UNIVERSITY OF CALIFORNIA
SANTA CRUZ

ECOLOGICAL AND EVOLUTIONARY DRIVERS OF MORPHOLOGY AND ALGINATE CONTENT IN GIANT KELP (*MACROCYSTIS PYRIFERA*)

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Sara T. Gonzalez

June 2022

The Dissertation of Sara T. Gonzalez is approved:

_____
Professor Peter T. Raimondi, Chair

_____
Professor Sylvain Faugeron

_____
Professor Kristy Kroeker

_____
Research Biologist Daniel Reed

_____
Peter Biehl
Vice Provost and Dean of Graduate Studies

Table of Contents

List of Tables

List of Figures

design of treatment placement (pyrifera [P], integrifolia [I], and mixed [M]) on 20 concrete parking bumpers arranged in two rows of 10 bumpers each (right). Length of a bumper is 1.2 m and distance between bumpers in all directions is approximately 5 m.

Figure 2.4. Measurements taken on kelp individuals: primary stipe length (red line), adapted from Brostoff (1988); and count of number of branches. The number of branches was counted as the greatest total number of branch points along any single stipe.

Figure 2.5. Illustrations of the holdfast morphology of *Macrocystis "pyrifera"* (A) and *M. "integrifolia"* (B) from Demes et al. (2009), and photographs of representative individuals from the pyrifera (C) and integrifolia (D) treatments in the present study, and the front and back of a holdfast conglomerate from a single mixed treatment replicate with both pyrifera-like (E1) and integrifolia-like (E2) forms in the present study.

Figure 2.6. Total height (top) and length of primary stipe (bottom) of the three treatments over the experimental period. Symbols above dates indicate significant ($p < 0.05$) differences in primary stipe length: *= between pyrifera and integrifolia, ^ = between pyrifera and mixed, ^{O} = between integrifolia and mixed.

Figure 2.7. Top panel: development of a single integrifolia individual over two months, demonstrating the progression of stipe tissue into holdfast structure. Bottom panel: development of a single pyrifera individual over two months, exhibiting little change in holdfast shape. Silver rod is for size reference with black lines spaced by 1 cm.

Figure 2.8. Lengths of primary stipe for all mixed treatment sporophytes on the final sampling date (9/15/21). Reference lines at 1.92 cm and 2.27 cm denote the minimum lower 95% confidence value for pyrifera treatment individuals and the maximum upper 95% confidence value for integrifolia treatment individuals, respectively, in the present study.

Figure 2.9. Mean number of branch points per individual by treatment.

Figure 2.10. Reproductive timing of pyrifera and integrifolia outplants with modeled probability of being reproductive (left y-axis) and empirical data of reproductive states (right y-axis; 1=reproductive, 0=not reproductive) of outplanted individuals sampled on 9/15/21.

Figure 3.1. Map of populations sampled for *integrifolia* (orange) and *pyrifera* (blue) in California and Chile. Site abbreviations are as follows, in alphabetical order: ALG=Algarrobo, ANC=Ancud, BAM=Bahía Mansa, BOC= La Boca,

CAY=Cayucos, CHO=Chome, DAL=Dalcahue, DOC=Las Docas, MON=Las Monjas, PCH=Punta Choros, PLB=Playa Blanca, STL=Stillwater Cove, TOT=Totorolillo, TUM=Tumbes.

Figure 3.2. Representative photographs of the *Macrocystis* ecomorphs, *pyrifera* (left) and *integrifolia* (right).

Figure 3.3. Linear regression associations between mean site sea surface temperature (SST) and latitude (left y-axis; blue), and between depth below mean lower low water (MLLW) and latitude (right y-axis; red).

Figure 3.4. Site mean sea surface temperatures (x-axis) and depths of kelp individuals (y-axis), colored by morph and distinguished with symbols by site.

Figure 3.5. Leverage plot of alginate yield (as proportion of dry algal weight) vs depth below mean lower low water (MLLW) generated from mixed model.

Figure 3.6. Least squares mean proportion G content in alginate samples for *integrifolia* and *pyrifera* samples.

Figure 3.7. Leverage plot of proportion G content vs mean sea surface temperature generated from mixed model.

Figure 3.8. Leverage plot of proportion G content vs depth below mean lower low water (MLLW) generated from mixed model.

Figure 3.9. Relationships between alginate guluronic acid (G) content and molecular weight for *integrifolia* (blue; o marker) and *pyrifera* (red; x marker) alginate samples. Only the relationship for *pyrifera* is statistically significant.

Abstract

Ecological and Evolutionary Drivers of Morphology and Alginate Content in Giant Kelp (*Macrocystis pyrifera*)

by

Sara T. Gonzalez

The giant kelp, *Macrocystis pyrifera,* exists as one species with distinct morphological variants—or "ecomorphs"—in different populations, yet the mechanism for this variation is unclear. One ecomorph (*"pyrifera"*) has a conical, mound-shaped base while the other ("*integrifolia*") has a flattened, elongated base that forms denser kelp beds. My research on the drivers of these distinct phenotypes can inform decisions about management and restoration of kelp populations, as well as selective breeding for certain traits in aquaculture. First, I assessed the patterns of giant kelp ecomorph spatial distribution and genetic differentiation across its Western Hemisphere range. I collected samples from 18 kelp populations in Chile and California and used whole-genome sequencing to evaluate the degree of genetic divergence among populations. My results demonstrate that *"pyrifera*" and *"integrifolia*" are genetically distinguishable, yet divergence patterns differ between Northern and Southern Hemispheres. Next, I empirically tested the effect of local environment on giant kelp morphology. I collected reproductive tissue from adult individuals of both ecomorphs naturally occurring at the same location, cultured the

spores in the laboratory, and outplanted the resulting baby kelp to the seafloor in a common ocean environment. Weekly monitoring revealed distinguishing morphological features at multiple developmental stages, ultimately culminating in morphologically distinct adults which also differed in reproductive timing. By including a mixed treatment with equal numbers of spores from each ecomorph, I observed the morphologies that emerge in a competitive context. This study also revealed for the first time how the characteristic "*integrifolia"* morphology forms in nature. Finally, I investigated ecomorph variation in production of alginate, one of the most commercially important chemicals from kelp. I compared alginate yield and composition of *"pyrifera"* and *"integrifolia"* populations across varied environments using kelp samples from 15 populations (a subset of those used in the genetic differentiation study). Alginate yield and composition were significantly different between the two morphs and were also related to the depth at which the kelp grew. Collectively, this work reveals a genetic basis to giant kelp morphology as well as eco-physiological divergence between the two ecomorphs, giving reason to consider them as distinct species.

Dedicated to:

My grandfather,

whose passion for science and scholarship inspired me every day,

and who made me feel like a rockstar with every small achievement.

Acknowledgements

This dissertation was made possible by contributions, guidance, and encouragement from many individuals. I am especially grateful to the following people:

My advisor, Pete Raimondi, for giving me the freedom to take every opportunity no matter where in the world it took me, spending endless hours helping me design experiments and patiently guiding me through analyses, and for trusting me to make the right decisions but always coming through when I needed his advice.

The members my dissertation reading committee, Sylvain Faugeron, Kristy Kroeker, and Dan Reed, for lending their valuable expertise and asking questions that challenged me to think critically about my research questions and results.

My collaborators, Filipe Alberto, Gary Molano, Finn Aachmann, Olav Aarstad, and Wenche Strand, for sharing their expertise and data, teaching me new laboratory and analytical methods, and without whom this dissertation would not have been possible.

Kersten Schnurle, for being the most amazing travel companion, field assistant, and friend as we took our 6-week tour of the Chilean coast to collect kelp samples that provided data for Chapters 1 and 3. Rosie Campbell, for being a stellar dive buddy

and laboratory assistant through multiple rounds of cultivating kelp, and whose assistance made Chapter 2 possible.

Giacomo Bernardi and Remy Gatins for lending their expertise in genetic analyses and bioinformatics. Kathleen Kay and Rachel Meyer for generously opening their genetics labs to me and providing access to laboratory equipment and training.

Steve Clabuesch and Dave Benet for assisting with diving research methods in Chile and California, boating operations, gear and training for all SCUBA operations, and, with Mark Carr, hauling 20 100-lb concrete parking bumpers out to sea for Chapter 2. Dive buddies Brynn O'Hara, Rachel Pausch, Sara Ebersole, Hillary Krumbholz, Ben Walker, May Roberts, Gina Contolini, and Niko Kaplanis, and Anna Voznyuk for laboratory assistance.

All of the researchers at the Pontificia Universidad Católica de Chile's Estación Costera de Investigaciones Marinas, especially Miriam Fernández, Bryan Bularz, and Alejandro Pérez-Matus for mentoring me during my Fulbright year in Chile and introducing me to the world of kelp. And all of the kelp harvesters who took time to speak with me about their work, welcomed me into their homes, and taught me about the value of kelp as a natural resource.

Iván Hinojosa and Evie Wieters for helping me coordinate diving operations at several field sites in Chile, and Juan Puño for generously taking in me and my kelp samples (for alginate) to dry at his kelp processing plant in San Jose de Maipo.

Maribel Solas of the Faugeron Lab and Gabriel Montecinos of the Alberto Lab for mentoring me in essential lab skills for culturing early life stages of kelp.

The Aachmann Lab for welcoming me into their lab, teaching me about alginate chemistry, and helping me have as many Norwegian experiences as possible under COVID-19 restrictions.

The UCSC Ecology and Evolutionary Biology Department staff, especially Betsy Steele, Nicci Hack, and Sylvie Childress for managing the facilities that allowed me to successfully culture kelp in the laboratory.

Funding sources that enabled me to travel to field sites, analyze samples, obtain materials for laboratory and field work, and support my living stipend during my time in graduate school: Graduate Assistance in Areas of National Need Fellowship (U.S. Department of Education), Fulbright Student Scholarship (U.S. State Department), Ford Foundation Predoctoral Fellowship, Lewis and Clark Fund for Exploration and Research (American Philosophical Society), the Dr. Earl H. Myers and Ethel M.

Myers Oceanographic & Marine Biology Trust, the UCSC Graduate Student Association, and the UCSC Ecology and Evolutionary Biology Department.

My Raimondi-Carr lab mates, especially my office-mates Kat Beheshti, Monica Moritsch, and Kate Melanson, for sharing their wisdom and giving great pep-talks.

Kat Dale, Rachel Pausch, and Wendy Bragg, for being incredible friends, going on adventures with me and always keeping my spirits high.

My friends since childhood, Abby, Colleen, Kat, and Naomi, for making me feel like my research was super cool and for so many laughs over video chats throughout the years.

My parents, who were simultaneously my constant cheerleaders and harshest reality-checkers, indulged my requests to read pieces of my chapters or grant proposals, and let me ramble on for hours over the phone about my research. All of my aunts, uncles, and cousins, who made my work feel valued and relevant by asking questions over Thanksgiving dinners and sending me news articles about kelp. And my brother, Jesse, for encouraging me when I needed it most, and for visiting me in Chile and carrying all of the heavy gear on our Patagonia trek.

Introduction

Along the rocky shores of the eastern Pacific Ocean, massive kelp forests create highly productive ecosystems composed of a rich biodiversity of fishes, invertebrates, birds, and marine mammals. These species depend on the kelp forest for habitat, food, breeding ground, and refuge from predators. Fish and invertebrate species of the kelp forest have been harvested for thousands of years, but more recently the kelp itself has emerged as a valued natural resource. Across the globe, kelp is cultivated and harvested for abalone feed and the raw material for alginates, polysaccharides which serve as emulsifying agents in many commercial products.

The giant kelp, *Macrocystis pyrifera*, is one of the most widespread and fastest-growing algae in the world, making it ideal for harvesting and cultivation. Much of the species' range occurs on the west coast of the Americas in temperate waters with sufficient nutrients. Giant kelp exists as distinct "ecomorphs" which are recognized based largely on variation in holdfast morphology—the two most common ecomorphs are known as *pyrifera* and *integrifolia* (and in a few locations two additional ecomorphs, *angustifolia* and *laevis,* can be found). For clarity, I will use the genus name *Macrocystis* to refer to all ecomorphs collectively, as the genus is monospecific. The mechanisms driving morphological variation within *Macrocystis* remain unclear, and previous research shows evidence for both environmentally-induced plasticity and well as a genetic basis to morphology. This dissertation focuses

on the *pyrifera* and *integrifolia* ecomorphs in North and South America and seeks to uncover the mechanisms driving this morphological variation.

In **Chapter 1**, I assess the patterns of *Macrocystis* ecomorph spatial distribution and genetic differentiation across its Western Hemisphere range. The recent and rapid dispersal of the ancestral *pyrifera* morph of *Macrocystis* from the Northern Hemisphere to the Southern Hemisphere (Macaya & Zuccarello 2010a) raises the question of whether the *integrifolia* morph exists via phenotypic plasticity in response to the environment, or whether it has evolved over time via local adaptation. The incongruous patterns of divergence between ecomorphs across hemispheres (Astorga et al. 2012) suggest that, if the *integrifolia* morph evolved from *pyrifera* over time, it may have evolved independently in each hemisphere. However, these studies are limited because they focus on neutral genes or gene regions rather than considering the entire genome. My study uses whole genome sequencing on 100 individuals from 18 populations (11 *pyrifera* and 7 *integrifolia*) to assess genetic divergence based on both neutral and adaptive alleles. Reconciling the genetic differences between ecomorph populations across California and Chile enhances our understanding of the evolutionary history and the influence of environment on this phenotypically variable species.

In **Chapter 2**, I experimentally investigate the factors controlling morphological variation within *Macrocystis.* It was previously believed that differences in form among the ecomorphs was entirely due to environmental conditions, namely depth and wave exposure. However, some research shows

evidence that genetics also play a role in *Macrocystis* morphology. Transplant experiments of *angustifolia* and control transplants of *pyrifera* into a population of *pyrifera* at Point Loma, CA, resembled original populations in basal stipe and holdfast appearance, providing evidence of a genetic basis for morphological differences (Brostoff 1988). Along the southern coast of South America, populations were clustered both genetically and morphologically by geographic region, suggesting that environmental conditions are driving evolutionary divergence between regions (Camus et al. 2018a). I conducted a common garden experiment where I cultivated the early life stages of both ecomorphs in the laboratory and then planted the emergent sporophytes to the bottom of the ocean where they grew for an additional five months. My experiment revealed that the ecomorphs maintain their parental morphology even when grown in identical conditions, indicating that morphology is genetically controlled.

In **Chapter 3**, I determine the influence of genetics and environment on alginate content and composition in *Macrocystis* ecomorphs. Since alginates are structural components of the algal cell, changes in morphology may relate to changes in alginate content. Differences in alginate content and composition can reflect functional differences of different algal tissues. For example, in *Durvillaea antarctica* and *D. willana* in southern New Zealand, Kelly & Brown (2000) found that blades and stipes had more mannuronic acid (M) and holdfasts had more guluronic acid (G), which aligns with the need for blades and stipes need to be more flexible under wave force while holdfasts must be more rigid. The proportions of M and G that comprise

the alginate also relate to their utility for commercial products depending on how elastic or rigid the material needs to be. I used Nuclear Magnetic Resonance to analyze the composition of alginates extracted from kelp individuals from 15 populations, a subset of the populations sampled in Chapter 1. I found that both alginate yield and composition differed significantly between the two ecomorphs even after accounting for site variability.

Understanding the relationship between genetics, environment, and phenotypic traits such as morphology and alginate content in algae will inform uses of *Macrocystis* in aquaculture and contribute to our broader understanding of how natural selection acts on phenotypically variable species. My dissertation examines the drivers of morphology from both ecological and evolutionary perspectives, and looks toward application of this foundational knowledge by investigating alginate content in giant kelp populations.

CHAPTER 1

Whole genomes distinguish the two most common giant kelp (*Macrocystis*) ecomorphs

Abstract

The giant kelp, *Macrocystis pyrifera,* exists as distinct morphological variants—or "ecomorphs"—in different populations, yet the mechanism for this variation is uncertain, and potential environmental drivers for either adaptive or plastic morphological features have not been identified. The two most common ecomorphs, *Macrocystis "pyrifera"* and *M. "integrifolia*," are distributed throughout temperate waters of North and South America but rarely overlap in geographic location and are believed to exhibit a parallel, rather than mirrored, distribution across the equator. This study evaluates the degree of genetic divergence between *M. "pyrifera"* and *M. "integrifolia"* by sampling 18 populations in Chile and California and using whole-genome sequencing and SNP markers. Our results based on a principal component analysis, admixture clustering by genetic similarity, and dendrogram inference demonstrate that the two ecomorphs of giant kelp, *M. "pyrifera"* and *M. "integrifolia,"* are genetically distinguishable. Analyses reveal separation by hemisphere and between morphs within hemispheres, suggesting that the convergent *"integrifolia"* morphology arose separately in each of the Northern and Southern hemispheres. This is the first study to use whole-genome sequencing to understand the genetic divergence of the giant kelp ecomorphs. Such understanding is needed to effectively manage natural kelp populations, as well as optimize farmed populations via selective breeding for specific traits. Future studies are needed to uncover the environmental forces driving local adaptation and the presumed convergent evolution of these morphs in the Northern and Southern hemispheres.

Key words: ecomorph; kelp; *Macrocystis integrifolia*; *M. pyrifera*; morphology; Phaeophyceae; whole-genome sequencing

Introduction

For species with phenotypically distinct populations that are geographically separated, it can be difficult to isolate effects of environment versus genotype on phenotype. For example, phenotypic variation can occur via speciation that arises from physical barriers or other disruptions to gene flow between populations. Phenotype may also be altered by environmental conditions within an individual's lifetime (phenotypic plasticity). Falsely attributing variation to phenotypic plasticity leads to misclassifications and underrepresentation of species diversity when genetic analyses would support splitting morphologically distinct taxa (e.g. Weber et al. 2017, Augyte et al. 2018).

Brown algae (Phaeophyceae) contain many globally distributed marine species with intraspecific morphological variation spread over both sympatric and reproductively isolated populations. Stark morphological differences among giant kelp (*Macrocystis pyrifera*) populations have been observed for nearly two centuries, spurring much debate and re-classification of populations as distinct species or alternative forms of the same species (see below). Presently, four morphological variants, or "ecomorphs," of the single species, *M. pyrifera,* are recognized globally:

M. 'pyrifera,' M. 'integrifolia,' M. 'angustifolia,' and *M. 'laevis.'* All four ecomorphs are considered the same species by many (Coyer et al. 2001, Macaya & Zucarello 2010a), and the most recent review supports the unification of these morphs under *M. pyrifera* and supports *Macrocystis* as a monotypic genus (Schiel & Foster 2015). Hereafter, "*Macrocystis*" refers to the species as a whole, and ecomorphs are referred to by only their specific epithets. The ecomorphs are distinguished by characteristics of the holdfast and blade (Howe 1914, Womersley 1954, Hay 1986). The two most common ecomorphs, *pyrifera* and *integrifolia*, differ in their holdfast morphology; *pyrifera* is characterized by having a conical, mound-shaped holdfast while *integrifolia* has a flattened, strap-like holdfast (Figure 1.1). The ecomorphs were molecularly indistinguishable based on analyses of internal transcribed spacer regions (Coyer et al. 2001); however, an assessment of genetic divergence based on whole-genome sequences has never been done. The mechanisms underlying morphological phenotypic variation among ecomorphs remain unknown.

The largest genetic break within *Macrocystis* is between populations from the Northern versus Southern Hemisphere, regardless of ecomorph (Coyer et al. 2001, Astorga et al. 2012). The previously reported low genetic divergence among Southern Hemisphere populations may be attributed to connectivity via floating kelp moved by the Antarctic Circumpolar Current (Macaya & Zuccarello 2010b). A phylogeny developed by Astorga et al. (2012) revealed genetic separation between *pyrifera* and *integrifolia* morphs from the Chilean coast; however, this trend was not consistent in the Northern Hemisphere. Some studies on populations in the Southern Hemisphere

describe genetic differentiation associated with ecomorph and genetic breaks potentially related to ecomorph adaptation to local environmental conditions (Macaya & Zuccarello 2010a, Camus et al. 2018a). While many of these studies noted the locations of samples collected, a phylogeny based on samples from known ecomorphs (confirmed with observation of the holdfast) has not been constructed.

The conceptual homogenization of the four ecomorphs into a single species began with genetic crossing experiments that showed all ecomorphs, except *laevis* which was not tested, were interfertile (Lewis et al. 1986, Lewis & Neushul 1994, Westermeier et al. 2007). The single-species view was further supported by genetic analysis showing that with internal transcribed spacer (ITS) sequences ITS1 and ITS2, it was not possible to differentiate between *integrifolia* and *pyrifera* (Coyer et al. 2001). Furthermore, in some cases *pyrifera* individuals from a single population in North America were more genetically distinct from each other than were individuals from distant populations of *angustifolia* and *pyrifera* in the Southern Hemisphere (Coyer et al. 2001). Demes et al. (2009) argued that holdfast morphology was dependent on the depth at which the sporophyte develops, so *Macrocystis* should be a monospecific genus with distinct "ecotypes." The authors claim that an individual sporophyte will follow one of three depth-dependent developmental routes toward a morphology resembling *pyrifera, integrifolia,* or *angustifolia.* Field observations show that *pyrifera* is generally found in deeper waters (4-70 m) while *integrifolia* is found in shallow waters or intertidal (0-10 m); however, the recorded maximum depth of *integrifolia* in the Southern Hemisphere is considerably deeper than that of

integrifolia in the Northern Hemisphere (Graham et al. 2007). Additionally, the authors' choice of the term "ecotype" (Demes et al. 2009) to describe these different forms is inconsistent with definitions of the term in which morphological attributes are hereditary, genotypic responses to the selective action of the environment (Turesson 1922, Gregor 1944). Thus, the morph variants have been grouped together as a single species despite a lack of empirical evidence that different morphologies are environmentally induced or that the ecomorphs interbreed in the wild.

It is still unclear whether a given *Macrocystis* ecomorph occurring in different areas of the globe evolved once or multiple times. The widespread global range of *Macrocystis* and its ecomorphs is curious given the short dispersal distance (usually <100 m from the parental plant) of its spores (Gaylord et al. 2002, Reed et al. 2004, Gaylord et al. 2006). The present-day *Macrocystis* ecomorphs are believed to have originated from a *pyrifera* morph ancestor in the Northern Hemisphere, likely on the northwest Pacific coast, which later colonized the southeast Pacific via rafting, ultimately extending range throughout the Southern Hemisphere (Coyer et al. 2001, Macaya & Zuccarello 2010b, Astorga et al. 2012). The ability for *Macrocystis* to extend its range via rafting likely enabled its recolonization of subantarctic islands following elimination by ice scour during the last glacial maximum (Macaya & Zuccarello 2010a). It is therefore possible that the *integrifolia* morph evolved independently in the Northern and Southern Hemispheres.

The *pyrifera* and *integrifolia* ecomorphs exhibit parallel distributions across the equator, rather than mirrored, as would be expected based on latitudinal gradients

of most physical and oceanographic factors (Figure 1.2). In the Northern Hemisphere *integrifolia* is reported to range from mid-high latitudes and *pyrifera* from mid-low latitudes, but this is based on limited observations of *integrifolia* in the field (see Macaya & Zucarello 2010a). The opposite distribution pattern is true for both ecomorphs in the Southern Hemisphere; *pyrifera* is found mainly at higher latitudes (plus a small range in Peru) and *integrifolia* is found at lower latitudes (Graham et al. 2007, Macaya & Zuccarello 2010a). Stillwater Cove, California, is one of the only observed locations in which both *pyrifera* and *integrifolia* ecomorphs are found (Graham et al. 2007, Hargarten 2015, Jeffries 2015), although the adjacent populations are separated by depth. No sites of co-occurrence of the two morphs in the Southern Hemisphere have been described; however, the existence of such sites is possible as genetic haplotypes based on microsatellites that tend to correspond with either *pyrifera* or *integrifolia* in Chile can be found co-occurring at sites in Peru (Salavarria et al. 2018). The absence of sites where both ecomorphs occur in true sympatry (without separation by depth) suggests a case of incipient speciation with selection against morphological, and likely also genetic, hybrids.

