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## Ecological and Reproductive Consequences of Morphological Variation in *Egregia Menziesii* (Turner) Areshoug

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ECOLOGICAL AND REPRODUCTIVE CONSEQUENCES OF MORPHOLOGICAL  
VARIATION IN *EGREGIA MENZIESII* (TURNER) ARESHOUG

A Thesis Presented to the  
Faculty of  
California State University, Monterey Bay

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
In  
Marine Science

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by  
Heather K. Fulton-Bennett  
Summer 2016

**CALIFORNIA STATE UNIVERSITY MONTEREY BAY**

The Undersigned Faculty Committee Approves the

Thesis of Heather K. Fulton-Bennett

ECOLOGICAL AND REPRODUCTIVE CONSEQUENCES OF MORPHOLOGICAL  
VARIATION IN *EGREGIA MENZIESII* (TURNER) ARESHOUG

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## ABSTRACT

### ECOLOGICAL AND REPRODUCTIVE CONSEQUENCES OF MORPHOLOGICAL VARIATION IN *EGREGIA MENZIESII* (TURNER) ARESHOUG

By Heather K. Fulton-Bennett

The extensive morphological plasticity of seaweeds has been appreciated since the first species were described, however, the costs and benefits of this strategy are not yet well understood. Studies have reported that morphological variants differ in reproduction, growth, and physiology, in addition to the ways in which they impact surrounding communities. This study examined the consequences of morphological plasticity in the intertidal kelp, *Egregia menziesii*, which is common along rocky shores from Baja California to British Columbia. *Egregia* is also considered to be a foundation species due to the dense canopy it forms and the numerous species that depend on it for food and shelter. This monospecific genus was previously described as several independent species due to variability in blade and midrib (or rachis) morphology. In central California, rachi can be either papillated or smooth, with papillated rachi being dominant north of Point Conception, and smooth rachi dominant south of Point Conception. The Monterey Bay area is home to populations of both morphological forms and this study evaluated the effects of rachis morphology on reproduction, desiccation resistance, grazing resistance, and growth patterns of *Egregia menziesii*. These questions were assessed using a suite of field surveys, field experiments, and laboratory experiments. The results indicated that reproductive output measured over the course of a year was not significantly different between the two morphological forms, however, some seasonal differences in the timing of reproductive output was observed. Smooth rachi appeared to have higher spore release, but lower settlement success than papillated rachi at the same site, indicating potential life-history trade-offs and a complicated interaction between morphology and environmental conditions. An obligate limpet was observed to occur at similar densities between the two rachis morphologies, however the grazing scars it created were significantly larger on smooth rachi, indicating that rachis papillations may offer some level of grazer resistance. Rachis morphology also significantly affected desiccation rates in the intertidal. Rachi with denser papillations exhibited slower rates of water loss over time, perhaps indicating a resistance to drying. This resistance to desiccation could also benefit other intertidal organisms sheltering under *Egregia* canopies. Evaluations of the effects of biomass loss on growth demonstrated that the intercalary meristem is much more diffuse than previously thought, providing *Egregia* with a mechanism to recover from physical damage that occurs along the rachis. This ability to repair and regrow following physical disturbance is a potential advantage in the high stress rocky environment. Overall, my results indicated that differences in reproduction, grazer resistance, and desiccation resistance occur among two distinct morphotypes of *Egregia menziesii*. While these two morphotypes may not be separate species, their unique characteristics result in physiological impacts, as well as impacts on the intertidal community.

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<b>Table of Contents</b>	<b>Page</b>
List of Figures.....	vi
List of Tables.....	ix
Introduction.....	1
Materials and Methods.....	8
Results.....	12
Discussion.....	18
Conclusions.....	25
Literature Cited.....	26
Figures.....	30
Tables.....	48

<b>List of Figures</b>	<b>Page</b>
Figure 1. Map of research sites in the Monterey Bay (from north to south): Mitchell's Cove, It's Beach, Pleasure Point, China Rock, and Stillwater Cove. Stillwater Cove has populations of both papillated and smooth individuals, and was used for all comparative experiments.	30
Figure 2. Thallus morphology of <i>Egregia menziesii</i> adapted from Friedland & Denny 1995.	31
Figure 3. Papillation density by site, arranged from North to South, with Stillwater Cove separated by morphology since both smooth and papillated individuals were present. There was no significant difference between Mitchell's Cove and China Rock, however papillated individuals at Stillwater Cove (SWC-P) had significantly lower papillation densities than either Mitchell's Cove or China Rock. Its Beach, Pleasure Point, and Stillwater smooth (SWC-S) were all populations of smooth individuals, with no papillations observed. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are $\pm$ SE.	32
Figure 4. A comparison of blade area per 1 cm of rachi between blade types (sporophylls and vegetative blades) as well as rachis type (smooth or papillated). There is no significant difference in sporophyll area between morphs ( $n = 85$ , $F_{1,167} = 0.1747$ , $p = 0.6765$ ) or in vegetative area between morphs ( $n = 85$ , $F_{1,167} = 0.5242$ , $p = 0.4701$ ). Vegetative blades have approximately 2.5 times the surface area per cm of rachis of sporophylls. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are $\pm$ SE.	33
Figure 5. A comparison of the average blade area per centimeter of rachis between blade type (vegetative and sporophyll) and season. There is a significant effect of blade type on blade area, but no significant effect of season. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are $\pm$ SE. See Table 2 for full ANOVA results.	34
Figure 6. A comparison of blade area by blade type (sporophyll, vegetative) and site (Table 1). Sporophyll area is significantly different by site ( $F_{4,164} = 3.6809$ , $p = 0.0067$ ), with China Rock having significantly higher sporophyll area than all other sites except Pleasure Point ( $p = 0.1020$ ). Vegetative blade did not differ significantly by site ( $F_{4,164} = 1.6266$ , $p = 0.1699$ ), despite China Rock have significantly higher blade area than Its Beach ( $p = 0.0248$ ). Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are $\pm$ SE.	35
Figure 7. Effects of morphology and date on two metrics of reproductive output: a) zoospore concentration and b) total reproduction (settlement success). Samples were taken approximately monthly from September 2014 through January 2016 at Stillwater Cove, dependent on tides and weather. Error bars are $\pm$ SE, replicated by rachis for each sampling date. ( $n = 5$ )	36

- Figure 8. Effect of morphology on average reproductive output across the total time sampled at Stillwater Cove, CA: a) zoospore output concentration (15 months) (t-test:  $t_{1,30}=1.39917$ ,  $p = 0.1720$ ) and b) total reproduction (settlement success, 12 months) (t-test:  $t_{1,24}=-0.45478$ ,  $p = 0.6534$ ). Error bars are  $\pm$ SE. 37
- Figure 9. Spore output concentration around the Monterey Bay, over 6 quarters, Fall 2014 to Winter 2015 from Mitchell's Cove, Its Beach, Pleasure Point, China rock, and Stillwater Cove (papillated and smooth). Error bars are  $\pm$ SE. (n = 3) 38
- Figure 10. Spore settlement success around the Monterey Bay from Winter 2014 through Winter 2015 at Mitchell's Cove, Its Beach, Pleasure Point, China Rock, and Stillwater Cove (papillated and smooth). Values exceed 100% settlement due to the variability in spore concentration estimates. Error bars are  $\pm$ SE. (n = 3) 39
- Figure 11. Effects of limpets on *Egregia* by rachis morph: A) The average limpet density between smooth and papillated rachi at Stillwater Cove, CA, with no significant difference between morphs (ANOVA:  $F_{1,96} = 1.3817$ ,  $p = 0.2427$ ). B) Average scarred area of rachis by rachis morph, with smooth rachi having significantly larger scars than papillated rachi (ANOVA:  $F_{1,22} = 9.4334$ ,  $p = 0.0056$ ). C) The percent rachis removed by rachis morph, with no significant difference seen between morphs (ANOVA:  $F_{1,22} = 0.6872$ ,  $p = 0.4160$ ). Error bars are  $\pm$ SE. 40
- Figure 12. Limpet morphometrics compared to the scar area on the rachis, with smooth rachi indicated using the ( $\circ$ ) and papillated rachi using the ( $\bullet$ ). A) Limpet mass was positively related to the scar area, with the  $r^2$  of papillated rachi equalling 0.693, while that of the smooth rachi is 0.474. The overall  $r^2$  value of all limpets and their scars was 0.392. See Table 4a for ANCOVA results. B) The area of the limpet's footprint is also positively correlated with the area of the scar. The  $r^2$  value for papillated rachi individuals is 0.609, while that for smooth individuals is 0.270, while the  $r^2$  value for all rachi is 0.249. See Table 4b for ANCOVA results. (n = 24) 41
- Figure 13. The decrease in % wet weight of the rachi over 2 hours at Stillwater Cove between papillated and smooth rachi. Error bars are  $\pm$ SE. (n = 5) 42
- Figure 14. The decrease in % wet weight of the rachi over 2.5 hours outside at Moss Landing Marine Laboratories. Error bars are  $\pm$ SE. Results of repeated measures ANOVA see Table 5a. (n = 7) 43
- Figure 15. The decrease in % wet weight of rachi over 2.5 hours outside of Moss Landing Marine Laboratories, compared between papillated and smooth rachi from Stillwater Cove and papillated rachi from China Rock. Error bars are  $\pm$ SE. Results of repeated measures ANOVA see Table 5b. (n = 7) 44



Figure 16. The decrease in % wet weight of rachis segments in a 60°C oven over A) 160 hours and B) the first 2.5 hours of the same experiment. Error bars are  $\pm$ SE. Results of ANCOVA see Table 7. (n = 10) 45

Figure 17. Growth rate (cm/day) along the rachis, from Zone 1 adjacent to the terminal lamina to Zone 4, 40 cm from the terminal lamina. Removed rachi had the terminal lamina and transition zone (Zone 0) removed at the start of the experiment. Short rachi were those shorter than 50 cm, while long rachi were those longer than 50 cm. Error bars are  $\pm$  SE. 46

Figure 18. Growth rates (cm/day) by zone of rachis, from the transition zone near the tip (Zone 1) to 40 cm from the transition zone towards the base of the rachis (Zone 5). Rachi with the terminal lamina removed are noted by the solid line, while rachi without the terminal lamina removed are noted with the dashed line. Error bars shown are  $\pm$  SE. 47

## List of Tables

	<b>Page</b>
Table 1. Field sites for seasonal reproductive output monitoring, from south to north.	48
Table 2. Results of a three-way Analysis of Variance (3-way ANOVA) comparing rachis morphology, blade type, and season effects on blade area around the Monterey Bay.	49
Table 3. Results of a 3-way ANOVA comparing the effects of site, blade type, and season on blade area around the Monterey Bay.	50
Table 4. Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and day effects on reproductive output from reproductive surveys at Stillwater Cove: a) average zoospore concentration, and b) average spore settlement success.	51
Table 5. Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and season effects on reproductive output from surveys around the Monterey Bay: a) average zoospore concentration, and b) average spore settlement success.	52
Table 6. Results of Analysis of Covariance (ANCOVA) tests comparing a) the effects of limpet mass and morph (papillated, smooth) on the rachis scar area (mm <sup>2</sup> ) and b) limpet foot area (mm <sup>2</sup> ) and morph (papillated, smooth) on the rachis scar area (mm <sup>2</sup> ).	53
Table 7. Comparisons of % Wet Weight remaining between rachis morphologies at Stillwater Cove over 160 hours.	54
Table 8. Comparisons of % Wet Weight remaining between rachis morphologies at Stillwater Cove over 160 hours.	55

## **Introduction**

Phenotypic plasticity is defined as the ability of an organism to change aspects of its form, ecology, or physiology as a result of differential expression of a single genotype, in response to environmental variation, or interactions between the two (Price 2003, Miner et al. 2005). Intraspecific variation in morphology is thought to increase the survival and productivity of organisms, especially in highly dynamic environments, where a flexible body plan could serve to enhance competitive abilities and ultimately influence the size or spatial distribution of a population (Gerard 1987, Slatkin 1987, Sultan 2001). Phenotypic plasticity may increase the range of habitable environments that can be occupied by an organism and this may be especially important in species with limited dispersal distances and when gene flow is limited between environments (Slatkin 1987). Morphological variability also has important consequences for an organism's surroundings, especially in foundation species, which provide habitat and energy to a community that is disproportionate to their abundance (Hughes 2010, Gaylord et al 2011).

In macroalgae, intraspecific morphological variation is extremely common, often resulting from the patchy and physically variable locations they inhabit (Pielou 1978, Gaines & Lubchenco 1982). Geographic ranges may stretch thousands of miles, and distributions along continental margins are often extremely elongate, limiting interactions between populations at extremes of the range (Reed et al. 1988). Dispersal distances in macroalgae are also commonly restricted due to short viability of spores in the water column (Gaines & Lubchenco 1982, Reed et al. 1988, Coyer et al. 1997). Populations inhabiting geographic extremes or in isolated habitat patches are often characterized by intraspecific variations in morphology (Chapman 1974, Dudgeon & Johnson 1992, Fowler-Walker et al. 2006), temperature tolerance (DeWreede 1978, Gerard & DuBois 1988), wave exposure tolerance (Wing et al. 2007, Wernberg & Vanderkilt 2010), photoadaptive responses (Gerard 1987), and responses to nutrient availability (Gagne et al. 1982). As a result, a single species may be adapted to local environmental conditions and only exhibit limited survival in certain areas of its range due to physical or ecological constraints such as herbivory or temperature stress (DeWreede 1978). Wave exposure is one of the most common factors that can induce morphological variation in macroalgae, where blades of sheltered populations can be wide, thin, and undulate, while wave exposed populations are often characterized by narrow, thick, and flat blades (Roberson & Coyer 2004, Fowler-Walker et al. 2006).

Intraspecific variation in macroalgal morphology has long been documented, but the consequences of such variants are still being explored (Wattier & Maggs 2001). Many studies have focused on the physiological consequences of morphological variation, from temperature, nutrient, and desiccation stresses to photosynthetic efficiency (DeWreede 1978, Gagne et al. 1982, Gerard 1988, Dudgeon & Johnson 1992, Wing et al. 2007). For example, Stewart and Carpenter (2003) observed that increased surface area to volume ratios in two species of brown algae in the California Channel Islands resulted in increased rates of biomass-specific net photosynthesis. In addition, morphological variation corresponded with changes in wave exposure, while net photosynthesis was associated with water flow speeds. There is also evidence that morphological variation can influence algal responses to hydrodynamic stress experienced during development, resulting in higher whole plant mortality among individuals with fronds requiring higher breakage forces (Kraemer & Chapman 1991, Demes et al. 2013). Hurd et al. (1996) looked at the effect of blade morphology on the uptake of inorganic nitrogen in different wave environments and found that blade morphology did not enhance nitrogen uptake in an area of low water motion, however blade morphology became important in areas of high water motion. While blade morphology can be locally adapted to increase survival (Hurd et al. 1996, Stewart & Carpenter 2003, Roberson & Coyer 2004, Fowler-Walker et al. 2006), the environmental cause of this variation is often difficult to discern.

Ecological and physiological consequences of morphological variation have been extensively studied in the kelps (Ochrophyta: Laminariales) due to their global distribution and status as foundation species in many marine ecosystems (Wattier & Maggs 2001, Roberson & Coyer 2004). One of the most conspicuous examples of the ways in which variable blade morphology can impact associated communities occurs in the giant kelp, *Macrocystis pyrifera* (L.). Individuals from wave-sheltered sites possess wide, thin, undulating blades while exposed sites are characterized by narrow, thick, flat blades (Hurd et al. 1996). *Macrocystis* is a well-known foundation species in coastal temperate waters around the world, forming dense forests that provide habitat and structure, while altering physical properties such as light and water movement through the bed (Jackson & Winant 1983, Schiel & Foster 1986, Graham et al 2007). Differences in blade morphology change the uptake of nutrients and flow of water within a kelp bed, affecting other primary producers as well as filter feeders (Jackson 1977, Hurd et al. 1996). Holdfast morphology also varies within this species, with the rhizomatous '*integrifolia*' form

commonly inhabiting shallow subtidal areas in this kelp's range. The *integrifolia* form is characterized by a flat, strap-like holdfast that contains less interstitial space and correspondingly provides much less habitat than the conically mounded holdfasts of the '*angustifolia*' or '*pyrifer*' forms that occur in deeper water (Andrews 1945, Scagel 1947, Druehl & Kemp 1982, Thiel & Vasquez 2000). The '*integrifolia*' form is also able to grow vegetatively in addition to reproducing sexually, potentially leading to greater genetic connectivity and resource-sharing within a *Macrocystis* bed (Graham et al. 2007). The effects of morphological plasticity within a single species are clear in *Macrocystis*, but less well understood in other kelp species.

