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Gametophyte Fitness and Costs of Self-Fertilization in the Giant Kelp Macrocystis Pyrifera

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GAMETOPHYTE FITNESS AND COSTS OF SELF-FERTILIZATION IN THE GIANT KELP *MACROCYSTIS PYRIFERA*

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Robert A. San Miguel

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GAMETOPHYTE FITNESS AND COSTS OF SELF-FERTILIZATION IN THE GIANT KELP *MACROCYSTIS PYRIFERA*

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ABSTRACT

Gametophyte Fitness and Costs of Self-Fertilization in the Giant Kelp, *Macrocystis pyrifera*
by
Robert A. San Miguel
Master of Science in Marine Science
California State University Monterey Bay, 2017

It is widely established that inbreeding can incur heavy costs in a variety of plants, animals, and algae. To date, ten species of kelp have been tested to ascertain the degree to which selfing reduces recruitment of juvenile sporophytes and of those ten species, seven have reduced recruitment when inbred. In this study, I set out to understand whether there is variability in response to self-fertilization among giant kelp gametophytes grown from multiple sites, what those differences are, and how it affects sporophyte recruitment. I collected reproductive sporophylls from fifteen *Macrocystis pyrifera* individuals in Point Loma, Leo Carrillo State Beach, Carpinteria, and Cayucos, CA. After inducing release of zoospores, I raised gametophytes in both polycultures and monocultures resulting in levels of self-fertilization of 7% and 100% respectively. I recorded the days it took to see the first sporophyte in each dish and a week later counted the number of sporophytes, female gametophytes, and eggs to standardize the data among replicates. I found that, when comparing the density between selfed and outcrossed recruits, there was a reduced number of recruits in selfed than in outcrossed cultures for 3 sites. There was no significant difference in relative cost of self-fertilization among sites. I also found that recruitment was delayed in selfed cultures, but the severity of the delay varied among sites. Eggs existed in an approximately 1:1 ratio to female gametophytes, with the exception of Carpinteria where eggs existed in an approximately 1:2 ratio to female gametophytes. This study demonstrates that *Macrocystis pyrifera* responds to self-fertilization differently at different sites, that the costs of self-fertilization do not vary among sites, and that self-fertilization results in slower recruitment than outcrossing in giant kelp.

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ACKNOWLEDGEMENTS

Choosing a graduate program is anything but easy. Deciding upon what to study and executing an actual thesis, however, is on the next level of difficult. My time at Moss Landing Marine Laboratories (MLML) is one I look back on fondly. It was difficult, but it was also so worth it thanks to the support from the community that is MLML.

For their continued emotional support throughout my time at MLML, I have to thank my friends and fellow graduate students. Firstly, I have to thank Andrea Launer, Melinda Wheelock, and Melissa Nehmens who lived with me throughout my first two years out here in California and were my first friends. Your patience is unending and simply admirable. Secondly, I need to thank my friends Diane Wyse and Catherine Drake. They both started a year before me, but the three of us became friends instantly and I would frequent their house weekly – I even gave myself the title of roommate. These five women were my family while at MLML and provided me the emotional support to undertake my thesis project.

The Phycology lab at MLML is also very special. We call ourselves the BEERPIGs The graduate students that were here during my time taught me so many things. Heather Fulton-Bennett taught me to work hard and never rest on my laurels. Mike Fox taught me how to be successful at science. Alexis Howard (now Troll) taught me to grow kelp in the laboratory even though I was anything but an easy student. Suzanne Christensen taught me to take a quiet step back and really examine the whole scope of my work. Sarah Jeffries taught me the importance of working without distracting others. Scott Gabara taught me how to be a scientific diver. Jasmine Ruvalcaba taught me how to laugh when things go wrong. Lindsay Cooper taught me not to judge a book by its cover. Maureen Wise taught me how to twerk. Sara Worden taught me my intertidal algae. Stephan Bitterwolf taught me that my tone while speaking matters. Steven Cunningham taught me that stress is not worth it. Cody Dawson taught me not to judge others based on what others have said about them. Angela Zepp taught me that even people from the Midwest can swim quite well. Last but not least, Jarred Klosinski taught me that you can live on rice and spaghetti alone.

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INTRODUCTION

Evolution, from a population-genetics perspective, is a change in the frequencies of alleles across generations (Freeman and Herron 2007). Since the early 1900s, evolutionary biologists have used the Hardy-Weinberg principle to understand the processes that shape a population's gene pool (Pierce 2012). The principle states that in a large random-mating population with no selection, mutation, or migration, the allele frequencies and the genotype frequencies are constant from generation to generation (Guo and Thompson 1992).

Nonrandom mating, specifically, can have profound indirect effects on evolution (Freeman and Herron 2007). Perhaps the most widely studied example of nonrandom mating is inbreeding: the mating between relatives. The main genetic consequence of inbreeding is the decrease in the frequency of heterozygotes and the increase in the frequency of homozygotes, termed homozygosis (Wright 1977). Homozygosis can then lead to two major genetic threats: the first is the erosion of quantitative genetic variation necessary for adaptive evolution if there happens to be a change in the selective landscape (Lande 1995); the second is the accumulation of deleterious, or harmful, mutations (Lynch et al. 1995). The subsequent reduction of fitness as a result of inbreeding has come to be known as inbreeding depression (Keller and Waller 2002).

Inbreeding depression can incur heavy costs in organisms (Charlesworth and Charlesworth 1987, Waser and Price 1994, Crnokrak and Roff 1999). Consequently, some species have developed barriers to reduce or avoid the occurrence of inbreeding. Angiosperms can possess genetically determined self-incompatibility systems ensuring that they do not produce seeds when fertilized with their own pollen, called homomorphic

incompatibility (Charlesworth and Charlesworth 1987). Heterostyly, the morphological variation between the carpel and stamen in flowers of the same species within a population, is another form of self-incompatibility that has been found to prevent self-pollination in 22 angiosperm families (Charlesworth and Charlesworth 1987). Even something as common as dioecy, having separate male and female plants, has been argued as a mechanism of preventing inbreeding in plants (Charlesworth and Charlesworth 1987). Self-incompatibility, however, is not always beneficial.

Kelp, like ferns, exhibit a heteromorphic diplohaplontic life history in which the macroscopic free-living stage is diploid and produces zoospores, or spores, while the microscopic free-living haploid stage produces the gametes (Raven et al. 2005, Graham et al. 2009). Additionally, kelp are self-compatible, weedy, and can grow vegetatively (Raimondi et al. 2004, Graham et al. 2007, Barner et al. 2011, Demes and Graham 2011). Thus, kelp may be model organisms for testing whether mating system hypotheses developed for vascular plants are broadly applicable. Of the kelps, only two of them have been well studied with regards to fitness and costs of self-fertilization: *Macrocystis pyrifera* (Raimondi et al. 2004, Westermeier et al. 2010) and *Postelsia palmaeformis* (Barner et al. 2011, Wooton and Pfister 2013).

The giant kelp, *Macrocystis pyrifera*, is the most widely studied of all the kelps due to its near global distribution, its importance as a foundation species in coastal habitats, and its role in aquaculture (Gutierrez et al. 2006, Graham et al. 2007). Studies conducted in the Santa Barbara channel examining the costs of self-fertilization in *Macrocystis pyrifera* concluded that *Macrocystis pyrifera* exhibited a high cost to self-fertilization (Raimondi et al. 2004). Raimondi et al. (2004) employed a combination of

laboratory and field studies to evaluate successful zygote production, age-specific survivorship, and adult fecundity of completely self-fertilized, partially self-fertilized, and outcrossed zoospore solutions. Their results showed that, as the level of self-fertilization increased, zygote production decreased linearly, survivorship was lowest for individuals in the self-fertilized treatment, and zoospore production was much lower in the completely self-fertilized treatment, while the partially self-fertilized and outcrossed treatments were similar (Raimondi et al. 2004). A more recent study conducted by Westermeier et al. (2010) confirmed heterosis, or hybrid vigor, in *Macrocystis pyrifera*. Clonal gametophytes of *Macrocystis pyrifera* from a 250km stretch of coastline in southern Chile were crossed in a factorial design and resulted in hybrids that yielded larger thalli than self-fertilized individuals (Westermeier et al. 2010).