Some studies show genetic similarity between ecomorphs, supporting the view that morphological variation in *Macrocystis* is due to phenotypic plasticity induced by environmental conditions. Based on molecular analyses of internal transcribed spacer sequences, *integrifolia* and *pyrifera* populations in California could not be genetically distinguished (Coyer et al. 2001). Using the DNA barcoding gene cytochrome c oxidase subunit I (COI) as a standardized marker for species ID in

macroalgae, Macaya & Zuccarello (2010a) showed shared haplotypes among distant sites around the Southern Hemisphere and between sympatric ecomorph populations of *integrifolia* and *pyrifera* in Stillwater Cove, California. The authors noted that the low genetic variation across the world and shared haplotypes among distant populations likely indicated that all ecomorphs have a very recent common ancestor, rapid dispersal, and/or high levels of gene flow between populations, suggesting that the differences in morphology between *integrifolia* and *pyrifera* are due to local environmental influences rather than genetics (Macaya & Zuccarello, 2010a). This conclusion is further supported by Salavarria et al. (2018) who found no genetic differences between samples of *pyrifera* and *integrifolia* based on the mitochondrial marker atp8-S.

Other research, however, shows evidence for natural selection on genotypes as the basis for morphological differences based on local adaptation to environmental factors, genetic similarity between distant populations of the same ecomorph, or geographic alignment of genetic and morphological clusters. In the Northern Hemisphere, Johansson et al. (2015) found that genetic clusters of *Macrocystis* were associated with environmental factors, with the light extinction coefficient being the best environmental predictor of co-ancestry clusters. Light intensity or related factors may be selective drivers of morphological adaptations, such that lateral holdfast growth with stipes covering a large area is favored in high light (shallow) habitats and upward holdfast and stipe growth is favored in low light (deep) conditions. In another genetic study using microsatellite loci, *pyrifera* populations in geographically distinct

areas were more genetically similar to each other based on F_{ST} values than were *pyrifera* and *integrifolia* populations occurring in the same area (Hargarten 2015). This suggests that morphology may be genetically controlled and that populations within an ecomorph have shared ancestry. In a third study, along the southern coast of South America, populations of *Macrocystis* were clustered both genetically and morphologically by geographic region, suggesting that environmental conditions are driving evolutionary divergence between regions (Camus et al. 2018a). However, the morphological groups were distinguished by features such as total length, weight, and number of blades, rather than the holdfast metrics which characterize ecomorphs. Collectively, these studies provide evidence for morphology resulting from local adaptation rather than plasticity induced by environmental factors.

Laboratory experiments have demonstrated that *pyrifera* and *integrifolia* can hybridize, and that lab-reared hybrids grown to juvenile sporophytes in the field exhibited holdfast morphology with both *pyrifera*- and *integrifolia*-type features (Westermeier et al. 2007). This would indicate that this ecomorphological trait is at least in part heritable. The body of knowledge on *Macrocystis* ecomorph hybrids in the literature remains limited, especially as the possible genes responsible for ecomorphology have not been identified.

Understanding the basis for ecomorphic variation is especially important in Chile, where ecomorphs exhibit high geographic separation and kelp harvesting is subject to different regulations by region. *Macrocystis* populations in the northern regions where the *integrifolia* morph dominates are overexploited (Vásquez 2008)

and in some areas the fishery has remained closed for years. In order to carry out successful repopulation of degraded forests or see natural recovery via spore dispersal from other nearby populations, we must understand the underlying genetic structure of the populations, including local adaptations to differing environmental conditions and variable survival across different genotypes.

The objectives of this study were to 1) determine the patterns of spatial and genetic structure within and between the *pyrifera* and *integrifolia* ecomorphs, and 2) determine whether certain genomic regions from whole-genome sequences can discriminate between the two ecomorphs. Based on the assumption (tested here) that ecomorphology is genetically determined, and the hypothesis that the *integrifolia* ecomorph arose separately in the Northern and Southern Hemispheres over evolutionary time, we make the following predictions: individuals will show 1) closer relatedness within versus between ecomorphs, at least within hemispheres, 2) closer relatedness among populations of the same ecomorph in the same hemisphere compared to across hemispheres, and 3) closer relatedness between different ecomorphs within a hemisphere than between the same ecomorphs between hemispheres. Because our sampling covers a large latitudinal range, we would expect to see populations diverge due to genetic drift. If there is no consistent genetic differentiation between ecomorphs, then this divergence should be random and unrelated to ecomorph boundaries. However, if ecomorphs are genetically distinguishable, then we should see a non-random divergence where all populations of a given ecomorph group more closely genetically with each other than with any

population of the other ecomorph, regardless of how proximal geographically those populations may be. Our results demonstrate clear genetic differences between ecomorphs with little mixing between populations, even among those in close geographic proximity.

Methods

Site selection

Sites were selected based on prior ground truthing and collections by other researchers, accessibility, and proximity to SCUBA air tank supplies. In total we sampled six Northern Hemisphere populations (central California: Stillwater Cove [two populations] and Cayucos; southern California: Arroyo Quemado, Catalina Island, and Camp Pendleton) and 12 Southern Hemisphere populations (Playa Blanca, Punta Choros, Totorolillo, Las Docas, Algarrobo, Las Monjas, La Boca, Chome, Tumbes, Bahía Mansa, Ancud, and Dalcahue) (Table 1.1, Figure 1.3). La Boca was identified by the author as the northernmost confirmed *pyrifera* morph site in Chile. Additional *integrifolia* sites near La Boca (Las Monjas and Algarrobo) were included to provide greater resolution near the ecomorph boundary. The northernmost *integrifolia* site selected in Chile was Playa Blanca; north of this site, *Macrocystis* is absent for approximately 600 km and reappears in Antofagasta (Vásquez 2008).

Sample collection, preparation, and storage

Tissue used in genetic analyses were collected via SCUBA diving. At each site, the divers selected an accessible portion of the kelp bed and collected blades from 5-9 individuals, each at least 2 m apart, over a distance of at least 30 m. Blades from different individuals were placed in separate Ziploc bags underwater. Ecomorph characterization of individuals was based on holdfast morphology. *Pyrifera* have conical holdfasts, while *integrifolia* have flattened, rhizomatous holdfasts (Macaya & Zucarello 2010a; Figure 1.1). In addition, the haptera of *pyrifera* holdfasts arise from all sides of the cylindrical segments, while haptera of *integrifolia* are produced only from the edges along two sides of the flattened holdfast (Setchell 1932, Womersley 1954).

Samples were transported on ice from the field site to the laboratory. Each sample was thoroughly rinsed in tap water, spun dry in a salad spinner, and then pat-dried with a paper towel. The blades in best condition (free of epibionts, holes, degradation, and discoloration) were selected for genetic analyses. A small piece of tissue (approximately 4-6 cm^2) was cut and placed into a disposable teabag, which was placed into a sealable plastic bag containing color-indicating silica gel beads and stored at room temperature until analysis (within 4 to 10 months). New silica gel was added if beads changed color in order to maintain samples as dry as possible. All samples were processed the same day as collected except for Cayucos and Las Monjas, which were processed the day after collection.

Morphological characteristics and depth

For each individual sampled in the Southern Hemisphere populations, we measured two morphological features that tend to quantify the qualitative differences between ecomorphs: 1) the holdfast diameter and 2) the number of branches along the stipe. The holdfast diameter was measured in two directions at the base of the holdfast: 1) lengthwise across the longest cross-section, and 2) perpendicular to the longest cross-section. The number of branches was counted as the greatest total number of branch points along any single stipe; a branch point was counted as any point on the stipe that bifurcated into two stipes with pneumatocyst-bearing blades, including the initial bifurcation point.

The depth of each kelp individual was measured at the holdfast using a dive computer. All depths were standardized to Mean Lower Low Water (MLLW) elevations by adjusting for the tide height at the time the depth was recorded.

Statistical analyses included all 12 Southern Hemisphere populations for number of branches and depth analyses; however, for holdfast diameter analysis, sites BOC (*pyrifera*) and PCH (*integrifolia*) were excluded because the highly wave exposed conditions and location of the kelp in the surf zone made it infeasible to take accurate holdfast measurements at these sites. For each site, measurements for 10-15 different kelp individuals each separated by at least 2 m were included in analyses. Holdfast diameter and depth were analyzed using mixed model ANOVAs with site nested within morph as a random effect, morph as a fixed effect, and either ratio of the longer diameter to the shorter diameter or depth as the response variable. A direct comparison of the number of branches between the two morphs was not possible

because there was no variance within *integrifolia* (all individuals had 1 branch point). Therefore, the distribution of values for number of branches in *pyrifera* and 95% confidence interval were used to evaluate whether the morph groups were significantly different.

Genetic analyses

DNA was extracted from approximately 10 mg of dried blade tissue using a Macherey-Nagel Nucleospin Plant II Genomic DNA Purification Kit, with some modifications in incubation time and number of buffer washes to increase purity and DNA yield (see Appendix A1 for details). Extracted DNA samples were sent to Texas A&M University AgriLife Genomics & Bioinformatics Service for library preparation and whole-genome sequencing (~18x coverage). DNA sequences were trimmed to remove adapters, polyG tails, and other low-quality reads using *Fastp* (Chen et al. 2018). We mapped trimmed sequences to a draft reference genome with 34 scaffolded contigs (G. Molano, *pers. comm.*) using the program BWA (Li and Durbin 2009), and we evaluated and formatted the mapped sequences using *SAMtools* (http://samtools. sourceforge.net/; Li et al. 2009). We used *BCFtools* (Danecek et al. 2021) to call variants on each individual and merge the variant call files (VCF), and we used *Plink* (Purcell 2007) to filter the VCF. We visualized the evolutionary history of the divergence among groups using *SNPRelate* (Zheng et al. 2012) that produced a dendrogram based on SNP dissimilarity and hierarchical cluster analysis. We analyzed variants to explore population structure and genetic clustering using a

principal components analysis (PCA) on the variants of all 100 samples using eigenvectors produced in *Plink* with commands --make-bed (which creates a binary fileset) and --pca. We also analyzed variants of the Northern Hemisphere and central California subgroups separately and used PCA on each of these groups. We then identified genetic clusters with *ADMIXTURE* (Alexander et al. 2009) and evaluated the most supported number of clusters based on cross-validation (CV) errors with *ADMIXTURE* using K-means.

Results

Morphological characteristics and depth

For Southern Hemisphere samples, the holdfasts of *pyrifera* were on average more symmetrical than *integrifolia*, as the mean ratio of the longest to shortest dimensions of *pyrifera* holdfasts (mean=1.31 ± 0.28) was smaller than that of *integrifolia* (mean=5.92 ± 0.37) (Mixed effects ANOVA, $F_{(1, 6.9)}$=11.452, p<0.0120). Number of branches for *integrifolia* was always 1, whereas the average number of branches for pyrifera was 3.1±0.12. There was no variance in number of branches within *integrifolia* (all individuals had 1 branch point). The mean number of branches for *pyrifera* was 3.1 ± 1.5, and since the 95% confidence interval (upper 95%=3.5; lower 95%=2.8) does not include 1, the number of branches were significantly different between the morph groups.

The average depth below the MLLW was not significantly different between *integrifolia* and *pyrifera* populations based on a mixed effects ANOVA. Individuals of both morphs were found as shallow as 1 m above MLLW.

Genetic analyses

A dendrogram with all samples based on SNP dissimilarity showed the southern California populations as an outgroup, with the rest of the populations separated first by a divergence between the Northern and Southern Hemispheres, and then a divergence between the *pyrifera* and *integrifolia* ecomorphs within each hemisphere (Figure 1.4).

The PCA retained 109,441 SNPs after pruning and showed that the most explanatory principal component (PC1) discriminated between all samples in the Northern versus Southern Hemisphere, and PC2 discriminated between the two morphs in the Southern Hemisphere (Figure 1.5, top). PC3 separated the southern California populations (AQ, CI, and CB) from the other Northern Hemisphere populations and also separated the southernmost population in the Southern Hemisphere (DAL) from all other Southern Hemisphere populations (Figure 1.5, bottom). The two morphs in the Northern Hemisphere did not separate along any PC in the analysis with all 100 samples. When Northern Hemisphere samples were analyzed separately in a new PCA, PC1 separated southern California from central California populations, and PC2 showed greater divergence among southern California populations than between morphs (Figure 1.6, top). When the southern

California populations were removed and central California samples were analyzed on their own in a third PCA, PC1 distinguished *integrifolia* from *pyrifera* individuals, even when from the same location at STL (Figure 1.6, bottom).

From the Admixture analysis, the most supported cluster grouping was K=2, and K=3 was also well supported (Figure 1.7). When Southern Hemisphere samples were analyzed separately, K=2 was the most supported clustering, separating all *integrifolia* samples from all *pyrifera*. When Northern Hemisphere samples were analyzed separately, K=1 was the most supported clustering, and when central California samples were analyzed separately the most supported clustering was K=2, distinguishing *integrifolia* at STL from *pyrifera* at both STL and CAY.

Discussion

Our analysis based on whole-genome sequencing across 18 *Macrocystis* populations demonstrates that the two ecomorphs of giant kelp, *Macrocystis "pyrifera"* and *M. "integrifolia,"* are genetically distinct. The most important genetic break among the populations in the Southern Hemisphere coincides with the geographic distributions of the ecomorphs, and in the Northern Hemisphere adjacent populations of *pyrifera* and *integrifolia* are as genetically distinguishable as distant populations of the same *pyrifera* ecomorph. By including the entire genome and populations from both Northern and Southern Hemispheres, the present study elucidates genetic divergence between the two ecomorphs that was not apparent in previous studies using microsatellites or specific markers (Coyer et al. 2001,

Salavarria et al. 2018). Our analyses showed that the largest genetic divergence among all samples was between the Northern and Southern Hemispheres, with subsequent divides by ecomorph. We provide evidence that *Macrocystis* in the Northern Hemisphere is genetically distinct from Southern Hemisphere populations, and that the *pyrifera* and *integrifolia* morphs are currently undergoing speciation in both the Northern and Southern Hemispheres in a likely case of convergent evolution.

As both an ecologically and economically important species, *Macrocystis* has been well-studied in terms of geographic distribution, morphological variation, and ecophysiology; yet the evolutionary history of the different ecomorphs and the interaction between genotype and phenotype remained unclear prior to this study. Among the many prior phylogenetic and biogeographic studies of *Macrocystis*, several theories on the origins of the species and its present-day ecomorphs have been proposed (Astorga et al. 2012, Lindberg 1991, Chin et al. 1991) and a clear consensus has yet to be reached. The most prominent hypothesis is that present-day *Macrocystis* ecomorphs originated from a *pyrifera* morph ancestor in the Northern Hemisphere and later migrated to the Southern Hemisphere (Coyer et al. 2001, Macaya & Zuccarello 2010b, Astorga et al. 2012). Our analyses offer new information by identifying specifically a southern California *pyrifera* origin, and indicating the independent evolution of *integrifolia* in each of the Northern and Southern Hemispheres. The evolutionary forces underlying the emergence of the *integrifolia* morph in both hemispheres and its non-mirrored distribution across the equator remain unclear.

The genetic clusters most supported by the Admixture analysis correspond to all Northern Hemisphere individuals and all Southern Hemisphere individuals (K=2); and Southern Hemisphere *pyrifera*, Southern Hemisphere *integrifolia*, and Northern Hemisphere *pyrifera* and *integrifolia* (K=3). Other supported groupings (K=4) revealed separation between central California and southern California in the Northern Hemisphere. Additionally, when Southern Hemisphere samples were analyzed separately the most supported clustering in the Southern Hemisphere was K=2 and corresponded to the *pyrifera-integrifolia* morph geographic boundary, similar to the analyses by Camus et al (2018a). However, in the Northern Hemisphere the most supported clustering was K=1. This indicates that the inter-morph divergence in the Northern Hemisphere, evident in the dendrogram and PCA outputs, follows a different evolutionary trajectory than the inter-morph divergence in the Southern Hemisphere, possibly caused by differences in the alleles involved, history of gene flow, or forces of selection.

The PCA mirrors the findings of the Admixture analysis, revealing that the most explanatory principal component (PC1) discriminates between the Northern and Southern Hemispheres, in concordance with previous research (e.g. Astorga et al. 2012), and the second most explanatory principal component (PC2) separates the two morphs in the Southern Hemisphere. Notably, the genetic separation on PC1 between southern California and central California populations (Figure 1.5, top), covering less than 4 degrees of latitude, is greater than the separation between any Southern Hemisphere populations across 14 degrees of latitude. The high genetic diversity

among Northern Hemisphere sites, especially in southern California, dwarfs the inter-morph differences, and may account for why previous genetic studies using COI (Macaya & Zucarello 2010a, 2010b) or ITS regions (Astorga et al. 2012) were not able to resolve clades that distinguish the two ecomorphs in the Northern Hemisphere. Our study found that after removing the wide genetic diversity encompassed by the southern California populations, *pyrifera* from Stillwater Cove were more genetically similar to a distant *pyrifera* population (CAY) than to *integrifolia* from Stillwater Cove. Even when all Northern Hemisphere populations were included in the PCA, the *integrifolia* population at STL can be identified as a distinct population even though it is geographically less than 100 m from the *pyrifera* STL population. While the genetic divergence between the two morphs in the Southern Hemisphere is consistent across a wide latitudinal range, the evolutionary history of the morphs in the Northern Hemisphere is more complex, perhaps due to a longer history of *Macrocystis* and of *pyrifera* in particular compared to the Southern Hemisphere.

Based on our dendrogram, a group of Northern Hemisphere *pyrifera* populations (southern California) were ancestral to all other populations, and the divergence between central California and Southern Hemisphere populations occurs before the divergences between *pyrifera* and *integrifolia* populations within each hemisphere. This indicates that the *integrifolia* morph evolved independently in each hemisphere. Previous research has shown that the ancestral *Macrocystis* haplotype corresponding to the *pyrifera* morph in Chile is also present in populations in

northern and central Peru, and that higher haplotype diversity in Peru compared to Chile suggests dispersal from the Northern to the Southern Hemisphere (Salavarria et al. 2018). It is possible that *Macrocystis* originated in southern California and this source population dispersed northward up the coast of California as well as southward to South America. The separation of DAL from other *pyrifera* populations in the Southern Hemisphere is in agreement with findings from Macaya & Zucarello (2010b), where populations on the west coast of Chiloe Island and further south had a distinct haplotype from other *pyrifera* populations on the east coast of Chiloe and northward. While previous studies suggest genetic haplotype groupings that tend to align with purported ecomorph populations, our study is the first to create a dendrogram using individuals that have been explicitly classified as either *pyrifera* or *integrifolia* in the field based on morphology. Our dendrogram aligns with the genetic groupings identified in the PCA and Admixture analyses (i.e. Northern Hemisphere *Macrocystis*, Southern Hemisphere *integrifolia,* and Southern Hemisphere *pyrifera*), but by incorporating all PCs it provides additional information about these groups originating from a set of the southern California *pyrifera* populations.

While some research proposes that all *Macrocystis* arose from an ancestral *pyrifera* morph (Astorga et al. 2012), other research presents an alternative perspective on the evolutionary history of *Macrocystis* ecomorphs. Chin et al. (1991) propose that *Macrocystis* originated from a polymorphic complex distributed globally, and that vicariant events explain the present-day biogeography of the species. Results from our study demonstrate that there are fixed morphological

differences, including holdfast diameter dimensions and number of branches, between *pyrifera* and *integrifolia* as well as clear genetic differentiation. Furthermore, when the two morphs were grown in co-culture and outplanted to the ocean, no morphological hybrids emerged (see Chapter 2). Therefore, it may be appropriate to classify these two morphs as separate species.

The existence of distant distributions of *pyrifera* and *integrifolia* with similar phenotypes in both the Northern and Southern Hemispheres could be explained by two processes: 1) each morph evolved once, most likely in the Northern Hemisphere, and then dispersed to the Southern Hemisphere, or 2) the morphs evolved separately in each hemisphere (convergent evolution). If the first process were true, we would expect to see greater genetic similarity within morphs—regardless of hemisphere—than between morphs, especially since the present study considers the whole genome including genes responsible for adaptive phenotypes. However, the results of our study reveal that different ecomorph populations within a hemisphere are more genetically similar than populations of the same ecomorph from different hemispheres. This indicates that within each hemisphere, a common ancestor led to the evolution of the two ecomorphs, whose morphologies across hemispheres are identical via convergent evolution. Further research is needed to understand the genes responsible for the phenotypes in both hemispheres and the underlying genetic causes of the convergence, which could arise from adaptive mutations that occurred in populations in both hemispheres (parallel evolution) or evolution of a shared ancestral polymorphic allele (Stern 2013).

The environmental factors that have selected for either *pyrifera* or *integrifolia* characters over evolutionary time remain unclear, and future studies are needed to investigate the factors driving the convergent evolution of *Macrocystis* morphologies in the Northern and Southern Hemispheres. Temperature is an unlikely driving factor given the incongruous latitudinal distributions of *pyrifera* and *integrifolia* in the Northern and Southern Hemispheres. Previous research has indicated that wave intensity may induce a plastic response in certain traits such as blade morphology; *Macrocystis* individuals from distinct environments transplanted to a common site converged in blade morphology over time (Druehl and Kemp 1982). However, those authors did not measure holdfast morphology which would be more indicative of ecomorph characterization. Globally, *pyrifera* tends to be found deeper than *integrifolia,* which is generally considered intertidal (Graham et al. 2007). However, in this study there was no difference in depth between the morphs, and there is empirical evidence that *integrifolia* maintains its holdfast morphology even when grown from embryonic sporophytes at 9 m depth (see Chapter 2). In Chile, the regions between 30-33 ºS and 40-42 ºS have been previously described as important biogeographic boundaries (Meneses et al. 2000, Camus et al. 2001) across which many marine species have genetic discontinuities (Haye et al. 2014). These breaks correspond to the clustering of Southern Hemisphere populations of *Macrocystis* found in the present study, but the ecomorph divergence in the Northern Hemisphere does not come from a clear geographic boundary. The specific natural selection

forces that favor either the *pyrifera* or *integrifolia* morph, and whether or not these factors are the same in both hemispheres, warrants further research.

Acknowledgements

I would like to thank Dr. Filipe Alberto and Gary Molano for providing access to their draft *Macrocystis* genome, supplying genetic data from kelp samples, and assisting with analyses; Kersten Schnurle for assisting with diving and sample collections; Dr. Sylvain Faugeron for assisting with dive site selection and transport and storage of samples, and advising genetic analyses; Dr. Kathleen Kay and Dr. Rachel Meyer for providing equipment and lab space for DNA extractions, and for preliminary discussions of genetic analyses which informed sampling design; and Dr. Giacomo Bernardi and Dr. Remy Gatins for consultations regarding bioinformatics.

Tables and Figures

Table 1. Kelp collection site information.

