Vadas 1972, Luning and Neushul 1978, Fain and Murray 1982, Schiel et al. 2004); yet, the various stages of kelp life histories can have different levels of susceptibility to changes in water temperatures (Fain and Murray 1982). Previous experiments have examined how the growth and development of various kelp species respond to increases in temperature; however, most studies have not tested the effects of temperature decreases and increases on the timing to egg production or between multiple competing species (Vadas 1972, Luning and Neushul 1978, Fain and Murray 1982). For example, nine different central California kelp species have a narrow thermal tolerance window for embryonic development, such that temperatures less than 12°C and greater than 17°C inhibit growth of these species to healthy embryonic sporophytes (Luning and Neushul 1978). Female fertility was also shown to decrease at temperatures higher or lower than 12°C in most of the species tested. In two other experiments, the highest temperature of 20°C resulted in decreased growth and survival of female gametophytes of *Nereocystis luetkeana* (Mert.) Postels and Ruprecht and no germination or survivorship in *Alaria marginata* Postels and Ruprecht (Vadas 1972, Hoffman et al. 2003). It is important to understand how each life history stage will be affected in

canopy-forming and understory kelps, especially gametogenesis, and give us insight into the demographic importance of each stage.

Giant kelp (*Macrocystis pyrifera*), bull kelp (*Nereocystis luetkeana*), and stalked kelp (*Pterygophora californica*) form extensive kelp forests in the Pacific Northwest and are foundation species for fishes, invertebrates, and other algae (Springer et al. 2006, Graham et al. 2007). *Macrocystis* and *Pterygophora* are perennial kelps and occur in the eastern Pacific from Baja California, Mexico, to southern Alaska and Vancouver Island, British Columbia (Abbott and Hollenberg 1976, Graham et al. 2007), whereas *Nereocystis* is an annual kelp found from central California to Alaska (Table 1, Setchell 1908, Miller and Estes 1989). *Macrocystis* is the dominant kelp from Baja California to northern California and becomes patchy and sparse in its distribution from northern California to Alaska, where *Nereocystis* is the dominant kelp (Edwards and Estes 2006).

Table 1. Relevant average temperatures at range limits for *Macrocystis pyrifera* and *Nereocystis luetkeana*, and the average temperature for their overlapping range (NOAA NODC & Hickey et al. 1991).

<i>Average Temperatures</i>			
	<i>Macrocystis pyrifera</i>	<i>Nereocystis luetkeana</i>	<i>Pterygophora californica</i>
<b>Northern limit</b>	<b>5.3°C (Southern Alaska)</b>	<b>2.9°C (Eastern Aleutians)</b>	<b>8°C (Vancouver Island)</b>
<b>Southern limit</b>	<b>20°C (Baja California)</b>	<b>12.7°C (Piedras Blancas)</b>	<b>20°C (Baja California)</b>
<b>Central CA (over-lapping range)</b>	<b>12°C</b>	<b>12°C</b>	<b>12°C</b>

*Pterygophora* is the dominant understory kelp throughout most of its range from Vancouver Island to Bahia Rosario, Baja California, Mexico (Matson and Edwards 2007). Coexistence of these three species within the same beds frequently occurs in

central California, which may result in intense competition for space (Dayton et al. 1984, Dayton 1985, Dayton et al. 1992, Schiel et al. 2004). Given the overlapping ranges and habitat requirements of *Macrocystis*, *Nereocystis*, and *Pterygophora*, they are the ideal kelps to study the effects of competition because they naturally co-occur and serve similar functions (foundation and canopy-forming species) in the ecosystem.

*Macrocystis* is adapted to warmer temperatures compared to other kelps and has been shown to colonize areas previously inhabited by *Nereocystis* and *Pterygophora* when water temperatures were too high for *Nereocystis* and *Pterygophora* to persist (Schiel et al. 2004). *Macrocystis* naturally out-competes *Nereocystis* in areas optimal for both species because *Macrocystis* is perennial and can recruit year-round, whereas *Nereocystis* is annual with a limited recruitment window (Dayton 1985, Schiel et al. 2004). *Pterygophora* is a perennial with limited recruitment windows; however, it has reproductive periods more than once a year (De Wreede and Klinger 1990). For the canopy-forming kelps, *Nereocystis* has been shown to be more tolerant of wave-exposed/shallow areas than *Macrocystis*; however, the two species do co-occur in beds from Piedras Blancas and up through northern California (Abbott and Hollenberg 1976, Edwards and Estes 2006, Graham et al. 2007). Macroscopic sporophytes of these species clearly compete for light and space but it is unclear whether competition occurs on the microscopic level during gametogenesis (the reproductive stage) in these areas and if there is an effect of temperature on competitive success.

The goal of this study, therefore, was to quantify the effects of temperature on the microscopic life stages of *Nereocystis*, *Pterygophora*, and *Macrocystis* and to determine

how temperature affects competition at the microscopic level among these species. If the competitive outcome between these taxa is affected by temperature, climate change could have drastic effects on kelp forest systems. Laboratory studies were focused on the microscopic life history stages of *M. pyrifera*, *P. californica*, and *N. luetkeana* and the effects of temperatures that encompass the upper and lower temperature limits of both kelps. Chemical competition among microscopic stages was quantified between *Macrocystis* and *Nereocystis*, and *Macrocystis* and *Pterygophora*, using varying ratios of the kelps in laboratory experiments to determine if the competitive effects of the relationship were asymmetrical (or simply density-dependent). All competitive experiments included *Macrocystis* as one of the species because it is the dominant kelp along the central California coast. Timing of egg production plays a major role in this competitive relationship and the time to maturation of each species at the various temperatures was also quantified. Determining the temperature tolerances for these three kelp taxa while considering their competitive relationship allows for a better understanding of how their ranges may change in the future. The goals of this study were addressed with the following questions: Are *Macrocystis pyrifera* and *Pterygophora californica*, as well as *Macrocystis pyrifera* and *Nereocystis luetkeana* competing at the microscopic level? Are these competitive relationships temperature-dependent? Does timing to egg production change with temperature?

## METHODS

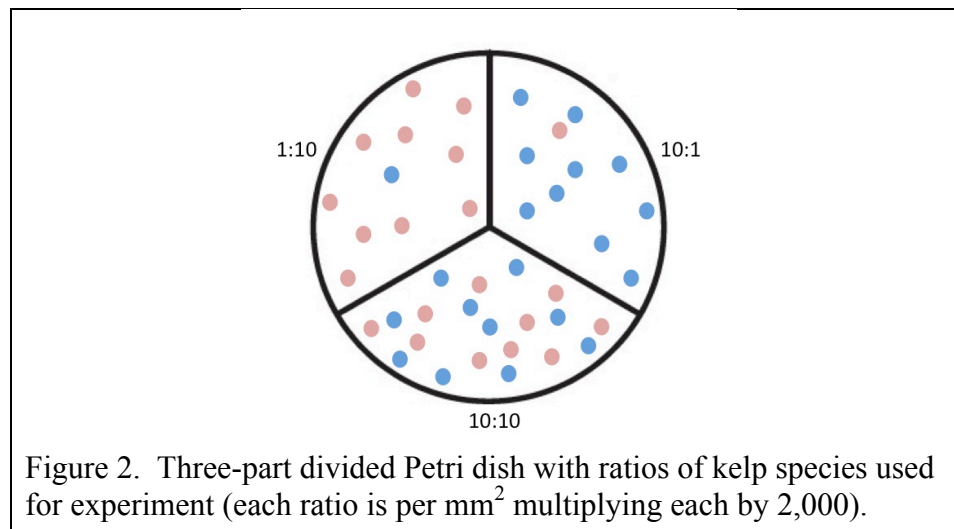
### *Competitive dynamics in microscopic stages of kelp*