The other kelp in which self-fertilization and fitness has been well studied is *Postelsia palmaeformis*. While the two kelps share a very similar life cycle, *Postelsia palmaeformis* is a high intertidal cumaphyte, whereas *Macrocystis pyrifera* is a subtidal species (Dayton 1973, Abbott and Hollenberg 1976, Paine 1979, Graham et al. 2007). This results in differences between the two species with respect to dispersal of zoospores. While *Macrocystis pyrifera*'s zoospores are dispersed into the water column (Gaylord et al. 2002, Graham 2003), those of *Postelsia palmaeformis* are dropped directly beneath the parents plant at low-tide, which can create a higher occurrence of inbreeding (Paine 1979, Blanchette 1996, Barner et al. 2011). This is supported by Barner et al.'s (2011) results that there were no barriers to self-fertilization in seven experimental populations of *Postelsia palmaeformis*. The rate of population decline of *Postelsia palmaeformis* over the summer was also not significantly explained by density, individual size, or whether

the plant was the result of self-fertilization (Barner et al. 2011). The results of their study demonstrated that for the populations of *Postelsia palmaeformis* on Tatoosh Island, the costs of self-fertilization are low (Barner et al. 2011). Another study found that time to extinction in populations of *Postelsia palmaeformis* was most strongly associated with population size, not self-fertilization (Wooton and Pfister 2013). Regardless of whether the populations had high incidences of selfing or not, smaller populations went extinct faster (Wooton and Pfister 2013).

The correlation between small population size and increased extinction risk has been well documented (Schaffer and Samson 1985, Lande 1988) and was also found in kelps (Wooton and Pfister 2013). Though there was no correlation between time to extinction and self-fertilization, Wooton and Pfister (2013) conducted their study on a kelp species that exhibits low costs to self-fertilization (Barner et al. 2011), which likely slowed the timing to extinction in small populations. Wooton and Pfister's (2013) conclusions led me to wonder whether self-fertilization will significantly predict timing to extinction in small populations of a kelp species that exhibit high costs to self-fertilization, like *Macrocystis pyrifera* (Raimondi et al. 2004), unlike populations of kelp species that have low costs to self-fertilization like *Postelsia palmaeformis* (Barner et al. 2011).

Historically, few studies report a quantitative estimate of lifetime fitness based on multiple components across the life cycle (Sexton et al. 2009), and of those that do, few conduct the study at multiple sites. There are two major breaks in the genetic diversity of *Macrocystis pyrifera* in coastal California separating the species into three distinct genetic groups, establishing a general trend that as distance along the coastline increases

northward from the equator, genetic diversity decreases as is expected with species range expansion following the last glacial maximum (Johansson et al. 2015). Though Raimondi et al. (2004) examined multiple fitness components across the life cycle of *Macrocystis pyrifera*, their study was confined to one area: the Santa Barbara Channel. Their results, therefore, may not be applicable to all populations of *Macrocystis pyrifera*. For my thesis, I built upon their work and expanded it to include multiple sites throughout the range of *Macrocystis pyrifera* in California.

While Raimondi et al. (2004) did examine zygote production, survival to adulthood, development of reproductive structures, and fecundity as separate fitness components, only one component occurred during the haploid gametophyte stage, while three occurred in the diploid sporophyte stage. The ratio of zygotes to female gametophytes was used as the sole fitness component to evaluate performance at the miscroscopic scale (Raimondi et al. 2004). Howard (2014) examined how temperature influenced the time to egg production in multiple kelp species and observed differences not only among species, but also in temperature responses. Unfortunately, gametophytes used by Howard (2014) all came from kelp populations in central California, but it led me to believe that timing to egg production, and therefore timing to fertilization, in female gametophytes is variable and when held at equal temperatures, may vary by population. It was also observed that female *Macrocystis pyrifera* gametophytes from Chile often produce multiple eggs in the laboratory without aeration (Muñoz et al. 2004), while those from the Santa Barbara channel have been observed to only produce one egg per female gametophyte unless aerated in the laboratory (Reed et al. 1991), indicating reproductive variability within the species.

There are certain advantages that come about as a result of variability in timing to egg production and female gametophyte fecundity. The shorter the time to fertilization the more quickly a small population of kelp can rebound after a disturbance. Additionally, the production of multiple eggs per female gametophyte allows for increased fertilization opportunities from unrelated male gametophytes, and a potentially higher concentration of lamoxirene, the hormone that induces spermatozoid release from male kelp gametophytes (Mamer 1984).

To better understand differences in the reproductive ecology of giant kelp gametophytes across the species' range in California, experimental cultures were grown in the laboratory. The experiments were designed and data was collected in a way to address the following hypotheses:

1) Selfed cultures from *Macrocystis pyrifera* will result in less recruitment than outcrossed cultures for all sites.
2) Selfed cultures from *Macrocystis pyrifera* will recruit slower than outcrossed cultures and will vary across sites.
3) The mean fecundity in subtidal *Macrocystis pyrifera* gametophytes will not vary among sites.

METHODS

Field sampling

Sporophylls were collected at four sites representative of all three genetic groups (Johansson et al. 2015) of *Macrocystis pyrifera* in coastal California (Figure 1). The sites sampled were Point Loma, Leo Carrillo State Beach, Carpinteria, and Cayucos. Divers entered the kelp beds by boat or by swimming from shore. Upon reaching the edge of the

kelp bed, divers sank to the bottom and swam along the longest axis of the kelp forest. Sporophyll bundles (Figure 2) were collected from twenty individuals spread approximately 10m apart to reduce levels of kinship among individuals (Johansson et al. 2013). Once twenty bundles were collected, the samples were placed in a cooler and driven back to the laboratory in Moss Landing, CA.

Laboratory experiments

Gametophytes of *Macrocystis pyrifera* were cultured in a blocked design using 2-part Fisherbrand™ Compartmentalized Petri Dishes (catalog number: FB08757150) at different self-fertilization ratios to test the early life history costs of self-fertilization in *Macrocystis pyrifera* at each site (similar to the work of Raimondi et al. (2004) with *Macrocystis pyrifera* from the Santa Barbara Channel). Each section was assigned a self-fertilization treatment of either Selfed (Treatment I) or Outcrossed (Treatment II).

Each sporophyll was cleaned by dipping it in a 1% iodine solution for 30s, followed by a 20s deionized water rinse, and a 60s salt water bath. Zoospore release was induced by laying the blades in a glass pan with moist pieces of paper towel between them and then placing the pan in a refrigerator overnight. The pan was removed 24 hours later and the sporophylls from each individual placed into a separate bowl with Instant Ocean™ (product no. SS15-10) seawater to stimulate sporulation. After an hour, a 10-μm mesh was used to strain out particles from the zoospore solution. The concentration of stock zoospore solutions was estimated using a hemacytometer and recorded. Each section of the dish was inoculated with a calculated volume of zoospore solution that aimed to yield a settlement density of approximately 25 zoospores/mm^2. Additional seawater (up to 10mL) was added to each portion of the petri dish to ensure that the

zoospores could settle throughout the dish homogenously. Sporophylls from each individual were paired with a specific petri dish to serve as independent replicates for the Selfed solutions for a total of 15 replicates (i.e. 15 petri dishes). The Outcrossed solution was created by using an equal number of zoospores from 15 individuals so that this treatment resulted in an expected self-fertilization rate of roughly 7% $\left(\frac{1\ individual}{15\ potential\ mates} = 6.67\%\ chance\ of\ mating\ with\ self\right)$. The concentration of the Outcrossed treatment zoospore solution was estimated using a 1mL sample and a hemacytometer before it was used to inoculate the remaining half of the petri dish. Petri dishes were placed overnight in a 12° C incubator with fluorescent tube lights (FLD20/18, 20 watt) set to a 14:10 hour light cycle with an irradiance of 35-40 μmol m^{-2} s^{-1}, to induce zoospore settlement. After settlement, the water was replaced within 24 hours with Provasoli's Enriched Seawater (Provasoli 1968) and changed every seven days until data were collected.

Data were collected using a Leica DM IL microscope. Counts were conducted at least weekly using 10 fields of view at 400x magnification to note the appearance of eggs on female gametophytes, the number of eggs produced on each female gametophyte, and the appearance of embryonic sporophytes. One week after the first embryonic sporophyte was sighted in a section for each respective dish, 10 fields of view were made at 400x magnification for that section of the dish. The number of sporophytes, female gametophytes, and eggs were recorded before concluding the experiment in that half of the dish. This is a method of standardizing the timing of data collection among sites and between treatments to account for differences in timing to fertilization among individuals.

Experiments lasted 90 days, and if no sporophyte appeared, a value of zero was given for that replicate's recruitment value and time to sporophyte production set at 90 days.