Site name	Site abbreviation	Coordinates	Hemisphere	Morph	Collected by
Stillwater Cove	STL	36.565058, -121.943553	North	Pyrifera and Integrifolia	S. T. Gonzalez
Cayucos	CAY	35.446456, -120.932394	North	Pyrifera	S. T. Gonzalez
Arroyo Quemado	AQ	34.47, -120.119	North	Pyrifera	F. Alberto
Catalina Island	CI	33.462, -118.511	North	Pyrifera	F. Alberto
Camp Pendleton	CB	33.157, -117.36	North	Pyrifera	F. Alberto
Playa Blanca	PLB	-28.1851111, -71.1651667	South	Integrifolia	S. T. Gonzalez
Punta Choros	PCH	-29.245712, -71.466174	South	Integrifolia	S. T. Gonzalez
Totorolillo	TOT	-32.022839, -71.508658	South	Integrifolia	S. T. Gonzalez
Las Docas	DOC	-33.139851, -71.708218	South	Integrifolia	S. T. Gonzalez
Algarrobo	ALG	-33.360022, -71.668728	South	Integrifolia	S. T. Gonzalez
Las Monjas	MON	-33.492179, -71.642485	South	Integrifolia	S. T. Gonzalez
La Boca	BOC	-33.903175, -71.834772	South	Pyrifera	S. T. Gonzalez
Tumbes	TUM	-36.623731, -73.093019	South	Pyrifera	S. T. Gonzalez

Chome	CHO	-36.806467, -73.177088	South	Pyrifera	S. T. Gonzalez
Bahía Mansa	BAM	-40.581556, -73.738197	South	Pyrifera	S. T. Gonzalez
Ancud	ANC	-41.865314, -73.831927	South	Pyrifera	S. T. Gonzalez
Dalcahue	DAL	-42.382756, -73.655023	South	Pyrifera	S. T. Gonzalez

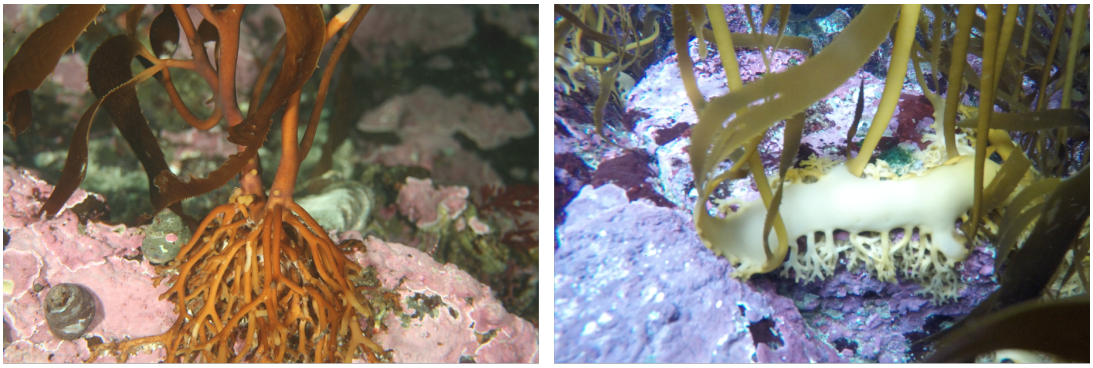


Figure 1.1. Representative photographs of *pyrifera* (left) and *integrifolia* (right). Left photo by Sam Richardson; right photo by S. T. Gonzalez.

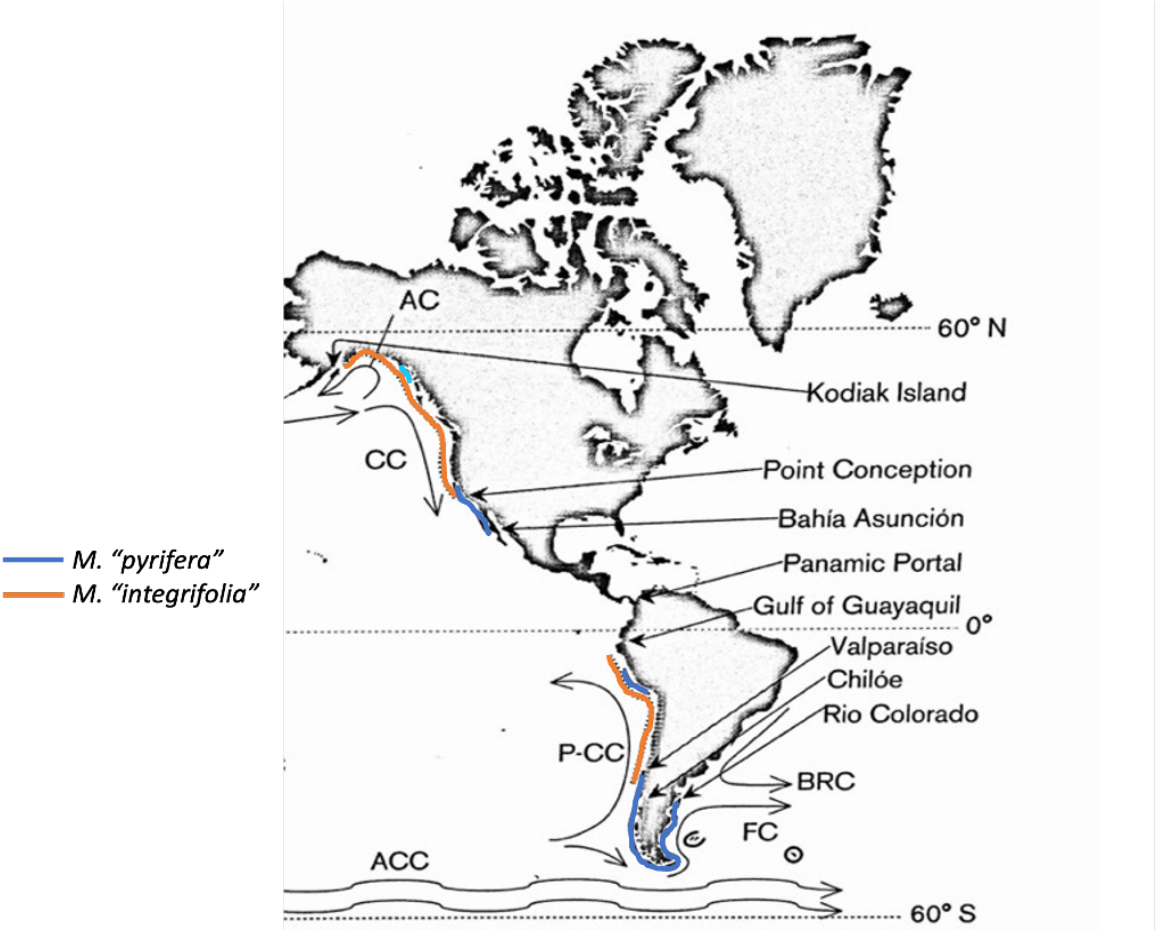


Figure 1.2. Map of *Macrocystis pyrifera* (blue) and *M. integrifolia* (orange) distribution in the Western Hemisphere. Adapted from Schiel & Foster (2015) with an additional *pyrifera* population (light blue) added in Sitka, Alaska (P. Raimondi, *pers. comm.*).

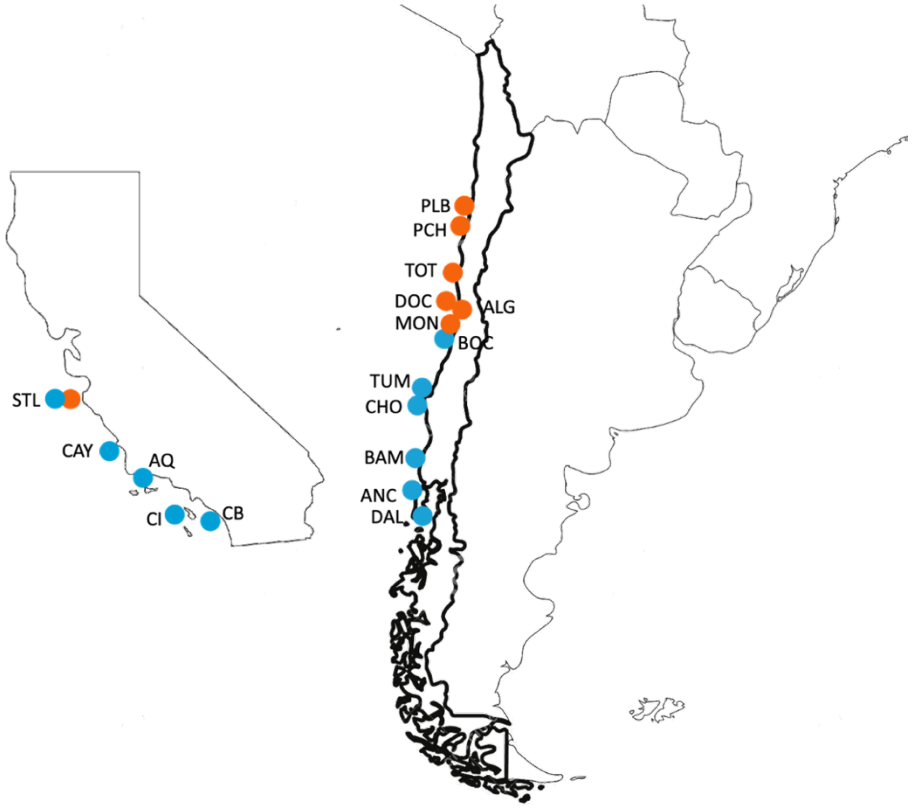


Figure 1.3. Map of populations sampled in the present study in California (left) and Chile (right). *Integrifolia* populations are in orange; *pyrifera* are in blue.

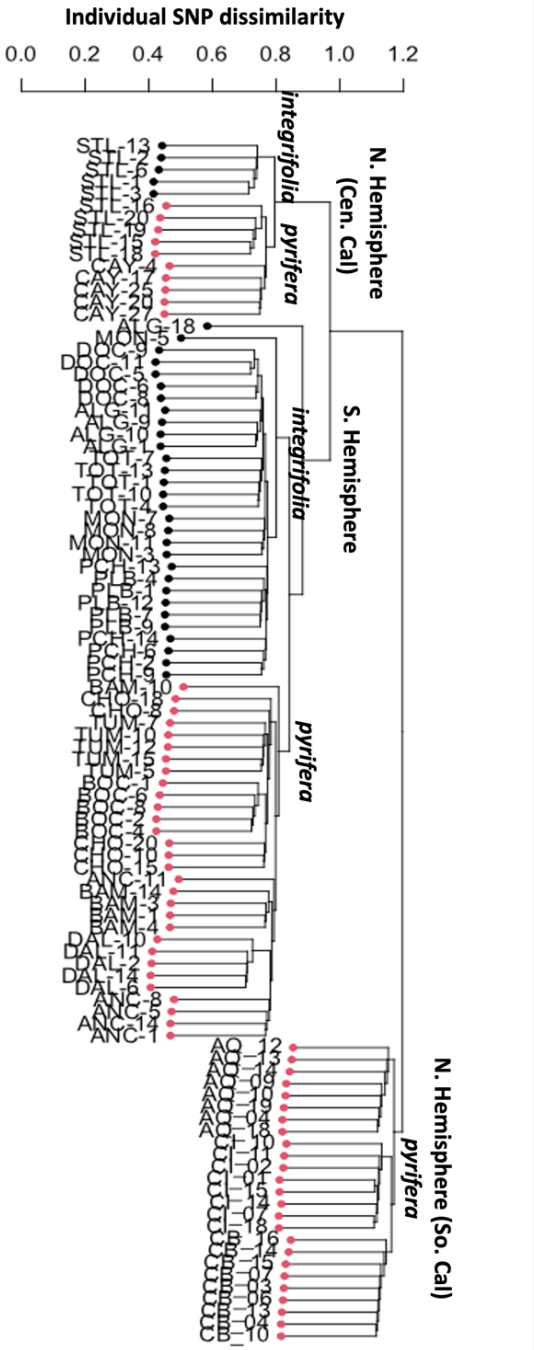


Figure 1.4. Dendrogram based on individual SNP dissimilarity for all samples. Z scores greater than 15 are displayed. *Pyrifera* samples are colored in red; *integrifolia* are colored in black.

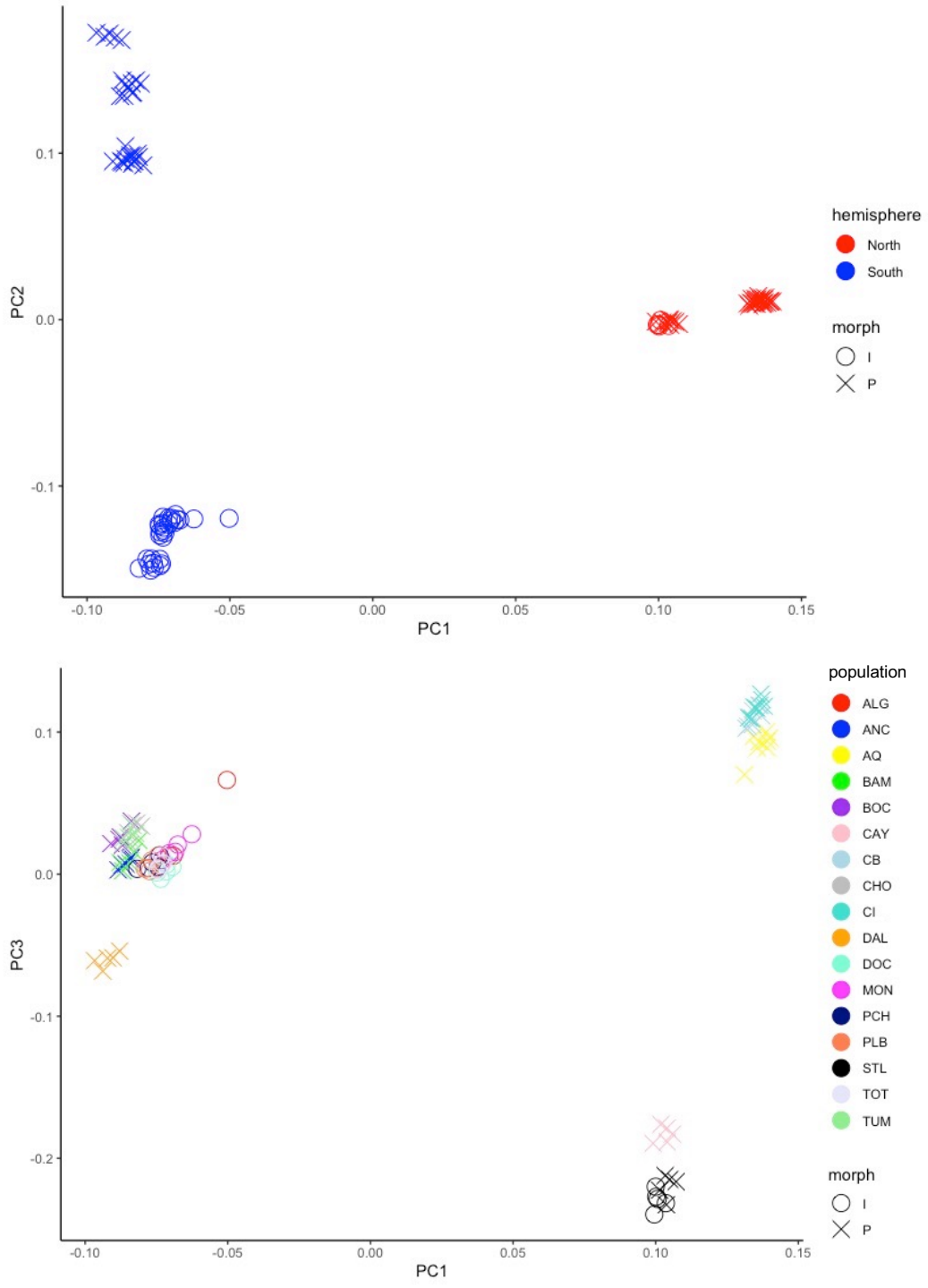


Figure 1.5. Principal component analysis for all samples. PC 1&2 (top) and PC 1&3 (bottom).

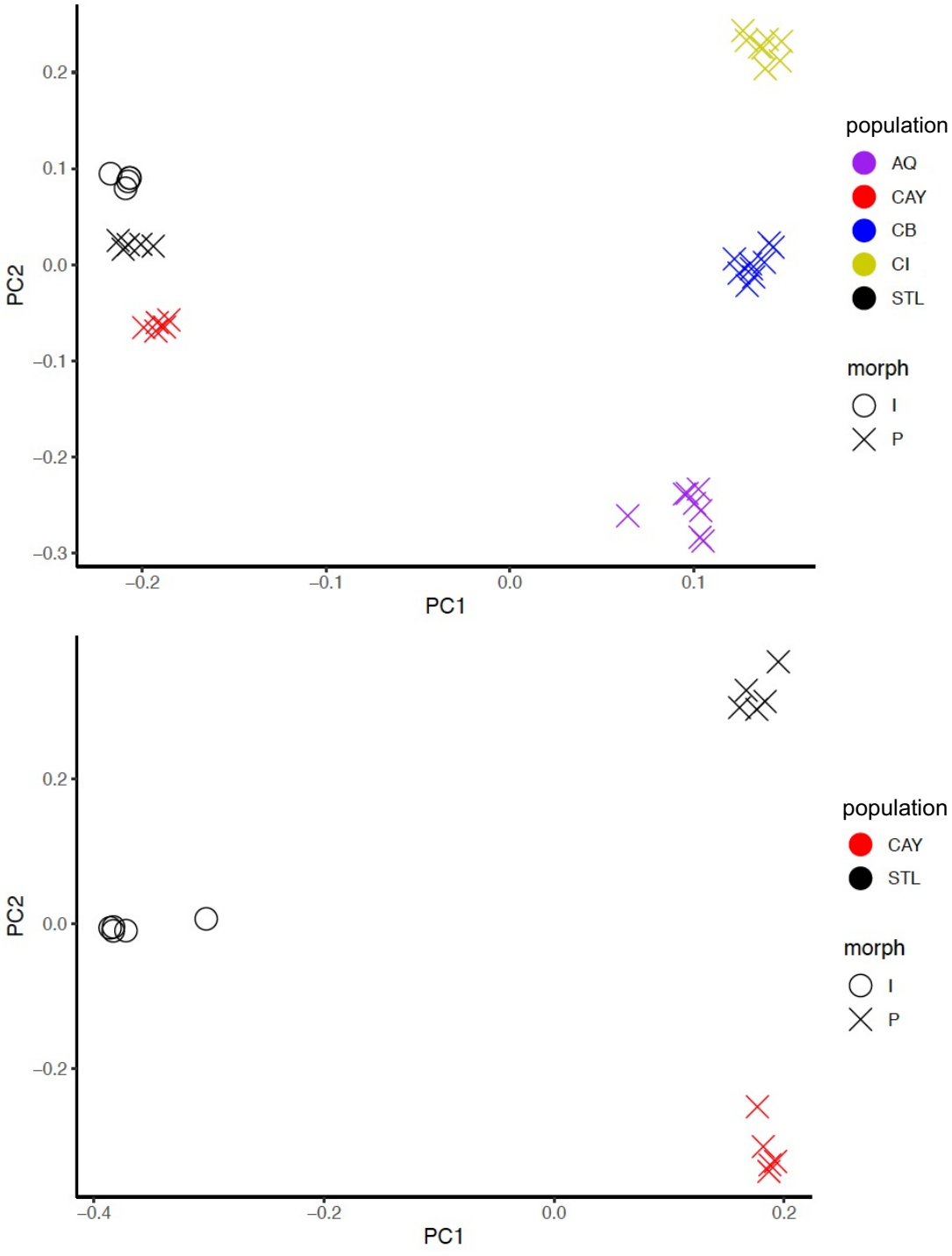


Figure 1.6. Principal component analyses for all Northern Hemisphere populations (top) and for central California populations only (bottom).

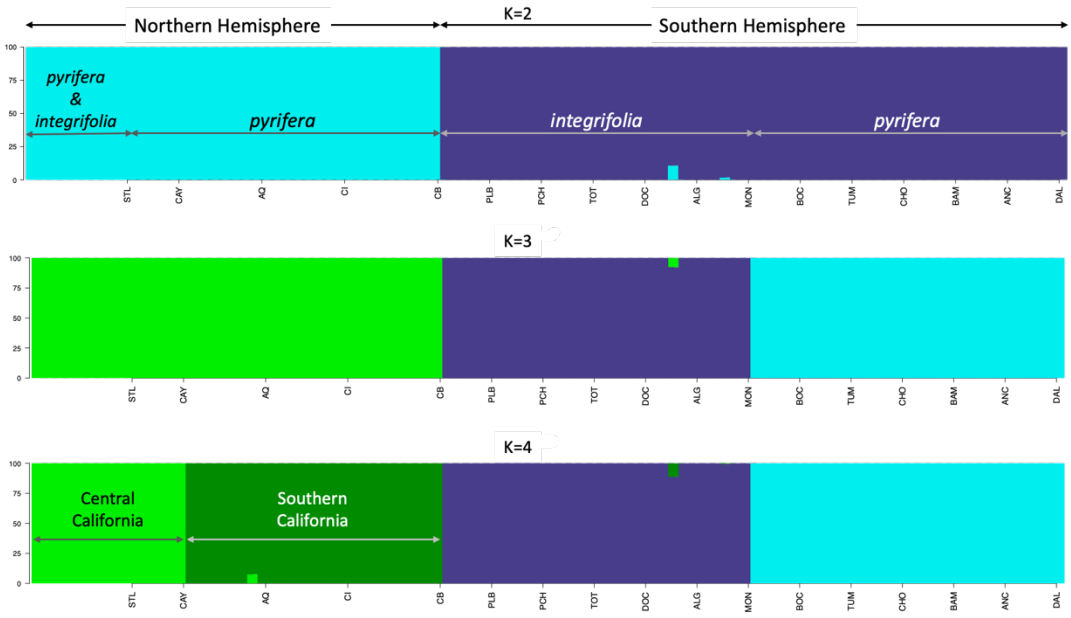


Figure 1.7. ADMIXTURE clustering for all 100 samples using K=2, K=3, and K=4 clusters. Important geographic groups include Northern Hemisphere, Southern Hemisphere, and southern California (AQ, CI, and CB). The most supported is K=2 clusters (top panel).

CHAPTER 2

Experimental assessment of environmental versus genetic influences on *Macrocystis* morphology

Abstract

The giant kelp, *Macrocystis pyrifera,* exists as distinct morphological variants—or "ecomorphs"—yet the mechanism for this variation is uncertain, and whether these morphological features are genetically fixed or malleable under different environmental conditions is unclear. The two most common ecomorphs, *Macrocystis pyrifera* and *M. integrifolia*, exhibit almost no geographic overlap across their range in temperate waters of North and South America, with few exceptions such as Stillwater Cove, CA; however, in laboratory settings they are able to interbreed. Our study for the first time directly compared the growth, development, and morphological transformations of all life stages of the two *Macrocystis* ecomorphs in a common garden experiment. We experimentally tested the influence of local environment on giant kelp morphology by rearing lab-cultured embryonic sporophytes from spores released by *M. pyrifera* and *M. integrifolia* sporophylls collected from multiple individuals at Stillwater Cove, CA. The spores were cultured in three treatments: *pyrifera* only, *integrifolia* only, and mixed (50:50 *pyrifera*:*integrifolia* spores). We outplanted the resultant embryonic sporophytes to concrete blocks installed at 7.5-9 m depth in the ocean and monitored the development of the sporophytes over five months. Our finding of distinct differences in morphology between the pyrifera and integrifolia treatments at multiple stages of development including reproductive adults, indicates that the morphological differences between the two ecomorphs are genetically determined rather than environmentally induced. We found that primary stipe length and number of branches

can be used as diagnostic traits for distinguishing the ecomorphs prior to the stage when adult sporophyte morphology can be definitively characterized. Additionally, no morphological hybrids were observed in the mixed treatment, and ultimately the mixed individuals were more often categorized as *integrifolia*-like than *pyrifera*-like.