One of the less studied members of the Laminariales is the monospecific genus, *Egregia*. Extensive variability in morphology originally resulted in the description of three species (and two subspecies) from what is now considered, based on genetics, to be a single species, *Egregia menziesii* (Turner) Areschoug (Chapman 1961, Guiry 2014). *Egregia* is found from southeast Alaska to Baja California, Mexico and is common along rocky intertidal and shallow subtidal habitats in both protected and wave-swept areas (Abbott & Hollenberg 1976). Studies on *Egregia*'s role in the intertidal community are limited, but Hughes (2010) reported significant negative effects on the cover of other algal species and diversity with the presence of *Egregia* at a wave exposed site. The strong whiplash motion of the fronds resulted in lower richness and total cover of other algal species, with higher cover of sessile invertebrates and crustose and articulated coralline algae (Hughes 2010). However, this effect differed at a lower wave energy site where the presence of *Egregia* served as a stress ameliorator and sand trap, positively affecting algal diversity and cover. As a foundation species, *Egregia* also provides habitat and food to many intertidal fish and invertebrates, in addition to affecting the surrounding algal community (Hughes 2010). Other studies have found that large, foliose brown algae have negative effects on the benthic invertebrates and algae through scouring and substrate shading (Kennelly 1989, Santelices 1990, Bertness et al. 1999, Taylor & Schiel 2005), but Hughes (2010) indicated that the relationship between foundation species and their communities is complex and merits further study.

While Hughes (2010) examined the impact of *Egregia* on the associated benthic community, the study failed to take into account variation in morphology that could potentially affect the interaction between *Egregia* and its intertidal community. *Egregia*'s unique thallus morphology is characterized by a thick midrib, or rachis, from whose margins hundreds of small

blades sprout (Abbott & Hollenberg 1976). The morphology of both the blades and the rachis is highly variable throughout its range, with blades ranging from unbranched filaments to highly divided to wide strap-like blades (Abbott & Hollenberg 1976, Henkel et al. 2007). The rachis can be smooth or covered in dense papillations, with some individuals showing intermediate morphs (Henkel et al. 2007). In California, these morphs were previously described as two different species: individuals with smooth rachi were termed *Egregia laevigata*, while papillated individuals were described as *E. menziesii* (Chapman 1961). These two morphs overlap in their distribution from California's Channel Islands to southern Washington, with papillated individuals dominant north of Point Conception and smooth individuals ('*laevigata*' form) more dominant south of Point Conception (Fig. 1; Henkel et al. 2007). Smooth individuals in the area of overlap are generally found in more protected habitats, but may grow alongside papillated individuals at some sites (Fig. 1). While several studies have examined the morphological differences in detail (Blanchette et al. 2002, Henkel & Murray 2007, Henkel et al. 2007), more work is needed to understand potential ecological differences related to *Egregia*'s morphological plasticity.

Current studies have found no genetic basis for the three species and two subspecies of *Egregia* described previously (Abbott & Hollenberg 1976, Lane et al. 2006, Henkel et al. 2007). Henkel et al. (2007) reported that *Egregia* has high genetic variability, however, there were no geographic patterns to that variation, and thus no evidence for speciation within the genus. Numerous studies have documented morphological variability as a function of latitude and attributed that variation to environmental factors such as nutrients and wave exposure (Blanchette et al. 2002, Henkel & Murray 2007, Henkel et al. 2007). Blanchette et al. 2002 conducted reciprocal transplant experiments around Point Conception to examine differential survivorship in the face of different nutrient regimes and wave exposure. The authors reported that growth of both morphologies (i.e., smooth and papillated) was highest at sites north of Point Conception, but that increased rates of breakage of individuals with smooth rachi, when transplanted north of Point Conception served to decrease their survivorship. The smooth morph of *Egregia* had lower breaking strength and higher drag, but higher surface area, and thus was better adapted to the low nutrient and low water motion environment south of Point Conception (Blanchette et al. 2002). However, numerous *Egregia* populations with smooth rachi persist north of Point Conception, and appear to influence the underlying intertidal community, yet the

environmental and ecological factors responsible for promoting the persistence of both morphologies in central California are not yet known.

Morphological plasticity has the potential to result in differences in physiological and reproductive capacity within a species. With both smooth and papillated populations of *Egregia* being present within meters of each other, it is possible that differences in reproductive timing have contributed to the separate populations and their success. Reproduction in *Egregia* has only been closely examined in southern California populations (Black 1974, Henkel & Murray 2007), and reproductive output and settlement success may be different north of Point Conception, perhaps correlating with morphological variation throughout the species' range. Studies of *Egregia* in southern California have observed that sporophyll production is highest in the winter, when water temperatures were lower and more nutrient rich. However, reproductive studies have not focused on *Egregia* north of Point Conception where water temperatures are colder year-round and nutrients are rarely limiting (Henkel & Murray 2007), yet where physical disturbance from waves is much greater in the winter and spring.

Growth rates in *Egregia* have been found to vary significantly depending on their surroundings (subtidal versus intertidal) as well as across the nutrient and temperature break that occurs around Point Conception (Chapman 1961, Black 1974). While growth rates differ, it is not known how well *Egregia* can recover from biomass loss as a result of physical disturbance. There is thought to be both a basal meristem found in the holdfast as well as an apical meristem in the terminal lamina and transition zone (Chapman 1961, Black 1974). The removal of this area should result in the termination of rachis growth, while in some species it may promote increased branching or the initiation of new frond growth from the basal meristem (Chapman 1961, Black 1974). In the intertidal environment, recovery from biomass loss is critical to the persistence of individual plants (Dethier 1984, Demes et al 2013, Poore et al 2013), though studies have found that self-pruning in *Egregia*, while resulting in decreased frond density, allows for higher survivorship of the entire plant by decreasing drag (Demes et al 2013). In *Egregia*, reaction to the removal of the terminal lamina may differ between morphotypes, resulting in the greater success of papillated individuals north of Point Conception where wave forces are considerably stronger (Blanchette et al. 2002, Henkel et al. 2007).

As a foundation species, *Egregia* provides habitat and food for a variety of organisms in the intertidal, with the most closely linked species being the obligate limpet *Lottia insessa*. Black

(1974, 1976) examined the effect of the limpet's home scars on *Egregia rachi* in Santa Barbara and found it to cause up to 30% of plant detachment. Biomechanical strength is thought to be higher in papillated individuals (Henkel & Murray 2007), so herbivory may play a different role in communities dominated by papillated individuals, while areas of co-occurrence may indicate a difference in limpet populations between morphological forms. However, *L. insessa* is only found from Baja California to southern Oregon, so the impacts of grazers are likely to change with geographic location (Kuo & Sanford 2013). Limpet scars are known to cause up to 36% of frond breakages, contributing significantly to rachis mortality and sublethal plant damage in southern California (Proctor 1968, Black 1976). Limpet scars can be extensive along the length of a rachis, and it is possible that rachis papillations may change the grazing patterns of limpets, perhaps slowing grazing or decreasing the damage to the medulla (Proctor 1968, Black 1976). While biomechanical consequences of differing rachis morphology have been documented, it is still unknown if other ecological differences may be related to morphological traits.

One of the major stresses in the intertidal is desiccation and while *Egregia* is susceptible to drying out, it also serves as cover for smaller invertebrates and algae (Hughes 2010). An organism's ability to hold water is usually related to its surface area as well as physiological characteristics (Dromgoole 1980). Under high temperatures, water retention can also help cool the thallus through evaporative cooling, while increased overlapping branches or blades can slow desiccation rates (Schonbeck & Norton 1979, Dromgoole 1980, Bell 1995). *Egregia*'s varying rachis morphology significantly alters the surface area of the rachis and may change the species' ability to resist desiccation, though it may be offset by differences in physiology that parallel morphological variation.

Morphology is important in determining both the ecological role of an organism, as well as its interactions with the surrounding community. As a foundation species, *Egregia*'s basic ecology is not well understood, with few studies conducted north of Point Conception, especially on the microscopic stages of the alga. While some studies have examined the causes of and trends in this species' morphology, little is known as to how the morphology may affect *Egregia*'s ecology, and this study hopes to address some of those areas of interest (Blanchette et al. 2002, Henkel et al. 2007, Henkel & Murray 2007). The primary objectives of the study are to evaluate differences between the papillated and smooth rachi of *Egregia* in terms of physiology, competitiveness, and reproduction. As an intertidal organism, *Egregia* is susceptible to the



pressures of grazing and desiccation (Black 1974). It is possible that the presence of papillations may change the retention of water in the thallus or alter grazing pressure by *Lottia insessa*. Further, if there are physiological differences between these morphologies, which may account for the distributional differences that presently occur. In locations with populations of both morphologies, ecological differences could be particularly interesting as the plants are exposed to the same environmental conditions, yet persist in different physical forms.

This study will focus on the basis for morphological variability in the rachis of *Egregia menziesii* and whether these morphotypes differ in reproduction, grazing resistance, and water retention. Specifically, I addressed the following questions: 1) does reproductive output differ between rachis morphologies? 2) does resistance to the grazing by the obligate limpet *Lottia insessa* vary between rachis morphologies? 3) does water retention differ between individuals of different rachis morphologies? and 4) does the loss of the terminal lamina and transition zone affect rachis growth?

To address the first question, I hypothesized that reproductive output would be higher among individuals with papillated rachi, due to its likely better adaptation to conditions north of Point Conception. Second, I hypothesized that papillated rachi would suffer less damage due to limpet grazing as the papillations would make it more difficult for the limpet to attach to the rachis and feed. Third, I hypothesized that papillated rachi would retain more water when desiccated due to the increased rachis thickness with the papillations and the hydrodynamic properties of the papillations that may serve to retain water. Fourth, I hypothesized that the removal of the terminal lamina and transition zone would cause the cessation of all rachis elongation as the intercalary meristem is thought to be located in the transition zone. The ultimate goal is that this study will increase our knowledge of kelp reproduction, as well as the population dynamics and interactions affecting a foundation species on California's rocky coasts.

## Materials and Methods

### *Study species and study system*

*Egregia*'s morphology has been a source of curiosity and taxonomic confusion for decades. While *Egregia* has been concretely classed in the Order Laminariales, its closest relatives remain unresolved due to uncertainty in taxonomic affinity based on analyses of characteristics such as mid rib and blade morphology, compared to genetic markers. *Egregia* has a relatively small, dense holdfast, with very little interstitial space between haptera. From the holdfast a single cylindrical stalk transitions into the flattened rachis, with branches occurring from this base and farther up the fronds. *Egregia*'s blades are found along both narrow edges of the flattened rachis, and extend along the entirety of the frond, which can reach lengths of 15 meters (Fig. 2; Abbott & Hollenberg). Blade morphology varies greatly, from wide strap-like blades to divided filiform blades to very small oval blades (Abbott & Hollenberg 1976, Henkel & Murray 2007). Blade and rachis variation was the basis for early descriptions of multiple species in the genus. While *Egregia* does possess pneumatocysts, higher densities of pneumatocysts have been reported in intertidal populations compared to deeper subtidal populations (Chapman 1961). Sporophylls appear similar to vegetative blades, though these are often slightly smaller or are shrivelled in texture, and they are also found along the entirety of the rachis (Abbott & Hollenberg 1976). At the end of the rachis is the terminal lamina, a flattened single blade with occasional outgrowths, but no distinct blades, sporophylls, or pneumatocysts (Fig. 2). This terminal lamina is thought to protect the meristem, found at the transition between the rachis and the terminal lamina, from erosion and other damage (Black 1974, Henkel & Murray 2007). The removal of this terminal lamina and the transition zone is thought to halt growth of the frond and promote branching from the base of the individual (Black 1974, 1976, Henkel & Murray 2007). Due to the robust nature of the rachis, whiplash motion from fronds has been reported to have significant impacts on the intertidal community structure and diversity (Hughes 2010).

While *Egregia* commonly occurs along rocky shores from the mid intertidal down to 20 meters depth (Abbott & Hollenberg 1976), this study focused on intertidal populations in the Monterey Bay region. In addition, the Monterey Bay area is unique in that both smooth and papillated rachi populations are present. Generally, smooth and papillated populations occur at

different locations along the central California coast, as a function of wave exposure (papillated forms are more common at wave exposed sites). However, at Stillwater Cove, Carmel Bay, the two morphologies overlap in their distribution, with papillated and smooth individuals growing co-occurring and growing directly alongside each other. This congruence made Stillwater Cove the ideal location to compare reproductive, physiological, and ecological impacts of morphology without the complication of different environmental conditions. In addition to Stillwater Cove, four other sites around the Monterey Bay were selected, with three on the north end of the bay, and one on the south end in addition to Stillwater Cove (Fig. 1, Table 1). These sites encompassed three sites with papillated rachi (Mitchell's Cove, China Rock, and Stillwater Cove) and three sites with smooth rachi (Its Beach, Pleasure Point, and Stillwater Cove). While Stillwater Cove served as the focus for this research, these additional sites were important in broadening our understanding of seasonal reproduction and local geographic variability in rachi morphology.

### *Morphology and Reproduction*

To determine whether spore release concentrations and settlement densities differed significantly between *Egregia* morphs, 5 reproductive rachi of each morph were collected monthly from Stillwater Cove. Rachi were kept dry and in the dark at 25° C for two to three hours between collection and processing. From each rachis, 10 sporophylls were haphazardly chosen from along the length of the rachis. Each sporophyll then had a 3.1 mm diameter circle of sorus tissue removed and all tissue circles for each rachis (n = 10) were placed in 5 mL of Provasoli's enrichment solution (PES; Provasoli 1968). After 1 hour at 20° C, the concentration of spores in each solution was determined using 6 fields of view on a haemocytometer (Joska & Bolton 1987). Spore concentrations were tracked for each morphology at Stillwater Cove monthly for 14 months. To obtain settlement density, spore solutions were diluted to the lowest concentration obtained from that month's sampling, usually 500/mL. These standardized spore solutions were then added in 5mL volumes to tripart petri dishes, with one partition representing a single individual, with 10 parts total from the two morphologies. Settlement density was calculated by counting the number of spores visible after rinsing the petri dishes with PES after 36 hours in an incubator at 10° C under 12:12 light dark cycle.

To determine whether spore release and settlement between morphs would also be dependent on geography, 3 reproductive rachi from 3 separate individuals were also collected from 5 sites, including Stillwater Cove, seasonally (Table 1). Spore release and settlement were induced and calculated using the methods described above for monthly sampling at Stillwater Cove. Reproductive output was compared using a 3-way ANOVA, with the factors month, site and morph replicated by individual (n=3).

### *Morphology and Grazer Damage*

Limpet populations were surveyed on both papillated and smooth rachi at Stillwater Cove to assess whether grazing pressure differed between the two rachis morphologies. The number of limpets and the number of rachi for each individual was counted, and the number of limpets per rachi calculated so as to standardize density by size of individual. Limpets were surveyed on 39 papillated individuals and 59 smooth individuals. The density of limpets per rachi was compared using a one-way ANOVA.

To determine whether limpet scars were more damaging to papillated or smooth rachi, scarred rachi were collected from Stillwater Cove. Twelve scars from each morph were collected and taken back to Moss Landing Marine Labs for analysis. Rachis type, width, and cross-sectional depth were measured, as well as the mass, length, and width of the limpet on the rachis. Once the limpet was removed from the scar (non-destructively), the length, width, and depth of the scar were recorded using calipers accurate to 0.02 mm. The scars were then cross-sectioned across the middle of scar. Using a microscope, the depth of the medulla, the unscarred half of the rachis, and the scar were measured to the nearest micrometer. The ratio of the depth of the scar to the depth of the unscarred half of the rachis was used as a proxy for the amount of damage potentially done by the limpet's home scar, and will be referred to as percent rachis removed in following analyses. To compare the damage done by the limpet scars, the scarred area (length x width) and percent rachis removed were compared between rachis morphs using one-way ANOVAs. The relationship between limpet mass and scar depth, limpet mass and scar area, limpet area and scar depth, and limpet area and scar area were analyzed using linear regressions.

### *Morphology and Desiccation*

To evaluate whether desiccation rates differed between the two rachis morphologies of *Egria menziesii*, two methods were used. The first test measured desiccation under completely controlled conditions. Nine rachi of different papillated and smooth individuals were collected from Stillwater Cove and kept for 24 hours in a water table to ensure full hydration was achieved. A 5 cm section was then cut from each rachis and any excess water spun off in a salad spinner (10 manual turns) to obtain a conservative estimate of water loss during low tide exposure. These segments were then placed in aluminum weigh boats of known mass and the total wet mass of each segment measured to 0.01 grams. The weigh boats were then placed in a drying oven set to 60°C and the mass of each segment measured at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 16.5, and 160 hours from the time placed in the drying oven. The rate of desiccation was compared between rachis morphs using a repeated measures ANOVA (RM-ANOVA).

The second method to calculate desiccation rates between the two morphological forms was less controlled (in an experimental sense) but more accurately replicated conditions *Egria menziesii* experiences naturally in the field. Seven rachi from 14 individuals of papillated and smooth rachi were collected from Stillwater Cove and kept in a flowing seawater water table for 24 hours to ensure full tissue saturation. Each rachis was then removed from the water table and excess water removed using a salad spinner (10 manual turns). Rachi were then weighed whole to 0.01 g accuracy and placed on concrete in full sun. The day this experiment was conducted PAR ranged from 500-1110 micromoles/s/m<sup>2</sup> (mostly sunny) with very light winds (2-3 mph) and an air temperature of approximately 17°C. Rachi were then weighed whole at intervals over two hours. The percent wet weight of the rachis segments was compared between rachis morphs using a one-way ANOVA. The decrease in percent wet weight over time was analyzed using RM-ANOVA by both rachis morph and site.