Data Analysis

Dishes in which the gametophyte density in either treatment was less than 25 gametophytes/mm^2 were removed from analysis to eliminate any density dependent recruitment effects caused by gametophytes being too far from each other for fertilization to occur (Reed 1990, Reed et al. 1991). The ratio of final sporophytes to final female gametophytes was used to determine the mean relative cost of self-fertilization for each individual site (Raimondi et al. 2004). The response variable was a ratio to account for any differences in initial densities among replicate trials conducted on different dates using different batches of zoospores (Raimondi et al. 2004). Two two-way analyses of variance (ANOVA) were used to test the effects of self-fertilization treatment (fixed) and site (random) on the ratios of final sporophytes to final female gametophytes and final sporophytes to final eggs. A relative cost of selfing was assigned to each site using the mean decline in offspring number: $\frac{(Selfed\ Recruit\ Density - Outcrossed\ Recruit\ Density)}{Outcrossed\ Recruit\ Density}$ (Collens 2009). This relative cost of self-fertilization was used to quantify the outcrossing advantage. A third two-way ANOVA was used to examine the differences in days to first fertilization both between sites (random) and treatments (fixed). In order to eliminate any artifacts related to being grown in monoculture, only outcrossed cultures were used in the analysis of female gametophyte fecundity.

Sixty total dishes were inoculated with zoospores and of those, three from Cayucos and one from Carpinteria did not meet the required gametophyte density which may have limited recruitment and thus were not included in analyses. Four one-way

ANOVAs were used, one for each site, to test the effect of the block (dish) on recruitment. Blocking was insignificant at all sites; i.e. variability among dishes was random and indistinguishable from natural variability.

RESULTS

Costs of Self-Fertilization

A two-way ANOVA (Table 1a, Figure 3) testing the effects of site and treatment on the ratio of sporophytes and the number of female gametophytes found the interaction between site and treatment to be significant ($F_{3,104} = 2.708$, $p = 0.04902$) as well as the effect of site alone ($F_{3,104} = 5.258$, $p = 0.00204$), but no significant effect of treatment ($F_{1,104} = 1.370$, $p = 0.24450$). This significant difference in the interaction term is caused by the greater recruitment seen in the selfed culture at Cayucos relative to the outcrossed cultures whereas for all other sites, the outcrossed cultures yielded more recruits than the selfed cultures. A pairwise t-test using the Holm modification was conducted to further investigate the differences among the interaction between site and treatment (Table 1b) and found significant differences between the outcrossed treatment from Leo Carrillo and the selfed treatment from Carpinteria ($p = 0.0029$) with greater recruitment in the outcrossed treatment of Leo Carrillo. Significance was also found between the selfed treatment from Point Loma and the outcrossed treatment from Leo Carrillo ($p = 0.0084$) with greater recruitment in the outcrossed treatment from Leo Carrillo. The outcrossed treatments between Leo Carrillo and Point Loma ($p = 0.0331$) were also significant with greater recruitment in the outcrossed treatment from Leo Carrillo. Lastly, there was also a mildly significant difference between the outcrossed treatments of Carpinteria and Leo Carrillo ($p = 0.0734$) with greater recruitment in the outcrossed culture from Leo Carrillo.

A second two-way ANOVA (Table 2a, Figure 4) testing the effects of site and treatment on the ratio of sporophytes and the number of eggs found a significant interaction between site and treatment ($F_{3,104} = 4.442$, $p = 0.00559$), site alone ($F_{3,104} = 2.805$, $p = 0.04337$) and treatment alone ($F_{1,104} = 6.210$, $p = 0.01428$). Again, the interaction term was significant and the data visualization demonstrates that yet again the pattern of outcrossed treatments resulting in better recruitment than selfed treatments was reversed for Cayucos. An additional pairwise t-test was conducted to tease apart the significant interaction between site and treatment (Table 2b). Significant differences were found between the outcrossed treatment in Carpinteria and the selfed treatment in Carpinteria ($p = 0.0073$) with the outcrossed treatment having more recruitment. The differences between the outcrossed treatment from Leo Carrillo and the selfed treatment from Carpinteria ($p = 0.0501$) were also significant with the outcrossed treatment from Leo Carrillo having greater recruitment. Recruitment also differed significantly between the selfed treatment from Point Loma and the outcrossed treatment from Leo Carrillo ($p = 0.0084$) with the outcrossed treatment from Leo Carrillo having higher recruitment. Additionally, the selfed treatment from Point Loma and the outcrossed treatment from Carpinteria ($p = 0.0132$) differed significantly as well with greater recruitment occurring in the outcrossed treatment from Carpinteria. Finally, the outcrossed treatment from Point Loma and the outcrossed treatment from Carpinteria ($p = 0.0215$) also significantly differed with Carpinteria's outcrossed treatment having more recruits than the outcrossed culture from Point Loma.

After calculating and plotting the mean relative cost of self-fertilization for each site (Figure 5), it appears as though Carpinteria has a noticeably higher mean relative cost

to self-fertilization compared to the other sites which have a mean value just above zero. This indicates a slight benefit as a result of self-fertilization for Cayucos, Leo Carillo, and Point Loma. A one-way ANOVA (Table 3) was run testing the effect of site on the relative cost of self-fertilization, however, and found no significant effect ($F_{3,52} = 1.444$, $p = 0.241$).

Timing to Fertilization

At the conclusion of 90 days, only ten total dish partitions did not show signs of any sporophyte recruitment, six from Carpinteria and four from Leo Carrillo, with all of them being in the self-fertilized treatment. The data showed that outcrossed cultures always produced their first sporophyte before the selfed cultures though the degree of that difference varied among sites. A three-way ANOVA tested the effects of site, dish, and treatment on the number of days until the first sporophyte was seen in each dish (Table 4a, Figure 6). A significant effect was found for the interaction of site and treatment ($F_{3,104} = 9.522$, $p < 0.001$), in addition to significant effects of site alone ($F_{3,104} = 61.178$, $p < 0.001$) and treatment alone ($F_{1,104} = 25.045$, $p < 0.001$).

A pairwise t-test with the Holm modification was conducted to better understand the relationship between all site and treatment combinations (Table 4b). 15 of the interaction terms were highly significantly different ($p < 0.001$): selfed Carpinteria vs selfed Cayucos with Carpinteria taking longer, selfed Carpinteria vs outcrossed Cayucos, outcrossed Carpinteria vs selfed Carpinteria, selfed Leo Carrillo vs selfed Cayucos with Leo Carrillo taking longer, selfed Leo Carrillo vs outcrossed Cayucos, selfed Leo Carrillo vs outcrossed Carpinteria, outcrossed Leo Carrillo vs selfed Cayucos, outcrossed Leo Carrillo vs outcrossed Cayucos with Leo Carrillo taking longer, outcrossed Leo Carrillo

vs outcrossed Carpinteria with Leo Carrillo taking longer, selfed Point Loma vs selfed Carpinteria with Carpinteria taking longer, selfed Point Loma vs selfed Leo Carrillo with Leo Carrillo taking longer, selfed Point Loma vs outcrossed Leo Carrillo, outcrossed Point Loma vs selfed Carpinteria, outcrossed Point Loma vs selfed Leo Carrillo, and outcrossed Point Loma vs outcrossed Leo Carrillo with Leo Carrillo taking longer than Point Loma. None of the other pairings were found to be significant. The difference between the mean number of days to the first sporophyte being sighted was also plotted and shows smaller differences between treatments at most sites with the exception of Carpinteria which had a mean difference of 35 days likely due to the selfed dishes that never produced sporophytes (Figure 7).

Female Gametophyte Fecundity

Throughout this study, multiple examples of female gametophytes with multiple eggs, no eggs, and even seemingly intercalary eggs were found (Figure 8). Of the four sites, Carpinteria was unique in that the mean ratio of eggs to female gametophytes was 1:2 whereas for the other three sites, it was roughly a 1:1 ratio. Using only data from the outcrossed cultures, a two-way ANOVA was used to test the effects of site and dish on the ratio of eggs to female gametophytes (Table 5a, Figure 9) and found a significant effect of site ($F_{3,52} = 13.21$, $p < 0.001$). A pairwise t-test (Table 5b) using the Holm modification was done to further examine the pairwise differences between sites and only found significant differences between Carpinteria and the other three sites ($p < 0.001$), but not among comparisons between Leo Carrillo, Cayucos, and Point Loma.