To test the hypothesis that *Macrocystis* competes with both *Pterygophora* and *Nereocystis* at a microscopic level, reproductive material (spore-bearing blades) was collected periodically (~2 months) for approximately 15 months from several (~10) adult *Macrocystis*, *Pterygophora*, and *Nereocystis* plants growing at Stillwater Cove, California (36°33'56.79"N, 121°56'35.88"W). Experiments testing temperature tolerances and competitive interactions were conducted in the laboratory to control light levels (40  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with 14:10 h light/dark photoperiod) (Luning and Neushul 1978, Reed et al. 1996), temperature, nutrients, and spore densities. To induce spore release, sporophylls were rinsed in a 1% iodine solution for 30 seconds, placed in D.I. water for 30 seconds, scrubbed with a final rinse in sterile sea water, wrapped in damp paper towels, and stored in a dark room at 10° C. After three hours, sporophylls were placed in 18° C sterile seawater for one hour under ambient room light, with the resulting spore solution density counted using a hemacytometer at 400x magnification and diluted to the desired concentration (Reed et al. 1991).

Monocultures were grown as positive controls and density estimates were made for the different microscopic life stages (e.g., settlement, germination, gametophytes, and sporophytes) of each kelp species. The spore solutions were cultured in three-part Petri dishes (one enclosure with only *Macrocystis*, one with only *Nereocystis*, and one with only *Pterygophora*). The spore solutions were replaced with nutrient enriched seawater solution (Provasoli 1968) to enhance spore growth and germination. Petri dishes were

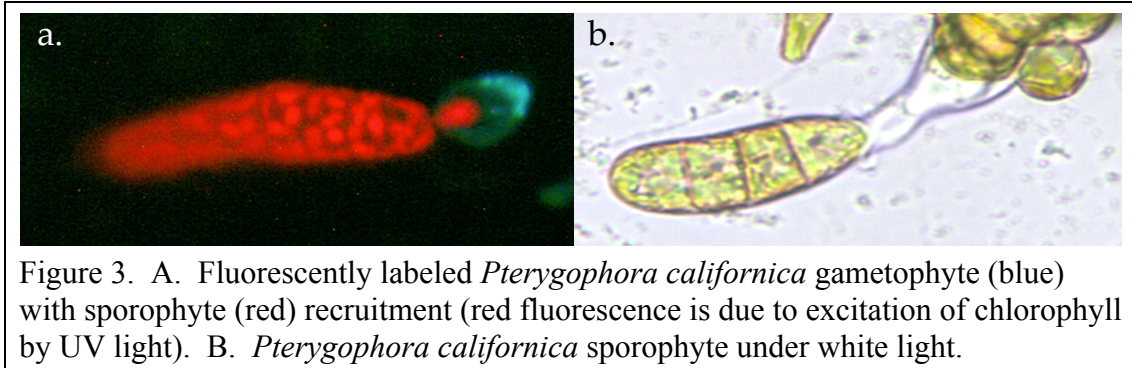
seeded with spore solution at  $\geq 1$  spore per  $\text{mm}^2$  (optimal  $\sim 50$  spores per  $\text{mm}^2$ ) concentrations (Reed 1991). In addition to the initial spore density and germination count (after 24 to 48 hours), gametophytes (7 to 10 days), and embryonic sporophytes (4 to 10 weeks) were estimated for each treatment. Density estimates were made using 40X magnification, with 15 haphazardly selected ocular quadrants to determine an average recruitment density for each treatment and life stage. Survival and maturation to sporophyte was quantified (counted) once there were no eggs left for fertilization or once there was no survival.

Treatments with two kelps settled on the same dish were performed to detect chemical competition according to the methods of Reed (1990). Three different ratios of the two species were seeded in three-part Petri dishes for competition trials to determine whether different ratios of the species (high-high, high-low, and low-high) affect competitive interactions. Seeding ratios included dishes with low densities of one species and high densities of the second species (1:10 and 10:1 spores per  $\text{mm}^2$ ), and high densities of both species (10:10 spores per  $\text{mm}^2$ ) as shown in Figure 2.



Petri dishes were seeded with spore solution at  $\geq 1$  spore per  $\text{mm}^2$  (optimal  $\sim 50$  spores per  $\text{mm}^2$ ) concentrations (Reed 1991).

To differentiate between species, one species was released first, allowed to settle for 24 hours, then dyed with 20% Fungi-fluor<sup>TM</sup> (0.01% calcofluor white stain) and sterile sea water for the next 24 hours. Fungi-fluor<sup>TM</sup> fluoresces when excited by UV light (240-400 nm; peak excitation 345-365 nm; Baselski and Robinson 1989) by binding a non-lethal bio-stain to beta-linked polysaccharides (Edwards 1999; Fig. 3).



The cell walls of microscopic stages of laminarian species have been previously stained using these methods (Cole 1964, Hsiao and Druehl 1973) and it was found to be non-toxic and have no effect on cell growth (Nakazawa et al. 1969). After this initial 48 hours, the dye was discarded and the settled spores were rinsed three times with sterile salt water to remove remaining dye. The second species was then allowed to settle for 24 hours in the dishes using the same spore release process. To account for the time difference between the settlement of the two species, the order in which the species were settled was switched between experiments. After the settlement period, the spore solutions were replaced with nutrient enriched seawater solution (Provasoli 1968) to



enhance spore growth and germination. The nutrient enriched solution was changed in the dishes once per week.

By varying seeding ratios of the competitor, I was able to test whether the two kelp species were having negative interspecific effects on the recruitment of one another and what level of competition was occurring. There were six replicates of each density ratio per trial that were averaged, for each of three trials. Univariate two-way fixed-factor analysis of variance (ANOVA) was conducted to test the competitive dynamics between species ( $n=3$ ), where the interaction between species and the seeding ratio was the output of interest. The competitive outcome was statistically tested for each interaction using pairwise comparisons with the Holm-modification applied (Holm 1979). The competitive interactions were the treatments with equal portions of both species present (10:10). Comparisons were also made for a species when there were high quantities of the competitor present versus low quantities of the competitor present (10:10 vs 10:1). The monocultures were analyzed by determining whether the results for the competitive interactions fell within the 95% confidence intervals of the monocultures.

#### *Effects of temperature on competitive dynamics*

To test the hypothesis that temperature changes the competitive relationship between *Macrocystis* and *Pterygophora/Nereocystis*, the methods from the previous section were repeated using incubators that allowed for temperature manipulations. Two incubators were used (M.R.C. Growth Chamber, Model LE-539) allowing two temperatures to be tested per trial. A total of four temperatures were tested: 4°C, 8°C,

12°C, and 16°C. A digital display continuously reported the temperature within the incubators, and temperature monitors recorded the temperature.