### *Sublethal Biomass Loss*

To simulate the effects of physical disturbance on *Egria* and to determine whether the loss of the terminal lamina results in the cessation of rachis elongation, the growth of plants with the lamina removed was compared to unmanipulated control plants. Twenty adult plants at Stillwater Cove were tagged and numbered using bird bands and zip ties. One rachis with a

terminal lamina was randomly selected from each plant, and plastic clothing tags were inserted into the rachis every 10 cm from the base of the rachis to the transition zone to the terminal lamina. Ten plants were then selected to have all terminal lamina removed. Growth of the rachis was measured approximately every 2 weeks for over 8 weeks by measuring the distance between the clothing tags in the rachis. If no growth occurred, the clothing tags would still be the original ten cm apart, but if more (or less) than ten cm distance was seen, then growth (or erosion) could be inferred. The total growth over 2 weeks was compared between individuals with and without terminal lamina using a one-way ANOVA. This experiment was also carried out with 6 long (>50cm) and 6 short (<50 cm) individuals, and a 2-way ANOVA was used to compare the effect of rachis length and presence of the terminal lamina.

## **Results**

### *Morphology and Reproduction*

To examine spatial variability in *Egremia* morphology, surveys of morphological parameters were conducted at five sites around the Monterey Bay area (Table 1, Fig. 1). With rachis morphology being of key concern to this study, the density of papillations per square centimeter of rachis was calculated for each site, as well as both smooth and papillated rachi at Stillwater Cove. Smooth rachi at Its Beach, Pleasure Point, and Stillwater Cove showed the expected zero papillations per square centimeter (Fig. 3). Papillated individuals were present at Mitchell's Cove, China Rock, and Stillwater Cove, however the density of papillations differed between these sites (Fig. 3; one-way ANOVA:  $F_{2,83} = 109.4005$ ,  $p < 0.0001$ ). Among the sites with papillated rachi, Mitchell's Cove and China Rock were not significantly different from each other (one-way ANOVA:  $F_{1,30} = 0.8204$ ,  $p = 0.3723$ ), however, Stillwater Cove had a significantly lower papillation density than either China Rock or Mitchell's Cove (Tukey's HSD:  $p < 0.0001$ ).

To evaluate spatial and temporal variability in reproductive traits between populations, the surface area of sporophylls and vegetative blades was examined as a function of rachis morphology (Fig. 4), season (Fig. 5), and site (Fig.6). A three-way ANOVA was used to examine the effects of season, blade type, and rachis type on the average blade area (Table 2).

Significant effects of both rachis type and blade type were found, but season was not significant (Table 2). There was also a significant effect of the Rachis\*Blade interaction term (3-way ANOVA:  $F_{1,322} = 14.4895$ ,  $p = 0.0002$ ), but no significant effect of any of the other interaction terms (Table 2). A Student's t-test on rachis type found that papillated rachi have significantly lower blade area than smooth rachi ( $t_{1,322} = 1.96736$ ,  $p < 0.0001$ ). As expected, sporophyll area was also significantly lower than vegetative blade area (Student's t-test:  $t_{1,322} = 1.96736$ ,  $p < 0.0001$ ). The interaction between blade type and rachis type was examined using a Tukey HSD pair-wise comparison and found that sporophylls of both smooth and papillated rachi had significantly lower blade area than vegetative blades of either rachis morph, but were not significantly different from each other (Table 2; Fig. 4). Vegetative blades, while significantly larger than sporophylls, were smaller on papillated rachi than on smooth rachi (Table 2; Fig. 4). While site could not be compared with rachis type due to the lack of both rachis types at each site, a 3-way ANOVA was run to examine the effects of site, season, and blade type on blade area (Table 3). A significant effect was found of both Site and Blade Type (as seen above), as well as significant interaction terms of Site\*Blade Type and Site\*Season (Table 3). In comparing site and season, vegetative blades in the fall had the highest area, followed by vegetative blades in spring, summer, and winter (Fig. 5). Sporophylls were consistently smaller than vegetative blades during all seasons (Fig. 5). A Tukey HSD test found that Pleasure Point had significantly higher blade area than any other site, followed by Stillwater Cove, while China Rock and Mitchell's Beach were significantly lower than the other sites but not significantly different from each other (Fig. 6, Table 3). The interaction term of Site\*Blade Type again found Pleasure Point vegetative blades to have significantly higher area than any other site but the blade area of other sites and types varied greatly (Fig. 6, Table 3). Overall, blade area was highest on smooth rachi found at Pleasure Point, with the highest blade area being found in the fall.

No significant differences in reproductive blade area were observed between rachis morphologies around the bay, however that metric fails to take into account the actual reproductive output of those blades. Instead, I used spore release concentration as an independent measure of reproductive output between the two morphologies. A 2-way ANOVA revealed that both date and morphology were significant factors affecting spore release concentration, however their interaction was not significant (Fig. 7a, Table 2). Date and morph together explained 37.7% of the variation in spore release concentration (Table 2, Fig. 7a). Spore release

concentrations pooled across the year were found to be higher in smooth rachi individuals than papillated individuals, although the effects were not significant (Fig. 7a, one-way ANOVA:  $F_{1,137} = 3.4620$ ,  $p = 0.0649$ ). The highest concentrations of spores released were seen in smooth rachi individuals during the fall and spring, while papillated rachi had lower outputs, but were highest in the fall (Fig. 7a). Comparing settlement success between morphs at Stillwater Cove also found significant effects of date ( $p = 0.002$ ), but not of morphology ( $p = 0.313$ ) (Table 2b, Fig. 7b). Settlement success was much more variable seasonally than spore release concentrations, and there was no significant difference in settlement success between morphs pooled through the year ( $p = 0.347$ ; Figs 7b, 8).

Reproduction was also measured quarterly at four other sites around the Monterey Bay: Mitchell's Cove, Its Beach, Pleasure Pt, and China Rock (Table 1, Fig. 1). A 2-way ANOVA of site and season, found a significant effect of season (2-way ANOVA:  $F_{1,3} = 2.8956$ ,  $p = 0.0015$ ), but no significant effect of site on spore release ( $p = 0.2589$ ; Table 3a, Fig. 9). The interaction term of site by season was not significant ( $p = 0.5989$ ; Table 3a), indicating that sites with high or low spore release were consistent across the seasons. Spore release concentrations varied seasonally and between sites around the Monterey Bay, however spore release at China Rock was significantly higher than all other sites (Tukey HSD ; Fig. 9). Season was also a significant factor in spore release concentration (One-way ANOVA:  $F_{3,104} = 5.5385$ ,  $p = 0.0014$ ), with fall and spring having significantly higher concentrations than winter or summer (Table 3a, Fig. 9). A 2-way ANOVA of settlement success by type and season found a significant effect of season (2-way ANOVA:  $F_{1,82} = 3.1478$ ,  $p = 0.0003$ ), but not of type ( $p = 0.5987$ ) or the interaction of season and type ( $p = 0.9717$ ; Table 3b). Spore settlement success was significantly higher during fall and winter than spring and summer (Student's T-test:  $t_{3,86} = 1.98793$ ,  $p$  values  $< 0.01$ ; Fig. 10). Site was not a significant factor in spore settlement success (one-way ANOVA:  $F_{4,85} = 0.8928$ ,  $p = 0.4719$ ).

### *Morphology and Grazer Damage*

At Stillwater Cove, there was no significant difference between the number of limpets per rachis as a function of rachis morphology (one-way ANOVA:  $F_{1,96} = 1.3817$ ,  $p = 0.2427$ ; Fig. 11a). On rachi collected from Stillwater Cove, dimensions of the limpet, rachis, and limpet's scar



were measured using digital callipers and a digital scale. While the number of limpets was not significantly different among rachis morphs (one-way ANOVA:  $F_{1,96} = 1.3817$ ,  $p = 0.2427$ ; Fig. 11a), the scarred area was significantly different between morphologies (one-way ANOVA:  $F_{1,22} = 9.4334$ ,  $p = 0.0056$ ; Fig. 11b). Smooth rachi had significantly larger scars by scar area than papillated rachi (one-way ANOVA:  $F_{1,22} = 9.4334$ ,  $p = 0.0056$ ; Fig. 11b). Further, there was also no significant difference in the percentage of the rachis removed by rachis type (ANOVA:  $F_{1,22} = 0.6872$ ,  $p = 0.4160$ ; Fig. 11c). Overall, there was a significantly higher grazing rate on the smooth rachi morphology, driven mainly by the larger scar area on smooth individuals paired with a similar area of rachis removed.

There was no significant difference in limpet mass between morphologies (one-way ANOVA:  $F_{1,22} = 0.8674$ ,  $p = 0.3360$ ). A regression of limpet mass (g) by scar area ( $\text{mm}^2$ ) found a significant positive relationship, with limpet mass explaining 39% of the scarred area (linear regression;  $\text{limpet mass} = 0.0856624 + 0.000365 * \text{scar area}$ ,  $F_{1,22} = 14.1631$ ,  $p = 0.0011$ ,  $r^2 = 0.391646$ ; Fig. 12a). An ANCOVA was used to compare the limpet mass on papillated rachi to scarred area on smooth rachi (Table 4a). The non-significant interaction term indicated that the slopes of the linear regressions of each morph are equal and therefore the limpet mass relates to scarred area in the same manner between rachis morphs (ANCOVA:  $F_{1,20} = 3.3474$ ,  $p = 0.0823$ ). Rachis type was significant suggesting that the y-intercepts (limpet mass with no scar) differed between the morphs (ANCOVA:  $F_{1,20} = 10.8416$ ,  $p = 0.036$ ). Finally, limpet mass was significant, indicating that regardless of rachis morph, scar area changed with limpet mass (ANCOVA:  $F_{1,20} = 9.7680$ ,  $p = 0.0053$ ; Table 4a).

A regression of limpet area ( $\text{mm}^2$ ) by scar area ( $\text{mm}^2$ ) also found a significant positive relationship, with limpet area explaining 25% of the scarred area (linear regression:  $\text{limpet area} = 50.489 + 0.0680 * \text{scar area}$ ,  $F_{1,22} = 7.2864$ ,  $p = 0.0131$ ,  $r^2 = 0.248$ ; Fig. 12b). To look at the amount of tissue removed from the rachis by a limpet, the percentage rachi removed was calculated as the percent of the total rachis depth that was missing in the middle of a limpet scar. An ANCOVA was used to compare the limpet area on papillated rachi to the scarred area on smooth rachi (Table 4b, Fig. 12b). The non-significant interaction term indicated that the slopes of the linear regression of each morph are equal and therefore limpet foot area relates to the scarred area in the same way on both papillated and smooth rachi (ANCOVA:  $F_{1,20} = 1.1610$ ,  $p =$

0.2941). Rachis type was again significant, indicating that the y-intercepts (limpet area with no scar) differed between the morphs (ANCOVA:  $F_{1,20} = 8.9490$ ,  $p = 0.0072$ ). Limpet foot area was also significant, indicating that regardless of rachis morph, scar area changed with limpet foot area (ANCOVA:  $F_{1,20} = 4.4398$ ,  $p = 0.0479$ ; Table 4b).

### *Morphology and Desiccation*

To examine the effect of morphology on desiccation, experiments were carried out at Stillwater Cove, under controlled outside conditions at Moss Landing Marine Labs, and in a drying oven at Moss Landing Marine Labs. At Stillwater Cove, the percent decrease in wet weight was measured over two hours every thirty minutes (Fig. 13). A comparison of the different rachis morphs found the desiccation rate of the rachis morphs to be significantly different (one-way ANOVA:  $F_{1,45} = 118.561$ ,  $p = 0.0344$ ), with the smooth morphology losing water more rapidly than the papillated morphology. In addition, the final percent of the wet weight also differed by morph (one-way ANOVA:  $F_{1,16} = 6.8593$ ,  $p = 0.0186$ ; Fig. 13), with the smooth morphology weighing less at the end of the simulated drying experiment.

To better control environmental factors, desiccation was also measured in a protected area outside at Moss Landing Marine Labs. Again, the percent change in wet weight was measured over two hours (Fig. 14). A comparison of the rate of desiccation revealed a significant difference between rachis morphs (one-way ANOVA:  $F_{1,100} = 203.775$ ,  $p = 0.0051$ ; Fig. 14), with the smooth rachi morphology losing water more rapidly than the papillated morphology. At Moss Landing Marine Labs, the desiccation rates of papillated rachi at China Rock was also compared to that of rachi from Stillwater Cove, and found that the desiccation rate of China Rock's heavily papillated rachi was significantly slower than the desiccation rate of the relatively less papillated individuals from Stillwater Cove ( $F_{2,20} = 285.3130$ ,  $p < 0.0001$ ; Fig. 15). A repeated measure ANOVA was used to compare the percent wet weight of different rachis morphs and sites throughout the 2 hour experiment. There was a significant effect of rachis type (RM-ANOVA:  $F_{1,18} = 15.0171$ ,  $p = 0.0001$ ) and time (RM-ANOVA:  $F_{4,18} = 501.6078$ ,  $p < 0.0001$ ), as well as a significant interaction of rachis type\*time (RM-ANOVA:  $F_{2,18} = 15.0171$ ,  $p = 0.0001$ ). The significant interaction term indicates that the wet weight of the rachis is changing

over time, but that the change differs between the rachis types. This result is likely driven by the differences in the rachis morphology by site, with rachi from China Rock drying out more slowly than those from Stillwater Cove, and to a higher percent wet weight (Fig. 15).

Finally, desiccation was examined in a completely controlled environment: a drying oven set to 60°C for 160 hours. As expected, a linear regression found a significant negative relationship between the percent wet weight and time in the dryer (linear regression: percent wet weight remaining =  $56.68 - 0.2913 * \text{Time (hours)}$ ,  $F_{1,176} = 564.995$ ,  $p < 0.0001$ ,  $r^2 = 0.2359$ ; Fig. 16). A comparison of percent wet weight over time found a significant difference between rachis morphologies at every time point measured, though the difference decreased as the rachi approached their dry weight (Table 6). Comparing the regression lines for each morphology over time found no significant difference between either the slopes ( $F_{1,176} = 2.007$ ,  $p = 0.158$ ) or the line intercepts ( $F_{1,176} = 2.071$ ,  $p = 0.152$ ). A repeated measures ANOVA examined the change in wet weight over time between rachis morphs and found a significant effect of rachis type (RM-ANOVA:  $F_{1,16} = 17.0583$ ,  $p = 0.0008$ ), time (RM-ANOVA:  $F_{9,8} = 21674.045$ ,  $p < 0.0001$ ), but interaction term of rachis type\*time was not found to be significant (RM-ANOVA:  $F_{9,8} = 2.6853$ ,  $p = 0.0895$ ). The significant effect of rachis type and time indicates that there is a difference between the rachis types, as well as the samples over time, however the lack of a significant interaction term indicates that the rachis sections change in the same manner over the course of the experiment. This result is contrary to that found in the controlled outdoor experiment discussed above, likely due to this laboratory study examining the differences of rachi solely at Stillwater Cove.

### *Sublethal Biomass Loss*

Living in the intertidal, *Egregia* is subjected to strong wave forces and persistent grazers that require the ability to recover easily from sub-lethal rachis damage. To determine the location of the meristem, or growth region, the terminal lamina and transition zone of rachi were removed from rachi of different lengths. Rachi were grouped as long, greater than 50 cm in length, or short, less than 50 cm in length, with half of each group having their terminal lamina and transition zone removed. Growth per day was compared between treatments across the top 40 cm

of the rachis (considered four ‘zones’; Fig. 17). Growth in all treatments decreased from the tip of the rachis (Zone 1) to 40 cm away (Zone 4; Fig. 17, 18). Despite the removal of the terminal lamina and transition zone, rachi with the ‘meristem’ removed did not have significantly different growth rates than those with the terminal lamina present (one-way ANOVA:  $F_{1,96} = 0.4911$ ,  $p = 0.4852$ ; Fig. 17, 18). Short and long rachi also did not have significantly different growth rates (one-way ANOVA:  $F_{1,96} = 2.8970$ ,  $p = 0.0920$ ; Fig. 17). As this experiment showed no effect of the length of rachis, a further experiment was carried out to clarify the location of the growth region along the rachis of *Egregia*. With further replication, there was found to be no difference in growth between rachi with their terminal lamina and transition zone removed and those that were left untouched (one-way ANOVA:  $F_{1,148} = 0.0181$ ,  $p = 0.8933$ ; Fig. 18, Table 8). There was no significant difference at any of the zones, from Zone 0 at the tip of the rachis to 40 cm from the tip (Zone 4) (Fig. 28, Table 8). Despite the removal of what was thought to be the meristem, growth rates did not change and elongation occurred well below the transition zone.

## Discussion

Morphological plasticity is pervasive in seaweeds and differences in morphological form within a species have been shown to influence growth, reproduction, and ecology (Chapman 1961, North 1971, Hurd 2000, Charrier et al. 2012). *Egregia menziesii* is one of the most morphologically and genetically variable kelps, so much so that it was previously described as several species and subspecies (Lane et al. 2006, Guiry 2016). Blade and rachis morphology in *Egregia* varies greatly along the California coast, with the Monterey Bay being unique as an area of significant overlap of the two morphologies (Blanchette et al. 2002, Henkel et al. 2007). Thus I used the Monterey Bay region to examine potential differences in reproduction, growth, and ecology between these two distinct rachis morphologies of *Egregia*.