Key words: common garden; ecomorph; kelp; *Macrocystis integrifolia*; *M. pyrifera*; morphology; outplant

Introduction

Giant kelp, *Macrocystis pyrifera*, is one of the most widespread kelp species in the world, with populations occurring in the Americas, Africa, Australia, New Zealand, and the sub-Antarctic islands. Much of its range occurs along the west coast of the Americas in temperate waters with sufficient nutrients (Macaya & Zucarello 2010a, Schiel & Foster 2015). Stark morphological differences among giant kelp populations have been observed for nearly two centuries, spurring much debate and re-classification of populations as distinct species or merely alternative forms of the same species.

Presently, four morphological variants—or "ecomorphs"—of the single species, *M. pyrifera,* are recognized globally: *M. "pyrifera," M. "integrifolia," M. "angustifolia,"* and *M. "laevis."* The ecomorphs are distinguished by characteristics of the holdfast and blade (Howe 1914, Womersley 1954, Hay 1986). Although all

four ecomorphs are currently considered the same species (Coyer et al. 2001, Macaya & Zucarello 2010a), the morph variants are still used in the literature (e.g. Schiel & Foster 2015). Hereafter, "*Macrocystis"* refers to the species as a whole, and ecomorphs are referred to by only their specific epithets. Of the four globally recognized ecomorphs, *pyrifera* and *integrifolia* have the widest distributions, while *angustifolia* is restricted to South Africa and Australia, and *laevis* is found only on the Marion Island, Prince Edward Islands (Perissinotto & McQuaid 1992), and one region in southern Chile (Aguilar-Rosas et al. 2003), which has been contested as a mis-identification of *pyrifera* (Gutierrez et al. 2006). The two most common ecomorphs, *pyrifera* and *integrifolia*, differ in their holdfast morphology; *pyrifera* is characterized by having a conical, mound-shaped holdfast while *integrifolia* has a flattened, strap-like holdfast (Figure 2.1). Historically, the morphs were molecularly indistinguishable based on analyses of internal transcribed spacer regions (Coyer et al. 2001); however, recent work using a whole-genome approach found clear genetic differentiation between *pyrifera* and *integrifolia* (see Chapter 1). The mechanisms underlying phenotypic variation among the ecomorphs remain unknown.

Macrocystis is believed to have originated from a *pyrifera* morph ancestor in the Northern Hemisphere, likely on the northwest Pacific coast, which then later colonized the southeast Pacific via rafting, ultimately extending range throughout the Southern Hemisphere (Coyer et al. 2001, Macaya & Zuccarello 2010a, Astorga et al. 2012). *Macrocystis*, like all kelps, exhibits a biphasic life cycle involving a diploid sporophyte and haploid gametophyte stage (Schiel & Foster 2015). Adult sporophytes

release microscopic spores produced by meiosis, which develop into either male or female gametophytes. Under proper environmental conditions, fertilization occurs and the resulting zygote develops into a juvenile sporophyte (reviewed in Schiel & Foster 2015). The ability for *Macrocystis* to extend its range via rafting likely enabled its recolonization of subantarctic islands following elimination by ice scour during the last glacial maximum (Macaya & Zuccarello 2010b).

The geographic distributions of the *integrifolia* and *pyrifera* ecomorphs are parallel across the equator rather than mirrored, as would be expected based on latitudinal gradients of most physical and oceanographic factors. Based on the literature, in the Northern Hemisphere *integrifolia* ranges from mid-high latitudes and *pyrifera* from mid-low latitudes, but in the Southern Hemisphere *pyrifera* generally occurs at higher latitudes while *integrifolia* dominates at lower latitudes (Figure 2.2; Graham et al. 2007, Macaya & Zuccarello 2010a, Schiel & Foster 2015). However, populations of *pyrifera* in the Southern Hemisphere have been observed as far north as Peru, and *integrifolia* populations have been observed in the Interior Sea of Chiloe in southern Chile (Camus et al. 2018a, S. Faugeron *pers. comm.*). The two morphs are almost never found in the same location, except for a couple of sites in California and possibly Peru (Graham et al. 2007). Stillwater Cove, California, is one of the rare locations where *pyrifera* and *integrifolia* have been found in sympatry (Graham et al. 2007, Hargarten 2015, Jeffries 2015), although the ecomorphs are separated by depth. While no sites of co-occurrence of the two morphs in the Southern Hemisphere have been explicitly described, the existence of such sites is plausible as genetic haplotypes

based on microsatellites that tend to correspond with either *pyrifera* or *integrifolia* in Chile can be found co-occurring at sites in Peru (Salavarria et al. 2018).

The evolutionary history of the four ecomorphs (*pyrifera, integrifolia, angustifolia,* and *laevis*) has been explored by multiple groups of researchers (see below), and the current theory considers *Macrocystis* as a single species because different ecomorph groups are not more genetically distinct than populations within a single ecomorph. Based on noncoding rDNA internal transcribed spacer regions (ITS1 and ITS2) the largest genetic break within *Macrocystis* is between populations from the Northern versus Southern Hemisphere, regardless of ecomorph (Coyer et al. 2001). Furthermore, ecomorph groups were found to be paraphyletic—populations of *pyrifera* within the same region in California were more genetically distinct from each other than were Southern Hemisphere populations of *pyrifera* and *angustifolia* separated by a distance two orders of magnitude larger (Coyer et al. 2001). The low genetic divergence among Southern Hemisphere populations may be attributed to connectivity via floating kelp moved by the Antarctic Circumpolar Current (Macaya & Zuccarello 2010b). A phylogeny developed by Astorga et al. (2012) revealed genetic separation between *pyrifera* and *integrifolia* morphs from the Chilean coast; however, this trend was not consistent in the Northern Hemisphere. These results suggest that either the *integrifolia* morph evolved independently in the Northern and Southern Hemispheres, or that ecomorphology is a plastic response to unidentified environmental drivers that are present in both hemispheres.

The conceptual homogenization of the four putative *Macrocystis* species into a single species began with genetic crossing experiments which showed that all putative species, except *M. laevis* which was not tested, were interfertile (Lewis et al. 1986, Lewis & Neushul 1994, Westermeier et al. 2007). Additionally, *pyrifera-integrifolia* hybrid individuals can produce fertile progeny (Murúa et al. 2021). While no quantitative information regarding relative fertility between intra- and inter-morph crosses has been reported, researchers note that the crops of sporophytes were "indistinguishable" between the two cross types (Westermeier et al. 2007) and that sporophytes of hybrid crosses "grew normally" (Lewis et al. 1986). The single-species view was further supported when Coyer et al. (2001) showed that, using internal transcribed spacer (ITS) sequences ITS1 and ITS2, it was not possible to differentiate between *M. integrifolia* and *M. pyrifera*. Controversy remains within the literature regarding whether ecomorphology in *Macrocystis* is an environmentally induced or a genetically fixed trait.

Previous research has shown evidence for environmentally-induced plasticity as the basis for general morphological differences among *Macrocystis* populations. Wave exposure and depth are believed to be the primary environmental variables responsible for morphological variation. When *integrifolia* individuals from four sites in Canada with different wave conditions were transplanted to an intermediate site, they converged on similar blade morphology and biomass over one year (Druehl & Kemp 1982). In the Northern Hemisphere, the *integrifolia* morph is typically found at shallower depths (0-10 m) toward the low intertidal, whereas *pyrifera* usually occurs

at deeper depths (4-70 m) (Graham et al. 2007). Based on this trend, Demes et al. (2009) argued that holdfast morphology was dependent on the depth at which the sporophyte develops, so *Macrocystis* should be a monospecific genus with distinct "ecotypes." The authors claimed that an individual sporophyte will follow one of three depth-dependent developmental routes toward a morphology resembling *M. pyrifera, M. integrifolia,* or *M. angustifolia*. Notably, Demes et al. (2009) chose the term "ecotype" to describe these different forms, although this is inconsistent with definitions of the term in which morphological attributes are hereditary, genotypic responses to the selective action of the environment (Turesson 1922, Gregor 1944). Contrary to the depth-dependent hypothesis, in the Southern Hemisphere, *integrifolia* can be found in the subtidal and intertidal, and among Chilean populations the maximum depth recorded of *integrifolia* is deeper than the maximum depth of *pyrifera* (reviewed in Graham et al. 2007).

The view that phenotypic plasticity induced by certain environmental conditions is primarily responsible for ecomorphological variation is supported by studies which found genetic similarity between ecomorphs, with a focus on the two most predominant ecomorphs, *pyrifera* and *integrifolia*. Based on molecular analyses of internal transcribed spacer sequences, *integrifolia* and *pyrifera* populations in California could not be genetically distinguished (Coyer et al. 2001). Using the DNA barcoding gene cytochrome c oxidase subunit I (COI) as a standardized marker for species ID in macroalgae, Macaya & Zuccarello (2010a) showed that adjacent ecomorph populations of *integrifolia* and *pyrifera* in Stillwater Cove, CA, shared the

same haplotype. Following this lack of genetic distinction between the two ecomorphs, a likely explanation for the morphological differences would be due to plasticity based on the different environments from which they were collected (intertidal versus subtidal). The authors noted that the low genetic variation across the world and shared haplotypes suggested that all ecomorphs have a very recent common ancestor, rapid dispersal, and/or high levels of gene flow between populations, and this suggests that the differences among morphs are due to local environmental influences (Macaya & Zuccarello, 2010a). However, it is difficult to make conclusions about plasticity based on genes that are not under selection, such as COI. A whole-genome approach would be more appropriate for identifying whether there are adaptive genes responsible for morphological differences that can discriminate between the two morphs (see Chapter 1).

Other research shows evidence for natural selection on genotypes as the basis for morphological differences. Kopczak et al. (1991) found that geographically isolated populations of *Macrocystis* have undergone genetic divergence that can be explained by ecotypic adaptation. Brostoff (1988) showed that transplants of standing *angustifolia* thalli and control transplants of *pyrifera* thalli into a population of *pyrifera* at Point Loma, CA, each resembled their respective natural populations in basal stipe and holdfast appearance. These results give insight into the lack of plasticity in morphology of established individuals but leaves the developmental paths of the two ecomorphs unresolved. In their seascape genetics study of Northern Hemisphere *Macrocystis,* Johansson et al. (2015) found that genetic clusters were

correlated with environmental factors, and the light extinction coefficient was the strongest environmental factor associated with co-ancestry clusters. Light intensity or related factors may be selective drivers of morphological adaptations. A genetic study using microsatellite loci found that *pyrifera* populations in geographically distinct areas were more genetically similar to each other based on F_{ST} values than were *pyrifera* and *integrifolia* populations occurring in the same area (Hargarten 2015). This suggests that morphology may be genetically controlled. Along the southern coast of South America, populations of *Macrocystis* were clustered both genetically and morphologically by geographic region, suggesting that environmental conditions are driving evolutionary divergence between regions (Camus et al. 2018a). However, the morphological groups were distinguished by features such as total length, weight, and number of blades, rather than the holdfast metrics which characterize ecomorphs. While these studies provide evidence that the two morphs are genetically isolated and that genetic clusters mirror morphological ones, they lack the empirical evidence to conclude whether morphology is genetically determined. Without a field experiment to test the effect of a common environment on the morphological development of both morphs, it is not possible to determine to what extent genetic versus environmental factors control morphology in *Macrocystis*, and whether these patterns and processes are consistent across hemispheres.

One set of intriguing but limited results come from laboratory experiments, which have demonstrated that *pyrifera* and *integrifolia* can hybridize (Lewis and Neushul 1994, Westermeier et al. 2007, Murúa et al. 2021), and the resulting holdfast

morphology was a combination of *pyrifera*- and *integrifolia*-type characters (Westermeier et al. 2007). These results suggest that there may be a genetic basis for the distinct ecomorphologies. However, the specific nature of the morphology and stipe branching pattern of the hybrids are unclear, and the experiment used gametophyte clones from only one male and one female individual of each morph type, making it difficult to establish a pattern of hybrid morphology. Murúa et al. (2021) showed that hybrids of *pyrifera* and *integrifolia* can produce fertile offspring which may perform better than their parents in harsh conditions of high temperature and light. Importantly, the crosses between female *integrifolia* and male *pyrifera* were not viable (Murúa et al. 2021). If hybrid crosses are only half as successful as pure crosses then this could contribute to selection against hybrids in the wild, except perhaps in areas with harsh environmental conditions. Given the ability for *pyrifera* and *integrifolia* to hybridize in the lab, and that in some situations hybrids may perform better than pure crosses, it is perplexing that hybrids have rarely been documented in the field. One way to resolve this is to create a co-culture of males and females from both ecomorphs and examine the rate of hybrid fertilization and survival compared to that of pure crosses in a competitive context.

The aim of the present study was to compare 1) survival and development of morphological features of *pyrifera* and *integrifolia* lab-reared cultivars subsequently grown in identical field conditions, and 2) survival and development of morphological features of a mixed *pyrifera* and *integrifolia* culture compared to pure-morph cultures. If morphology is controlled by genotype, then genetically distinct

sporophytes will retain their parental morphology regardless of environmental conditions. Alternatively, if morphological differentiation is due to plasticity, with differential gene expression induced by environment, then the morphology of kelp outplants derived from different parental ecomorphs will conform to the same morphology when grown in the same environment. Regarding *pyrifera-integrifolia* hybrids, we hypothesize that the two morphs will be interfertile and able to produce a hybrid sporophyte, but hybrids will be outcompeted by intramorph sporophytes grown in co-culture. This study was the first to test the influence of local environment on morphology of *Macrocystis* sporophytes derived from known parental ecomorphs, and to test how hybrid individuals fare in competition with "pure" ecomorph crosses. We identified distinguishing features of *Macrocystis* ecomorphs at multiple developmental stages, providing evidence for genetically based morphological differences.

Methods

Site selection for sporophyll collection and sporophyte outplanting

Parent sporophylls for the treatment crosses were collected from kelp at Stillwater Cove, CA (Figure 2.3, "sporophyll collection site"). Stillwater Cove is one of the only locations in the world where both *pyrifera* and *integrifolia* occur in adjacent populations. By using sporophylls from these populations, we minimized confounding factors such as differing environmental conditions that would influence the quality of the spores.

After culturing the three treatments in the lab (see below), the sporophytes were outplanted off the coast of Del Monte Beach, Monterey, CA (Figure 2.3, "outplanting site"), which is approximately 6.5 km from Stillwater Cove and is similarly wave-protected, has a sandy bottom substrate suitable for installing the outplanting substrate (concrete parking bumpers), and did not have any existing wild stands of kelp. A lack of existing kelp ensured that our outplant surfaces would not become contaminated with spores from other standing kelp populations and that our outplanted kelp would not be influenced by chemical signals or associated fauna with a particular ecomorph of an existing kelp population.

Field collection and cleaning of sporophylls

Pyrifera and *integrifolia* gametophytes were cultivated in the laboratory from wild-harvested sporophylls in three experimental treatments: pyrifera, integrifolia, and mixed pyrifera and integrifolia (50% of each). Reproductive sporophylls were collected from eight *pyrifera* and eight *integrifolia* individuals at Stillwater Cove, CA via scuba diving on February 25, 2021. Ecomorph characterization of individuals was based on holdfast morphology (Figure 2.1). *Pyrifera* have conical holdfasts while *integrifolia* have rhizomatous holdfasts (Macaya & Zucarello 2010). In addition, the haptera of *pyrifera* holdfasts arise from all sides of the cylindrical segments, while haptera of *integrifolia* are produced only from the edges along two sides of the flattened holdfast (Setchell 1932, Womersley 1954). *Integrifolia* samples were collected between 0-1.5 m below the mean lower low water line (MLLW), and

pyrifera were collected between 3-6 m below MLLW to avoid sampling of potentially cryptic hybrids at the boundary between the two ecomorphs. Sporophylls were collected from individuals at least 2 m apart to minimize the probability of collecting closely related individuals. The total area over which all individuals were collected was approximately 18,000 m^2. At the collection site, we wrapped each sporophyll in a paper towel dampened with sterile (filtered 0.2 μm) seawater, and placed the sample into a Ziploc bag. Sporophylls were transported in a cooler with ice to the laboratory (approximately 1.5 h drive).

Laboratory kelp culturing

At the lab, each sporophyll was cleaned of epiphytes by pouring cold tap water onto the sporophyll and gently brushing it with a paintbrush. A small piece (~2-4 cm^2) of each sporophyll was cut and placed in its own 50 mL falcon tube with 35 mL of Provasoli Enriched Seawater (PES) media using sterile natural seawater to release spores (see Appendix A2 for details on PES preparation). The falcon tubes were kept in a refrigerator (no lights, 4°C) for 2.5 hours. Spore concentrations in each tube were measured using a hemocytometer, and volumes containing 1.7-4.7 x 10^5 spores per tube were combined into spore solutions for each morph. Spore solutions were diluted with PES to achieve spore densities of 40-46 spores/mm^2 in the settlement containers (see below), which is within the optimal density range for *Macrocystis* gametophyte growth and development (Reed et al. 1991).

For each treatment, spores were settled onto 60 6-cm long pieces of nylon string (3.2 mm diameter) laid on the bottom of a 28.4 x 15.3 x 11.7 cm clear plastic container. Each container was filled with 600 mL of the appropriate spore solution for the treatment— *pyrifera* only*, integrifolia* only, or a mixed 50:50 solution of *pyrifera* and *integrifolia* spores. The depth of the spore solution in each container was ~1 cm to the surface of the nylon strings. The containers were placed in an incubator (12ºC, no lights) overnight for 14 h. The following morning a 12:12 h light cycle was initiated in the incubator with white fluorescent lights at 52-63 µmol m^{-2} s^{-1} photon flux density (variation is due to small inconsistencies across the surface of the incubator). Incubator conditions were determined based on previously identified optimal conditions (Reed et al. 1991, Raimondi et al. 2004, Gutierrez et al. 2006, Westermeier et al. 2006, Plá & Alveal 2012, Camus & Buschmann 2017, Camus et al. 2018b).

After 48 h, the strings were removed from the settlement containers and placed into Petri dishes (6 strings per dish) filled with 35 mL of PES enriched sterile seawater. The dishes were maintained in the incubator (12ºC, 12:12 h L:D, 52-63 µmol m^{-2} s^{-1} photon flux density) for 5 weeks and the dishes were refreshed with new PES enriched media every 3-4 days. Two weeks prior to outplanting to the ocean, we began refreshing the dishes using only sterile natural seawater (without PES media) and reduced the light to 15-21 µmol m^{-2} s^{-1} photon flux to acclimate the kelp to the level of nutrients and light in the ocean at the outplanting site. Five days prior to outplanting to the ocean, the strings were moved into 57-L aquaria (one for each

treatment), each with approximately 20 L of sterile natural seawater and bubblers inside the incubator with light maintained at 15-21 μmol m^{-2} s^{-1} photon flux.

Outplanting young sporophytes

We used concrete parking bumpers (1.2 m x 0.15 m x 0.15 m) as the attachment substrate for the cultivated kelp sporophytes. Underwater using SCUBA, we arranged 20 bumpers in two parallel rows of 10 bumpers, each 5 m apart, at the bottom of the ocean (25-30 ft) at the outplanting site, Del Monte Beach, CA (Figure 2.3).

Sporophytes were approximately 3-5 mm in length at the time of outplanting to the ocean. Each 6-cm long piece of nylon string was interwoven into an 8-cm long 6.4 mm diameter twisted line. The strings with sporophytes were attached to the concrete bumpers by divers using nylon zipties (see Appendix A3). On each concrete bumper, one string of each treatment was fastened to the top of the bumper at the left, center, or right position following a Latin squares design (Figure 2.3). In total, 20 strings of each of the three treatments were outplanted across the 20 bumpers.

Monitoring and measuring outplants

The strings were outplanted on 10 April 2021 and the outplants were monitored approximately once per week between 10 April 2021 until 15 September 2021. Over the first six weeks, outplants were checked for survival and additional strings with sporophytes of the appropriate treatment were added to locations where

no surviving sporophytes were visible. These additional strings were seeded at the same time and from the same batch of spores as the initial outplants, and sporophyte length did not exceed 5 cm. After 21 May 2021, no additional strings were outplanted. By the final sampling date, there was no significant difference in ages of individuals (calculated as number of days since outplanting) among the three treatments.

Morphological features were measured as they became apparent throughout the course of the kelp's development. For the majority of juvenile sporophyte development, the holdfast is very small and indistinct, so we used several other metrics to quantify the morphology prior to when the holdfast could be characterized. Beginning on 30 April 2021, **total plant height** was measured for the tallest sporophyte at each outplant location. The length of the primary stipe was measured starting on 28 May 2021, when a small holdfast consisting of emergent haptera had formed. The exposed **primary stipe** was measured following the description in Brostoff (1988), that is, from the top of the holdfast (where haptera had connected into the mound) to the first bifurcation, or to the base of the emergent blade if a bifurcation had not yet formed (Figure 2.4). The **number of branches** was counted as the greatest total number of branch points along any single stipe; a branch point was counted as any point on the stipe that bifurcated into two stipes with pneumatocyst-bearing blades, including the initial bifurcation point (Figure 2.4). For mixed treatment replicates, the highest number of branches on any individual for that replicate was recorded.

Beginning on 28 May 2021, the smaller individuals on pyrifera and integrifolia replicates were cut at the base of the primary stipe just above the holdfast. Multiple individuals were maintained on a single replicate as long as they were growing far enough apart that they were not growing on top of each other. This thinning was performed to facilitate observation of the emergent morphologies without the confusion of multiple individuals growing on top of each other. The thinned individuals were cut rather than pulled so as not to disturb any haptera from the remaining focal individuals that may have been intertwined with the cut individuals. Importantly, mixed treatment replicates were never thinned, since we wanted to observe the ratio of resulting cross types (pure pyrifera, pure integrifolia, or hybrid).

The sporophylls on outplants were inspected each week for presence of sori, indicating reproductive maturity. If differences exist in reproductive timing among the treatments, this could lead to prezygotic isolation between *pyrifera* and *integrifolia*, and may help us understand the spatial pattern of ecomorph distribution and lack of hybrid forms.

Statistical analyses

Morphological measurements were compared among treatments using mixed models with bumper as a random effect, and treatment, date, and the interaction of treatment and date as categorical predictor variables for the response variables of primary stipe length and total height (log-transformed). Date, rather than age, was

used in analyses because the different outplanting cohorts grew at different rates, and since plants were only monitored once per week, only certain ages were captured in each cohort. Growth was highly influenced by ocean conditions such that the changes between dates dwarfed differences between ages on a given date. For total height, the data were log-transformed to meet the assumption of homoscedascity.

The number of branches were compared among all treatments using an analysis of covariance with treatment, date, and the interaction between treatment and date as categorical predictor variables, and number of branches as the response variable. The number of branches ranged from 0-4.

Reproductive timing was analyzed using a nominal logistic model with treatment as a categorical predictor variable and age of the individual (in days from date of outplanting) as a continuous predictor variable, and presence/absence of reproductive sporophylls as the response variable. Only data collected on the last day of sampling (15 September 2021) were included in the analyses to capture the maximum number of reproductive individuals over the monitoring period. We generated a random output table from the model to examine the probability of being reproductive at different ages. Mixed treatment individuals were excluded from the formal analysis of reproductive timing since there were multiple individuals on a single mixed treatment string, and this would result in a higher probability of finding reproductive individuals. Only one reproductive status was assigned per replicate string in the mixed treatment because as holdfasts from different individuals became intertangled it was not feasible to distinguish which sporophylls belonged to which

individuals. Therefore, the reproductive status per replicate in the mixed treatment was not comparable to the pyrifera and integrifolia treatments.