Survival and maturation were tested for each microscopic stage of *Macrocystis*, *Pterygophora* and *Nereocystis* in response to exposure to the four temperature levels. There were six replicates of each species interaction and temperature treatment per trial for *Macrocystis* versus *Pterygophora* and *Macrocystis* versus *Nereocystis*. Each trial was run three times during the 15-month period that collections occurred and the replicates within each trial were averaged and the mean was used as a replicate. Each trial was used as a replicate to test each temperature and species interaction. A univariate two-way fixed-factor ANOVA was conducted to test the effect of each temperature on the competitive outcome between species, where the interaction between species and the seeding ratio was the output of interest ( $n = 3$ ), similar to the previous test. The *Macrocystis/Pterygophora* and *Macrocystis/Nereocystis* competitive treatment experiments at 4°C had zero recruitment in one treatment level in all three trials; therefore, this temperature was analyzed using a univariate one-way fixed-factor ANOVA. The competitive outcome was statistically tested for each interaction using pairwise comparisons with the Holm-modification applied (Holm 1979). To test for the effect of temperature on sporophyte recruitment ( $n = 3$ ), a pairwise comparisons test was done for the competitive treatment (10:10) with equal settlement densities of each species at each temperature.

### *Timing of egg production*

The monocultures used as positive controls were also used to test the hypothesis that time to egg production changes with temperature. Monocultures were grown at all four temperatures (4°C, 8°C, 12°C, and 16°C) for each species (*Macrocystis*, *Nereocystis*, and *Pterygophora*) and sampled to determine the percentage of female gametophytes with released eggs for each kelp species with six replicates per experiment. One experiment was run for each species at each temperature. Fifty randomly-chosen females were sampled every one to three days to determine the ratio of females with eggs to females without eggs. This sampling was conducted before females began producing eggs and continued until all eggs had become sporophytes or the gametophytes had died. Comparisons between time of egg production for *Nereocystis*, *Pterygophora*, and *Macrocystis* at the different temperatures were analyzed by comparing differences in the time until 80% females had produced eggs. To analyze the effect of temperature on the time of egg production for each species, a one-way univariate analysis of variance (ANOVA) was conducted with temperature being the fixed factor and day at which 80% egg production was reached being the dependent variable ( $n = 6$ ). This 80% time point was used to allow temperature to affect the rate at which eggs were produced. The difference to 80% egg production of each species at each temperature was statistically tested for using pairwise comparisons with the Holm-modification applied (Holm 1979). To analyze the effect of species on the time of egg production at each temperature, a one-way univariate analysis of variance (ANOVA) was conducted with species being the fixed factor and day at which 20% egg production was reached being the dependent

variable ( $n = 6$ ). This 20% time point was chosen because it would allow me to determine which species would have the initial advantage by releasing eggs before the other species. The difference to 20% egg production between species at each temperature was statistically tested for using pairwise comparisons with the Holm-modification applied (Holm 1979).

## RESULTS

### *Competitive dynamics in microscopic stages of kelp and the effects of temperature*

#### *Macrocystis pyrifera vs. Pterygophora californica*

Three trials were analyzed as replicates of the competition experiment at each temperature (4°C, 8°C, 12°C, 16°C). All trials had successful sporophyte recruitment at all treatment levels, except 4°C (Fig. 4). At 4°C, both *Macrocystis* and *Pterygophora* showed low sporophyte recruitment and *Macrocystis* had zero recruitment when seeded at densities of 1 zoospore/mm<sup>2</sup> (Fig. 4d). *Macrocystis* showed a greater decline in recruitment than *Pterygophora* with the presence of *Pterygophora* in the mixed species treatments (Fig. 4d). The effect of seeding ratio on sporophyte recruitment was significant ( $P = 0.004$ ; Table 2a). *Pterygophora* had significantly ( $P = 0.001$ ) greater recruitment densities than *Macrocystis* when equal quantities of both species were present (i.e., 10M:10P; Table 3). Recruitment densities of *Pterygophora* did not differ with varying quantities of *Macrocystis* (i.e., 10M:10P vs. 1M:10P;  $P = 0.067$ ). Recruitment densities of *Macrocystis* differed with varying quantities of *Pterygophora* (i.e., 10M:10P vs. 10M:1P), only by a small margin ( $P = 0.050$ ).

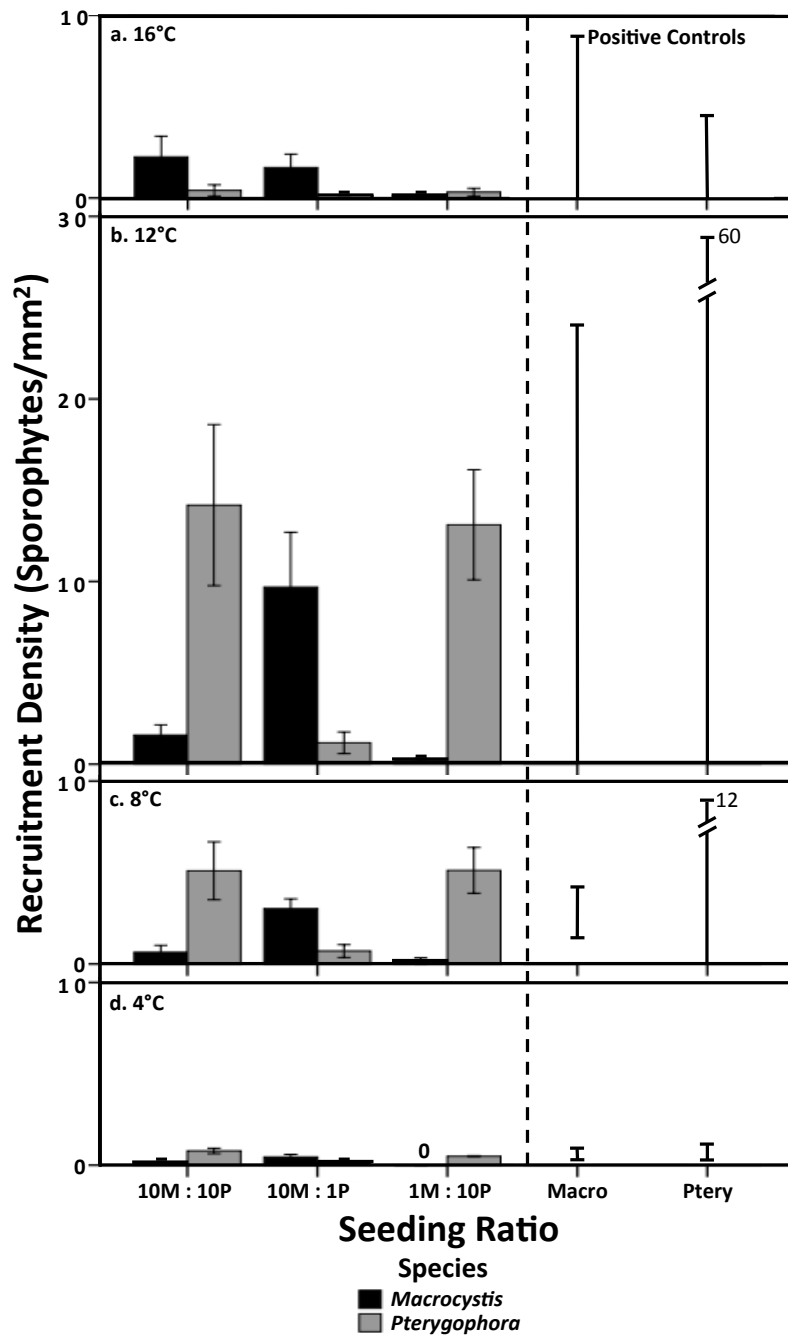


Figure 4. Recruitment densities of *Macrocyctis pyrifera* and *Pterygophora californica* in mixed-species treatments and monocultures at 4°C (d), 8°C (c), 12°C (b), and 16°C (a). The variables on the x-axis represent the seeding ratios of either 10 spores/mm<sup>2</sup> or 1 spore/mm<sup>2</sup> of *Macrocyctis* (M) or *Pterygophora* (P). Values are means  $\pm$  1 SE. Monocultures of each species show 95% CI to compare to mixed species treatments.