While smooth and papillated rachi were clearly significantly different by design, papillation density was observed to vary by site, with Stillwater Cove having lower papillation density than either China Rock or Mitchell’s Cove. This may be due to the lower wave exposure of the Stillwater Cove population, which is shielded from some winter swells by the Monterey Peninsula (Fig. 1). Previous studies on the California coast have found papillations to correlate

with more wave exposed areas, however within site variation has not been well quantified (Henkel et al. 2007).

This study found no significant difference in the area of vegetative or reproductive blades between the rachis morphologies, however, there were seasonal and geographical differences in this measurement. Sporophyll blade area peaked in the spring, whereas vegetative blade area peaked in the summer, indicating that sporophylls may develop or mature earlier than vegetative blades. This finding parallels Henkel & Murray's (2007) reported similarly that sporophyll production in southern California was greatest during periods of colder water and shorter day length, though the Monterey Bay population did not show the summer decline described in that study. While reproductive area was highest in the spring, it does not necessarily mean that reproductive output is highest at this time, as the sporophylls may not all be ripe at that time. Sporophyll area was also highest at China Rock, despite the tiny size of the sporophylls, and at Pleasure Point, with much larger sporophylls. This suggests that small sporophyll size can be paired with high spore density, resulting in greater reproductive potential than sporophyll size would suggest on its own. Despite having a similar blade and rachis morphology to Pleasure Point, sporophyll area was lowest at Its Beach. These results essentially uncouple any suggestions that the reproductive blade area differs due to the presence or absence of papillations, but instead is more dependent on blade density, which is likely determined by different wave exposures, temperature regimes, and nutrient availability at each site. Using plant density and size estimates collected from my sites the year prior to these experiments, it was possible to calculate the density of spores produced per square meter. These metrics yielded the highest estimate of spore production at China Rock, followed by Pleasure Point, Its Beach, Stillwater Cove, and Mitchell's Cove. Spore density at the site level was primarily driven by the average rachis length and the sporophyll area per cm of rachis. China Rock and Pleasure Point differ in a number of characteristics, from wave exposure, to substrate type, to *Egregia* morphology, so it is likely multiple factors that are contributing to the high reproductive output at these sites. Pleasure Point is inside the Monterey Bay protected from the worst of the winter swells, while China Rock faces directly northwest into the face of the winter weather. Temperature regimes are also drastically different, with Pleasure Point being bathed by part of a warm eddy in the upwelling shadow in the northern part of Monterey Bay, while upwelling is much stronger along the exposed edge of the Monterey Peninsula where China Rock is located.

These sites also have different rachis and blade morphologies, with Pleasure Point having smooth individuals with wide, spatulate blades, while the population at China Rock is made up of densely papillated individuals with narrow, dense blades. It may be that these two sites provide the best combination of conditions for each of these morphologies to be reproductively successful.

While sporophyll area varied geographically and seasonally, this does not necessarily correspond with reproductive output. Spore concentration from a standardized area of sorus and the success of the settlement of these spores are often used as a measure of reproduction in kelps (Neushul 1963, Reed 1990, Reed et al. 1996). Similar to reproductive blade area, spore release concentrations and settlement success varied across the Monterey Bay, however, spore release was again highest at China Rock, especially during the spring and fall. Settlement success of these spores showed a much less clear pattern, but with the high sporophyll area and high spore output, China Rock appears to be one of the most reproductively successful sites of those I sampled. This site is exposed to heavy surf, so despite the evident reproductive success, the density of plants is similar to other sites, indicating high levels of mortality between spore settlement and adulthood. High levels of spore release also correlated to the seasons of highest sporophyll area, indicating that spring and fall are the times of highest reproductive output, likely due to the increase in available nutrients from seasonal upwelling, as well as the decrease in temperatures and day length from the summer months. This is similar to patterns found by Henkel & Murray (2007) as well as Black 1974. This high output is followed by a huge bloom in juvenile plants in the late winter to early summer, especially at more protected sites (Black 1974, Henkel & Murray 2007). One interesting point of note is also that *Egregia* in the Monterey Bay area is reproductive year-round (this study, Muth pers. comm.), without as dramatic a peak in reproduction as reported in southern California populations, including spore output and settlement success (Henkel & Murray 2007). This continual reproduction is likely due to not being limited by nutrients as is common south of Point Conception (Black 1976, Henkel & Murray 2007).

To better compare rachis morphologies without the environmental and geographic variables, Stillwater Cove offered an excellent setting, due to having populations of both papillated and smooth rachi present alongside one another. With spore output and settlement

success quantified monthly, smooth rachi had slightly higher spore release concentrations and lower settlement success than papillated rachi, however, these differences were not significant across the year. As higher spore release concentrations did not appear to correspond to settlement success, it may be that environmental conditions were less favourable for spores from smooth individuals during this study or that the quantity of spores was offset by the quality.

Environmental conditions may be more favourable at different life stages of the morphs as well, for example low temperatures may favour spore release and settlement, but egg production may require warmer temperatures to occur (Luning 1980). It is also possible that individual variation overwhelmed any potential differences between the rachis morphologies. Increased replication may also have clarified these results, but would have decimated reproductive populations monthly, and at some sites it was difficult to find even three reproductive rachi due to shore contours, sand movement, and low plant density at some sites, especially Stillwater Cove. High wave exposure and steep intertidal benches made collecting especially difficult at Mitchell's Cove during the winter months.

While this study found clear trade-offs in spore production and settlement success, it also found clear differences in the seasonality and geographic patterns of reproduction add to our understanding of this kelp's population dynamics north of Point Conception. With the Monterey Bay's mix of rachis morphologies, these populations do not appear to be separated by reproductive barriers such as timing or success, but more likely by environmental conditions present when reproducing, though both morphs are successful throughout the area. A common garden experiment to determine whether offspring of the rachis morphologies would maintain the same rachis type was attempted, but was unsuccessful, thus further research on *Egregia* rachis morphology would greatly benefit from the completion of a similar experiment. These findings are in contrast to a recent study on reproduction in *Macrocystis pyrifera* and *M. integrifolia* forms at Stillwater Cove, where significant differences in the reproductive output of each form were found, though timing was not significant (Jeffries 2015). *Macrocystis* has recently been synonymised into a single species based on morphological plasticity, so its reproductive differences are crucial in understanding the role these different morphs play along our coasts.

While reproduction differs significantly between rachis morphologies, these physical properties may also have important impacts on *Egregia*'s interactions with other intertidal species. The obligate limpet grazer *Lottia insessa* is found grazing on *Egregia* rachi from Baja California through central Oregon, causing numerous scars that can result in frond breakage or even whole plant mortality under persistent limpet infestations (Black 1974, 1976, Kuo & Sanford 2013). While limpets were not found in significantly different densities on rachi with or without papillations, their ability to graze and create home scars may be affected by the presence of the papillations. The area of the rachis that was damaged by limpet scars was significantly higher on rachi without papillations, perhaps indicating a protective effect of the papillations for the individual. This could also be one factor in *L. insessa* not being found consistently north of central Oregon, despite *Egregia* being found as far north as Sitka, Alaska (Kuo & Sanford 2013), because only papillated morphologies occur in the northern part of the range (Abbott & Hollenberg 1976). This pattern of increased scarring on smooth rachi did not correspond to larger limpets being found on these rachi, so it seems that papillated rachi were simply less damaged by limpets of the same size than smooth rachi. Despite the significantly greater scarred area, the percent of rachis removed was not significantly different between the morphs, indicating that though the amount of rachis removed was similar, smooth rachi had much larger, shallower scars, while papillated rachi had smaller but deeper scars. While large scars damage more of the rachis surface, the smaller deeper scars on papillated rachi may damage the medulla, inhibiting the transport of nutrients through the rachis. Black (1976) found *L. insessa* to become more common on *Egregia* rachi through the spring and summer, paralleling the period of fastest growth. The infestation of these grazers can have significant effects on the reproduction of the population as the rachi mature (or break before maturation). Sublethal damage from isopod grazing was found to have similar lasting, population-level effects on reproduction in *Macrocystis pyrifera* (Graham 2002). Grazing was also found to have lasting impacts on *Ecklonia radiata* biomass in relation to morphological differences (Steinberg 1995, Wernberg et al 2003). The effect of these different types of scars is still unclear, but the clear difference in scarring by morphology could be an important factor in breakage and biomass loss in *Egregia* populations from Baja California to central Oregon.

Limpet grazers are only one of numerous hazards to the persistence of *Egregia* along central California coasts. Biomass loss has been well studied in kelps, due to their habitat-



forming characteristics, as well as their various methods of recovery (Graham 2002, Thompson 2010, Geange 2014). Regrowth and regeneration in kelps occurs from the meristem, but the location of this region varies with kelp morphology and taxonomy (Graham 2002). In *Egregia*, the meristem was thought to be intercalary between the tip of the rachis and the terminal lamina (Black 1974, Abbott & Hollenberg 1976). The removal of this region, by wave activity, grazer damage, or other means, was thought to cause the rachis to cease elongation (Black 1974). Growth of the plant would continue with new fronds growing from the basal meristem near the holdfast, branching of fronds, as well as the growth of fronds where the meristem was still intact (Black 1974, 1976). This study found the meristem to be much more diffuse than the narrow band between the rachis and the terminal lamina. Elongation of the rachis was found to persist even after 40 cm of the end of the rachis was removed (Fig. 20). This diffuse meristem is important in allowing *Egregia* to persist through much higher biomass loss in response to disturbance than was previously thought. This adaptation may be important in the intertidal and shallow subtidal that *Egregia* inhabits, due to the intense water motion and abrasion, and resulting tissue damage that can occur following contact with rough rocky substrates (Dethier 1984, Steneck & Dethier 1994, Poore et al. 2013). The diffuse meristem does bring into question the importance of the terminal lamina, as its erosion does not necessarily signal the end of the rachis elongation as had been thought (Black 1974). This terminal lamina has been found to have significantly higher photosynthetic capacity, however, so it may still serve to take advantage of good growing conditions early in the growing season before it is eroded away (Chapman 1961).

With *Egregia* being able to withstand substantial biomass loss due to damage from limpets or waves, it also faces stresses that cause less obvious damage, such as drying out during low tides. As an intertidal foundation species, *Egregia*'s presence has a significant effect on the community around it due to its whiplash effect, sand retention, and shading (Hughes 2010). As a subcanopy or canopy forming species, *Egregia* forms dense mats directly on the substrate at low tides, shading the substrate and underlying species from sun exposure (Black 1974, Friedland & Denny 1995), but also preventing water loss and photosynthesis (Dayton 1975, Hughes 2010). While this shading and abrasion may inhibit the growth of some species (Hughes 2010), it may also protect many from harsh sun exposure and desiccation common during low tides (Hughes 2010). The papillations on *Egregia* rachi increase the surface area of the rachis, but also shade the rachis interior better. The presence of papillations may have significant impacts on the

amount of water retained by *Egregia* during low tides, to the benefit of both *Egregia* and many surrounding species. This study found that rates of desiccation differed between smooth and papillated rachi, with smooth rachi losing a higher percentage of their total wet weight and reaching a lower percentage of the total wet weight than papillated rachi. This indicates that papillated rachi may retain water better during desiccation events than smooth rachi. However, the salad spinner method used gives only a conservative estimate of desiccation as water loss would occur more gradually in the intertidal as the tide recedes. Tidal ranges generally increase northward throughout *Egregia*'s geographic range, leaving the intertidal exposed for longer periods of time. Conversely, *Egregia* is found at deeper depths in the southerly portions of its range, reaching depths of 45 feet in San Diego and the California Channel Islands (Chapman 1961, Abbott & Hollenberg 1976). This change in depth range is likely due to increased light and nutrient availability, as well as decreased temperature stress in southern waters. Studies in another intertidal algae, *Mastocarpus papillatus*, found thallus temperature to be highly dependent on the thickness and roughness of the thallus, possibly due to the enhanced evaporative cooling provided by the increased roughness or the increased amount of fluid trapped between papillae (Bell 1992, 1995). With papillated individuals being found more commonly at wave exposed sites, the water retention could also play a factor in allowing *Egregia* to survive higher in the intertidal as it does in the more northern parts of its range. It is possible that the smooth morphology has simply doesn't develop papillations under certain environmental conditions that include low water motion and less desiccation stress. If these conditions occurred at a point in early development before morphology was fixed, it could help explain the mixed populations at Stillwater Cove, where wave exposure is not extreme, and dense eel grass beds retain water over the soft substrate. There are likely other effects of *Egregia*'s rachis morphology on its physiology, but this study's findings already indicate the importance of morphology in determining an organism's population dynamics, physiology, and community interactions.

While there were no clear differences in reproduction between morphs, there were clear ecological impacts of *Egregia*'s rachis morphology on its resistance to grazing and desiccation. *Egregia*'s diffuse intercalary meristem and persistent rachis elongation allow it to better withstand the stresses of its intertidal habitat. Morphology in kelp is an incredibly plastic trait, but its adaptations can tell us far more about an organism than simply its shape and species, and

is an important area for further research, as we seek to better understand changes in our oceans and how kelps and other algae will respond to these changes.

## Conclusions

Morphological plasticity is one of the hallmarks of marine algae, especially in foundation species like many kelps. While well studied in terrestrial systems, the effects of morphological variability are not well understood in marine environments (Price et al. 2003, Miner et al. 2005). This study adds to our knowledge of how phenotypic plasticity affects organisms and their surrounding communities, in an intertidal foundation species, *Egregia menziesii* (Hughes 2010, Charrier et al. 2012). This study also clarifies the morphological variation in this species in an area of high variation, as well as comparing the effects at a site with both morphs present under the same environmental conditions.

This study found that *Egregia* with smooth rachi produce more spores, but have lower settlement success than papillated individuals. This difference in reproduction may be indicative of advantageous environmental conditions occurring at different reproductive stages, or a difference in the reproductive strategy of these different morphs. Papillated individuals also had smaller scars from the obligate limpet grazer than smooth individuals, indicating an advantage against these grazers over individuals with smooth rachi. This study also found that heavily papillated rachi lost less of their wet weight during desiccation than smooth individuals, a clear advantage in persisting in the mid intertidal. The meristem of *Egregia* was also found to be more diffuse than previously thought, a further advantage against biomass loss from storm damage or self-entanglement (Demes et al 2013).

As *Egregia* forms important habitat and plays a large role in structuring intertidal communities, it is important to understand the differential roles these rachis morphs may play along our shores. Further studies are needed to understand the basis for this variation, and whether it is a heritable trait, as well as better understanding of the morph's roles in the intertidal community.