DISCUSSION

Costs of Self-Fertilization

The results from these experiments support Raimondi et al. (2004) who found that selfed cultures yield less sporophytes than outcrossed cultures regardless of the response variable used. The exception to this trend, however is Cayucos. Cayucos lies north of Pt. Conception which is a well-known biogeographic break for many species and also functions as a genetic barrier for *Macrocystis pyrifera* (Johansson et al. 2015). The results from the pairwise t-tests examining recruitment differences among sites indicated that there was a significant difference in recruitment density between Carpinteria and Leo Carillo as well as between Leo Carillo and Point Loma. The reason for greater recruitment from Leo Carillo is unknown. Johansson et al. 2015 found that Leo Carillo is a site located in the transition zone between three genetic population clusters along the California coastline and actually had near equal assignment to three clusters (Santa Barbara, Channel Island, and Southern California clusters) in the population genetics program, STRUCTURE (Pritchard et al. 2000).

Interestingly, despite the site and treatment level differences in recruitment, there was no significant difference in the relative cost of self-fertilization among any of the four sites contradicting the results from the earlier analyses. Site specific differences in response to self-fertilization was not statistically significant despite there being graphical support for such differences. These results demonstrate that some populations of *Macrocystis pyrifera* appear to be better at purging deleterious mutations than others and recruit better when faced with self-fertilization. However, this is not the first kelp to have demonstrated this, however, as Collens (2009) also found seemingly large differences in relative selfing cost in two populations of *Postelsia palmaeformis* from Tatoosh,

Washington. The results indicate that only at Carptineria is there a negative relative cost to self-fertilization but that largely there is not a relative recruitment cost or benefit associated with self-fertilization in *Macrocystis pyrifera* at Cayucos, Point Loma, and Leo Carrillo in California. Several experiments were attempted using samples from more northern sites including Santa Cruz, Stillwater Cove, and Big Creek but for unknown reasons, perhaps relating to the "Warm Blob" event (Bond et al. 2015), the cultures did not yield sporophytes and so data was unable to be incorporated from other sites north of Pt. Conception during this study. This may also have been due to high levels of inbreeding depression as those sites are part of a region with the least amount of genetic diversity and allelic richness in the entire state of California (Johansson et al. 2015).

Despite the lack of evidence for a cost to self-fertilization, the data from the other analyses support the notion that south of Pt. Conception, *Macrocystis pyrifera* does not perform or recruit as well in self-fertilized cultures whereas north of Pt. Conception the kelp grown in monocultures recruit as well or better than those in outcrossed cultures as is expected according to Baker's law (Baker 1955). I predict that should experiments be conducted on kelp samples from additional sites in central California, the results would be similar to those from Cayucos supporting the hypothesis that the percent density of recruits in selfed cultures would decrease northward from the equator.

Timing to Fertilization

The time until fertilization in the cultures indicated that there was not only variability among sites with regard to how long it takes for fertilization to occur in gametophytes from these populations, but also that selfed cultures generally took longer

to yield recruits than outcrossed cultures. These findings suggest that there may be strong barriers to self-fertilization in *Macrocystis pyrifera* and that it may vary among different populations along the coast.

Four dishes total did not yield sporophytes and all of them were in the self-fertilized cultures suggesting that these barriers may actually be very strong. The female gametophytes within these dishes produced eggs and the male gametophytes appeared to be healthy as well. The delays or differences among sites may also be due in part to when the sporophylls from each site were collected. Daniel C. Reed (pers. comm.) reported that in the Santa Barbara channel there are peak times for zoospore release and recruitment in May and July. This may mean that the sporophylls collected earlier than those months of the year did not contain fully developed zoospores ready for release at the time of my experiment, which may have led to underdeveloped gametophytes or gametophytes that were slower at reproduction. This likely also contributed to the recruitment differences in my self-fertilization experiments among the four sites. The experiments were not all conducted at the same time so it would be worth investigating in the future how seasonality affects the recruitment of kelp sporophytes.

Additionally, Collens (2009) observed parthenogenesis in six species of kelp and thus it is not unreasonable to believe that parthenogenesis is possible in *Macrocystis pyrifera* gametophytes as well. Several of the sporophytes that were examined in this study were malformed, which can be an indicator of parthenogenesis (Collens 2009), but these observations were not documented. It is worth noting as well that there are also many observations of seemingly normal parthenogenic recruits according to the literature (Kemp and Cole 1961, Motomura 1991, Gall et al. 1996, Druehl et al. 2005) and thus

parthenogenesis may occur in both selfed and outcrossed treatments. Though parthenogenesis could not be examined with this experimental design without the assistance of genetics, it could be an explanation for the delay in recruitment in the selfed cultures. The egg itself releases the hormone lamoxirene which causes the male gametophytes nearby to release chemotactic spermatozoids (Mamer 1984) that would seek out the egg so to prevent the actual fertilization, there would need to be a mechanism on the molecular level that blocks fertilization of the egg like a human egg cell does post fertilization to prevent polyspermy.

Female Gametophyte Fecundity

For three of the four sites sampled, the ratio of female gametophytes to eggs in each dish was very close to a 1:1 ratio indicating that for the most part, each female gametophyte extrudes about 1 egg. The exception to this was Carpinteria. which had a ratio of about 2 female gametophytes in a dish for every 1 egg. The pairwise t-test results showed that it was Carpinteria, specifically, that drove the significant response found in the 1-way ANOVA.

As mentioned before, despite *Macrocystis pyrifera* being a perennial species, it does exhibit peaks in zoospore production. The sporophylls collected from this site were done so in February for Carpinteria, which is not a peak recruitment period, and may have resulted in decreased fitness of released zoospores and the resulting gametophytes. The other sites were sampled later in the calendar year when the sporophytes had longer photoperiods which may increase the quality of zoospore production.

The temperature at which the cultures were grown in may have also been a factor. According to the results from Muñoz et al. (2004), the number of eggs per female

gametophyte not only differed among populations and the type of growth media they are given, but that temperature also has a large effect on the number of eggs a given female gametophyte will produce, such that gametophytes grown in colder temperatures and enriched with Provasoli's Enriched Seawater (1968) produce a higher number of eggs than those grown in warmer temperatures and with filtered seawater. It would be interesting to see if a similar response occurs as a result of temperature differences in growing conditions for gametophytes from the North American continent as well, as it remains to be studied and may be important given increasing ocean temperatures as a result of climate change.

CONCLUSION

The mixed mating system of *Macrocystis pyrifera* gives the species a unique opportunity to reproduce in a variety of different ways, with relatively little to no significant costs in terms of recruitment. There are also apparent differences in recruitment among sites populated by the species, which may be indicative of either local reproductive adaption or seasonal differences in reproductive potential. There was no statistical difference, however, in the relative cost of self-fertilization among the four sites studied. There were delays in timing to reproduction of self-fertilized cultures, but the extent of the delay varied among sites, which may be caused by self-incompatibility or it may also indicate incidences of parthenogenesis, which may be slower than sexual reproduction. These differences may also be site-specific or seasonal, but in any case the differences do exist and serve as evidence of plasticity in reproductive timing within *Macrocystis pyrifera*. Fecundity of female gametophytes in *Macrocystis pyrifera* was

also variable, but on average there was a 1:1 ratio of eggs to female gametophytes, with the exception of gametophytes cultured from Carpinteria. The reason for this difference may be due to seasonal differences in zoospore maturity and viability that lead to growth of less fit gametophytes. Therefore, using recruitment as a function of either the number of eggs or female gametophytes present is appropriate.

Table 1a. ANOVA on the effects of site and treatment (selfed/outcrossed) and dish on recruitment (# sporophytes / # female gametophytes).

Source	df	MS	F value	P
Site	3	0.18898	5.258	0.00204
Treatment	1	0.04924	1.370	0.24450
Site x Treatment	3	0.09733	2.708	0.04902
Error	104			

Table 1b. Pairwise t-test with the Holm modification applied showing direct comparisons of recruitment (# sporophytes / # female gametophytes) between all potential site and treatment pairings represented in p-values.

Interaction	Selfed Cayucos	Outcrossed Cayucos	Selfed Carpinteria	Outcrossed Carpinteria	Selfed Leo Carrillo	Outcrossed Leo Carrillo	Selfed Point Loma
Outcrossed Cayucos	1	-	-	-	-	-	-
Selfed Carpinteria	0.1980	1	-	-	-	-	-
Outcrossed Carpinteria	1	1	1	-	-	-	-
Selfed Leo Carrillo	1	1	1	1	-	-	-
Outcrossed Leo Carrillo	1	0.1128	0.0029	0.0734	0.4227	-	-
Selfed Point Loma	0.4227	1	1	1	1	0.0084	-
Outcrossed Point Loma	1	1	1	1	1	0.0331	1

Table 2a. ANOVA on the effects of site and treatment (selfed/outcrossed) on recruitment (# sporophytes / # eggs).