At 8°C, *Macrocystis* sporophyte recruitment decreased with increasing quantities of *Pterygophora* present (i.e., 10M:1P vs. 10M:10P) in the mixed-species treatments (Fig. 4c). The interaction between species and seeding ratio was significant ( $P = 0.002$ ; Table 2b). Recruitment of *Pterygophora* was significantly higher than *Macrocystis* with equal quantities of both species present (i.e., 10M:10P;  $P = 0.004$ ; Table 3). Recruitment densities of *Macrocystis* did not differ with varying quantities of *Pterygophora* (i.e., 10M:1P vs. 10M:10P;  $P = 0.079$ ), nor did recruitment densities of *Pterygophora* differ with varying quantities of *Macrocystis* (i.e., 1M:10P vs. 10M:10P;  $P = 0.985$ ).

Table 2. Univariate one-way analysis of variance for 4°C (a) and two-way analysis of variance for 8°C (b), 12°C (c), and 16°C (d) looking at sporophyte recruitment densities of *Macrocystis pyrifera* and *Pterygophora californica* at different seeding ratios.

a. 4°C				
<i>Source</i>	<i>df</i>	<i>MS</i>	<i>F value</i>	<i>P</i>
Seeding ratio with species	4	0.241	7.953	0.004*
Error	10	0.030		
b. 8°C				
<i>Source</i>	<i>df</i>	<i>MS</i>	<i>F value</i>	<i>P</i>
Species	1	25.139	10.796	0.007*
Seeding Ratio	2	1.654	0.710	0.511
Species*Seeding Ratio	2	24.712	10.612	0.002*
Error	12	2.329		
c. 12°C				
<i>Source</i>	<i>df</i>	<i>MS</i>	<i>F value</i>	<i>P</i>
Species	1	143.805	7.522	0.018*
Seeding Ratio	2	8.995	0.470	0.636
Species*Seeding Ratio	2	226.280	11.835	0.001*
Error	12	19.119		
d. 16°C				
<i>Source</i>	<i>df</i>	<i>MS</i>	<i>F value</i>	<i>P</i>
Species	1	5.017	5.052	0.044*
Seeding Ratio	2	2.026	2.040	0.173
Species*Seeding Ratio	2	2.059	2.074	0.168
Error	12	0.993		

At 12°C, all experiments had successful sporophyte recruitment at all treatment levels. *Macrocystis* sporophyte recruitment declined with the presence of *Pterygophora* in the mixed species treatments relative to monoculture treatments (Fig. 4b). The interaction between species and seeding ratio was significant (P = 0.001; Table 2c). *Pterygophora* had significantly greater recruitment densities than *Macrocystis* when equal quantities of the two species were present (i.e., 10M:10P; P = 0.004; Table 3). Recruitment densities of *Pterygophora* did not differ significantly with varying densities of *Macrocystis* (i.e., 1M:10P vs. 10M:10P). Recruitment densities of *Macrocystis* differed significantly with varying settlement densities of *Pterygophora* (i.e., 10M:1P vs. 10M:10P; P = 0.042); however, only by a small margin.

Table 3. Multiple comparisons test with the Holm-modification applied between *Macrocystis pyrifera* and *Pterygophora californica* showing competitive factors of interest and all temperatures tested.

	4°	8°	12°	16°
Macro (10M:10P) vs. Ptery (10M:10P)	P=0.001*	P=0.004*	P=0.004*	P=0.044*
	Ptery	Ptery	Ptery	Macro
Macro (10M:10P) vs. Macro (10M:1P)	P=0.050*	P=0.079	P=0.042*	P=0.486
Ptery (10M:10P) vs. Ptery (1M:10P)	P=0.067	P=0.985	P=0.769	P=0.907

Results at 16°C differed from those observed at 4°C, 8°C, and 12°C. In the three replicate trials, *Macrocystis* outcompeted *Pterygophora* (Fig. 4a). *Pterygophora* had decreased recruitment in all treatments when compared to *Macrocystis*. The interaction between species and seeding ratio was not significant (P = 0.168; Table 2d), although the effect of species on sporophyte recruitment was significant (P = 0.044). *Macrocystis* had significantly greater sporophyte recruitment density than *Pterygophora* with equal

quantities of both species present (i.e., 10M:10P;  $P = 0.044$ ; Table 3). Recruitment densities of *Macrocystis* did not significantly differ with varying quantities of *Pterygophora* present (i.e., 10M:1P vs. 10M:10P;  $P = 0.486$ ), nor did recruitment densities of *Pterygophora* significantly differ with varying quantities of *Macrocystis* (i.e., 1M:10P vs. 10M:10P;  $P = 0.907$ ).

#### Macrocystis vs. Nereocystis

To test the hypothesis that temperature changes the competitive interaction between *Macrocystis* and *Nereocystis*, three trials were analyzed as replicates of the competition experiment at four different temperatures (4°C, 8°C, 12°C, 16°C). All trials had successful sporophyte recruitment at all temperatures, except 4°C; at 4°C, both *Macrocystis* and *Nereocystis* experienced an overall decline in sporophyte recruitment and *Nereocystis* had zero recruitment when seeded at densities of 1 zoospore/mm<sup>2</sup>. *Nereocystis* recruitment decreased with the presence of *Macrocystis* in the mixed species treatments (Fig. 5d). The effect of seeding ratio on sporophyte recruitment was significant ( $P < 0.001$ ; Table 4a). Recruitment of *Macrocystis* was significantly greater than *Nereocystis* when equal quantities of both species were present (i.e., 10M:10N;  $P < 0.001$ ; Table 5). Recruitment densities of *Macrocystis* differed significantly with varying quantities of *Nereocystis*; however, this seems due to high variability among experiments (i.e., 10M:1N vs. 10M:10N;  $P = 0.001$ ). Whereas recruitment densities of *Nereocystis* did not differ with varying quantities of *Macrocystis* (1M:10N vs. 10M:1N;  $P = 0.496$ ).