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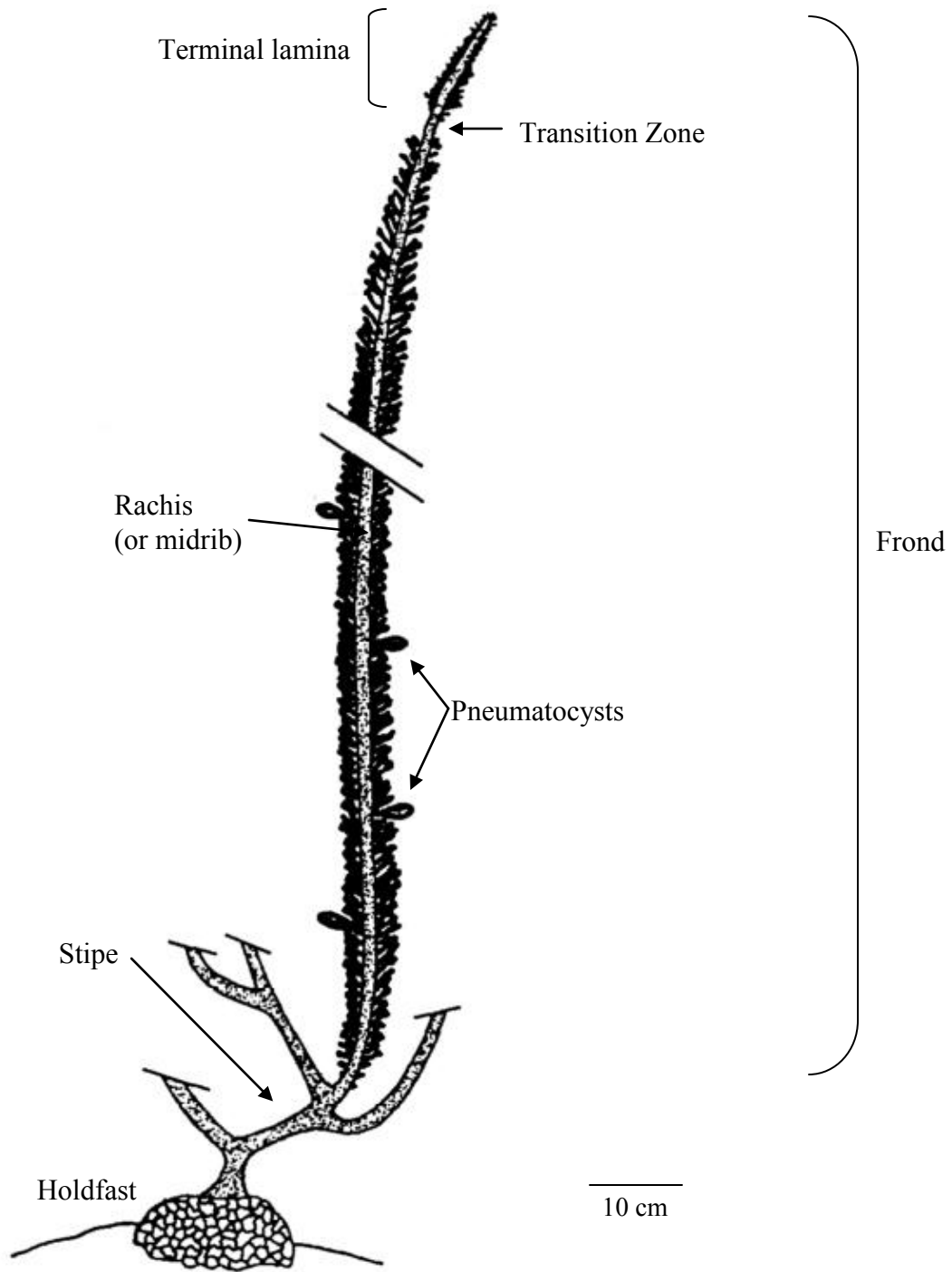
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## Figures

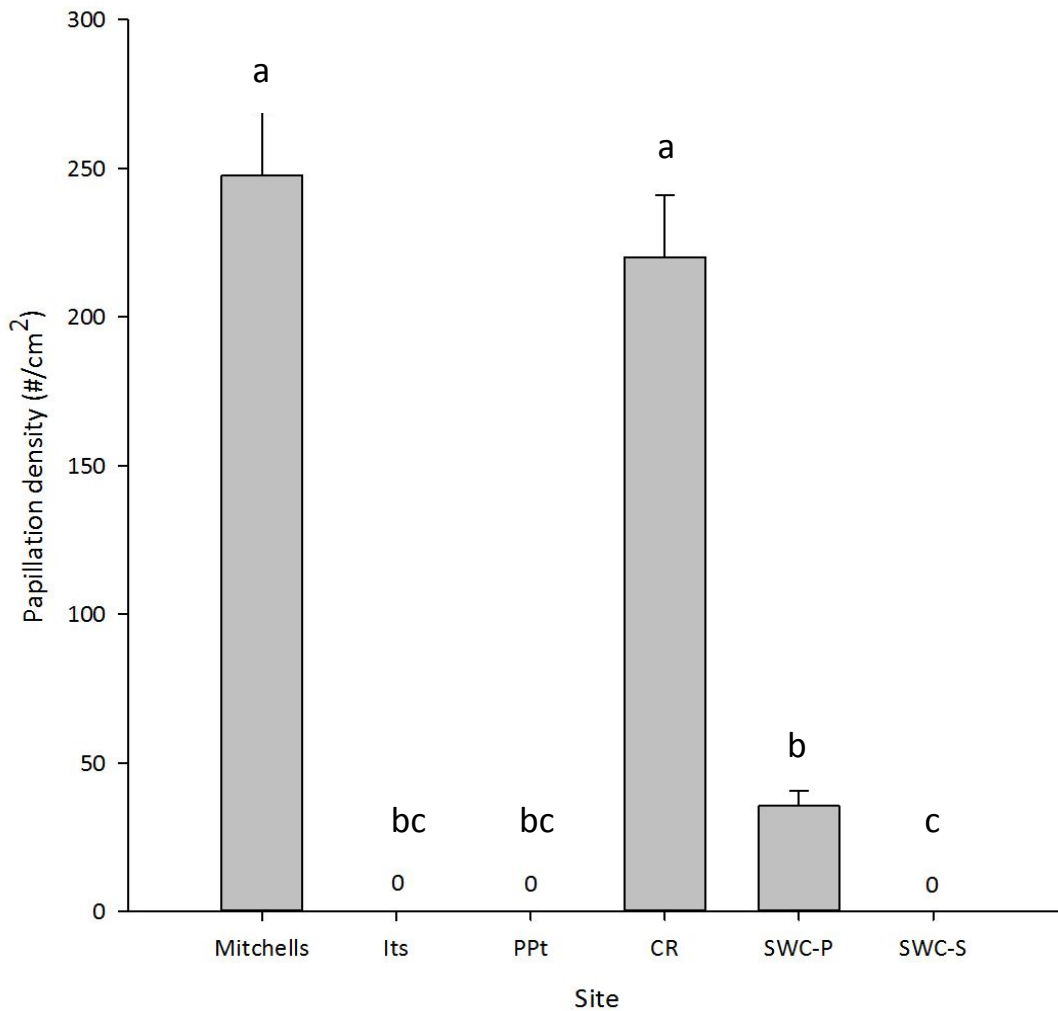


**Figure 1.** Map of research sites in the Monterey Bay (from north to south): Mitchell's Cove, It's Beach, Pleasure Point, China Rock, and Stillwater Cove. Stillwater Cove has populations of both papillated and smooth individuals, and was used for all comparative experiments.

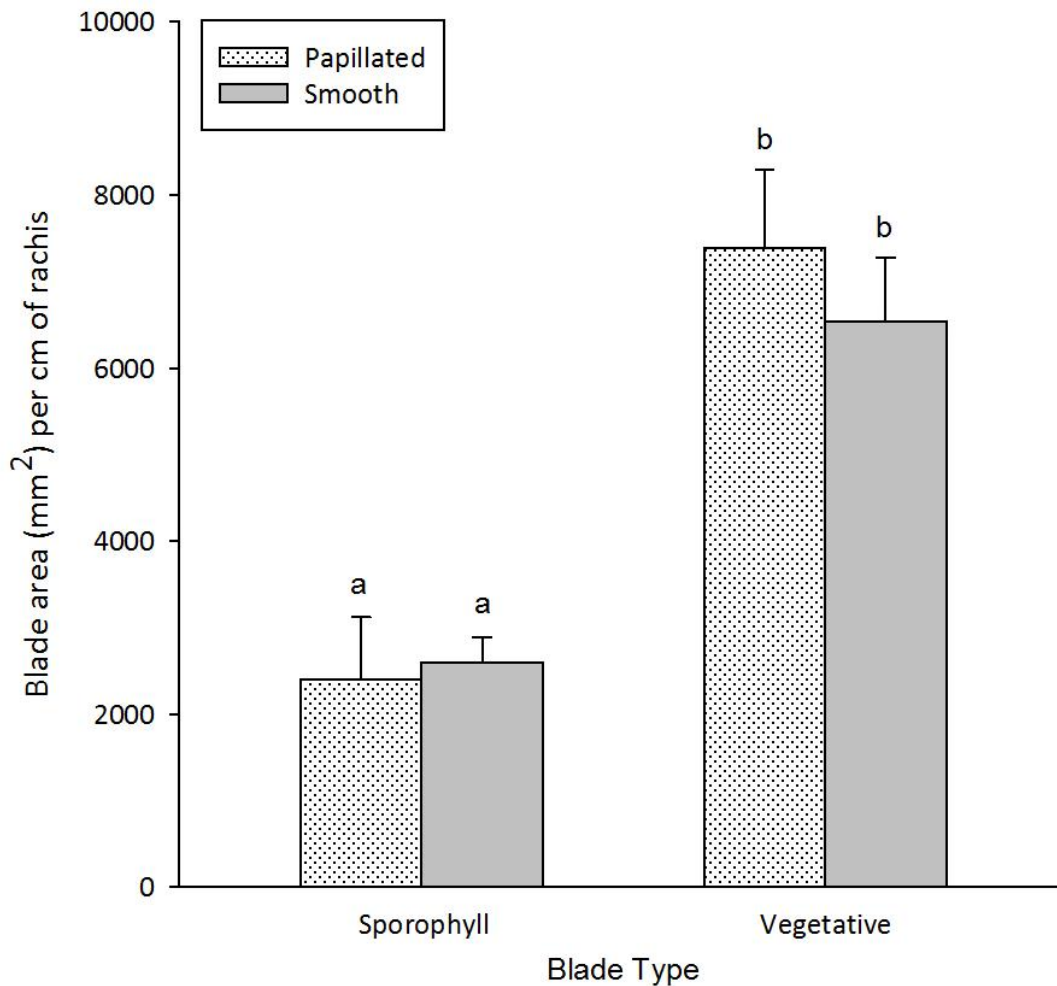




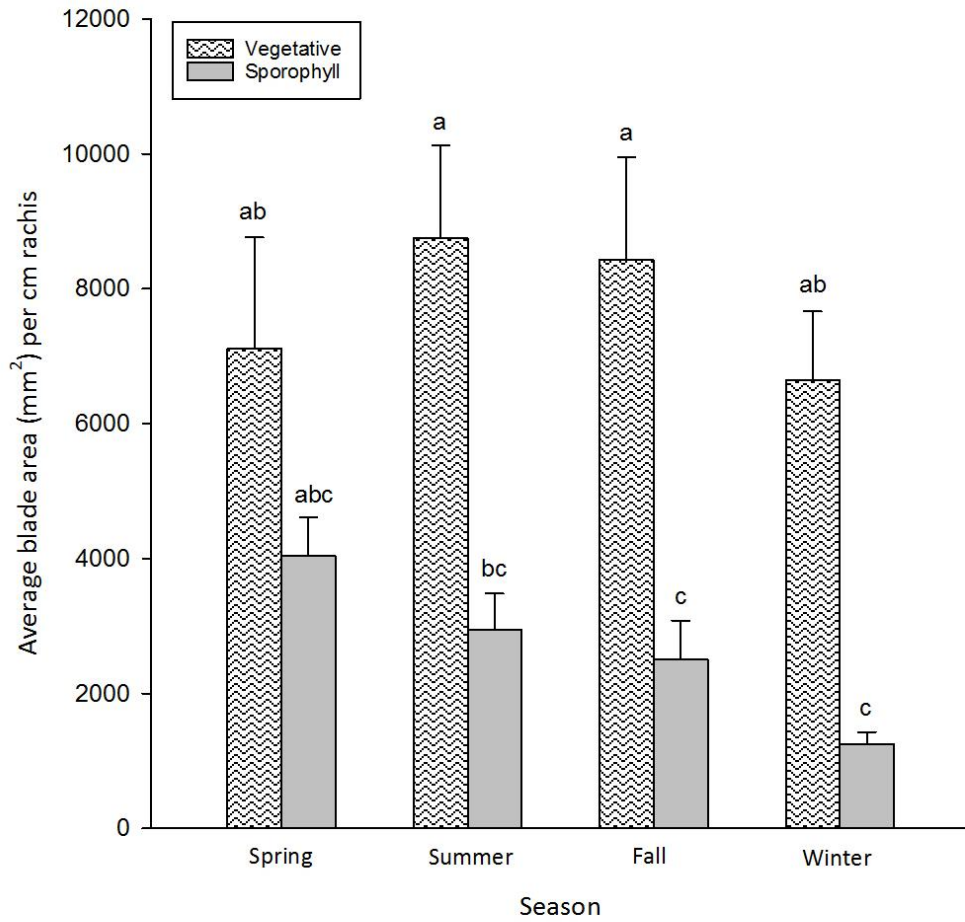
**Figure 2.** Thallus morphology of *Egregia menziesii* adapted from Friedland & Denny 1995.



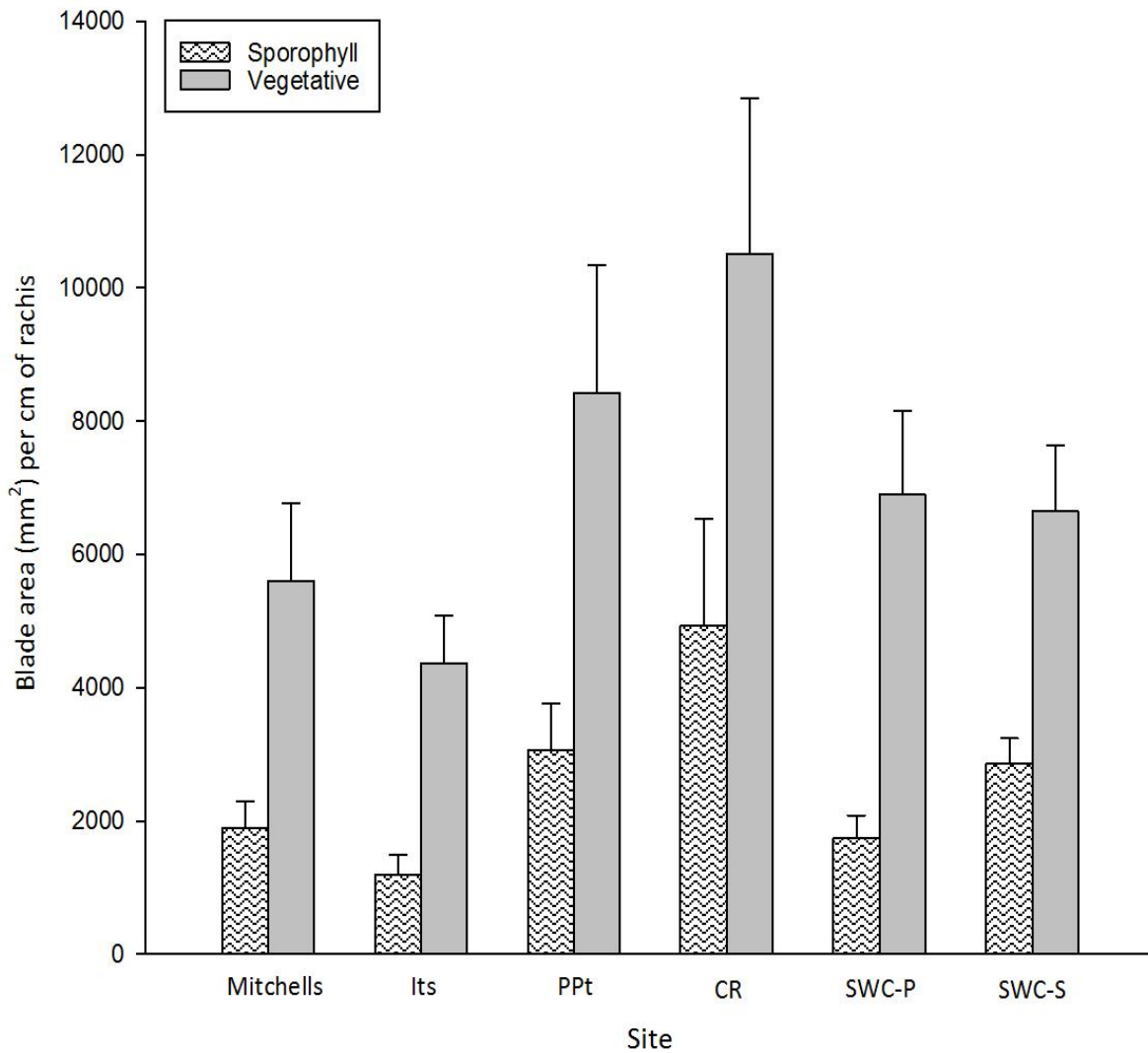
**Figure 3.** Papillation density by site, arranged from North to South, with Stillwater Cove separated by morphology since both smooth and papillated individuals were present. There was no significant difference between Mitchell’s Cove and China Rock, however papillated individuals at Stillwater Cove (SWC-P) had significantly lower papillation densities than either Mitchell’s Cove or China Rock. Its Beach, Pleasure Point, and Stillwater smooth (SWC-S) were all populations of smooth individuals, with no papillations observed. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are  $\pm$  SE.