Source	**df**	**MS**	**F value**	**P**
Site	3	0.07263	2.805	0.04337
Treatment	1	0.16080	6.210	0.01428
Site x Treatment	3	0.11501	4.442	0.00559
Error	104	0.02589		

Table 2b. Pairwise t-test with the Holm modification applied showing direct comparisons of recruitment (# sporophytes / # eggs) between all potential site and treatment pairings represented in p-values.

Interaction	Selfed Cayucos	Outcrossed Cayucos	Selfed Carpinteria	Outcrossed Carpinteria	Selfed Leo Carirllo	Outcrossed Leo Carrillo	Selfed Point Loma
Outcrossed Cayucos	1	-	-	-	-	-	-
Selfed Carpinteria	1	1	-	-	-	-	-
Outcrossed Carpinteria	1	0.1003	0.0073	-	-	-	-
Selfed Leo Carrillo	1	1	1	0.2027	-	-	-
Outcrossed Leo Carrillo	1	0.4538	0.0501	1	0.9210	-	-
Selfed Point Loma	1	1	1	0.0132	1	0.0084	-
Outcrossed Point Loma	1	1	1	0.0215	1	0.1272	1

Table 3. ANOVA on the effects of site on relative cost of self-fertilization ((selfed recruit density – outcrossed recruit density) / outcrossed recruit density).

Source	df	MS	F value	P
Site	3	4.240	1.444	0.241
Error	52	2.937		

Table 4a. ANOVA on the effects of site and treatment (selfed/outcrossed) on time to first sporophyte (days).

Source	df	MS	F value	P
Site	3	10384	61.178	< 0.001
Treatment	1	4251	25.045	< 0.001
Site x Treatment	3	1616	9.522	< 0.001
Error	104	170		

Table 4b. Pairwise t-test with the Holm modification applied showing direct comparisons of timing to first sporophyte (days) between all potential site and treatment pairings represented in p-values.

Interaction	Selfed Cayucos	Outcrossed Cayucos	Selfed Carpinteria	Outcrossed Carpinteria	Selfed Leo Carrillo	Outcrossed Leo Carrillo	Selfed Point Loma
Outcrossed Cayucos	1	-	-	-	-	-	-
Selfed Carpinteria	< 0.001	< 0.001	-	-	-	-	-
Outcrossed Carpinteria	1	1	< 0.001	-	-	-	-
Selfed Leo Carrillo	< 0.001	< 0.001	1	< 0.001	-	-	-
Outcrossed Leo Carrillo	< 0.001	< 0.001	1	< 0.001	1	-	-
Selfed Point Loma	1	1	< 0.001	1	< 0.001	< 0.001	-
Outcrossed Point Loma	0.23	0.67	< 0.001	1	< 0.001	< 0.001	1

Table 5a. ANOVA on the effects of site on mean female fecundity (# egg / # female gametophyte).

Source	**df**	**MS**	**F value**	**P**
Site	3	2.0262	13.21	< 0.001
Error	52	0.1534		

Table 5b. Pairwise t-test with the Holm modification applied showing direct comparisons of mean female fecundity between all potential site pairings represented in p-values.

Site	Cayucos	Carpinteria	Leo Carrillo
Carpinteria	< 0.001	-	-
Leo Carrillo	1	< 0.001	-
Point Loma	1	< 0.001	1

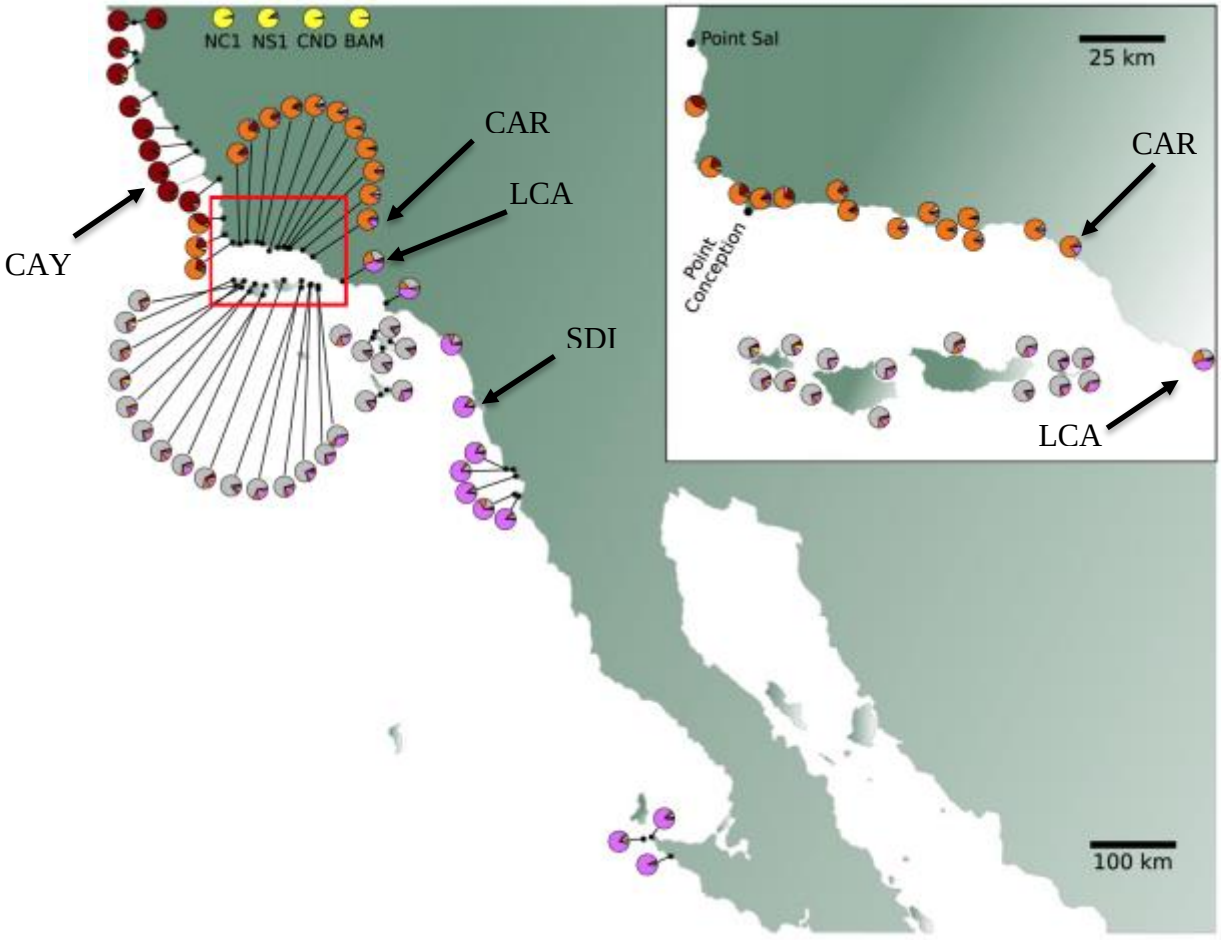

Figure 1 – Map of population genetic clusters of *Macrocystis pyrifera* taken from Johansson et al. (2015). Arrows added to indicate sampling site locations for this study. CAY = Cayucos, CAR = Carpinteria, LCA = Leo Carillo, SDI = San Diego (Point Loma).

Figure 2 – Image of a sporophyll bundle. Note that all sporophylls originate from a central point. This ensures that they are all from one individual kelp specimen and prevents the collection of sporophylls from different individuals that may have colonized the same holdfast structure.