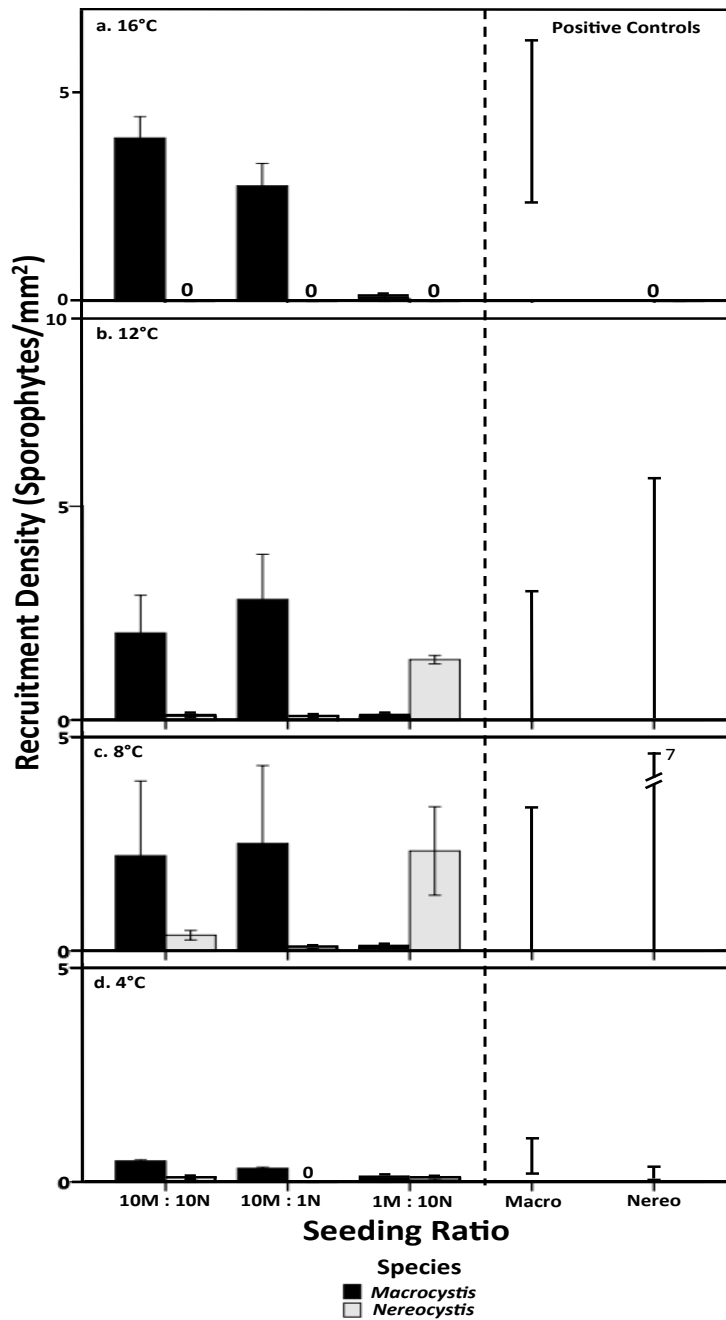


Figure 5. Recruitment densities of *Macrocyctis pyrifera* and *Nereocystis luetkeana* in mixed-species treatments and monocultures at 4°C (d), 8°C (c), 12°C (b), and 16°C (a). The variables on the x-axis represent the seeding ratios of either 10 spores/mm<sup>2</sup> or 1 spore/mm<sup>2</sup> of *Macrocyctis* (M) or *Nereocystis* (N). Values are means  $\pm$  1 SE. Monocultures of each species show 95% CI to compare to mixed species treatments.

At 8°C, both *Macrocystis* and *Nereocystis* had greater variability in all treatments than at 4°C, though *Macrocystis* remained competitively dominant (Fig. 5c). The effects of species, seeding ratio, and the interaction between the two factors were not significant (Table 4b). This is likely due to the increased variability among experimental replicates because two of the trials had much lower recruitment densities for *Macrocystis* than the third experiment did. The pairwise comparison between *Macrocystis* and *Nereocystis* was not significant when there were equal quantities of both species present (i.e., 10M:10N) likely due to the increased variability (Table 5). Recruitment densities of *Macrocystis* did not differ with varying quantities of *Nereocystis* (i.e., 10M:1N vs. 10M:10N), nor did recruitment densities of *Nereocystis* differ with varying quantities of *Macrocystis* (i.e., 1M:10N vs. 10M:10N).

Table 4. Univariate one-way analysis of variance for 4°C (a) and two-way analysis of variance for 8°C (b) and 12°C (c) looking at sporophyte recruitment densities of *Macrocystis pyrifera* and *Nereocystis luetkeana* at different seeding ratios.

a. 4°C

Source	df	MS	F value	P
Seeding Ratio with species	4	0.134	75.300	<<0.001*
Error	10	0.002		

b. 8°C

Source	df	MS	F value	P
Species	1	1.998	0.539	0.477
Seeding Ratio	2	0.026	0.007	0.993
Species*Seeding Ratio	2	10.155	2.742	0.105
Error	12	3.704		

c. 12°C

Source	df	MS	F value	P
Species	1	5.928	6.225	0.028*
Seeding Ratio	2	0.653	0.686	0.522
Species*Seeding Ratio	2	7.068	7.422	0.008*
Error	12	0.952		

At 12°C, all trials had successful sporophyte recruitment at all treatment levels. *Nereocystis* sporophyte recruitment declined in the presence of *Macrocystis* in the mixed species treatments relative to monoculture treatments (Fig. 5b). The interaction between species and seeding ratio was significant (P = 0.008; Table 4c). *Macrocystis* had significantly higher recruitment densities than *Nereocystis* when equal quantities of both species were present (i.e., 10M:10N; P = 0.031; Table 5). Recruitment densities of *Macrocystis* did not differ with varying quantities of *Nereocystis* (i.e., 10M:1N vs. 10M:10N). Recruitment densities of *Nereocystis* did not differ with varying quantities of *Macrocystis* (i.e., 1M:10N vs. 10M:10N); however, only by a small margin.

Table 5. Multiple comparisons test with the Holm-modification applied between *Macrocystis pyrifera* and *Nereocystis luetkeana* showing factors of competitive interest and all temperatures tested.

	4°	8°	12°
Macro (10M:10N) vs. Nereo (10M:10N)	P<0.001*	P=0.260	P=0.031*
	Macro	NS	Macro
Macro (10M:10N) vs. Macro (10M:1N)	P=0.001*	P=0.860	P=0.343
	Macro	NS	NS
Nereo (10M:10N) vs. Nereo (1M:10N)	P=0.496	P=0.235	P=0.123
	NS	NS	NS

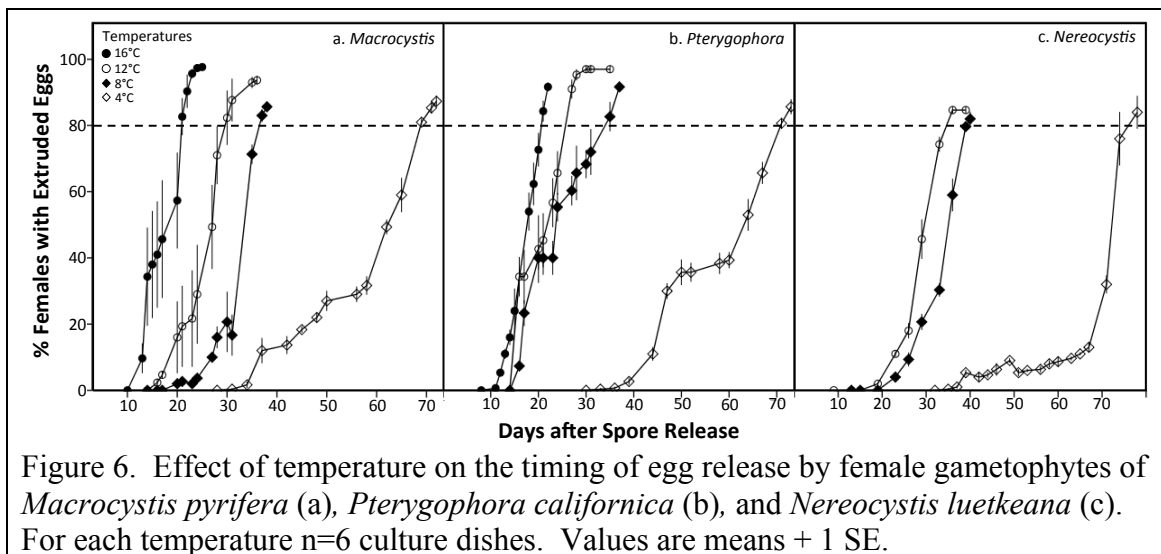
The results at 16°C were generated from a single experiment, and were not analyzed because there was no survival of *Nereocystis* in any of the treatments. Results for *Macrocystis* recruitment densities were presented graphically (Fig. 5a). A summary of competitive outcomes for each species interaction and temperature are given for the experiments with equal quantities of both species present (i.e., 10M:10P and 10M: 10N; Table 6).