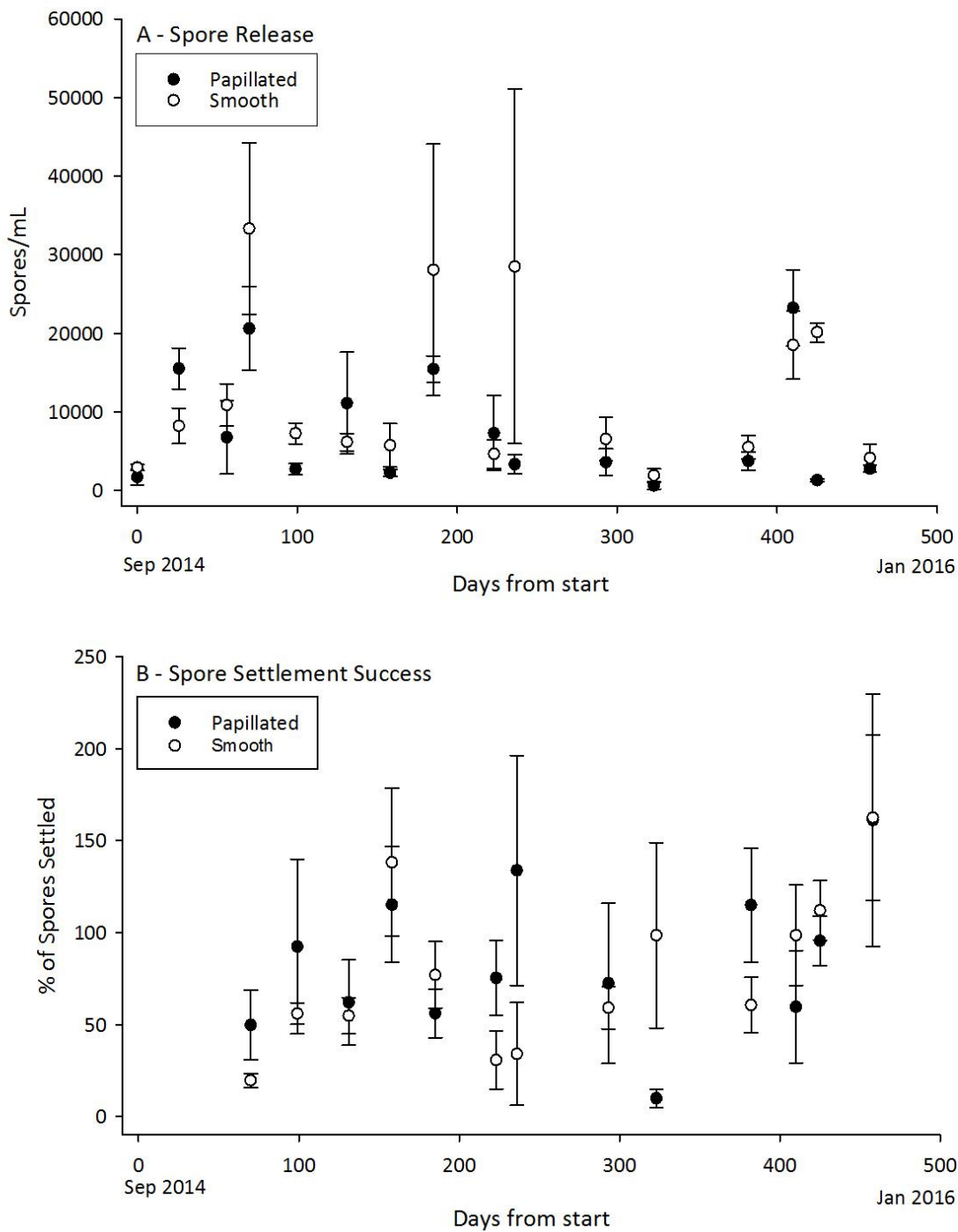
**Figure 4.** A comparison of blade area per 1 cm of rachi between blade types (sporophylls and vegetative blades) as well as rachis type (smooth or papillated). There is no significant difference in sporophyll area between morphs ( $n = 85$ ,  $F_{1,167} = 0.1747$ ,  $p = 0.6765$ ) or in vegetative area between morphs ( $n = 85$ ,  $F_{1,167} = 0.5242$ ,  $p = 0.4701$ ). Vegetative blades have approximately 2.5 times the surface area per cm of rachis of sporophylls. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are  $\pm$  SE.



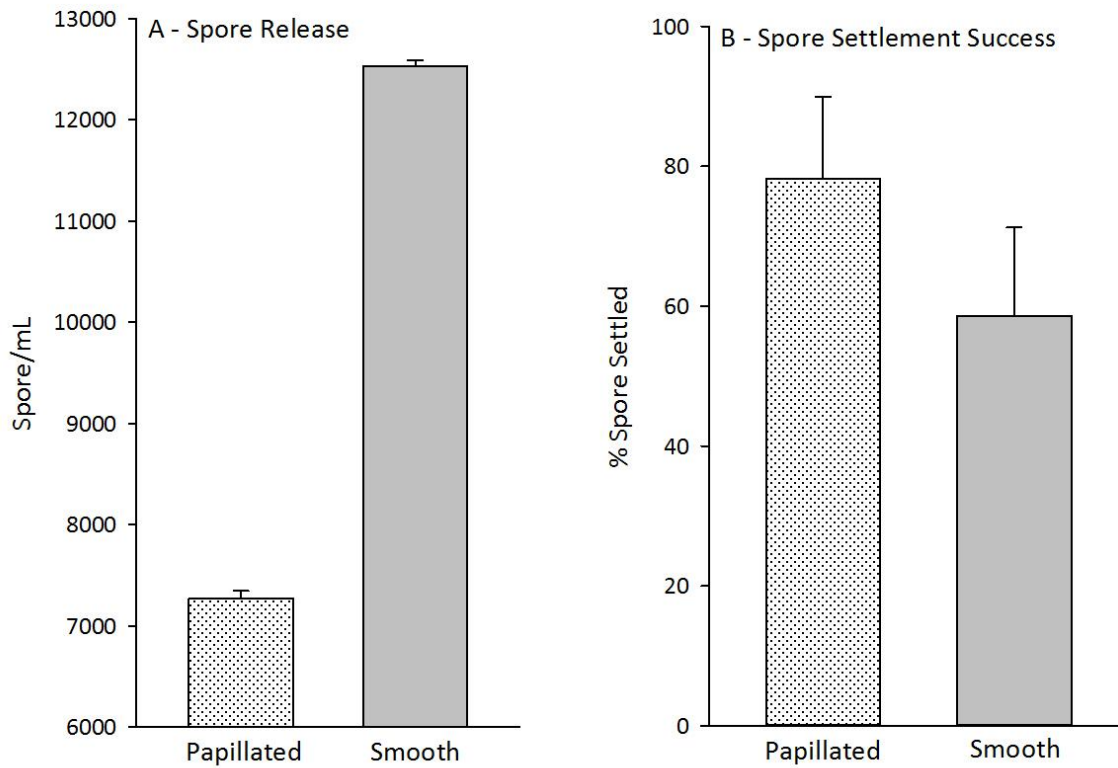
**Figure 5.** A comparison of the average blade area per centimeter of rachis between blade type (vegetative and sporophyll) and season. There is a significant effect of blade type on blade area, but no significant effect of season. Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are  $\pm$  SE. See Table 5 for full ANOVA results.



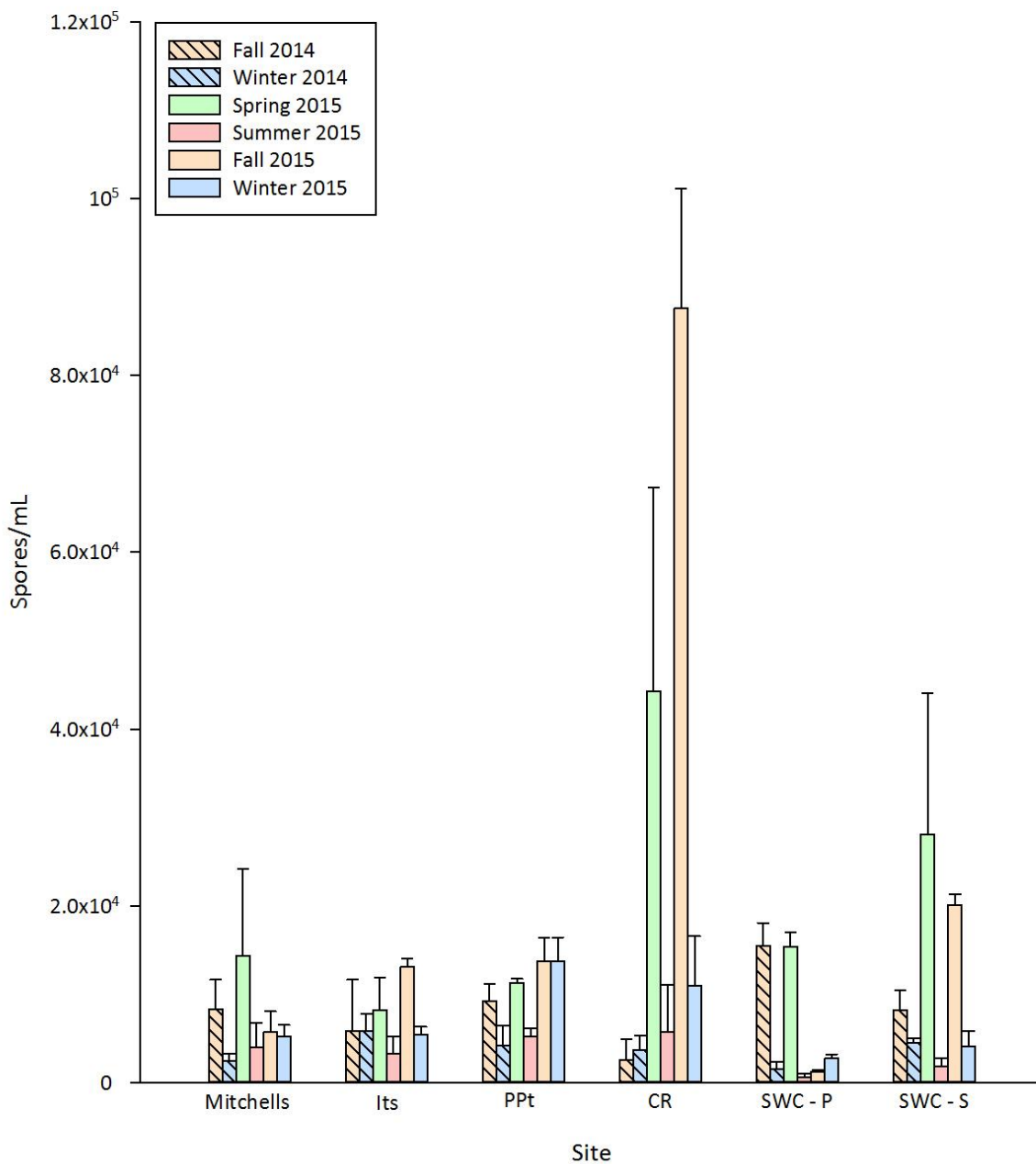
**Figure 6.** A comparison of blade area by blade type (sporophyll, vegetative) and site (Table 1). Sporophyll area is significantly different by site ( $F_{4,164} = 3.6809$ ,  $p = 0.0067$ ), with China Rock having significantly higher sporophyll area than all other sites except Pleasure Point ( $p = 0.1020$ ). Vegetative blade did not differ significantly by site ( $F_{4,164} = 1.6266$ ,  $p = 0.1699$ ), despite China Rock have significantly higher blade area than Its Beach ( $p = 0.0248$ ). Letters above bars represent significant differences ( $p < 0.05$ , Tukey HSD). Error bars are  $\pm$ SE.



**Figure 7.** Effects of morphology and date on two metrics of reproductive output: a) zoospore concentration and b) total reproduction (settlement success). Samples were taken approximately monthly from September 2014 through January 2016 at Stillwater Cove, dependent on tides and weather. Error bars are  $\pm$ SE, replicated by rachis for each sampling date. (n = 5)

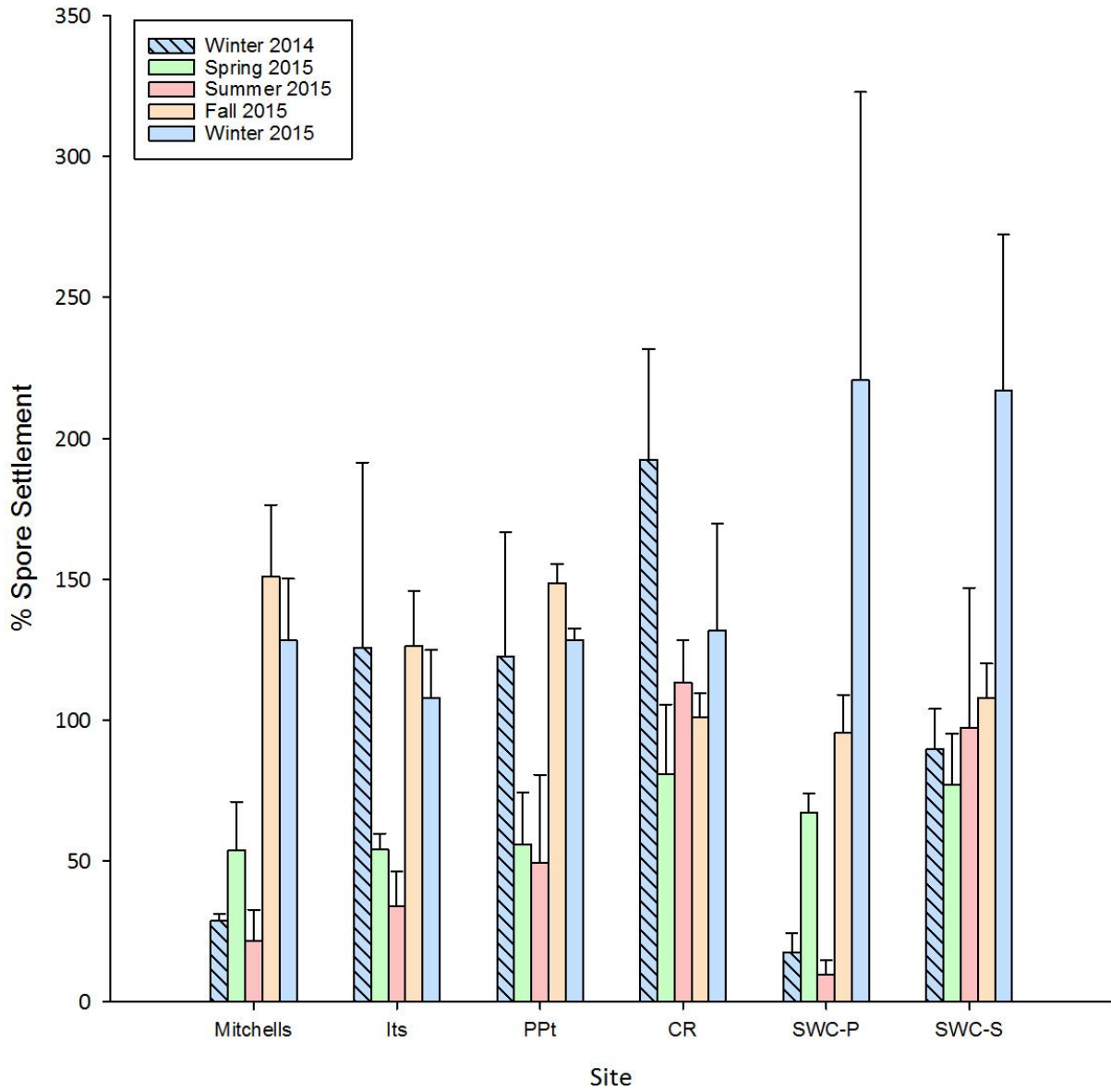


**Figure 8.** Effect of morphology on average reproductive output across the total time sampled at Stillwater Cove, CA: a) zoospore output concentration (15 months) (t-test:  $t_{1,30}=1.39917$ ,  $p = 0.1720$ ) and b) total reproduction (settlement success, 12 months) (t-test:  $t_{1,24}= -0.45478$ ,  $p = 0.6534$ ). Error bars are  $\pm$ SE.