Results

In the laboratory, all strings produced sporophytes. Sporophyte densities were similar across treatments (~15 sporophytes/cm of string). After approximately 5 months of growth in the field, on the final sampling date 09/15/21, the number of replicate strings outplanted with at least one surviving kelp individual was 14/20 (70%) for pyrifera, 14/20 (70%) for integrifolia, and 9/20 (45%) for mixed. All pyrifera and integrifolia outplants ultimately developed a form traditionally recognized as *pyrifera* (conical holdfast with upper primary stipe free of haptera) and *integrifolia* (flattened, rhizomatous holdfast lacking a haptera-free primary stipe) in the literature (e.g. Neushul 1971, Brostoff 1988), while mixed replicates developed forms similar to *pyrifera* or *integrifolia* with no apparent intermediate forms (Figure 2.5).

While the primary stipe lengths were initially similar among all treatments, this trend changed over time. Pyrifera had significantly longer primary stipes on average compared to integrifolia starting on 06/18/21 (9 weeks after outplanting; LSMeans Contrast, $F_{(1, 534.1)}$=4.5606, p=0.0332) and continuing through the end of the experiment (Figure 2.6, bottom graph). The mixed model (bumper as a random effect) with primary stipe length as the response variable contained treatment, date, and the interaction of treatment and date as predictor variables; all of these were

significant predictors of primary stipe length (Mixed model, $F_{(2, 534.6)}$=138.274, p<0.0001 (treatment); $F_{(15, 534.2)}$=8.639, p<0.0001 (date), $F_{(30, 534)}$=2.172, p=0.0004 (treatment*date)). By 08/12/21, there was no overlap in primary stipe length between the pyrifera and integrifolia treatments (Figure 2.6). By 08/20/21, all integrifolia replicates from the first outplanting cohort (04/10/21) had a primary stipe length of 0 cm, meaning the haptera had completely overgrown the primary stipe and first bifurcation. Haptera grew closer up the primary stipe toward the primary bifurcation in integrifolia than pyrifera treatment individuals, often surpassing the primary bifurcation and growing on secondary stipes. By this point, the secondary stipes on these individuals had flattened out as haptera growing beyond the second and third bifurcations pulled these stipes down to become part of the holdfast (Figure 2.7). Over the same time period, the holdfasts of pyrifera individuals did not change much except for additional layering of haptera (Figure 2.7).

The mixed model with log-transformed total height as the response variable contained bumper as a random effect and date, treatment, and the interaction of date and treatment as predictor variables; of these, date and treatment were significant predictors of height (Mixed model, $F_{(11, 525.7)}$=95.271, p<0.0001 (date); $F_{(2, 549)}$=6.668, p=0.0014 (treatment)). A Tukey HSD test showed that the mixed treatment had a significantly shorter mean height compared to the other two treatments, but pyrifera and integrifolia were not significantly different in height (Figure 2.6, top graph).

The morphologies of the mixed treatments can be quantified and characterized using primary stipe length after the height at which primary stipe length became

distinguishing between the pyrifera and integrifolia treatments. The mean total height of the pyrifera and integrifolia treatments at which the mean pyrifera and integrifolia primary stipe lengths were first observed to be significantly different (sampling date 06/18/21) was 34 cm. The maximum upper 95% confidence interval for integrifolia primary stipe length between 06/18/21 through the end of the sampling period was 2.27 cm, and the minimum lower 95% confidence interval for pyrifera over the same time period was 1.92 cm. All mixed replicate strings that survived to the end of the sampling period had total heights above 34 cm by the last date of height measurements (07/20/21). On the final sampling date (09/15/21), 9 replicate mixed treatment strings had sporophytes present, with a total of 26 individuals across all replicate strings. Out of these 26 mixed treatment individuals, 8 (~31%) had primary stipe lengths >2.27 cm, 15 (~58%) had primary stipe lengths <1.92 cm, and 3 (~12%) had primary stipe lengths between 1.92 and 2.27 cm (Figure 2.8).

The analysis of variance with number of branches as the response variable contained date, treatment, and the interaction of date and treatment as predictor variables; all predictor variables were significant (ANOVA, $F_{(2, 268)}$=27.415, p<0.0001 (treatment); $F_{(7, 268)}$=5.702, p<0.0001 (date); $F_{(14, 268)}$=3.633, p<0.0001 (treatment*date)). Thus, the treatments differed in their number of branches and the magnitude of this difference increased over the sampling period. By week 19 (08/20/2021), the number of branches was significantly lower in integrifolia compared to pyrifera, and after this point the mean number of branches continued to increase in pyrifera but decreased in integrifolia (Figure 2.9).

Reproductive timing differed between the pyrifera and integrifolia treatments. The first reproductive individuals were observed on 09/08/21. The nominal logistic model with presence of reproductive sporophylls as the response variable contained treatment as a categorical predictor variable and age of the individual (in days) as a continuous predictor variable; both of these were significant (Nominal logistic model, $X^2_{(1)}=6.881, p=0.0087$ (age); $X^2_{(1)}=4.658, p=0.0309$ (treatment)). The predicted values generated from a nominal logistic model indicated that at age 158 days (about 5 months), the probability of a pyrifera individual being reproductive was 84%, while the probability of an integrifolia being reproductive was only 40% (Figure 2.10).

Discussion

This is the first study to directly compare the growth, development, and morphological transformations at all life stages of the two *Macrocystis* ecomorphs in a common garden experiment. The clear morphological differences between *M. "pyrifera"* and *M. "integrifolia"* at multiple life stages of *Macrocystis* development suggest that these two morphs have intrinsic biological differences, and that characteristics such as primary stipe length and number of branches can be used as diagnostic traits for distinguishing the ecomorphs prior to when adult sporophyte morphology can be definitively characterized or in the absence of side-by-side comparison between the two morphs.

Given the results of this study, in accordance with genetic differences between the two morphs (see Chapter 1), we suggest that *M. "pyrifera"* and *M. "integrifolia"*

should be considered separate species. The collective evidence in support of distinct species includes 1) complete genetic separation between the two morphs within both Northern and Southern Hemispheres based on whole-genome sequencing, 2) empirical evidence that the offspring of pure-bred crosses within each morph grow up to exhibit their respective characteristic ecomorphologies even when growing in identical environments, and 3) empirical evidence that when given the opportunity to interbreed in a mixed pyrifera-integrifolia spore crossing treatment, the resulting offspring only resemble either characteristic pyrifera or integrifolia morphology (resulting in mean values for primary stipe length and number of branches that are intermediate between pyrifera and integrifolia treatments) and do not exhibit any hybrid features. Despite the ability for *pyrifera* and *integrifolia* to interbreed in the laboratory as demonstrated in previous research (e.g. Lewis et al. 1986, Westermeier et al. 2007), it is apparent that in nature the two morphs behave as reproductively, morphologically, and genetically isolated species.

The present study demonstrates that the holdfast morphology that characterizes the morphs, which can be observed qualitatively and quantified by primary stipe length and number of branch points, is a genetically fixed trait that does not exhibit a plastic response based on local environment. This finding is in contrast to previous theory that the holdfast morphology is determined by the depth at which the individual grows (Demes et al. 2009). Furthermore, we found that different aspects of the characteristic adult morphologies can be diagnostic at distinct stages of development. Primary stipe length was a distinguishing feature between the pyrifera

and integrifolia treatments in the juvenile sporophyte stage, while the overall appearance of the holdfast (mounding vs. flattened) was not distinguishing until later. The primary stipe length was first observed to be significantly different between pyrifera and integrifolia treatments on 06/18/21, and after this point, the primary stipe length for integrifolia plateaued. Since the mean height for pyrifera and integrifolia individuals on 06/18/21 was 34 cm, based on our data any individual with a total height of at least 34 cm and a primary stipe length >2.27 cm can be characterized with 95% confidence as a pyrifera individual, and a primary stipe length <1.92 cm can be characterized with 95% confidence as an integrifolia individual. Using these reference values, it may be possible to characterize a juvenile kelp sporophyte in the field as either *pyrifera* or *integrifolia* if it is at least 34 cm in height by measuring the height of its primary stipe.

Based on a qualitative assessment of holdfast forms, all mixed treatment sporophytes appeared to be either pyrifera-like or integrifolia-like with no intermediate morphologies, and based on our empirical data on primary stipe length as a diagnostic trait for ecomorphology, integrifolia-like forms were almost twice as common as pyrifera-like forms among the mixed individuals. Although it was sometimes impossible to distinguish heights of different individuals on the same replicate string due to intertangling of stipes, it is reasonable to assume that most, if not all, surviving individuals had reached at least 34 cm by the final sampling date (09/15/21) based on previous measurements. The breakdown of ecomorphologies of mixed individuals across all replicate strings at the end of the experiment can be

characterized as 58% integrifolia-like, 31% pyrifera-like, and 12% undefined. Given the rhizomatous nature of the integrifolia morphology, it is possible that individuals with this holdfast type tended to overgrow the pyrifera-like individuals, ultimately causing them to die. Thus, individuals with the integrifolia-like holdfast may have a competitive advantage over pyrifera-like individuals when growing side-by-side. However, it is also possible that the integrifolia-like forms grew such that they obscured the holdfasts of pyrifera-like individuals, and only ostensibly incorporated their stipes into the holdfasts of the integrifolia-like individuals. Genetic testing on the mixed treatment sporophytes to evaluate parentage would elucidate whether the sporophytes included any hybrids and if so, whether one of the ecomorphologies was genetically dominant.

In addition to primary stipe length, the number of branch points along the stipe was another distinguishing feature between the treatments and paralleled the pattern seen in the primary stipe length. While integrifolia and pyrifera treatments were similar in number of branches at the beginning of the experiment, the number of branches began decreasing in integrifolia as the stipes were over-grown by haptera and became part of the holdfast and as primary stipe length shrunk to 0 cm. Therefore, number of branches provides another quantitative metric of the characteristic ecomorphologies. Since number of branches is not a continuous metric, and ranged only from 0-4, it was difficult to identify a threshold value that distinguishes the morphs. Additionally, since the mixed individuals were growing in more crowded conditions than the other treatments, they may have developed more

slowly, and thus number of branches was not as reliable as primary stipe length for characterizing the morphologies of the mixed treatment individuals.

There was no difference in survival or height between the pyrifera and integrifolia treatments, demonstrating that integrifolia individuals can grow successfully even at depths that are considered more aligned with *pyrifera* habitats in nature (Graham et al. 2007). The mixed treatment individuals exhibited lower survivorship and shorter mean heights compared to pyrifera and integrifolia treatments, likely because, unlike with pyrifera and integrifolia, we did not remove any individuals from mixed treatment strings as the experiment progressed. This means that the mixed individuals were more crowded than the other treatments, which may have stunted their growth and reduced survival. While we removed individuals from crowded replicate strings in pyrifera and integrifolia treatments to increase likelihood of survival, we wanted to be able to observe the proportions of individuals with distinct morphologies that arose in the mixed treatment and therefore we did not trim any individuals from this treatment. Importantly, we did not observe any hybrid morphologies in the mixed treatment, which aligns with the lack of documented hybrids in natural populations.

It is well-documented that *pyrifera* and *integrifolia* can produce hybrid sporophytes when experimentally crossed under laboratory conditions (Lewis et al. 1986, Westermeier et al. 2007). However, a morphological hybrid individual has never been reported in the field, even in the rare locations where the ecomorphs co-occur. Hybrids may be unlikely to occur if gamete physiology differs between the two

morphs and favors intra-morph fertilization, and sporophytes resulting from successful hybrid fertilization may have reduced fitness and be out-competed by intra-morph sporophytes. Thus, prior to this study, it was unknown whether cryptic hybrids occur in the wild and take on the morphology of either *pyrifera* or *integrifolia*, or whether physiological or ecological factors make them absent in nature entirely.

As expected, the number of reproductive individuals in our study increased over time as the kelp aged, but for a given age sampled, pyrifera were more likely to be reproductive than integrifolia. However, at the end of the experiment, the probability of reproduction in pyrifera had begun to plateau, while it was still increasing exponentially for integrifolia. Therefore, it is possible that later in the season integrifolia would reach a level of reproductivity equivalent to that of pyrifera, but there would be a mismatch in the timing of peak reproductivity between the two morphs. This timing difference may be due to differences in development such that integrifolia reach reproductive maturity at a later age, or differential reproductive response to environmental cues that vary across the season such as day length and nutrient levels. Overall, the differences in patterns of reproductive timing between the two treatments demonstrated here may contribute to divergence of the two morphs in natural populations. Further research is warranted to understand whether the patterns in reproductive timing shown here are consistent across different depths and how these patterns align with those found in wild populations of both ecomorphs.

Our documentation of the growth of integrifolia individuals from embryonic sporophyte to adult reveals how the characteristic strap-like holdfast develops. As a juvenile sporophyte, the *integrifolia* morph develops similar structures as the *pyrifera* morph, including a primary stipe from which subsequent stipes branch, and a small mound of haptera that forms at the base of the primary stipe. The strap-like holdfast is later formed from stipe tissue that has been pulled down by haptera growing off of the stipe, adhering the stipe to the basal mound of haptera. The stipe tissue then flattens and widens over the basal haptera mound, creating an anchoring base for the rest of the stipes. It is possible that the mound of haptera that formed in integrifolia treatment individuals under the typical strap-like holdfast formed because they were grown at a deeper depth than where *integrifolia* typically grows. We were not able to do a shallow reciprocal outplanting due to logistical constraints of securing outplants in that habitat.

On a global scale, *integrifolia* is generally found at shallow depths while *pyrifera* is found at deeper nearshore depths, although there is overlap in the reported depths of the two morphs (Graham et al. 2007), and *pyrifera* can even be found in the intertidal (S. T. Gonzalez, *pers. obs.*). When the morphs occur in the same geographic location, they tend to stratify by depth with *integrifolia* shallower and adjacent *pyrifera* in deeper water (Jeffries 2015; S. T. Gonzalez, unpublished data). This study demonstrates that *integrifolia* can grow as successfully as *pyrifera* at depths beyond its typical range and maintains its strap-like morphology, so there are other factors that must preclude the natural occurrence of *integrifolia* at deeper depths when

pyrifera is also present. The precise factors that perpetuate the reproductive isolation between these two morphs are unknown and should be subjects of further investigation.

Acknowledgements

I would like to thank Rose Campbell and Rachel Pausch for assisting with diving and monitoring of kelp outplants; Dr. Filipe Alberto and Gabriel Montesinos for advising sporophyll collection and laboratory rearing protocols; and Dr. Mark Carr, Dave Benet, and Steve Clabuesch for assisting with boating operations and underwater set up of the concrete for outplant attachment substrate. Sara T. Gonzalez was supported by a U.S. Department of Education Graduate Assistance in Areas of National Need Fellowship, a Ford Foundation Predoctoral Fellowship, and the UC Santa Cruz EEB Department. This research was supported by the Lewis and Clark Fund for Exploration and Research and the Dr. Earl H. Myers & Ethel M. Myers Oceanographic & Marine Biology Trust.

Figures

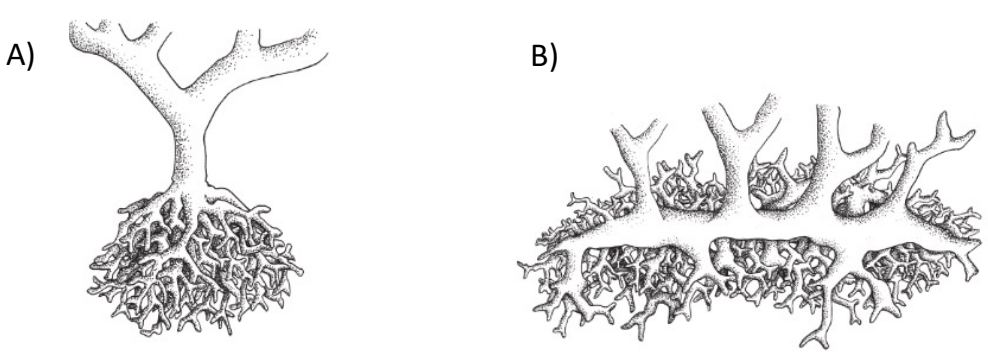


Figure 2.1. Illustrations of the two *Macrocystis* morphs: *pyrifera* (A) and *integrifolia* (B) from Demes et al. (2009).

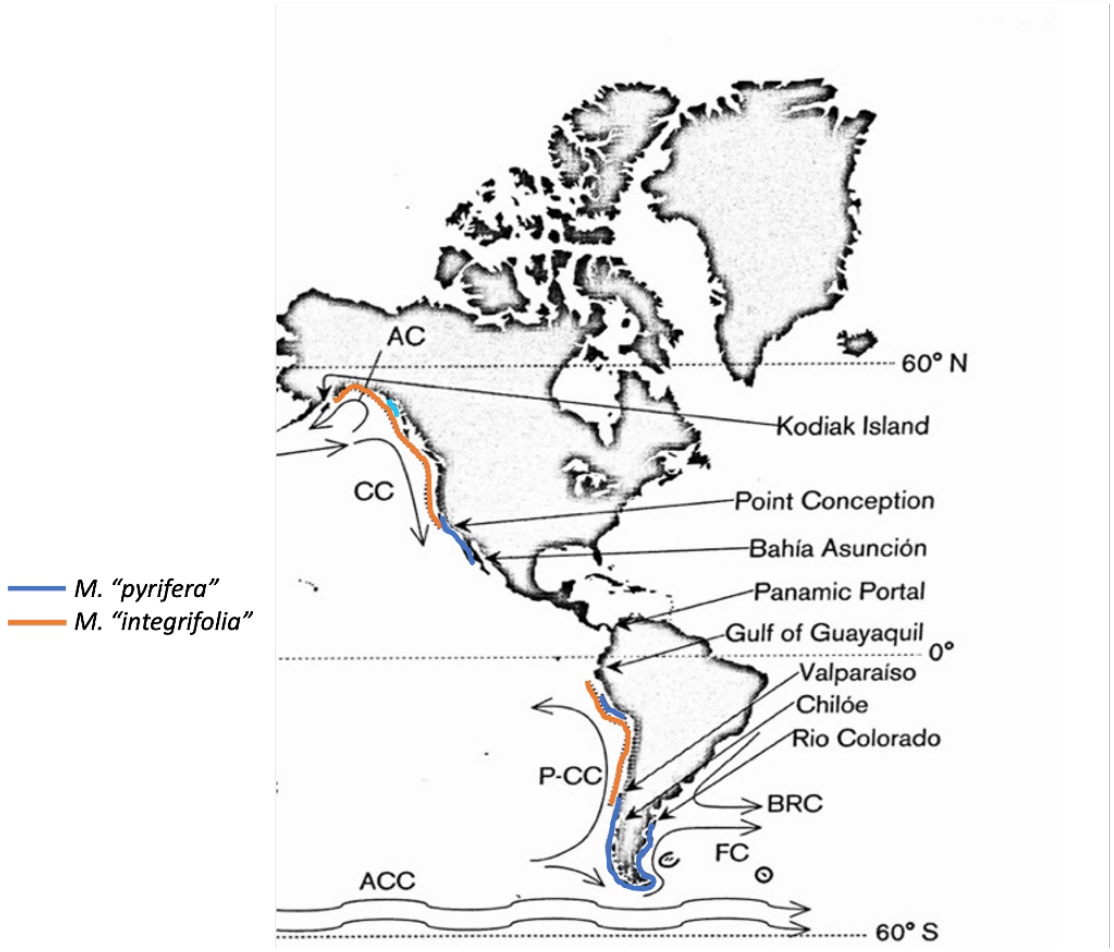


Figure 2.2. Map of *Macrocystis "pyrifera"* (blue) and *M. "integrifolia"* (orange) distributions in the Western Hemisphere. Adapted from Schiel & Foster (2015) with an additional *pyrifera* population (light blue) added in Sitka, Alaska (P. T. Raimondi, *pers comm.*).

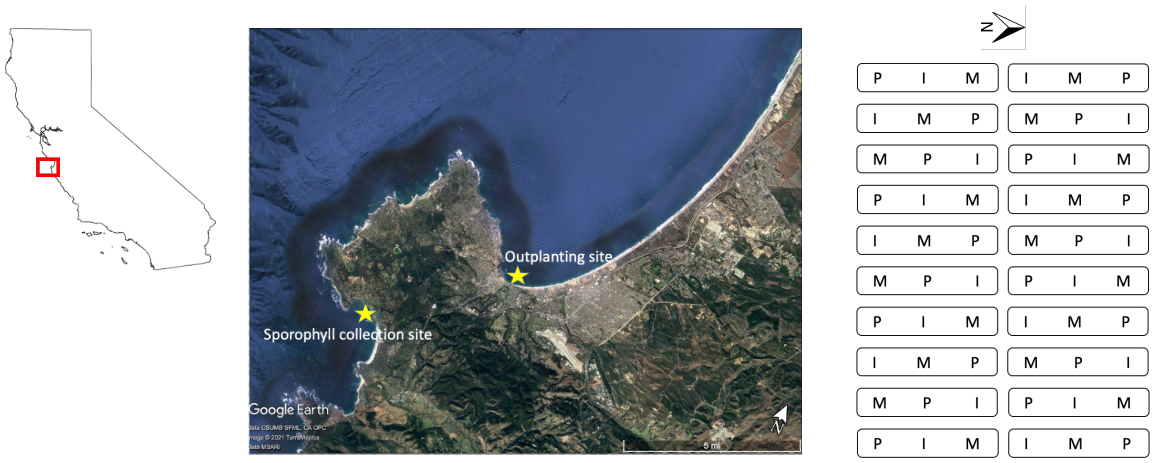


Figure 2.3. General location of study sites in California (left, red square), map of sporophyll collection site and outplanting site (center), and diagram of experimental design of treatment placement (pyrifera [P], integrifolia [I], and mixed [M]) on 20 concrete parking bumpers arranged in two rows of 10 bumpers each (right). Length of a bumper is 1.2 m and distance between bumpers in all directions is approximately 5 m.

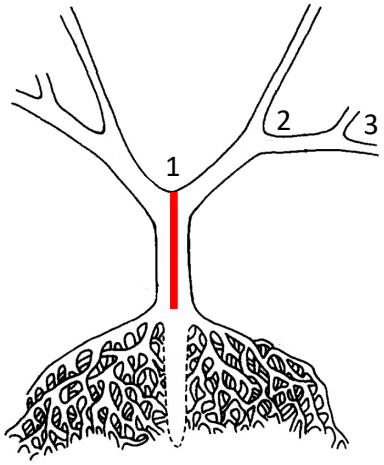


Figure 2.4. Measurements taken on kelp individuals: primary stipe length (red line), adapted from Brostoff (1988); and count of number of branches. The number of branches was counted as the greatest total number of branch points along any single stipe.