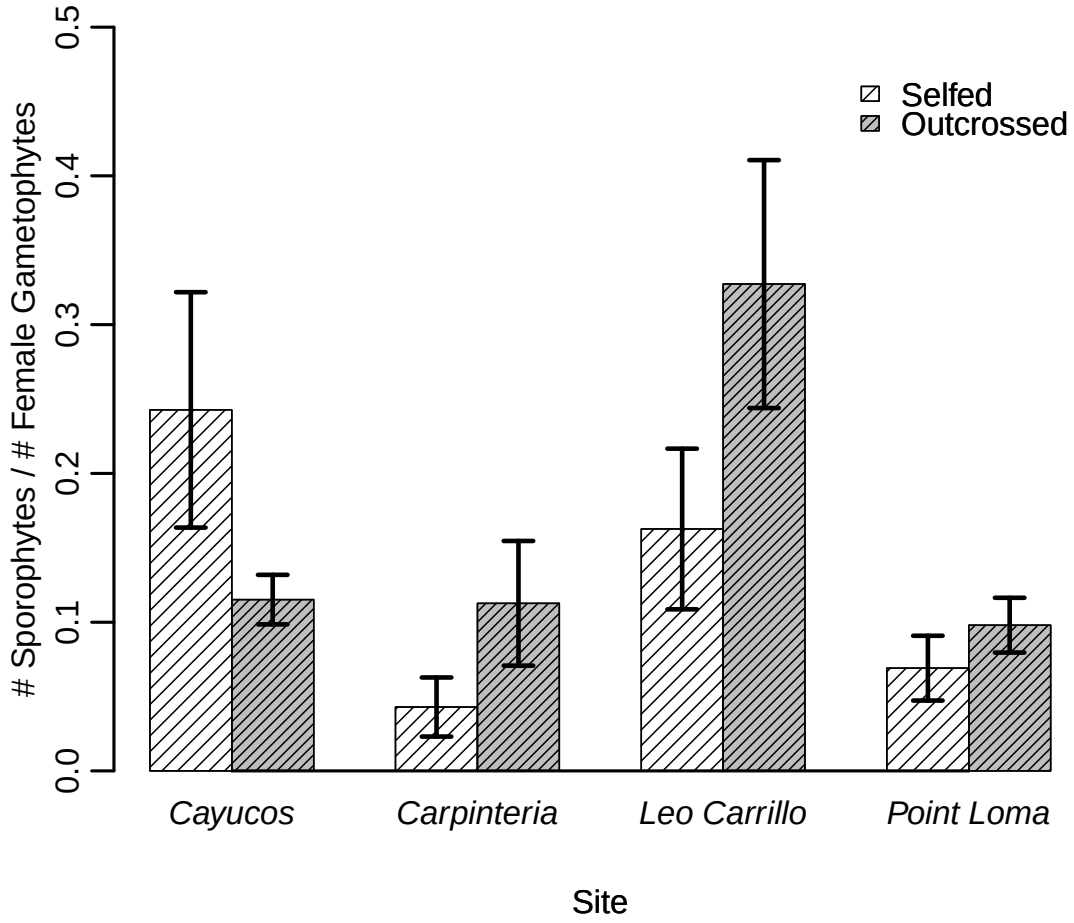

Figure 3 – Mean recruitment ratio (#Sporophytes/# Female Gametophytes) ± SE of *Macrocystis pyrifera* using the total number of sporophytes divided by the total number of female gametophytes in selfed and outcrossed cultures across 4 sites: Cayucos (n = 12), Carpinteria (n = 14), Leo Carrillo (n = 15), Point Loma (n = 15).

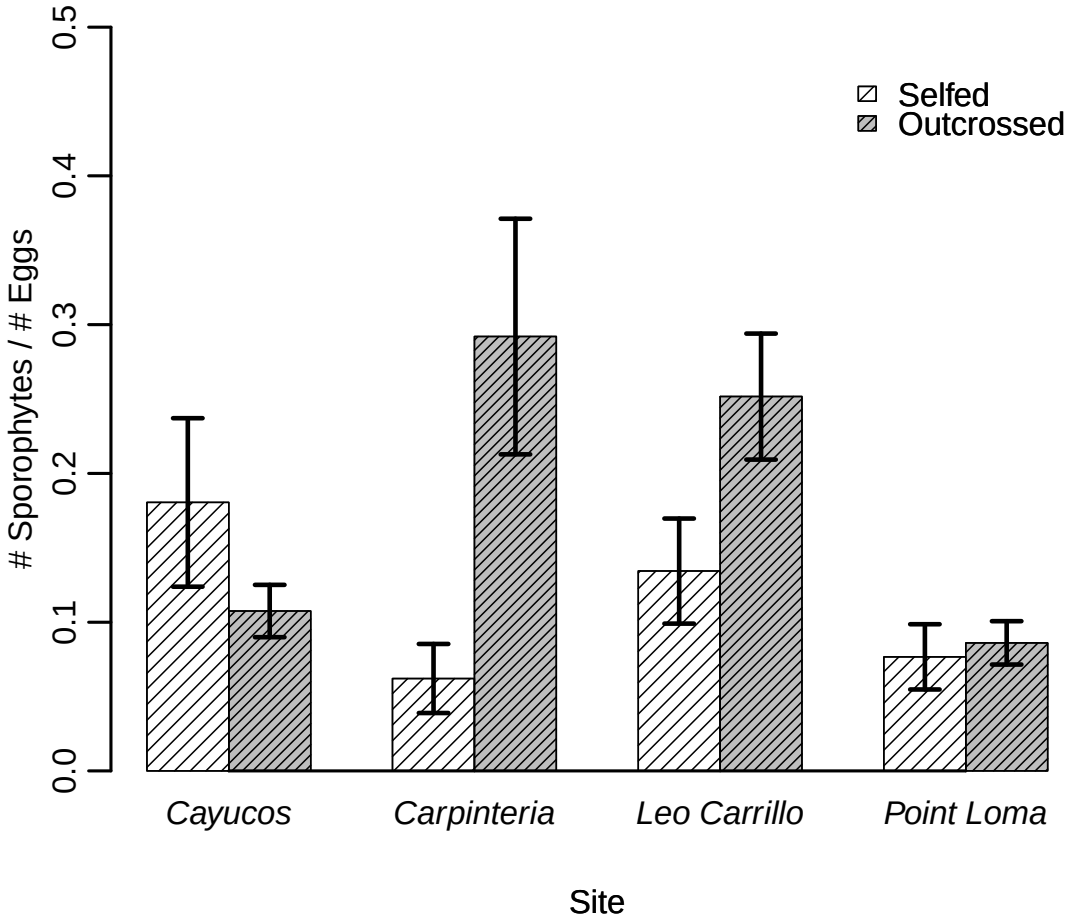

Figure 4 – Mean recruitment ratio (#Sporophytes/# Eggs) ± SE of *Macrocystis pyrifera* using the total number of sporophytes divided by the total number of eggs in selfed and outcrossed cultures across 4 sites: Cayucos (n = 12), Carpinteria (n = 14), Leo Carrillo (n = 15), Point Loma (n = 15).