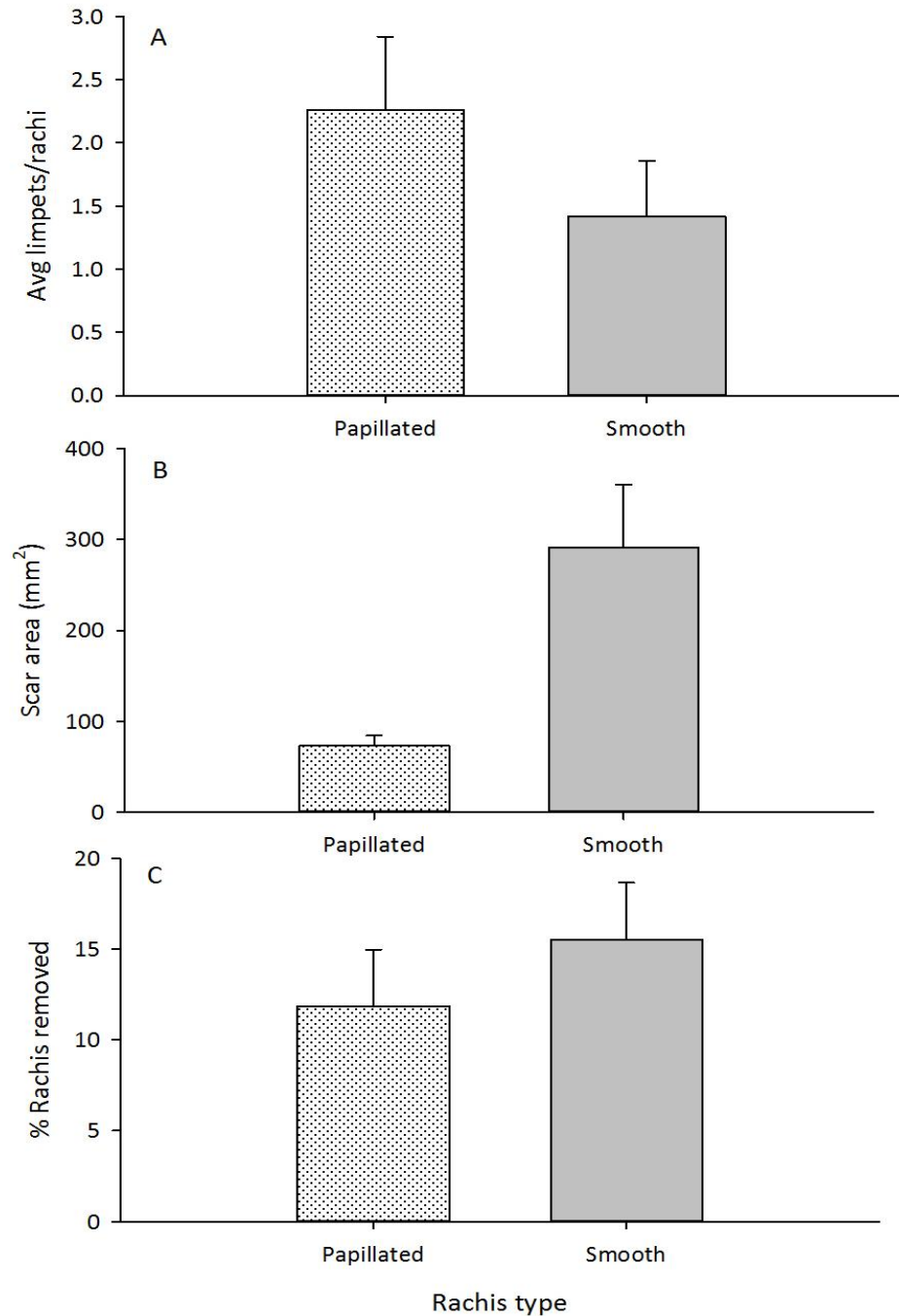


**Figure 9.** Spore output concentration around the Monterey Bay, over 6 quarters, Fall 2014 to Winter 2015 from Mitchell’s Cove, Its Beach, Pleasure Point, China rock, and Stillwater Cove (papillated and smooth). Error bars are  $\pm$ SE. (n = 3)

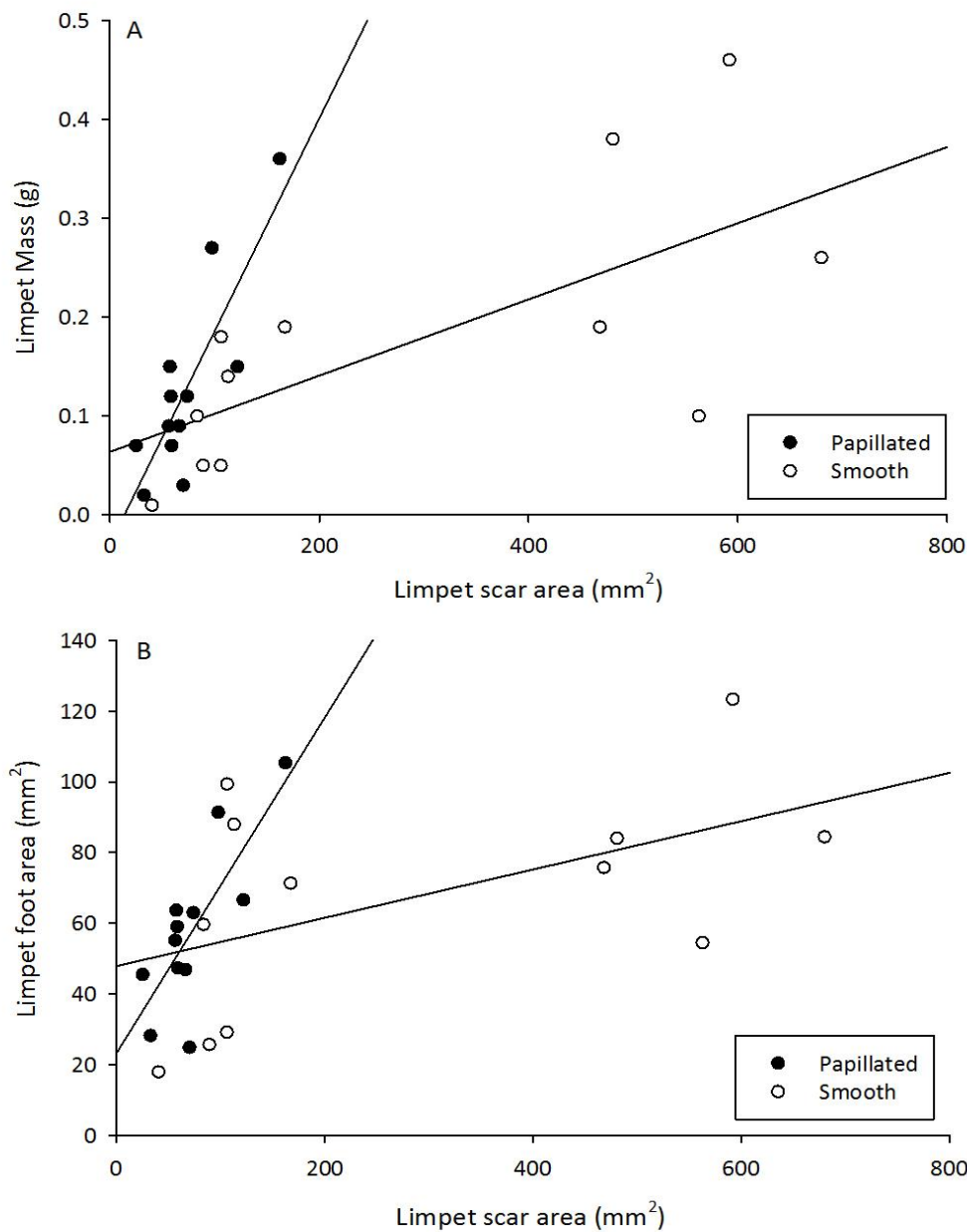




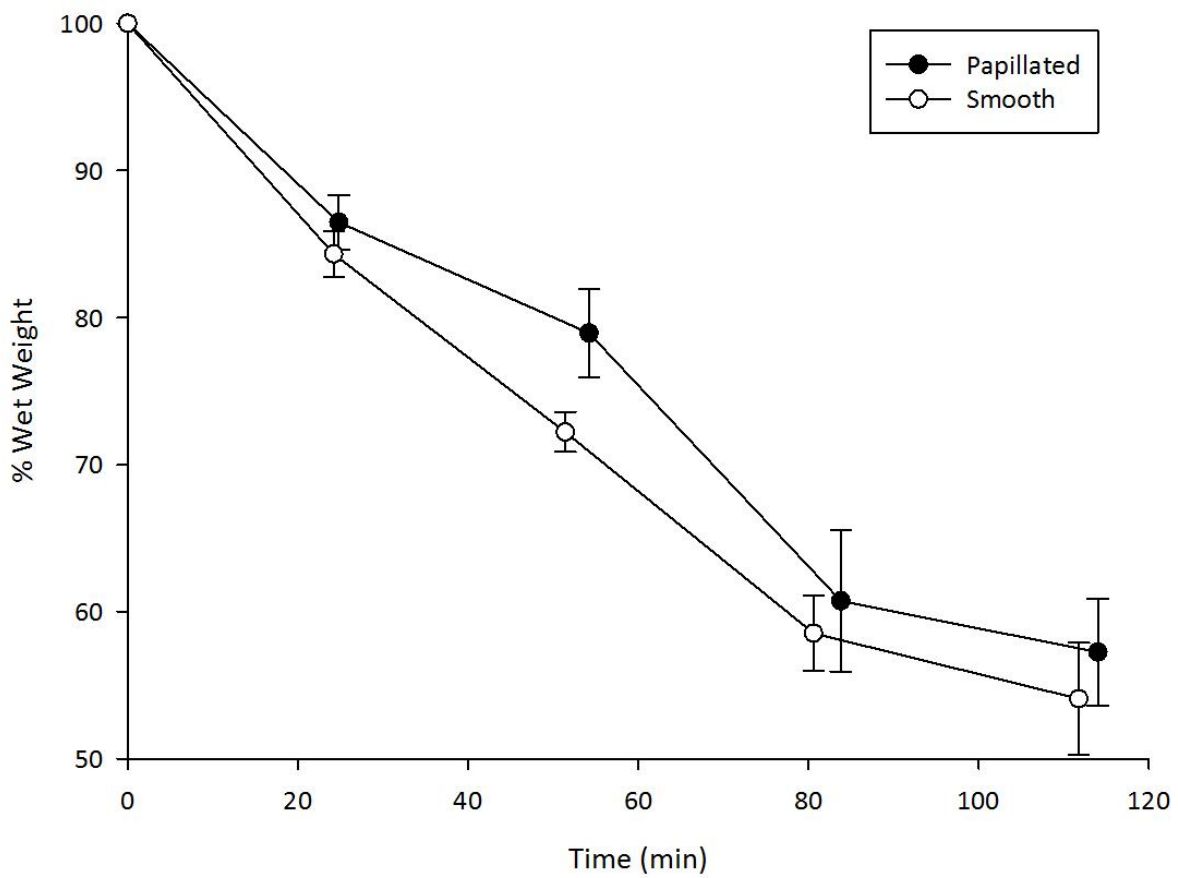
**Figure 10.** Spore settlement success around the Monterey Bay from Winter 2014 through Winter 2015 at Mitchell’s Cove, Its Beach, Pleasure Point, China Rock, and Stillwater Cove (papillated and smooth). Values exceed 100% settlement due to the variability in spore concentration estimates. Error bars are  $\pm$ SE. (n = 3)



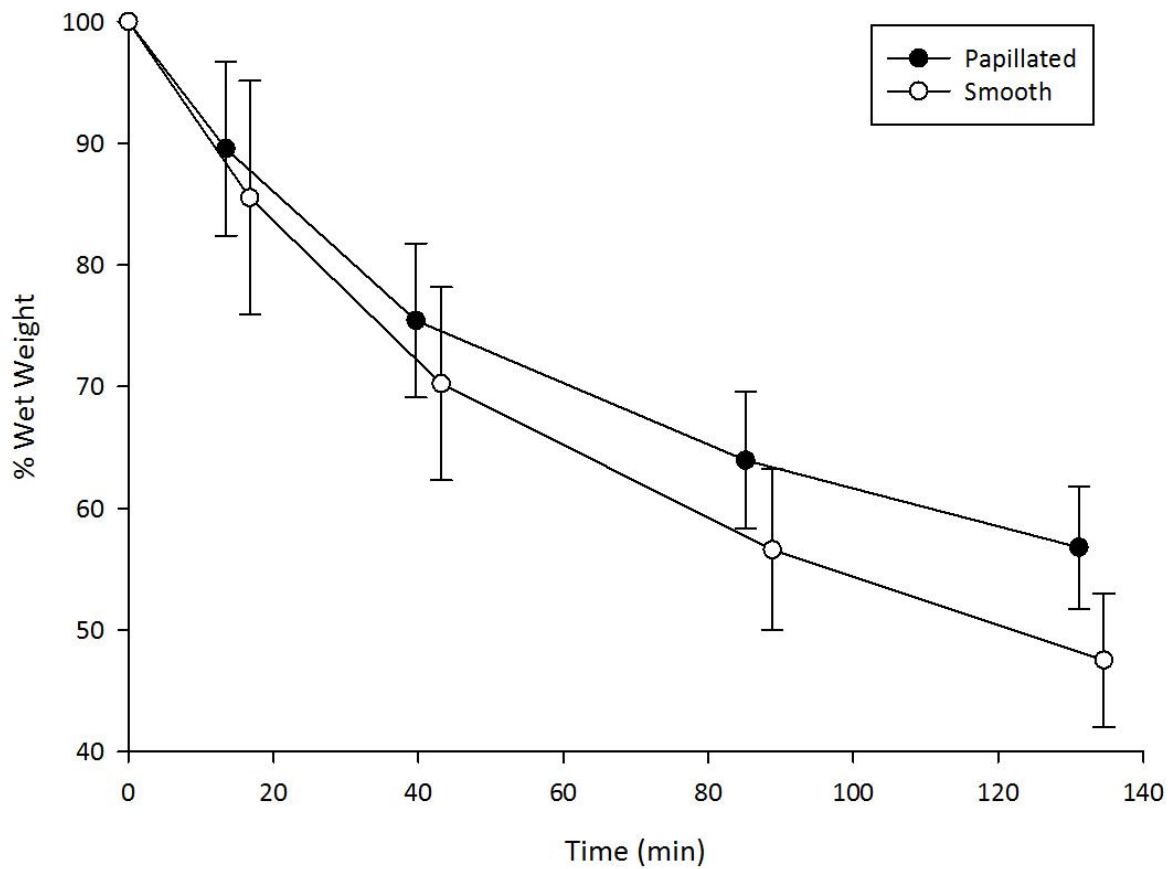
**Figure 11.** Effects of limpets on *Egregia* by rachis morph: A) The average limpet density between smooth and papillated rachi at Stillwater Cove, CA, with no significant difference between morphs (ANOVA:  $F_{1,96} = 1.3817$ ,  $p = 0.2427$ ). B) Average scarred area of rachis by rachis morph, with smooth rachi having significantly larger scars than papillated rachi (ANOVA:  $F_{1,22} = 9.4334$ ,  $p = 0.0056$ ). C) The percent rachis removed by rachis morph, with no significant difference seen between morphs (ANOVA:  $F_{1,22} = 0.6872$ ,  $p = 0.4160$ ). Error bars are  $\pm$ SE.



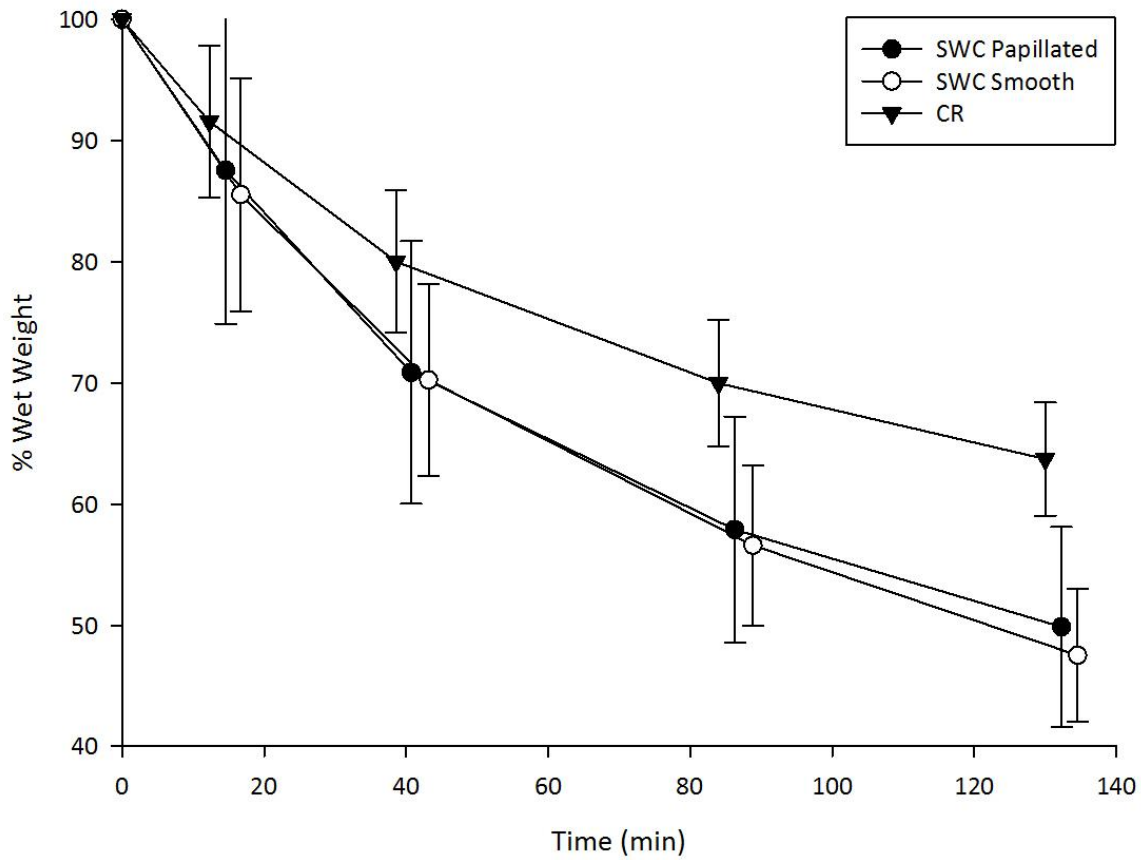
**Figure 12.** Limpet morphometrics compared to the scar area on the rachis, with smooth rachi indicated using the (○) and papillated rachi using the (●). A) Limpet mass was positively related to the scar area, with the  $r^2$  of papillated rachi equalling 0.693, while that of the smooth rachi is 0.474. The overall  $r^2$  value of all limpets and their scars was 0.392. See Table 6a for ANCOVA results. B) The area of the limpet's footprint is also positively correlated with the area of the scar. The  $r^2$  value for papillated rachi individuals is 0.609, while that for smooth individuals is 0.270, while the  $r^2$  value for all rachi is 0.249. See Table 6b for ANCOVA results. (n = 24)