Table 6. Pairwise comparisons using the Holm-modification to determine which species was the competitive dominant when there were equal quantities of both species present at each temperature. Comparisons between *Macrocystis pyrifera* to *Pterygophora californica* and *Nereocystis luetkeana*.

Competition	Temperature	Significance	Outcome Ratio (10:10)	Outcome
Macro vs Ptery	4°C	P=0.001*	1:6	Ptery
Macro vs Ptery	8°C	P=0.004*	1:8	Ptery
Macro vs Ptery	12°C	P=0.004*	1:9	Ptery
Macro vs Ptery	16°C	P=0.044*	5:1	Macro
Macro vs Nereo	4°C	P<0.001*	20:1	Macro
Macro vs Nereo	8°C	P=0.260	6:1	NS
Macro vs Nereo	12°C	P=0.031*	22:1	Macro
Macro vs Nereo	16°C	N/A	N/A	Macro

*Effects of temperature on timing of egg production*

Female gametophytes of *Macrocystis* and *Pterygophora* successfully produced eggs for fertilization at all temperatures (4°C, 8°C, 12°C, and 16°C), while *Nereocystis* produced eggs at only 4°C, 8°C, and 12°C (Fig. 6). Timing to egg production had a negative relationship with temperature (Fig. 6); gametophytes exposed to the warmest



temperature (16°C) produced eggs the fastest for both *Macrocystis* and *Pterygophora*, and timing to egg production increased with decreasing temperature for all species (Fig.

6). Timing to 80% egg production was compared for the four temperatures for *Macrocystis* and *Pterygophora*, and the three temperatures for *Nereocystis*.

Table 7. Univariate one-way analysis of variance of the effect of temperature on the timing to egg release by female gametophytes of *Macrocystis pyrifera* (a), *Pterygophora californica* (b), and *Nereocystis luetkeana* (c). For each temperature n=6 culture dishes.

a. <i>Macrocystis pyrifera</i>				
Source	df	MS	F value	P
Temperature	3	2528.944	363.877	<<0.001*
Error	20	6.950		
b. <i>Pterygophora californica</i>				
Source	df	MS	F value	P
Temperature	3	2843.833	344.707	<<0.001*
Error	20	8.250		
c. <i>Nereocystis luetkeana</i>				
Source	df	MS	F value	P
Temperature	2	2770.667	1061.106	<<0.001*
Error	15	2.611		

Timing to 80% egg production was significantly different among treatments for all species ( $P < 0.001$ ; Table 7). When using pairwise comparisons, timing to 80% egg production was significantly different between the four temperatures for *Macrocystis* and the three temperatures for *Nereocystis* (Table 8). For *Pterygophora*, timing to 80% egg production was significantly different between 4°C, 8°C, and 12°C ( $P < 0.001$ ); however, the timing to 80% egg production was not significantly different between 12°C and 16°C ( $P = 0.766$ ; Table 8).

Table 8. Multiple comparisons test with the Holm-modification applied showing if temperature on time to 80% egg production for *Macrocystis pyrifer*, *Pterygophora californica*, and *Nereocystis luetkeana*.

	4° to 8°	8° to 12°	12° to 16°
<i>Macrocystis</i>	P<0.001*	P<0.001*	P=0.006*
<i>Pterygophora</i>	P<0.001*	P<0.001*	P=0.766
<i>Nereocystis</i>	P<0.001*	P<0.001*	N/A

To be able to better compare each species at the different temperatures, time to 20% egg production was displayed for each temperature with all species (Fig. 7). At 4°C, *Macrocystis* and *Pterygophora* gametophytes did not significantly differ in time to 20% eggs released (P = 0.256); however, *Nereocystis* was significantly different from both *Macrocystis* (P < 0.001) and *Pterygophora* (P < 0.001; Table 9 and 10). At 8°C and 12°C, *Macrocystis* and *Pterygophora* gametophytes were significantly different in time to 20% eggs released (P < 0.001, P < 0.001), but *Macrocystis* and *Nereocystis* were not

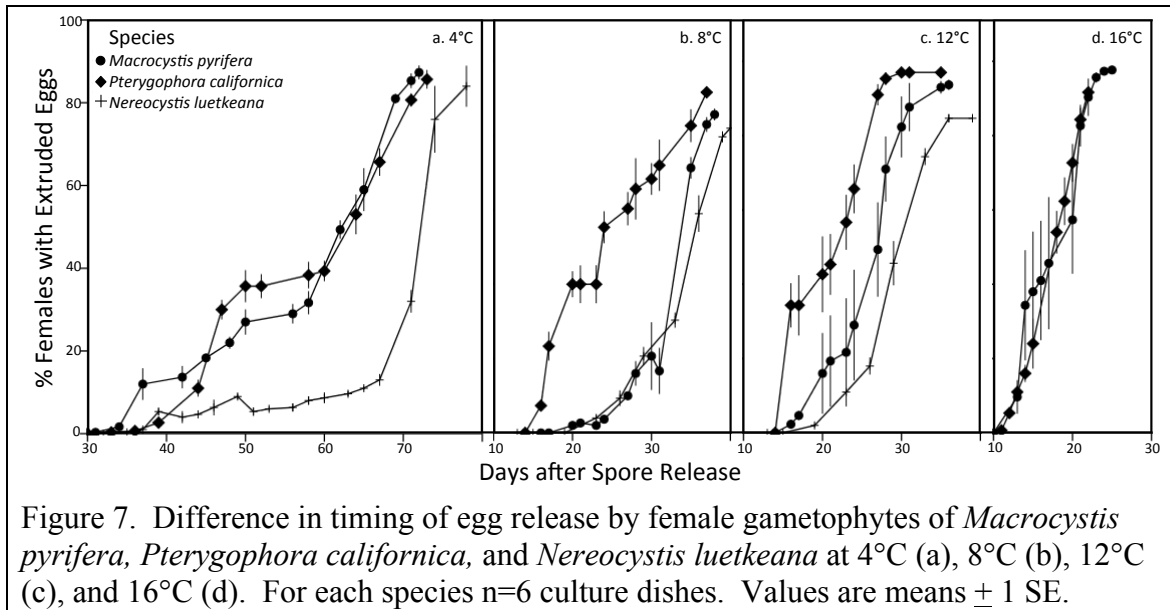


Figure 7. Difference in timing of egg release by female gametophytes of *Macrocystis pyrifer*, *Pterygophora californica*, and *Nereocystis luetkeana* at 4°C (a), 8°C (b), 12°C (c), and 16°C (d). For each species n=6 culture dishes. Values are means  $\pm$  1 SE.