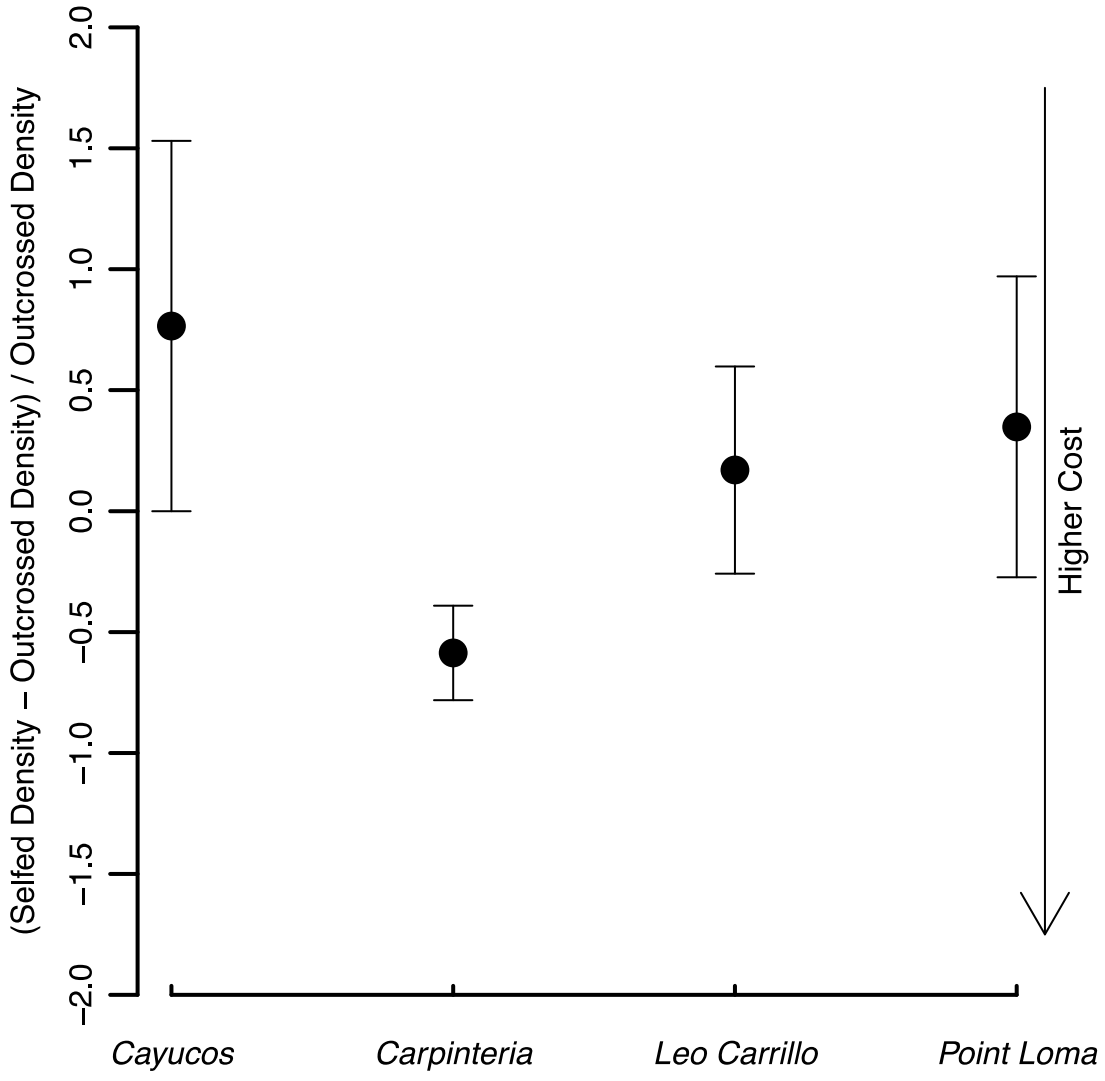

Figure 5 – Mean relative cost of self-fertilization (± SE) of *Macrocystis pyrifera* using the difference between the selfed and outcrossed recruit densities divided by the outcrossed recruit density across 4 sites: Cayucos (n = 12), Carpinteria (n = 14), Leo Carrillo (n = 15), Point Loma (n = 15).

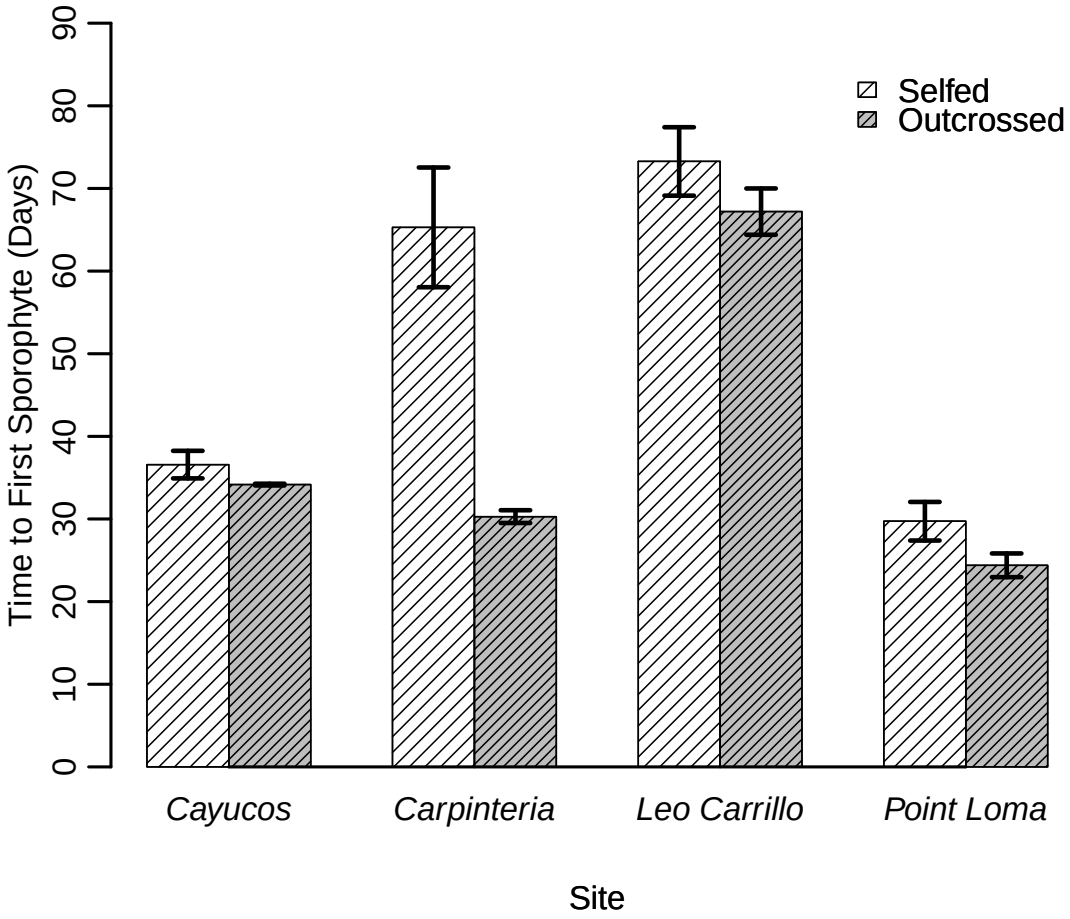

Figure 6 – Mean time to fertilization ± SE of *Macrocystis pyrifera* using the total number of days until the first sporophyte was sighted in each dish in selfed and outcrossed cultures across 4 sites: Cayucos (n = 12), Carpinteria (n = 14), Leo Carrillo (n = 15), Point Loma (n = 15).

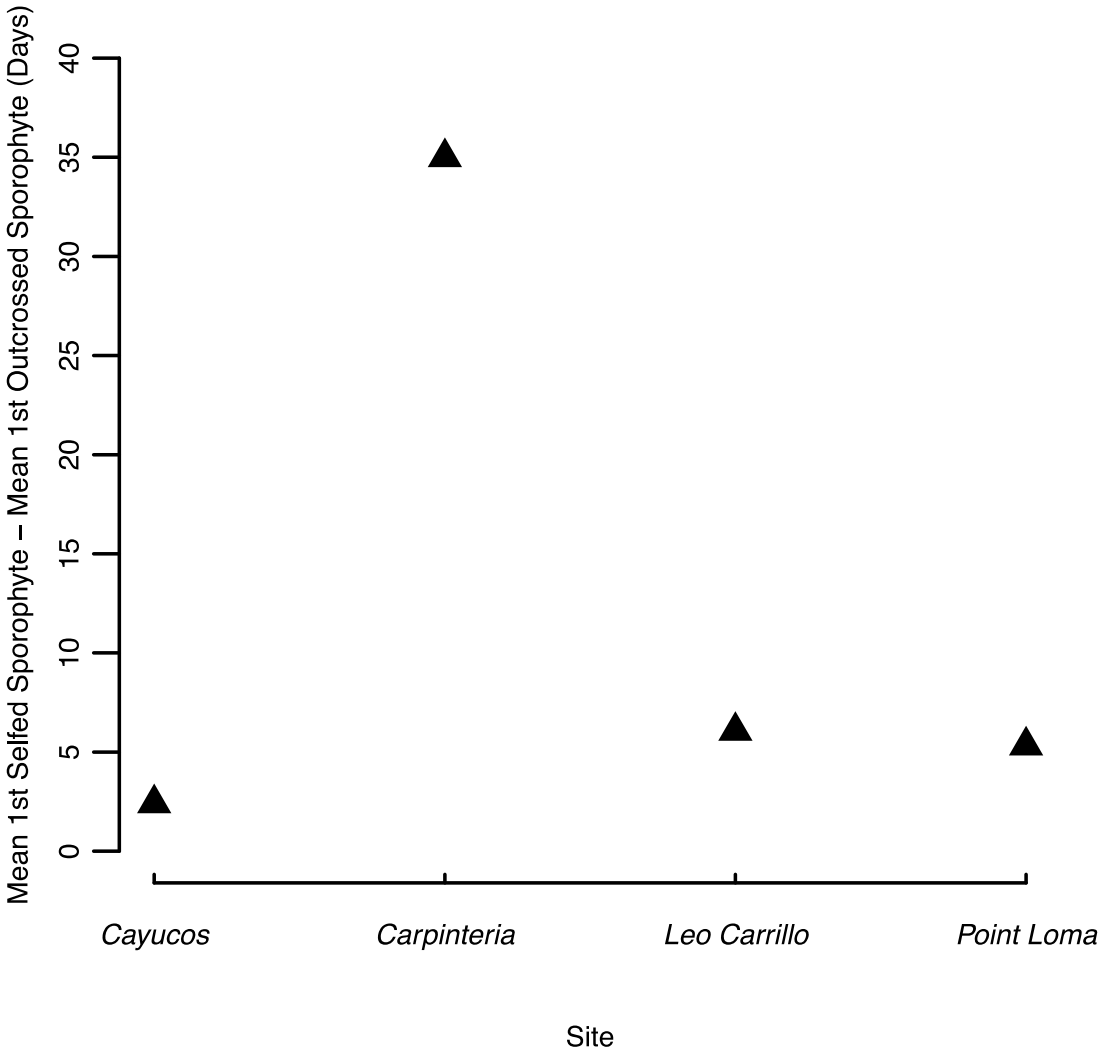

Figure 7 – Difference in mean time to first fertilization of *Macrocystis pyrifera* using the total number of days until the first sporophyte was sighted in each dish in selfed and outcrossed cultures across 4 sites: Cayucos, Carpinteria, Leo Carrillo, Point Loma.