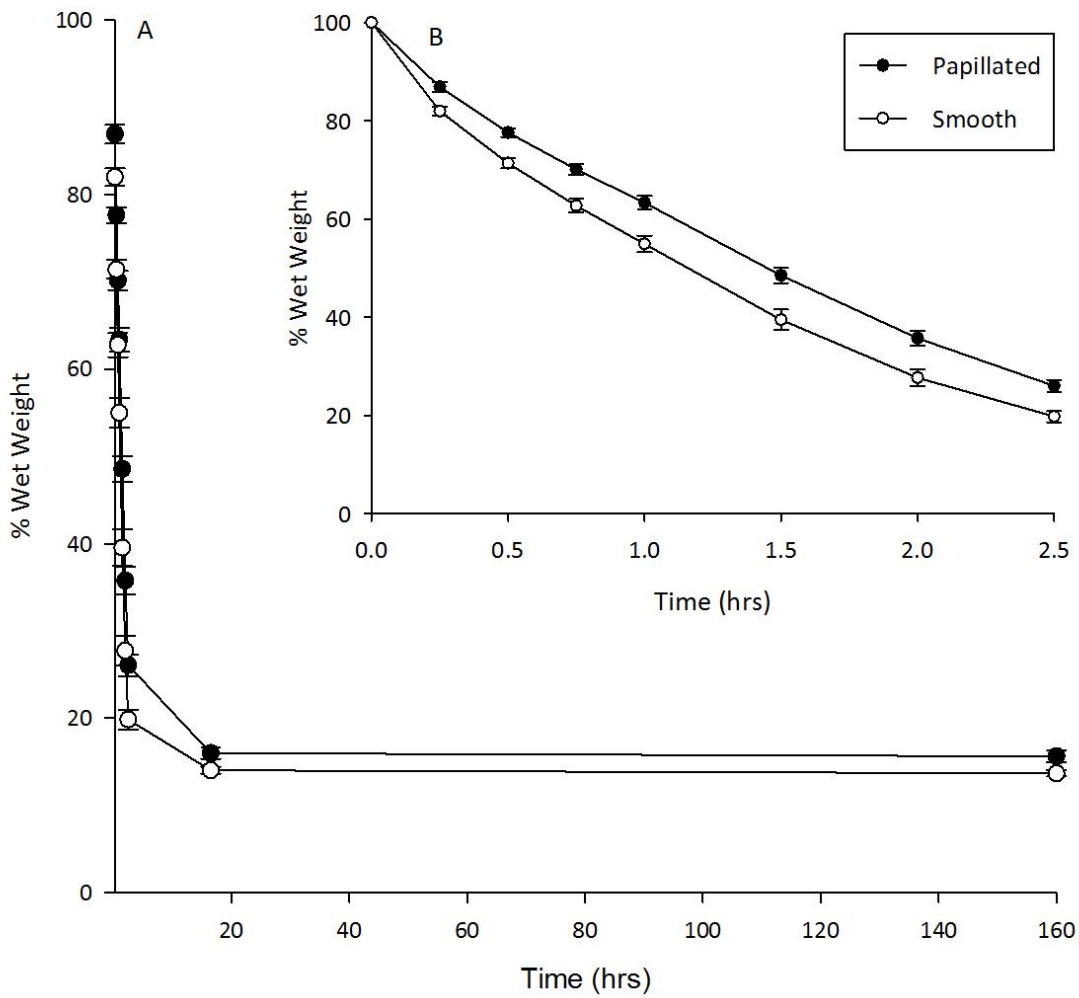
**Figure 13.** The decrease in % wet weight of the rachi over 2 hours at Stillwater Cove between papillated and smooth rachi. Error bars are  $\pm$ SE. (n = 5)



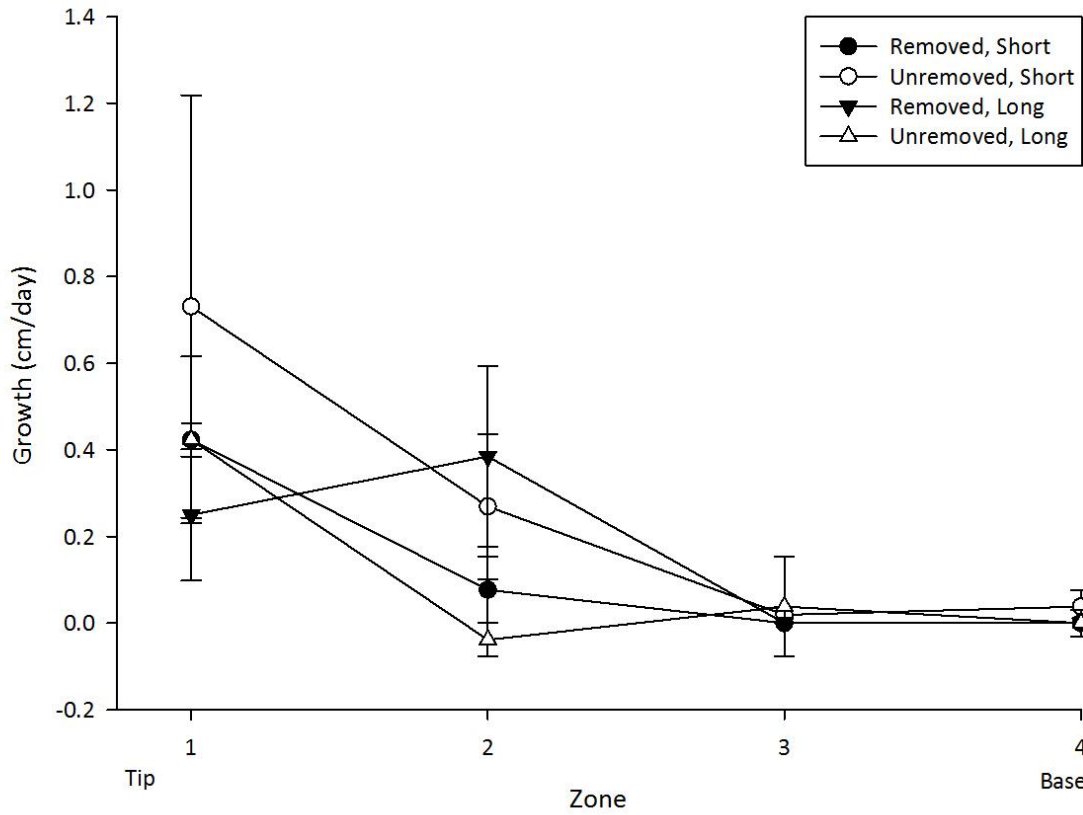
**Figure 14.** The decrease in % wet weight of the rachi over 2.5 hours outside at Moss Landing Marine Laboratories. Error bars are  $\pm$ SE. Results of repeated measures ANOVA see Table 5a. (n = 7)



**Figure 15.** The decrease in % wet weight of rachi over 2.5 hours outside of Moss Landing Marine Laboratories, compared between papillated and smooth rachi from Stillwater Cove and papillated rachi from China Rock. Error bars are  $\pm$ SE. Results of repeated measures ANOVA see Table 5b. (n = 7)

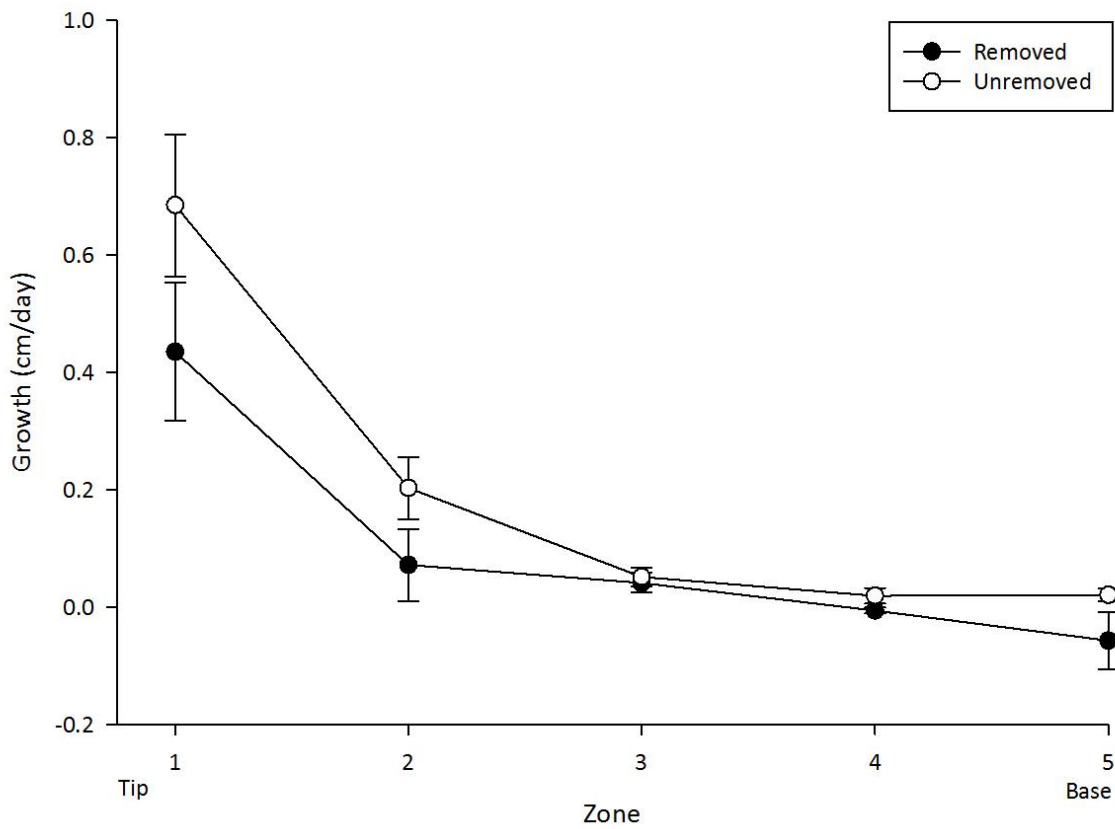


**Figure 16.** The decrease in % wet weight of rachis segments in a 60°C oven over A) 160 hours and B) the first 2.5 hours of the same experiment. Error bars are  $\pm$ SE. Results of ANCOVA see Table 7. (n = 10)



**Figure 17.** Growth rate (cm/day) along the rachis, from Zone 1 adjacent to the terminal lamina to Zone 4, 40 cm from the terminal lamina. Removed rachi had the terminal lamina and transition zone (Zone 0) removed at the start of the experiment. Short rachi were those shorter than 50 cm, while long rachi were those longer than 50 cm. Error bars are  $\pm$  SE.





**Figure 18.** Growth rates (cm/day) by zone of rachis, from the transition zone near the tip (Zone 1) to 40 cm from the transition zone towards the base of the rachis (Zone 5). Rachi with the terminal lamina removed are noted by the solid line, while rachi without the terminal lamina removed are noted with the dashed line. Error bars shown are  $\pm$  SE.

## Tables

**Table 1.** Field sites for seasonal reproductive output monitoring, from south to north.

Site	Rachis Type(s)	Location	Wave Exposure	Average Plant Density per sq meter	Average Plant Size (# of rachi)
Stillwater Cove	Smooth, Papillated	36° 33' 55.21" N 121° 56' 43.32" W	Medium-low	0.61	9.8
China Rock	Papillated	36° 36' 18.49" N 121° 57' 36.93" W	High	1.25	17.5
Pleasure Pt	Smooth	36° 57' 16.11" N 121° 58' 26.70" W	Low	3.69	6.9
Its Beach	Smooth	36° 57' 5.25" N 121° 1' 53.86" W	Medium	1.47	9.7
Mitchell's Cove	Papillated	36° 57' 0.88" N 121° 2' 40.27" W	High	2.44	16.1

**Table 2.** Results of a three-way Analysis of Variance (3-way ANOVA) comparing rachis morphology, blade type, and season effects on blade area around the Monterey Bay.

	df	MS	F	p
Rachis Type	1	18873435	39.9392	< 0.0001
Blade Type	1	48787530	103.2421	< 0.0001
Rachis*Blade	1	6847076	14.4895	0.0002
Season	3	306886.67	0.6494	0.5838
Season*Rachis	3	597256.67	1.2639	0.2868
Season*Blade	3	167923.33	0.3554	0.7853
Season*Rachis*Blade	3	245892.33	0.5203	0.6686
Error	322	472554		

Tukey HSD Multiple Comparison Test:

			p
Smooth, vegetative	v	Papillated, sporophyll	< 0.0001
Smooth, vegetative	v	Smooth, sporophyll	< 0.0001
Smooth, vegetative	v	Papillated, vegetative	< 0.0001
Papillated, vegetative	v	Papillated, sporophyll	< 0.0001
Papillated, vegetative	v	Smooth, sporophyll	0.0349
Smooth, sporophyll	v	Papillated, sporophyll	0.2862

**Table 3.** Results of a 3-way ANOVA comparing the effects of site, blade type, and season on blade area around the Monterey Bay.

	df	MS	F	p
Site	4	6952814	16.4588	< 0.0001
Blade Type	1	21040851	49.8082	< 0.0001
Site*Blade Type	4	2123881	5.0277	0.0006
Season	3	1034620	2.4492	0.0638
Site*Season	12	988113	2.3391	0.0071
Blade Type*Season	3	362072	0.8571	0.4637
Site*Blade Type*Season	12	432479	1.0238	0.4267

**Table 4.** Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and day effects on reproductive output from reproductive surveys at Stillwater Cove: a) average zoospore concentration, and b) average spore settlement success.

a) Average spore concentration

	df	MS	F	p
Date	15	500462893	3.0566	0.0004*
Morph	1	664831382	4.0605	0.0464*
Date*Morph	15	160091674	0.9778	0.4835
Error	107	163732742		

a) Average spore settlement success

	df	MS	F	p
Date	12	13944.08	2.8288	0.0022*
Morph	1	5070.22	1.0286	0.3128
Date*Morph	12	4064.70	0.8246	0.6249
Error	104	4929.29		

**Table 5.** Results of two-way Analysis of Variance (ANOVA) tests comparing morphology and season effects on reproductive output from surveys around the Monterey Bay: a) average zoospore concentration, and b) average spore settlement success.

a) Average zoospore output

	df	MS	F	p
Season	3	1523483024	5.5226	0.0015*
Morph	1	355654450	1.2882	0.2589
Season*Morph	3	219583504	0.7960	0.4989
Error	100	275861960		

a) Average spore settlement success

	df	MS	F	P
Season	3	30825.190	7.1414	0.0003*
Morph	1	1204.586	0.2791	0.5987
Season*Morph	3	336.845	0.0780	0.9717
Error	82	4316.416		

**Table 6.** Results of Analysis of Covariance (ANCOVA) tests comparing a) the effects of limpet mass and morph (papillated, smooth) on the rachis scar area (mm<sup>2</sup>) and b) limpet foot area (mm<sup>2</sup>) and morph (papillated, smooth) on the rachis scar area (mm<sup>2</sup>).

a)

	df	MS	F	p
Morph	1	186390.60	10.8416	0.0036*
Limpet Mass (g)	1	167933.91	9.7680	0.0053*
Morph*Limpet Mass	1	57549.91	3.3474	0.0823
Error	20	17192.23		

b)

	df	MS	F	p
Morph	1	2624.0269	5.0405	0.0362*
Limpet Foot Area (mm <sup>2</sup> )	1	4573.4727	8.7852	0.0077*
Morph*Foot Area	1	2563.0998	4.9325	0.0382*
Error	20	2385.80		

**Table 7.** Comparisons of % Wet Weight remaining between rachis morphologies at Stillwater Cove over 160 hours.

Time (hrs)	Papillated Avg % Wet Weight	Smooth Avg % Wet Weight	ANOVA results
0.25	86.8964	81.9642	$F_{1,16} = 11.2641, p = 0.0040^*$
0.5	77.6219	71.3840	$F_{1,16} = 20.2031, p = 0.0004^*$
0.75	70.1180	62.7399	$F_{1,16} = 17.6725, p = 0.0007^*$
1	63.3565	54.9541	$F_{1,16} = 15.3631, p = 0.0012^*$
1.5	48.5363	39.5287	$F_{1,16} = 12.2891, p = 0.0029^*$
2	35.7440	27.7115	$F_{1,16} = 11.7583, p = 0.0034^*$
2.5	26.0567	19.8304	$F_{1,16} = 13.2679, p = 0.0022^*$
16.5	15.9854	14.0223	$F_{1,16} = 6.4003, p = 0.0223^*$
160	15.6167	13.6719	$F_{1,16} = 6.8593, p = 0.0186^*$



**Table 8.** Comparisons of growth (cm/day) between rachi with the terminal lamina and transition zone removed and control rachi.

Zone	Unremoved Growth (cm/day)	Removed Growth (cm/day)	ANOVA Results
0	0.596970	0.496970	$F_{1,28} = 0.3690, p = 0.5485$
1	0.124242	0.136364	$F_{1,28} = 0.0280, p = 0.8683$
2	0.048485	0.033333	$F_{1,28} = 0.5087, p = 0.4816$
3	-0.00303	0.01818	$F_{1,28} = 2.7661, p = 0.1074$
4	-0.03030	0.01818	$F_{1,28} = 2.2770, p = 0.1425$