Table 9. Univariate one-way analysis of variance of the effect of species on the time to 20% egg release by female gametophytes of *Macrocystis pyrifera*, *Pterygophora californica*, and *Nereocystis luetkeana* at 4°C (a), 8°C (b), 12°C (c), and 16°C (d; *Macrocystis* and *Pterygophora*). For each temperature n=6 culture dishes.

a. 4°C				
Source	df	MS	F value	P
Species	2	1190.222	138.041	<<0.001*
Error	15	8.622		
b. 8°C				
Source	df	MS	F value	P
Species	2	282.722	74.619	<<0.001*
Error	15	3.789		
c. 12°C				
Source	df	MS	F value	P
Species	2	214.889	43.170	<<0.001*
Error	15	4.978		
d. 16°C				
Source	df	MS	F value	P
Species	2	8.333	2.747	0.128
Error	15	3.033		

significantly different ( $P = 0.254$ ,  $P = 0.090$ ; Table 10). At 16°C, *Macrocystis* and *Pterygophora* gametophytes did not significantly differ in time to 20% eggs released, and there were no eggs produced by *Nereocystis* at this temperature ( $P = 0.128$ ; Table 10).

Table 10. Multiple comparisons test with the Holm-modification applied showing if temperature on time to 20% egg production for *Macrocystis pyrifera*, *Pterygophora californica*, and *Nereocystis luetkeana*.

	M to P	M to N	P to N
4°C	P=0.256	P<0.001	P<0.001
8°C	P<0.001	P=0.254	P<0.001
12°C	P<0.001	P=0.090	P<0.001
16°C	P=0.128	N/A	N/A

## DISCUSSION

Kelp recruitment can be affected by a variety of biotic and abiotic factors (Luning and Neushul 1978). Successful recruitment depends on the proper temperature, light, nutrients, salinity, and settlement densities. This study addressed the effects of temperature on kelp recruitment success, interspecies competition, and timing of egg production by female gametophytes. Although the effects of temperature on recruitment and interspecies competition have been studied previously, the combined effects had yet to be explored. It is important to look at the combined effects of various factors because it gives us a better picture of what may happen in the natural environment (Edwards 2004).

The effects of temperature on the microscopic life stages and recruitment of kelps has been studied in many species; however, most studies have not tested the effects of temperature change on the timing to egg production or between multiple competing species (Vadas 1972, Luning and Neushul 1978, Fain and Murray 1982, Schiel et al. 2004). Previous studies showed that temperatures less than 12°C and greater than 17°C inhibited growth of central California kelps to healthy embryonic sporophytes, and that female fertility decreased at temperatures higher or lower than 12°C (Luning and Neushul 1978). This effect of temperature on development and recruitment was then applied to the sexual competition previously observed between *Macrocystis pyrifera* and *Pterygophora californica* (Reed 1990). Chemical competition experiments among microscopic stages of *Pterygophora californica* and *Macrocystis pyrifera* showed that *Macrocystis*' recruitment inhibition by *Pterygophora* was asymmetrical (Reed 1990);



*Pterygophora* inhibited *Macrocystis* and this relationship never switched. This competitive advantage was likely caused by *Macrocystis* male gametophytes sensing the pheromone (lamoxirene) released by *Pterygophora* female gametophytes before *Macrocystis* female gametophytes were mature (Fig. 7; Reed 1990, Maier et al. 2001). This chemical warfare between these species could cause *Macrocystis* to miss the fertilization of an entire cohort (Reed 1990).

My study looked at this question of chemical competition between kelps with the addition of variation in temperature. The effect of temperature on development and recruitment was then applied to the sexual competition between *Macrocystis pyrifera* and *Pterygophora californica* (Reed 1990), as well as *Macrocystis pyrifera* and *Nereocystis luetkeana*. My study showed that this sexual competition among species was indeed affected by temperature in more than one way.

First, I looked at the effect of temperature on the competitive dynamics between *Macrocystis* and *Pterygophora*. *Pterygophora* was the competitive dominant over *Macrocystis*, and this competitive hierarchy remained consistent at 4°C, 8°C, and 12°C, with the greatest germination and survival at 12°C (Fig. 4). Temperature above or below the ambient (12°C) temperature had a significant affect on germination and survival. Experiments at the lowest temperature tested (4°C) yielded low recruitment densities due to the low germination rates for both species (Fig. 4). This decreased survival at the lowest temperature tested was expected because neither of these species commonly occur in areas where temperatures get that low.

In the 16°C treatment, the competitive relationship between *Macrocystis pyrifera* and *Pterygophora californica* changed (Fig. 4). *Pterygophora* had overall lower recruitment densities at this higher temperature and *Macrocystis* appeared more tolerant of higher temperatures than *Pterygophora californica*. However, egg development and release did not significantly vary between these two species at this temperature ( $P = 0.128$ ). This was surprising since both *Macrocystis* and *Pterygophora* extend their ranges into southern California and even into Baja California. It is likely, however, that *Macrocystis* outcompetes *Pterygophora*, but that recruitment of *Pterygophora* was simply reduced in all treatments at this higher temperature. *Macrocystis* recruit density did not differ as a function of seeding density between the 10M:10P and 10M:1P treatments at 16°C when it was competitively dominant. In contrast, at all other temperatures *Macrocystis* greatly increased its recruit density in the 10M:1P treatment relative to others with higher *Pterygophora* seeding ratios, indicating that this response was due to competitive release.

Competitive hierarchies were also observed by looking at the recruitment densities of each species when there were high or low quantities of the other species present. At 4°C, 8°C, and 12°C, *Pterygophora* had similar recruitment densities whether the competitors densities were high ( $\sim 10/\text{mm}^2$ ), low ( $\sim 1/\text{mm}^2$ ), or absent (Fig. 4). This demonstrated that *Pterygophora* was not negatively affected by the presence or absence of *Macrocystis* in the culture. When looking at the recruitment densities of *Macrocystis* at 4°C, 8°C, and 12°C, it was negatively affected by the settlement density of *Pterygophora* in the culture. *Macrocystis* had higher recruitment densities when there

was zero or small quantities ( $\sim 1/\text{mm}^2$ ) of the competitor (*Pterygophora*) present in mixed species treatments. *Macrocystis* had significantly lower recruitment densities when there were high ( $\sim 10/\text{mm}^2$ ) quantities of the competitor (*Pterygophora*) present in the culture. The interaction between species and seeding ratio was significant at 8°C, and 12°C, showing that the effectiveness of the competitor is determined by the seeding density (Table 2). Reed (1990) had observed this same relationship; however, he grew his cultures in the field at the ambient temperature (15°C) for Santa Barbara, CA.

After observing the impact that temperature had on the competitive dynamics between *Macrocystis* and *Pterygophora*, I looked at the same effects of temperature and competition between *Macrocystis* and *Nereocystis*. At the ambient temperature of 12°C, *Macrocystis* outcompeted *Nereocystis* in competition experiments. The competitive hierarchy between *Macrocystis* and *Nereocystis* remained consistent at all temperatures (4°C, 8°C, 12°C, and 16°C), with the greatest germination and survival at 12°C again (Fig. 4). Temperature above 12°C or below 8°C had a significant affect on germination and survival. Experiments at the lowest temperature tested (4°C) yielded low recruitment densities due to the low germination rates among both *Macrocystis* and *Nereocystis* (Fig. 5). *Nereocystis luetkeana* had decreased germination at this lower temperature, likely due to its adaptation to local, higher temperatures. Another possible reason for *Nereocystis*' overall lower recruitment densities at all temperatures could be that it is the one species that drops its sori from the reproductive blades (sporophylls) to settle onto the benthos, as opposed to staying attached to the adult sporophyte and gradually releasing spores from the sori like *Macrocystis* and *Pterygophora* do. This likely leads to

extremely high settlement densities in the areas surrounding the sori (Abbott and Hollenberg 1976). This could then indicate that *Nereocystis* needs extremely high zoospore densities compared to other kelps in order to have successful germination and eventual recruitment (Amsler and Neushul 1989).