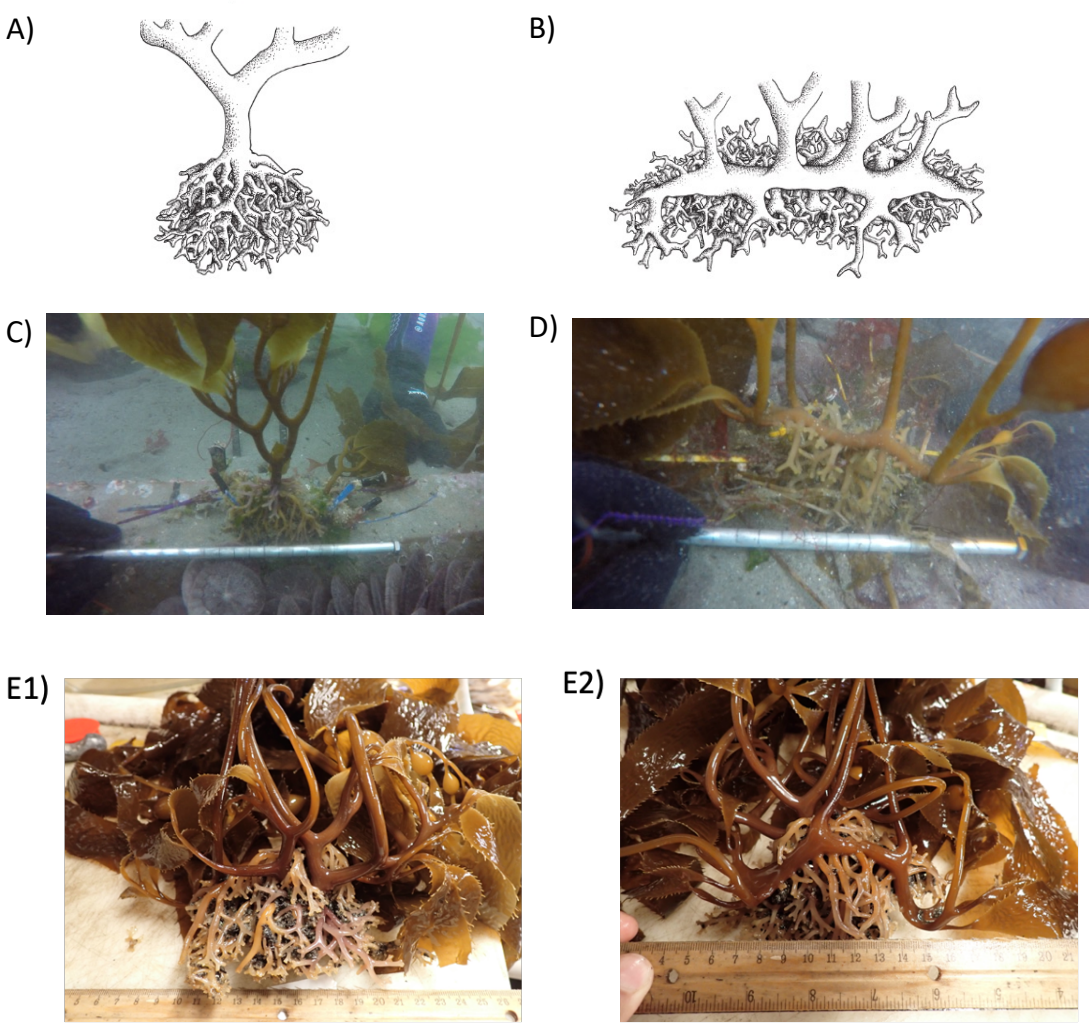


Figure 2.5. Illustrations of the holdfast morphology of *Macrocystis "pyrifera"* (A) and *M. "integrifolia"* (B) from Demes et al. (2009), and photographs of representative individuals from the pyrifera (C) and integrifolia (D) treatments in the present study, and the front and back of a holdfast conglomerate from a single mixed treatment replicate with both pyrifera-like (E1) and integrifolia-like (E2) forms in the present study. Photos by S. T. Gonzalez.

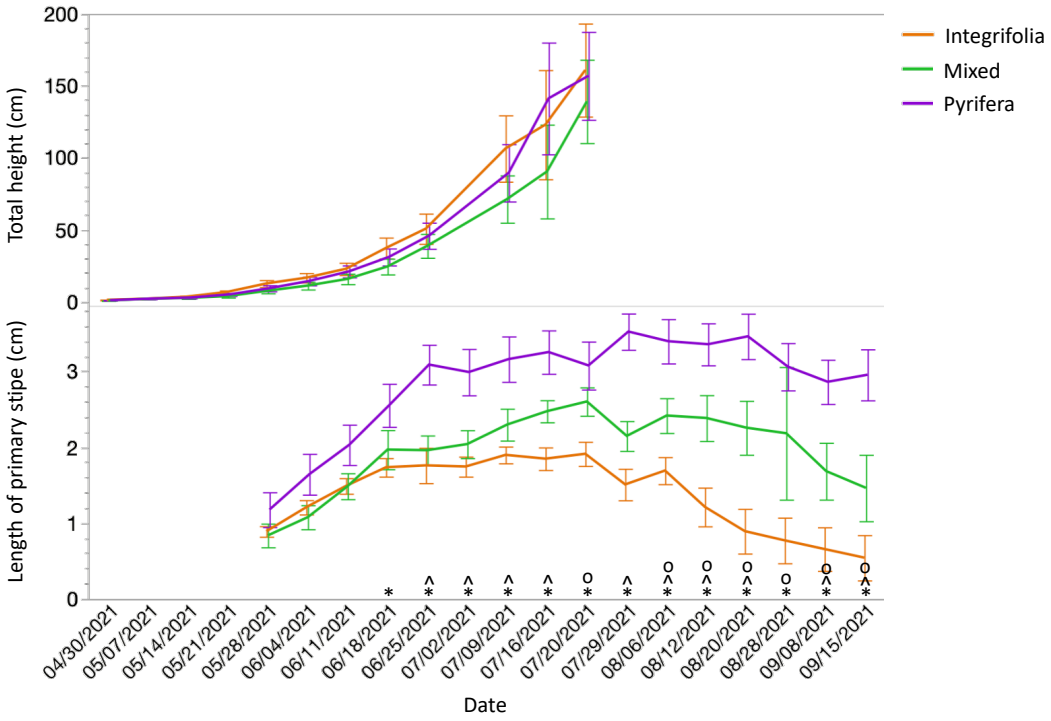


Figure 2.6. Total height (top) and length of primary stipe (bottom) of the three treatments over the experimental period. Symbols above dates indicate significant ($p < 0.05$) differences in primary stipe length: *= between pyrifera and integrifolia, ^ = between pyrifera and mixed, ^{O} = between integrifolia and mixed.

Figure 2.7. Top panel: development of a single integrifolia individual over two months, demonstrating the progression of stipe tissue into holdfast structure. Bottom panel: development of a single pyrifera individual over two months, exhibiting little change in holdfast shape. Silver rod is for size reference with black lines spaced by 1 cm. Photos by S. T. Gonzalez.

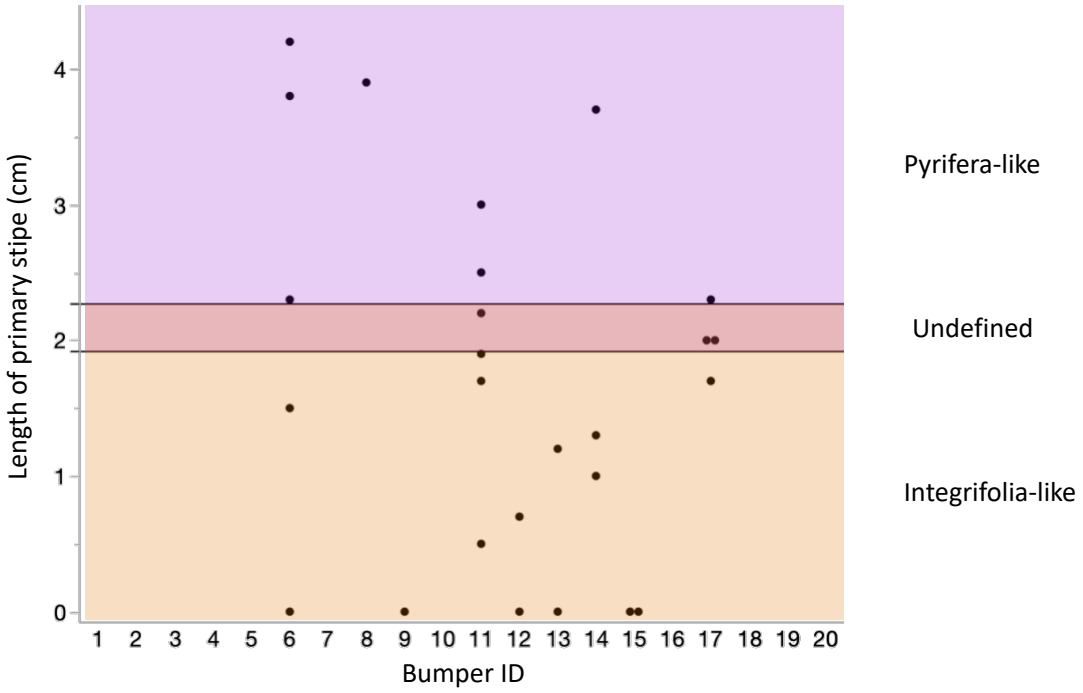


Figure 2.8. Lengths of primary stipe for all mixed treatment sporophytes on the final sampling date (9/15/21). Reference lines at 1.92 cm and 2.27 cm denote the minimum lower 95% confidence value for pyrifera treatment individuals and the maximum upper 95% confidence value for integrifolia treatment individuals, respectively, in the present study.

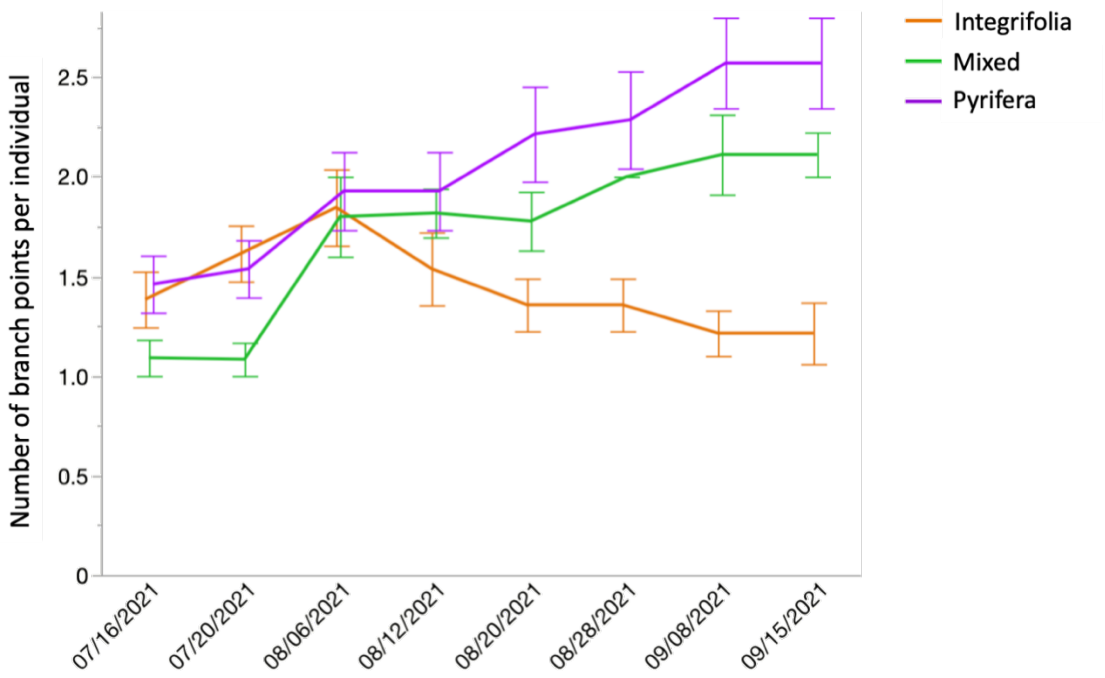


Figure 2.9. Mean number of branch points per individual by treatment.

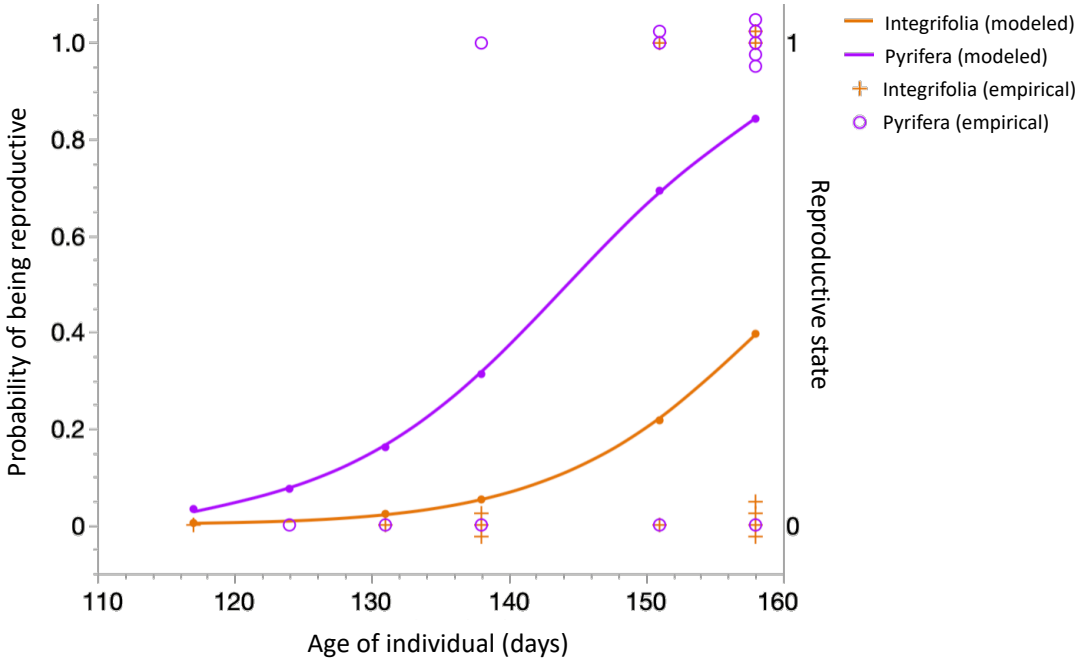


Figure 2.10. Reproductive timing of pyrifera and integrifolia outplants with modeled probability of being reproductive (left y-axis) and empirical data of reproductive states (right y-axis; 1=reproductive, 0=not reproductive) of outplanted individuals sampled on 9/15/21.

CHAPTER 3

Alginate yield and composition differ in two ecomorphs of giant kelp (*Macrocystis*)

Abstract

Variation in alginate composition of mannuronic (M) and guluronic (G) acids in seaweeds has important implications for their applications as gels or emulsifying agents in foods, beverages, pharmaceuticals, cosmetics, and industrial products. The relative proportions of M and G building blocks determine the flexibility of the algal tissue and the physical properties of the gel that can be manufactured from the alginate. Giant kelp (*Macrocystis pyrifera*) is one of the fastest-growing species of macroalgae with up to 45% alginate content by dry weight, making it an excellent candidate for alginate extraction. Giant kelp exists in four distinct morphological forms, termed "ecomorphs." The two most common ecomorphs, *M. "pyrifera"* and *M. "integrifolia,"* grow throughout the temperate west coasts of North and South America with almost no geographic overlap. This study investigated the relationship between morphology, environment, and alginate production in giant kelp. We collected *Macrocystis* blades from 15 populations: eight populations of *M. "pyrifera"* and seven populations of *M. "integrifolia"* along the coast of Chile and central California. Alginates were extracted from 76 individuals across the 15 populations, and their compositions were characterized. Alginates from all populations were M-rich, but G content was significantly higher in *M. "integrifolia"* individuals compared to *M. "pyrifera*." In addition, alginate composition was related to depth and sea surface temperature. Alginate yield did not differ between the two morphs but was related to depth of the kelp. These results suggest distinct applications for alginates from the two different giant kelp ecomorphs, as the M/G ratio is indicative of gelling

capacity and gel elasticity. More elastic alginate gels can be used for making textiles and wound dressings while more rigid alginate gels are used in pharmaceuticals, cosmetics, and foods.

Key words: alginate; ecomorph; guluronic acid; kelp; *Macrocystis integrifolia*; *M. pyrifera*; mannuronic acid

Introduction

Across the globe, kelp are cultivated and harvested for the raw material for alginate extraction to produce gels and emulsifying agents. Alginates are polysaccharides that serve as structural components in the algal cell wall and intercellular regions. Alginates give kelps their strong yet flexible design, enabling them to withstand persistent wave action in the ocean (Moe et al. 1995).

Alginate is a linear copolymer of 1→4 linked β-D-mannuronic acid (M) and its epimer α-L-guluronic acid (G) differing only at C-5. The content and composition of alginates varies across species of brown algae, and the relative proportion of the monomer M and G building blocks determines the flexibility of the algal tissue and the physical properties of the gel that can be manufactured from the alginate (Haug et al. 1966, Draget et al. 2005). Alginates with a low M/G ratio and large proportion of guluronic blocks tend to form a strong and rigid gel, while low amounts of guluronic blocks and a high M/G ratio yields a soft and elastic gel (Venegas et al. 1993, Rehm

& Moradali 2018). Molecular weight can also affect alginate properties, as gel strength increases with increasing molecular weight for samples with low viscosity (Martinsen et al. 1989, Smidsrød et al. 1996). Different species of brown algae contain differences in alginate composition and thus distinct potential uses for industry (Onsøyen 1996, Draget et al. 2005, Imeson 2010, Rehm & Moradali 2018).

The proportion of algal dry weight that alginate comprises and its composition vary substantially among and within species (Imeson 2010, Rehm & Moradali 2018). Alginate content can be related to tissue age, with higher content in younger kelp blades (Frei and Preston 1962, McKee et al. 1992). In addition, tissue type affects alginate yield and composition, as stipes tend to have higher amounts of alginates rich in guluronic acid compared to blades, and holdfasts tend to have higher amounts of guluronic acid compared to stipes (Cheshire and Hallam 1985, Venegas et al. 1993, Kelly & Brown 2000). This variation reflects functional differences among tissue types, where blades and stipes should be more flexible (higher mannuronic acid) and holdfasts need to be more rigid (higher guluronic acid; Kelly & Brown 2000).

The yield and composition of alginates in algae is also influenced by environmental factors. For example, sporophytes of *Lessonia trabeculata* growing on an exposed beach contained alginate with a lower M/G ratio and richer in guluronic acid compared to sporophytes growing in a protected bay (Frei and Preston 1962, Venegas et al. 1993). In addition, transplanted *L. trabeculata* blades from the protected to exposed habitat decreased in M/G ratio and increased in percent guluronic blocks, while the opposite occurred in transplants from the exposed to

protected habitat. This is consistent with the hypothesis that a higher guluronic acid content contributes to a more rigid algal form which is beneficial in the wave-exposed habitat (Venegas et al. 1993). Seasonal variation in alginate yield was observed for species of *Laminaria, Saccharina,* and *Alaria* in Scotland (Schiener et al. 2015), but not for species of *Sargassum* and *Turbinaria* in Tahiti (Zubia et al. 2008), although alginates tended to be elevated during austral winter and varied by species and location sampled. In Chile, *Macrocystis* alginate content is highest in autumn and declines in summer (Westermeier et al. 2012), while similar (Whyte & Englar 1978) as well as opposite patterns (Rosell & Srivastava 1984) have been observed in the Northern Hemisphere, suggesting that alginate content may be influenced by complex interactions of environmental factors. In addition, Westermeier et al. (2012) found differences in *Macrocystis* alginate content that were not related to environmental variables, suggesting genetic traits as a potential relevant factor.

The giant kelp (*Macrocystis pyrifera)* ecomorph complex presents a valuable opportunity to explore the variation in alginate content and composition among different morphological variants of the same species. Alginates comprise on average about 25% of the dry weight in *Macrocystis*, and can be as high as 45% (Whyte & Englar 1978, Hernandez-Carmona et al. 1999, Westermeier et al. 2012), so *Macrocystis* is one of the primary species harvested for alginate extraction. Giant kelp exists as distinct morphological variants—or "ecomorphs"—in different populations, yet the mechanism for this variation is uncertain, and the extent to which morphological features are genetically fixed or malleable under different

environmental conditions is unclear (but see Chapters 1 and 2). The two most common ecomorphs, *Macrocystis "pyrifera"* (hereafter *pyrifera*) and *M. "integrifolia*," (hereafter *integrifolia*) exhibit almost no geographic overlap across their range in temperate waters of North and South America (Graham et al. 2007). Since alginate is a structural component of brown algal cells, it may relate to ecomorph morphology. Previous studies on hybrid crosses among *Macrocystis* populations found that the different ecomorphs could interbreed (Mackenzie 1993, Lewis & Neushul 1994, Westermeier et al. 2007). However, these studies did not measure alginate composition in the resulting sporophytes, and it remains unclear how alginate content relates to morphology.

The objectives of this study were to determine 1) how alginate content and composition varies among *Macrocystis* populations with different environmental conditions, and 2) the extent to which alginate properties differ between ecomorphs. We hypothesized that genetics and environment interact to determine alginate content and composition in *Macrocystis.* Based on the genetic basis to morphology (Camus et al. 2018a, see Chapter 1), and the observed relationship between morphology and alginate composition, it is plausible that alginate composition is at least in part genetically determined. Environmental factors could also cause the ecomorphs to differ in alginate composition. *Integrifolia* tends to occur at shallower depths than *pyrifera* where it is more prone to experience higher wave energy, and such conditions could cause *integrifolia* to have a higher guluronic acid content than *pyrifera*. It is also possible that environmental factors and morphology interact to

influence alginate composition causing shallow-water *pyrifera* to have higher guluronic content than deeper-water *pyrifera*, but lower guluronic content than *integrifolia* found at the same depth.

Methods

Study sites

We sampled a total of 15 populations; eight *pyrifera* and seven *integrifolia,* which spanned 1.1 degrees of latitude in North America and 14.2 degrees of latitude in South America (Figure 3.1). Sampling in North America took place during April-June (northern spring) of 2019, and sampling in South America was during October-November (austral spring) of 2019. Sites representative of the Western Hemisphere distribution of *Macrocystis* were selected based on prior ground truthing of ecomorphology of different populations by other researchers, accessibility, and proximity to SCUBA air tank supplies. The majority of sites were located in the Southern Hemisphere, where *Macrocystis* populations are continuous over a larger portion of coastline compared to North America and where the ecomorph populations are well-documented. An additional site, La Boca, was identified in the present study as the northernmost extent of the *pyrifera* morph in Chile. Additional *integrifolia* sites near La Boca were added to provide greater resolution near the morph boundary. The northernmost *integrifolia* site selected in the Southern Hemisphere was Playa Blanca; north of this site, *Macrocystis* is absent for approximately 600 km and reappears in Antofagasta (Vásquez 2008).

Sample collection, preparation, and storage

Kelp blade tissue samples were collected in plastic bags using SCUBA or by snorkeling. Blades were collected at the mid-canopy level and were selected for the least damage and fewest epiphytes. At each site, we selected an accessible portion of the kelp bed and collected tissue from 5 individuals each separated by at least 2 m. At site CAY, 10 individuals were sampled to encompass both shallow and deep areas of the kelp bed, and at STL six individuals were sampled including both *pyrifera* and *integrifolia* individuals. At all sites, the individuals were identified as either *pyrifera* or *integrifolia* morph based on their holdfast morphology. *Pyrifera* individuals have conical holdfasts with haptera arising from all sides of the cylindrical segments, while *integrifolia* have rhizomatous holdfasts with haptera produced only from the edges along two sides of the flattened holdfast (Setchell 1932, Womersley 1954, Macaya & Zucarello 2010a; Figure 3.2). At least five blades were selected per individual to have enough material for alginate analyses. Blades were carefully selected from the same stipe or from multiple stipes that were confirmed to be from the same individual; multiple stipes were considered to be from the same individual if the stipes either emanated from a common point on the basal stipe or stemmed from different points on the same continuous holdfast. Blade samples were transported on ice from the field site to the laboratory. Each sample was thoroughly rinsed in tap water and spun dry in a salad spinner (some samples were pat dry with a paper towel if necessary),

and visible epibionts were removed. The sample was placed in a dry plastic bag, sealed, and frozen at -18ºC until drying.