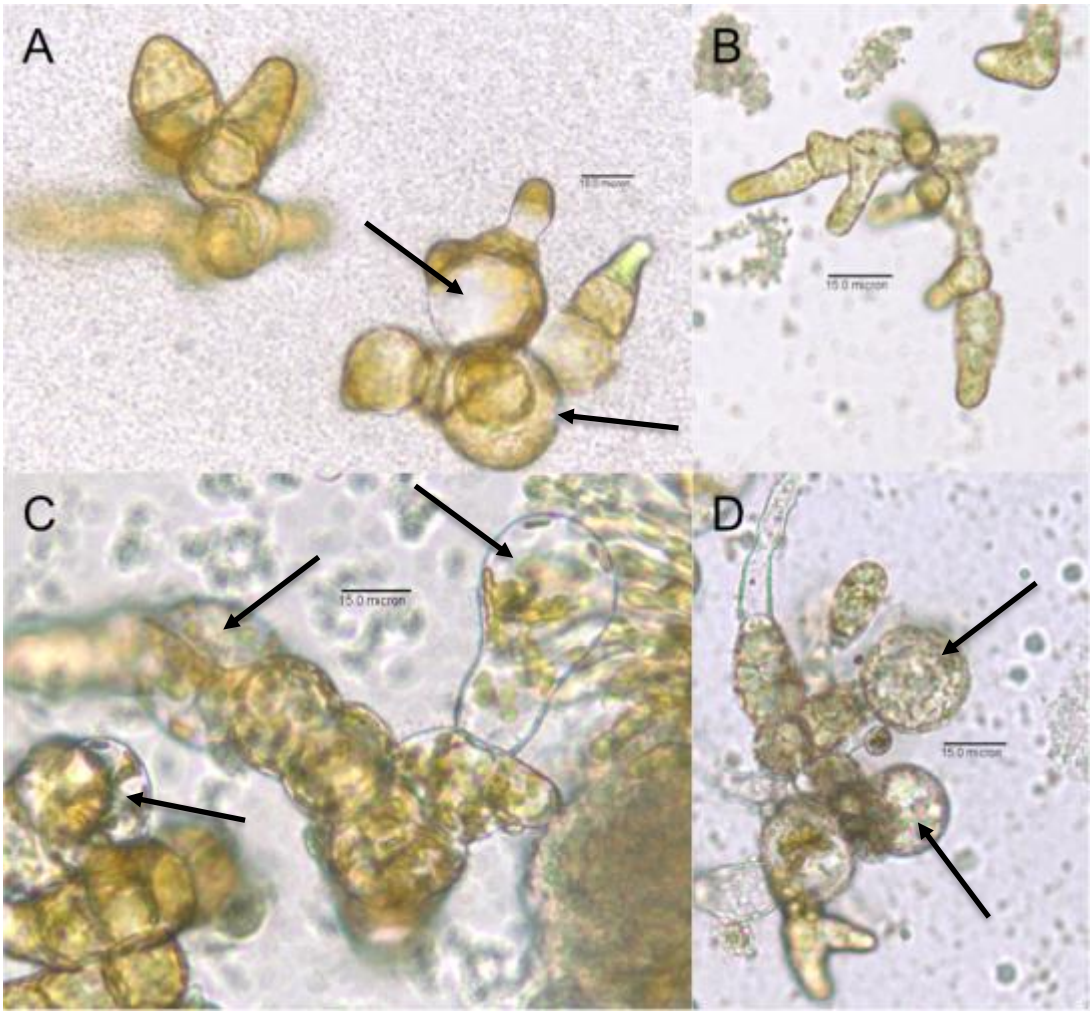

Figure 8 – Images of reproductive female gametophyte plasticity. Eggs seemed to sometimes appear intercalary (A), some female gametophytes were barren (B), others had unusually shaped eggs (C), and several were even seen to have multiple extruded eggs (D).

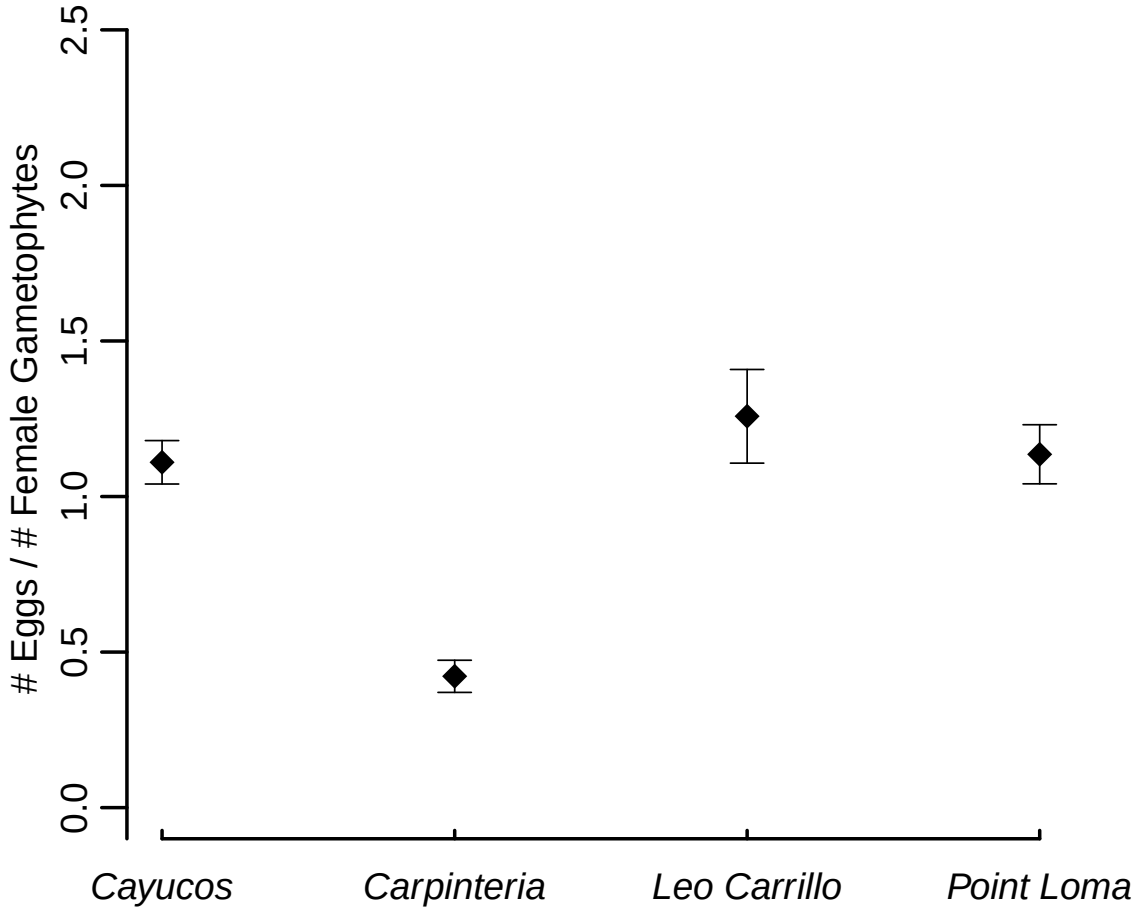

Figure 9 – Mean female gametophyte fecundity (± SE) of *Macrocystis pyrifera* using the number of eggs divided by the number of females in outcrossed cultures across 4 sites: Cayucos (n = 12), Carpinteria (n = 14), Leo Carrillo (n = 15), Point Loma (n = 15).

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