Competitive hierarchies were also observed by looking at the recruitment densities of *Macrocystis* and *Nereocystis* when there were high or low quantities of the other species present. At 4°C, 8°C, and 12°C, *Macrocystis* had similar recruitment densities whether the competitors (*Nereocystis*) densities were high (~10/mm<sup>2</sup>), low (~1/mm<sup>2</sup>), or absent (Fig. 4). *Nereocystis* had fairly low recruitment densities in all treatments and at all temperatures but appeared to have further decreased recruitment with the presence of *Macrocystis*. The interaction between species and seeding ratio was significant at 4°C and 12°C, supporting the hypothesis that sporophyte recruitment is affected by the presence of a competitor and that that affect can change depending on the seeding densities of the two species (Table 4). The effect of seeding ratio on sporophyte recruitment was significant at 4°C and 12°C supporting the hypothesis of a competitive interaction between *Macrocystis* and *Nereocystis*. The interaction between *Macrocystis* and *Nereocystis* was not significant at 8°C when there were equal quantities of both species present; however, there the mean recruitment was higher for *Macrocystis* than *Nereocystis*. The interaction was not significant likely due to the increased variability because one experiment had a much higher recruitment density than the other two experiments. Vadas (1972) showed that the fertility of female gametophytes was primarily affected by light intensity; however, temperature seems to have played a bigger

role in my experiments with *Nereocystis* that were run in optimal light conditions (40  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with 14:10 h light/dark photoperiod).

The overall competitive trend among these three kelp species showed that *Pterygophora californica* outcompeted *Macrocystis pyrifera*, which outcompeted *Nereocystis luetkeana*. This relationship was then tested further by looking at the timing of egg production by female gametophytes in these three kelp species (Fig. 6 and 7). At 8°C and 12°C, *Pterygophora* appeared to produce eggs a few days earlier than *Macrocystis*, and at a faster rate (Fig. 7). This is similar to what Reed et al. (1991) found as well; however, in his experiments, the rate at which eggs were produced was much faster for both species, meaning that the gametophytes went from zero eggs to maximum egg production much faster than in my experiments. This could be due to the aeration he used in his cultures, allowing females to put more of their energy into reproduction because of the increased availability of nutrients. At 4°C and 16°C, *Macrocystis* and *Pterygophora* were not significantly different in the time to egg production. In this study, it seems that fertility of female gametophytes did not decrease with temperature as suggested by Luning and Nueschul (1978); it was the timing to egg production that was affected. Since their study only observed female gametophytes after 2 weeks, they may have not waited long enough to observe fertility because it appears that the timing of fertility was actually affected.

The results for the timing to fertility among female gametophytes indicates that temperature had a stronger effect on *Macrocystis* than *Pterygophora* at 8°C, 12°C, and 16°C (Fig. 6). *Pterygophora* appeared to only change the timing to maturation slightly

between those three temperatures, while *Macrocystis* had no overlap in timing between the three temperatures. This could support the observation that *Macrocystis* seems to be so prolific along the California coastline where all of these temperatures occur.

*Macrocystis* may be outcompeted by *Pterygophora* during sexual competition; however, because of its tolerance to a larger range of temperatures, it has the potential for range expansion with future changes in the climate.

Looking at the difference in time to egg production for *Macrocystis* compared to *Nereocystis*, there was no significant difference between the two at 8°C and 12°C likely due to the high variable in *Macrocystis* (Fig. 7). However, at 4°C, *Nereocystis* did not reach 20% egg production until at least twenty days after *Macrocystis*. A possible reason for this could be that *Nereocystis* may wait for periods of upwelling relaxation for germination to occur. At 16°C, *Nereocystis* did not produce eggs or have any recruitment, possibly because this temperature is higher than the thermal tolerance of *Nereocystis*.

*Nereocystis* appeared to become reproductive much later than both *Macrocystis* and *Pterygophora*, which could explain why this kelp is more opportunistic in where it grows. Many of the *Nereocystis* beds along California's coast tend to be in more wave-exposed and harsh areas; however, these areas are usually prime upwelling centers as well. It is possible that *Nereocystis*' reproduction is triggered by bouts of cooler waters due to upwelling, or periods of storms causing their sori to fall and settle onto the benthos (Setchell 1908).

Kelp recruitment is dependent on a variety of factors. Zoospore densities, distance to competitors, and temperature can all affect the successful recruitment of kelps and their population's persistence. The timing to reproduction can either give a kelp species the competitive advantage or loss of an entire cohort (Reed 1991). Distance between zoospores is not only critical for fertilization within species, but it is also important for interspecies competition during reproduction (Reed 1990). Temperature has also shown to affect female gametophyte fertility among many kelp species; however, the mechanism was unclear (Luning and Neushul 1978). This study suggests that timing to reproduction was the factor driving competitive hierarchies but this competitive pressure could be lifted with changing temperatures. It is important to understand clearly all the factors affecting kelp recruitment because successful recruitment is necessary for populations to persist and provide the structure and foundation for the dynamic kelp forest environment (Graham et al. 1997).

The impacts of climate change, and specifically temperature, may affect population dynamics for older life stages through competition at the microscopic level. Shifts from kelp forests to turf-forming algae mats due to changes in temperature and CO<sub>2</sub> has drastically changed the underwater landscape in affected areas of southern Australia (Connell and Russell 2010). Increased temperature has also been shown to inhibit the reproduction of *Pterygophora californica* in Baja California as well (Matson and Edwards 2007). Kelp populations have shifted through ten years of induced ocean warming in Diablo Cove, California, nearly exterminating both *Nereocystis* and *Pterygophora* from an area where they were previously abundant (Schiel et al.

2004). This study is one more example of how climate change may shift existing populations and their potential persistence. Understanding how all the life stages of kelps and their competitive hierarchies might be affected by temperature and the other impacts of climate change is essential before these effects become irreversible.

## CONCLUSIONS

The results from this study provide insight into how different microscopic life stages of *Macrocystis pyrifera*, *Pterygophora californica*, and *Nereocystis luetkeana*, and the potential chemical competition between these kelps, may be affected by changes in water temperature. This chemical competition between kelp species needs to be better understood because this interaction may play a larger role in species and community dynamics than previously thought. The chemical warfare taking place between co-occurring species of kelp allows us to learn which species are dominant and what the resulting competitive hierarchies within the system may be. Understanding how the different life stages of kelps react to an increase or decrease in water temperature will allow predictions of how population ranges of *Macrocystis pyrifera*, *Pterygophora californica*, and *Nereocystis luetkeana* may shift in the future. This is important to study because range shifts may be partially determined by viability or competition between microscopic life stages. Such shifts will likely affect species diversity and productivity of kelp forest communities, and the economy of people that depend on these organisms for fisheries, medicine, and food. The results from this study indicate that *Macrocystis pyrifera* may become an even more dominant species within the kelp forest system as our



climate changes as it is released from its competitive disadvantage with *Pterygophora* at increasing temperatures. Studying how these kelp species compete with one another and how this relationship will be affected by climate change is essential given the role played by *Macrocystis*, *Pterygophora* and *Nereocystis* as biogenic habitat and ecosystem engineers within the kelp forest.

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