All samples from Chile were sun-dried over two days to a constant weight in 28-31ºC and ~23% humidity at Productos Concentrados algae processing plant in San Jose de Maipo, Chile. Samples collected in California were dried in an oven at 60ºC for at least 2 days, since the humid climate did not permit sun-drying. Each sample was ground to a fine particle size using a hand-powered mill or coffee grinder, sealed inside a paper envelope, and placed into a plastic bag with silica gel beads until analysis.

Site environmental data collection

To examine the extent to which the content and physical properties of alginate in *Macrocystis* were related to environmental factors we recorded the depth of each kelp individual and analyzed the mean sea surface temperature (SST) for each collection site. For each individual sampled, the depth of the holdfast at the time of collection was recorded and then adjusted to account for the height of the tide relative to the Mean Lower Low Water (MLLW) line. Mean SST was calculated for each site using raster images from the NOAA ERD and CoastWatch West Coast Regional Node, which had a spatial resolution of 0.01°. We used mean monthly SSTs from 2010 to 2020. Using these images, we obtained the SST value for the closest pixel to that of each site coordinate. The mean SST value for each site was the overall mean from 2010-2020.

Alginate extraction

Alginates were extracted and analyzed for composition based on established acid-base extraction protocols (e.g. Arvizu-Higuera et al. 2002) with modifications by the author for maximum yield. For extraction, 1 g of dried and ground algae sample was placed in a 50 mL centrifuge tube with 25 mL ultrapure water and heated to 70^{o}C in a hot water bath with slow agitation for 2 h. The sample was centrifuged (15 min, 10,000 x g, 20^{o}C) and the supernatant was drained. 25 mL HCl was added and the sample was incubated at room temperature overnight with shaking. The following day, the sample was centrifuged (15 min, 10,000 x g, 20^{o}C) and the supernatant was drained. 40 mL ultrapure water was added and the sample was shaken for 30 min before centrifuging (15 min, 10,000 x g, 20^{o}C) and draining the supernatant. 40 mL 0.2M $NaHCO_3$ was added and the sample was incubated at room temperature for 2 h with shaking to dissolve and convert alginates to sodium alginates. After centrifuging (15 min, 10,000 x g, 20^{o}C), the viscous sodium alginate solution was decanted into a beaker. NaCl was added at 0.2 % w/v, and the alginate was precipitated by adding an equal volume of 96% ethanol to the solution and stirring with a glass rod. The alginate was collected and remaining solution saved in a centrifuge bottle for collection of residual alginate. After this initial collection, the algae sample was resuspended in 40 mL ultrapure water for a second wash and shaken for 1 h before centrifuging (15 min, 10,000 x g, 20^{o}C) and collecting the sodium alginate solution. The alginate was precipitated and collected as described above, and the sample was

resuspended in 40 mL ultrapure water for a third wash. After all alginate was collected from the three washes, the remaining solution was collected in a centrifuge bottle, centrifuged (15 min, 10,000 x g, 20°C), and the precipitated alginate was collected. The alginate was washed three times in 70% ethanol and once in 96% ethanol. Finally, the alginate was transferred to a weigh boat and dried in a fume hood for at least 24 h, and then stored in a sealable plastic bag at room temperature.

Characterization of alginate composition

Dried alginate samples were prepared for ^{1}H NMR analysis by mild acid hydrolysis based on the ASTM F2259 (Ertesvåg and Skjåk-Bræk 1999). Specifically, 10 mg of alginate was dissolved in 20 mL ultrapure water, and the pH was adjusted to 5.8 using HCl. The sample was incubated in a water bath at 95°C for 1 h and then immediately cooled in a cold water bath. The pH was adjusted to 3.8 and the sample was incubated in a water bath at 95°C for 50 minutes and immediately cooled. The pH was neutralized to 6.8 and the sample was frozen and freeze-dried for at least 36 h until completely dry.

The freeze-dried alginate was dissolved in 600 mL D_2O (99.9% Sigma-Aldrich). 20 µL TTHA (triethylenetetraminehexaacetic acid, 0.3 M in. D2O, pH 7.0) was added as a chelator, and 2.5 µL 1 % TSP (3-(Trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt; Aldrich, Milwaukee, WI) in D_2O, was added as chemical shift reference. 550-575 µL of the sample was placed into an NMR tube. The NMR spectra were recorded using a BRUKER NEO 600 MHz equipped with 5 mm iProbe, a 30-

degree pulse flip angle, 64 scans and a spectral width of 10 ppm. All spectra were recorded at 83ºC to decrease sample viscosity for increased resolution, and to move the solvent peak away from the chemical shift region of alginate. NMR spectra were analyzed using TopSpin 4.1.0 (Bruker BioSpin). The alginate composition was determined as relative fractions of M and G blocks (mono, di and triades) (Grasdalen 1983, Ertesvåg and Skjåk-Bræk 1999).

To further examine the properties important for alginate gelling capacity, strength, and elasticity, we also quantified the molecular weight of each alginate sample. The dried samples were dissolved in mobile phase (0.15 M $NaNO_3$ with 10 mM EDTA, pH 6.0), diluted to 0.5 mg/ml and filtered with a syringe filter (pore size 0.45 μm) before injection. Size-exclusion chromatography (SEC) with online multi-angle light scattering (MALS) were performed at ambient temperature on an HPLC system consisting of a solvent reservoir, prominence degasser (shimadzu), LC-10 AD vp isocratic pump (shimadzu) infinity II autosampler (Agilent), Dawn HELEOS-II multi-angle laser Dawn Heleos II light scattering photometer (Wyatt) ($\lambda 0$ =663.8 nm) followed by an Optilab T-rEX differential refractometer (Wyatt). Separations were done on serially connected OHpak LB-G 6Bguard column and OHpak LB 806 and OH pak LB 805 columns (Shodex). The eluent was 0.15M $NaNO_3$ with 10 mM EDTA (pH=6.0) with a flow rate of 0.5 mL/min. Samples were analyzed twice with injection volumes 50 and 100 μL. Data were collected and processed (with dn/dc = 0.150 mL/g and A2 = 5x 10-3) using the Astra (v. 7.1.3) software (Wyatt, U.S.A.).

We selected a subset of *integrifolia* and *pyrifera* samples (four *pyrifera* and six *integrifolia* alginate samples) that had similar G content within each morph and formed them into gels (8 gel cylinders per sample) to test their strength and elasticity. We degraded each morph's sample from their starting molecular weights of 542 kDa (*pyrifera*) and 581 kDa (*integrifolia*) to a lower molecular weight of 177 kDa. The gels were formed by combining 1% alginate, 15 mM $CaCO_3$ (4µm) and 30 mM GDL, then setting for 24 h. The solution was saturated with Ca^{2+} for 24 h at 4°C in 50 mM $CaCl_2$ w/ 200 mM NaCl. Gradient measurements were obtained using a Texture Analyzer from Stable Micro Systems with Probe P/35 and used to calculate Young's modulus corrected for syneresis and rupture strength. Young's modulus = gradient x gel height / surface area of gel. We compared both *pyrifera* and *integrifolia* gels to reference samples from other brown algae and commercial *Macrocystis pyrifera* (Sigma-Aldrich).

Statistical analyses

While samples from both California and Chile are discussed in this paper, only samples from Chile were used in the statistical analyses. Only two sites were sampled in California (number of samples = 16 individuals), so it was not statistically appropriate to incorporate the environmental covariates (SST and depth) that were important to include for the Chilean sites.

Alginate composition was analyzed in terms of the proportion of G content, as the proportion of G and M monomers sums to one. We used analyses of covariance

with alginate yield or proportion G content as the response variables, SST and depth as continuous predictor variables, morph as a categorical predictor variable, and interactions between morph and SST and morph and depth as predictor variables to examine the extent to which morph and environmental factors explained variation in alginate content and composition. All non-significant ($p \geq 0.05$) interaction terms were removed to create the final reduced models. Site and latitude were not included in the model because they strongly co-varied with SST and depth for both morphs, and including them in the model would have interfered with identifying relationships between the environmental variables and alginate content. Site latitude was positively correlated with SST and negatively correlated with depth (Linear regression, $F_{(1, 58)}=4108.96$, $p<0.0001$ (SST); $F_{(1,58)}=7.73$, $p=0.0073$ (depth); Figure 3.3). We also used an analysis of covariance with molecular weight as the response variable; depth, SST, and G content as continuous predictor variables; morph as a categorical predictor variable; and the interactions between morph and depth, morph and SST, and morph and G content as predictor variables. All non-significant interaction terms were removed to create the final reduced model.

Results

Across the 12 Southern Hemisphere sites, mean SST ranged from 11.7-15.8^{o}C, and depths of kelp individuals ranged from -3 m to 0.72 m from MLLW. There was no overlap in SST between the two morphs, but the depths of individual

kelps from the two morphs were contained within the same range except for a few deeper individuals at site PLB (Figure 3.4).

The average total alginate yield for all Southern Hemisphere samples (N=60) was 28.2±3.1% of algal dry weight, with a range from 23.0% to 37.5%. The reduced model with alginate yield as the response variable contained depth, SST, and morph as predictor variables; of these, only depth was a significant predictor of alginate yield, where shallower kelp had higher alginate yield (Figure 3.5; Standard Least Squares, $F_{(1, 56)}$=6.848, p=0.011).

The reduced model with G content as the response variable contained morph, SST, and depth as predictor variables; all of these were significant predictors of G content (Standard Least Squares, $F_{(1,56)}$=26.769, p<0.0001 (morph); $F_{(1,56)}$=9.162, p=0.0037 (SST); and $F_{(1,56)}$= 8.272, p=0.0057 (depth)). After accounting for differences in G content driven by depth and SST, the least squares mean proportion G in *integrifolia* (0.494 ± 0.010) was approximately 24% higher than the least squares mean proportion G in *pyrifera* (0.398 ± 0.010) (Figure 3.6). The model also showed that G content was negatively related to both SST (Figure 3.7) and depth (Figure 3.8).

All samples had high molecular weight (mean = 741.7±17.5 kDa), indicating their utility for forming gels. The reduced model with molecular weight as the response variable contained depth, SST, morph, G content, and the interaction between morph and G content as predictor variables; of these, the interaction between morph and G content was a significant predictor of molecular weight (Standard Least Squares, $F_{(1,54)}$=5.621, p=0.0214). For *pyrifera*, there was a negative relationship

between molecular weight and G content (Linear regression, $F_{(1, 28)}$=20.62, p<0.0001), whereas no significant relationship existed for the *integrifolia* morph (Figure 3.9).

Mean gradient measurements from the Texture Analyzer were high for both *pyrifera* (714.23 ± 19.95 N/m [542 kDa sample] and 474.82 ± 21.11 N/m [177 kDa sample]) and *integrifolia* (1209.66 ± 31.59 N/m [581 kDa sample] and 758.12 ± 31.78 N/m [177 kDa sample]). The Young's modulus, corrected for syneresis, tended to increase with increasing G content but did not relate to molecular weight. Mean rupture strength was higher for both *pyrifera* (14.16 ± 0.70 kg [542 kDa sample] and 6.10 ± 1.22 kg [177 kDa sample]) and *integrifolia* (12.28 ± 0.81 kg [581 kDa sample] and 7.49 ± 0.55 kg [177 kDa sample]) compared to other reference samples, and tended to be lower for lower molecular weight samples within each morph.

While only samples from Chile were used in analyses, the alginate samples from California kelp were within two standard deviations of the average values for G content obtained for each morph in Chile, with one exception for one individual from CAY with a proportion G of 0.59. Alginate yields for the California samples also fell within the range of yields for the Chile samples.

Discussion

Alginates are important structural polysaccharides in brown algae, but the composition of the alginate and distribution of guluronic and mannuronic acid blocks can differ between species or among populations within a species. These

compositional differences result in different elasticity of the algal tissue and the materials that can be manufactured from the alginates. In this study the two ecomorphs of *Macrocystis*, *pyrifera* and *integrifolia*, had similar alginate yields but differed in composition. The total alginate yield obtained in this study was similar to previous studies with *Macrocystis* (Gomez et al. 2009). *Integrifolia* alginate samples had a higher guluronic acid content compared to *pyrifera* samples even after accounting for environmental differences among sites. In industry, alginate from kelp individuals with more guluronic acid would produce gels suitable for use as food thickeners or molds, while alginate with more mannuronic acid would be useful for textiles and wound dressings (Qin 2006, Imeson 2010). Within a subset of samples, *pyrifera* alginates had a lower Young's modulus (indicating higher elasticity) compared to *integrifolia*, likely due to their higher M/G ratio. The G content increased with depth and was negatively related to average SST, where G content could increase 4-5% due to just a 1^{o} change in latitude or a 1 m change in depth. Thus, if *integrifolia* grew in more typically *pyrifera*-like habitats, this would likely maximize the G content possible for the species. Therefore, both ecomorph type and local environment may be important to consider when harvesting or growing giant kelp depending on the downstream application of the alginates.

While the California samples covered far fewer populations and degrees of latitude than the Chile samples, the G content and yields of the California samples fell largely within the range of values found for Chile samples. This indicates some evidence of consistency in alginate content across hemispheres despite large genetic

differences (Astorga et al. 2012; see Chapter 1). As alginate is a crucial part of the algal cell and contributing factor to kelp tissue properties, it follows that the yield and general structure of alginates would be preserved even across distant kelp populations.

All samples had high molecular weight, averaging approximately 2-20 times higher than the industry averages of 30-400 kDa (Rehm and Moradali 2018), and were well above the minimum 100-300 kDa needed for ionic cross-linking (Draget et al. 2005), indicating their utility for forming gels. The negative relationship between molecular weight and G content in *pyrifera* is worth noting given that low molecular weight fragments with short G-blocks do not contribute to gel strength (Moe et al. 1995). The trend for *pyrifera* blades to increase in G content as molecular weight decreases may serve to preserve the utility of low molecular weight alginates in industry. The positive relationship between molecular weight and rupture strength may be due to longer polymer chains prior to degradation. The relatively high stability (high rupture strength) of both the *pyrifera* and *integrifolia* alginate gels suggests the presence of long sequences of alternating M/G blocks (Donati et al. 2005). While higher molecular weight gels had higher rupture strength, they also tended to have a higher percent syneresis, which may indicate a trade-off in performance. Resisting deformation (low percent syneresis) is an important quality for alginates used as structural materials such as hydrogels.

During alginate biosynthesis, the mannuronic acid residues form first, and then guluronic acid forms from epimerization of the mannuronic acid (Nyvall et al.

2003). Therefore, as the kelp tissue ages, more guluronic acid forms and thus the M/G ratio can be dependent on age (McKee et al. 1992). While it was not possible to determine the age of the kelp individuals or blades sampled in this study, *integrifolia* populations in northern Chile tend to be perennial whereas *pyrifera* populations can be perennial in wave-exposed areas but tend to be annual in wave-protected areas (Buschmann et al. 2004). Based on the characterization of exposure used in Buschmann et al. (2004), the *pyrifera* sites sampled for this study encompass both exposed and protected sites. This indicates an inherent difference in alginate composition between the two morph types unrelated to wave exposure or age. While environmental factors can impact the life spans of blades (Rodriguez et al. 2016), many of these factors such as nitrogen, chlorophyll a, and photon flux are correlated with SST or depth, and G content of alginate samples in this study was still significantly predicted by morph even after accounting for SST and depth. Future studies could further examine the relationship between alginate composition, ecomorph, and other environmental or age-related factors.

Even after accounting for the effect of morph, alginate composition was also correlated with depth and temperature. Both *pyrifera* and *integrifolia* were sampled across similar depths, but the two morphs had no overlap in average sea surface temperature due to their latitudinal separation. In a study with *Laminaria digitata* and *Saccharina latissima* populations, temperature was the only environmental variable that correlated with alginate M/G composition, although there were no discernable seasonal patterns across sites (Manns et al. 2017). Interestingly, in our study deeper

individuals had higher G content, in contrast to current theory that more wave-exposed, and by extension shallower, kelp would have higher G to withstand greater wave force (Moe et al. 1995). It is possible that having higher M content can also be adaptive in shallower waters because greater flexibility of tissues would prevent breakage in waves. On the other hand, all of the populations sampled were relatively shallow compared to the global average depth of the species (Graham et al. 2007), so future research could investigate whether this pattern holds across a larger depth gradient.

Acknowledgements

I would like to thank Dr. Finn Aachmann, Dr. Olav Aarstad, and Wenche Strand for collaborating on this work and providing laboratory access, materials, equipment, and training for alginate extraction and analysis. I would also like to thank Kersten Schnurle for assisting with diving for kelp tissue sample collection, Cristobal Castañeda for assisting with SST data analyses, Dr. Sylvain Faugeron for helping with site selection for the different ecomorphs, and Dr. Pete Raimondi for advising statistical analyses. Sara T. Gonzalez was supported by a Ford Foundation Predoctoral Fellowship and the UC Santa Cruz EEB Department. This work has been supported by The Research Council of Norway via grants 294946 (SBP-N) and 226244 (NNP) and the Lewis and Clark Fund for Exploration and Research.

Figures

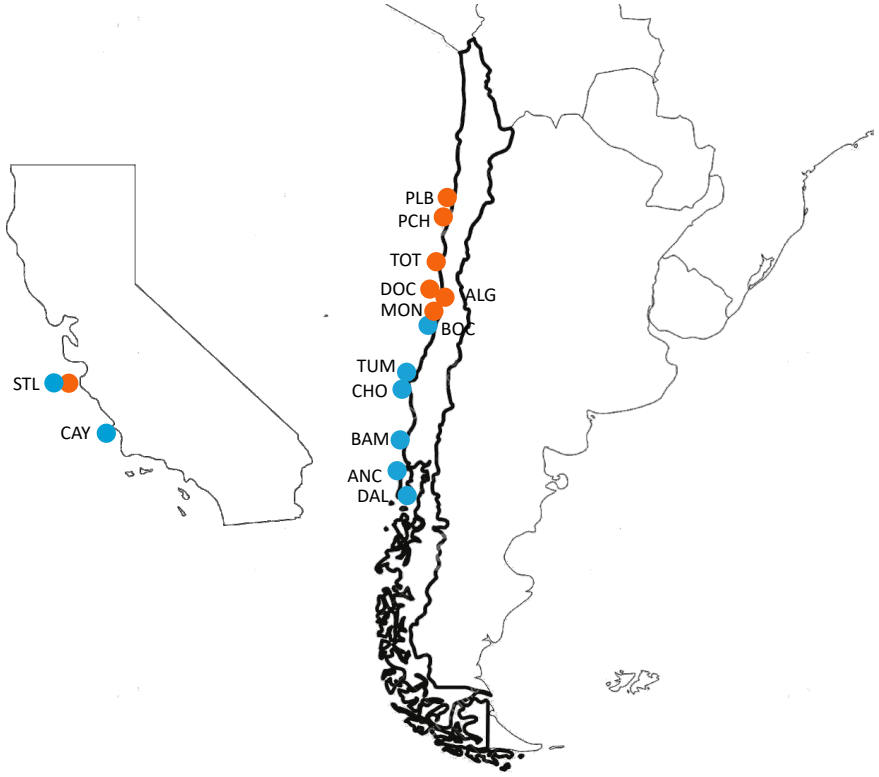


Figure 3.1. Map of populations sampled for *integrifolia* (orange) and *pyrifera* (blue) in California and Chile. Site abbreviations are as follows, in alphabetical order: ALG=Algarrobo, ANC=Ancud, BAM=Bahía Mansa, BOC= La Boca, CAY=Cayucos, CHO=Chome, DAL=Dalcahue, DOC=Las Docas, MON=Las Monjas, PCH=Punta Choros, PLB=Playa Blanca, STL=Stillwater Cove, TOT=Totorolillo, TUM=Tumbes.

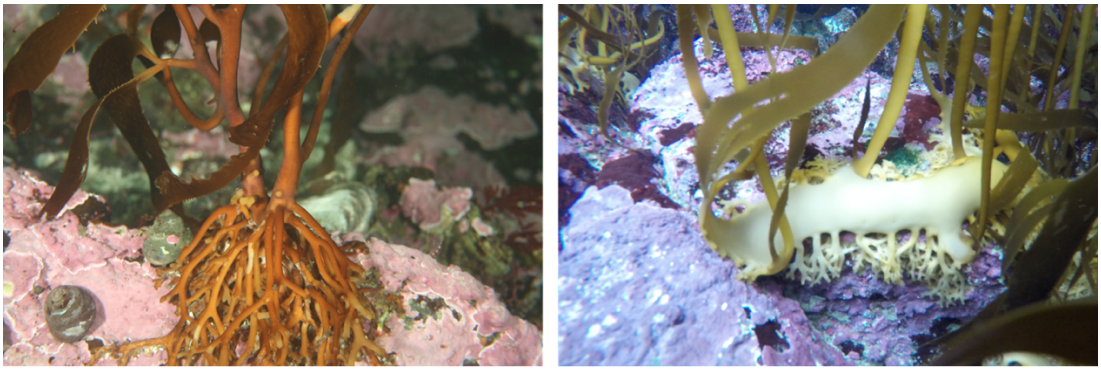


Figure 3.2. Representative photographs of the *Macrocystis* ecomorphs, *pyrifera* (left) and *integrifolia* (right). Left photo by Sam Richardson; right photo by S. T. Gonzalez.

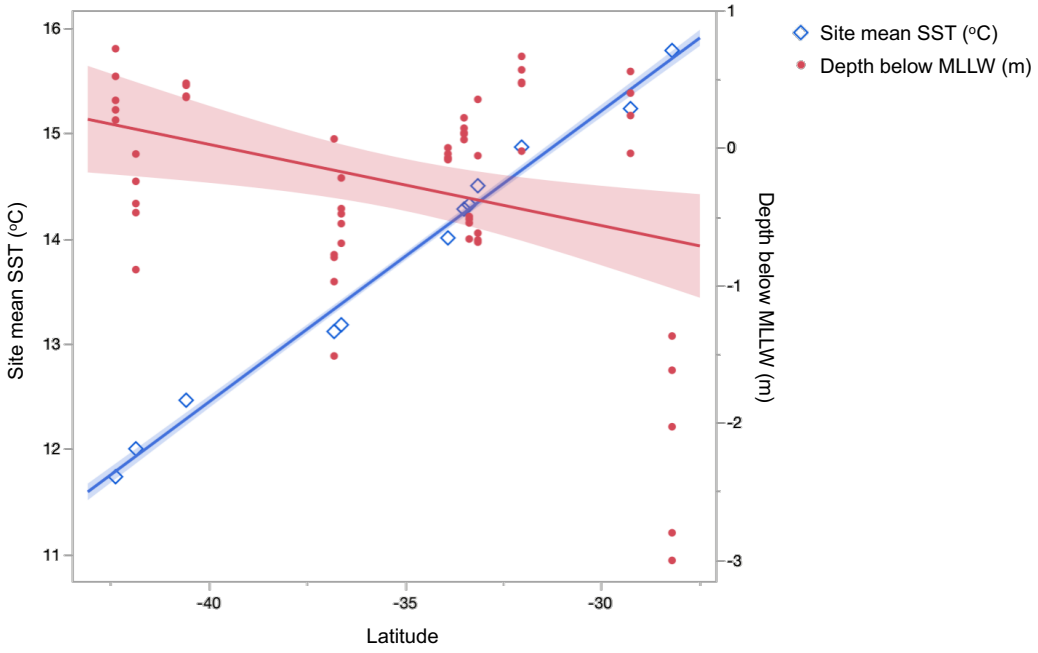


Figure 3.3. Linear regression associations between mean site sea surface temperature (SST) and latitude (left y-axis; blue), and between depth below mean lower low water (MLLW) and latitude (right y-axis; red).

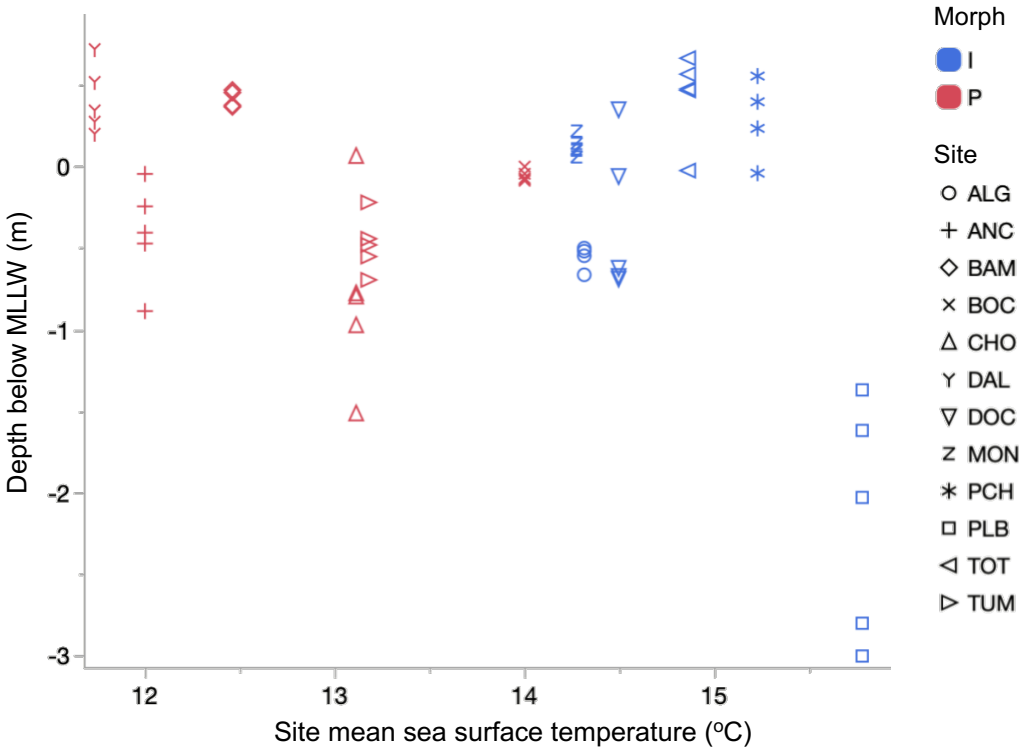


Figure 3.4. Site mean sea surface temperatures (x-axis) and depths of kelp individuals (y-axis), colored by morph and distinguished with symbols by site.

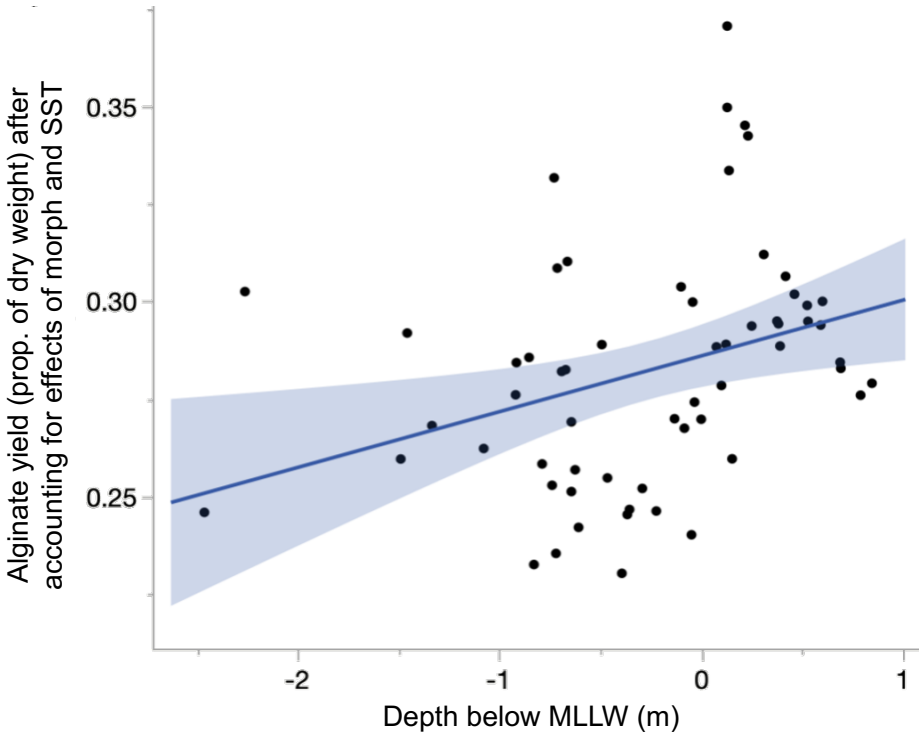


Figure 3.5. Leverage plot of alginate yield (as proportion of dry algal weight) vs depth below mean lower low water (MLLW) generated from mixed model.

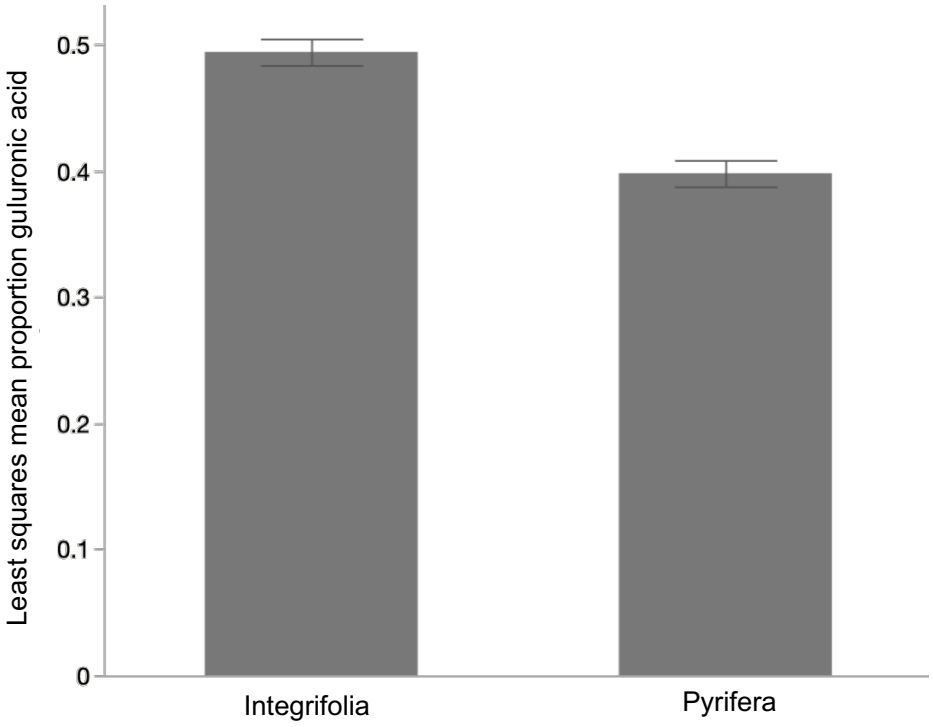


Figure 3.6. Least squares mean proportion G content in alginate samples for *integrifolia* and *pyrifera* samples.

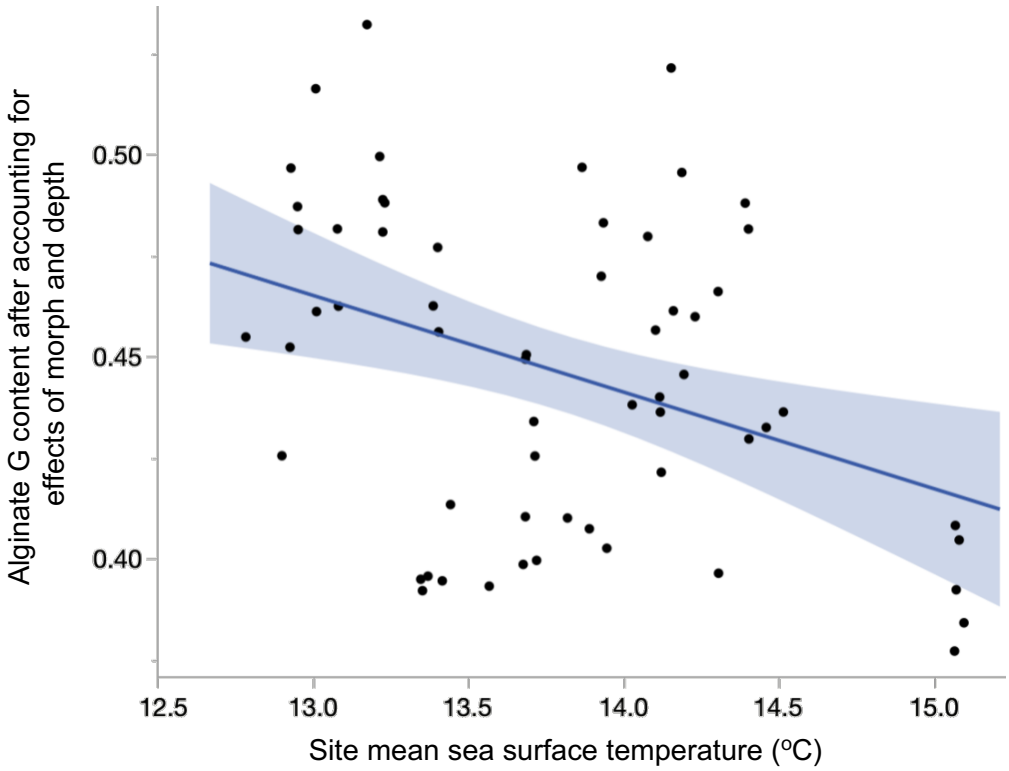


Figure 3.7. Leverage plot of proportion G content vs mean sea surface temperature generated from mixed model.

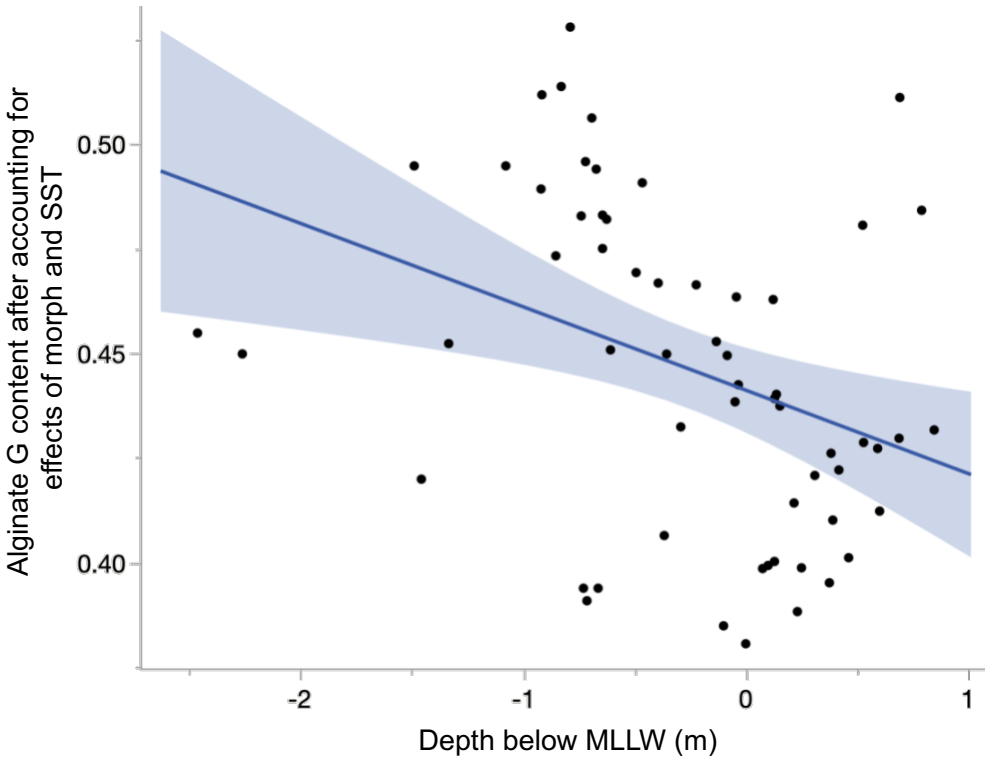


Figure 3.8. Leverage plot of proportion G content vs depth below mean lower low water (MLLW) generated from mixed model.

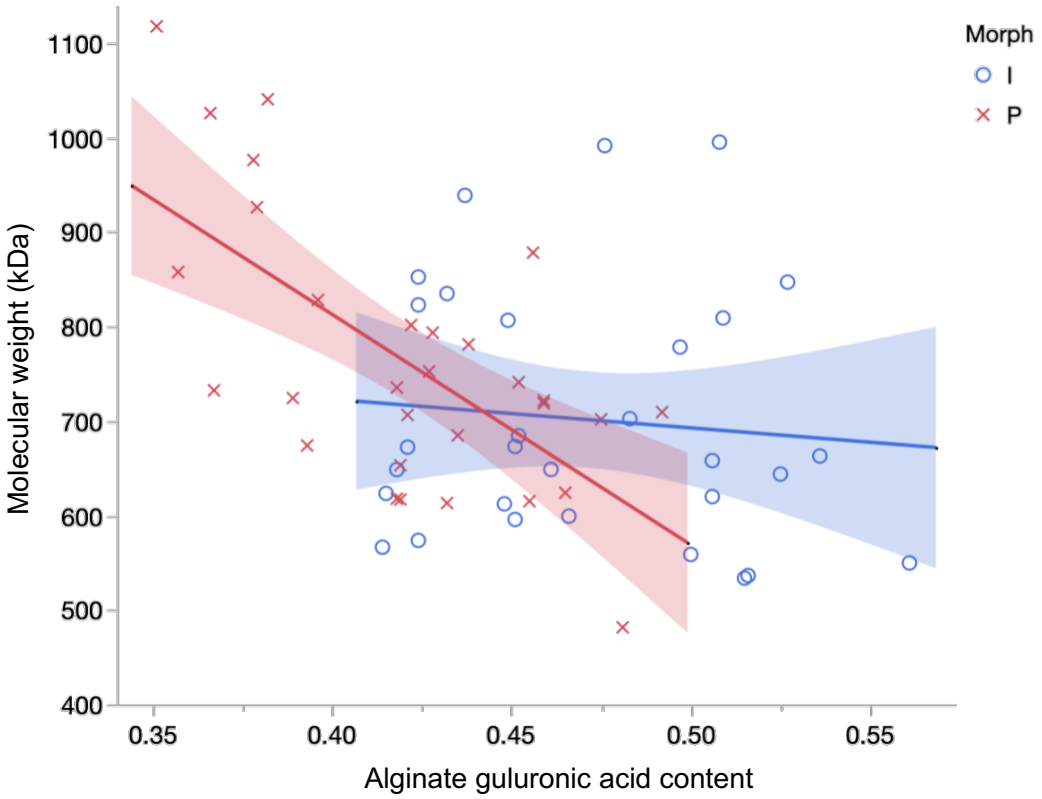


Figure 3.9. Relationships between alginate guluronic acid (G) content and molecular weight for *integrifolia* (blue; o marker) and *pyrifera* (red; x marker) alginate samples. Only the relationship for *pyrifera* is statistically significant.

Conclusion

Toothpaste, ice cream, probiotic capsules, lip balm, wound dressings—all of these everyday items share a common, essential ingredient: seaweed. Certain seaweeds known as brown algae produce the polysaccharide alginate, which gives the algal tissues both strength and flexibility (Draget et al. 2005), and these same properties are useful for commercial products that require a gelling or emulsifying agent. Kelp, a type of brown algae, is harvested and cultivated around the world for extraction of alginate, and giant kelp (*Macrocystis*) is one of the largest and fastest-growing kelp and one of the main species used for alginate extraction. Yet despite giant kelp's global prevalence, we have lacked an understanding of a crucial aspect of its natural history and development. Giant kelp exists in different forms—termed "ecomorphs"—in different places across its global range. The mechanisms underlying this variation are unclear, with some research attributing morphology to environmental influences (Demes et al. 2009) given a lack of genetic divergence between ecomorphs (Coyer et al. 2001), while others propose that morphological variation is correlated with genetic variation (Camus et al. 2018a). The motivation for this dissertation stems from a desire to resolve a decades-long controversy over the evolutionary origins of the morphologically distinct forms of giant kelp known as the *pyrifera* and *integrifolia* ecomorphs, and to explore the functional consequences of these different forms.

This dissertation shows that ecomorphology in *Macrocystis* is not environmentally induced, as held by previous theory, but rather it is genetically fixed. This work also demonstrates that the two ecomorphs should be viewed distinctively from an industry perspective, since their compositional differences in alginate may make each ecomorph suited for distinct material applications. As one of the most commercially important chemicals extracted from kelp, alginate as a global market is estimated to be worth at least $920 million by 2025 and as much as $1.1 billion by 2028 (Million Insights 2017, Research and Markets 2021, Grand View Research 2021, KBV Research 2021), so even small differences in alginate composition between kelp groups may have profound importance on an industrial scale.

In Chapter 1, I established foundational knowledge for the subsequent chapters by examining genetic differences between ecomorphs and global hemispheres. This work was made possible through access to a newly assembled *Macrocystis* genome developed by my collaborators. For this chapter I sampled giant kelp from a large portion of its Southern Hemisphere range as well as several representative Northern Hemisphere populations and used whole-genome sequencing to reveal that the two morphs are genetically separated in both Northern and Southern Hemispheres. Unlike previous genetic studies on giant kelp ecomorphs that used gene markers or specific sections of the genome, my research examined the entire genome which allowed for analyzing not only neutral alleles but also potentially adaptive alleles that would distinguish the morphs. Interestingly, the genetic divergence between ecomorphs was much clearer in the Southern Hemisphere compared to the

Northern Hemisphere, and the biggest genetic break overall was between hemispheres, indicating that these two world regions likely have different evolutionary trajectories that ultimately led toward an identical outcome of the same two distinct ecomorphs.

In Chapter 2, I conducted a common garden experiment, which is one of the most classic methods for disentangling effects of genetics versus environment on a phenotype, like holdfast morphology. The experiment showed complete morphological divergence between the two pure-bred ecomorph treatments, demonstrating that the characteristic morphologies are genetically pre-determined and re-enforcing the findings in Chapter 1. Prior to this experiment, it was unknown how the strap-like holdfast of *integrifolia* develops, but my work revealed that the holdfast structure is actually composed of stipe tissue that becomes overgrown by haptera and pulled down to the base of the plant.

In Chapter 3, I explored a possible functional consequence of morphology by analyzing the chemical composition of alginate, one of the most important commercial chemicals that is extracted from *Macrocystis*. Even after accounting for site-specific differences, the two ecomorphs still significantly differed in their alginate compositions, where *integrifolia* had a higher guluronic acid content than *pyrifera*, allowing it to make stronger gels. The more elastic alginate gels are used for making textiles and wound dressings while more rigid alginate gels are used in pharmaceuticals, cosmetics, and foods. Since uronic acid block proportions and distributions can have profound effects on the gelling capacity and elasticity of the

gels produced by these alginates, the two morphs may have distinct applications for industry. Environmental conditions like depth and sea surface temperature also related to alginate composition, so it may be important to consider both morph type and local environment for seaweed harvesting or cultivation for alginate extraction.

The collective results of all three chapters show intrinsic genetic, morphological, and chemical differences between the two ecomorphs, warranting their consideration as separate species. These considerations are important not only for protecting genetic diversity, but they also have relevant applications to kelp aquaculture and restoration. Recognizing ecological and developmental differences between kelp populations will influence decisions about which group is best suited for a particular restoration site or industry use. Additionally, advancing genetic work on kelp can help us assess how kelp populations are responding to ocean warming and identify local adaptations to climate stress such as high thermal tolerance, which is particularly relevant for selecting cultivars for farming.

While this dissertation provides novel findings in *Macrocystis* genetics, morphological development, and alginate properties, there are many new questions raised by these findings and several directions in which future research could further advance knowledge in these fields. The environmental conditions that may have selected for either *pyrifera* or *integrifolia* as the evolutionarily adaptive form in different regions are still unknown. We now know that *integrifolia* can survive with equal success as *pyrifera* in depths of up to 9 m, but perhaps toward the extremes of where giant kelp is found—as deep as 68 m and as shallow as 0 m (Schiel & Foster

2015) —one morph may have a competitive advantage. It is also unclear why locations with the two morphs existing in sympatry, such as Stillwater Cove, CA, are not more common across the species' global range. Finally, it would be important for aquaculture purposes to examine potential differences in other commercially important chemicals such as fucoidan and mannitol, and whether the morphs differ in biomass yield per meter of aquaculture line due to their different growth forms. Seaweed aquaculture is the fastest-growing aquaculture sector in the United States (NOAA 2020), and kelp has great potential to be a prominent global crop in the future, as most of its cultivation takes place in the ocean, where it requires no freshwater, fertilizer, feed, antibiotics, or pesticides. But in order advance breeding programs, technology, and large-scale ocean farms for growing kelp, we need to have a comprehensive understanding of kelp ecology and natural history, and a constant curiosity to fuel research investigations.

Appendices

A1. DNA extraction protocol (adapted from Macherey-Nagel Nucleospin Plant Kit II Genomic DNA Purification Kit)

Modifications to the original protocol are written in bold.

1. Homogenize **10 mg** dry weight (lypholized) plant material. Proceed will cell lysis using Buffer PL1.
2. Transfer the resulting powder to a new tube and add 400 µL Buffer PL1. Vortex the mixture thoroughly. Add 10 µL RNase A solution and mix sample thoroughly. Incubate the suspension for **30 min** at 65°C.
3. Place a NucleoSpin® Filter (violet ring) into a new Collection Tube (2 mL) and load the lysate into the column. Centrifuge for **10 min** at 11,000 x *g*, collect the clear flow-through and discard the NucleoSpin® Filter. Transfer the clear supernatant to a new 1.5 mL microcentrifuge tube.
4. Add 450 µL Buffer PC and mix thoroughly by vortexing.
5. Place a NucleoSpin® Plant II Column (green ring) into a new Collection Tube (2 mL) and load a maximum of 700 µL of the sample. Centrifuge for **5 min** at 11,000 x *g* and discard the flow-through. For higher sample volumes repeat the loading step.

6. 1st wash: Add 400 µL Buffer PW1 to the NucleoSpin® Plant II Column. Centrifuge for **2 min** at 11,000 x *g* and discard flow-through. 2nd wash: Add 700 µL Buffer PW2 to the NucleoSpin® Plant II Column. Centrifuge for **2 min** at 11,000 x *g* and discard flow-through. 3rd wash: Add another **700 µL** to the NucleoSpin® Plant II Column. Centrifuge for **10 min** at 11,000 x *g* and discard flow-through.
7. **Repeat step 6.**
8. Place the NucleoSpin® Plant II Column into a new 1.5 mL microcentrifuge tube. Pipette 50 µL Buffer PE (65ºC) onto the membrane. Incubate the NucleoSpin® Plant II Column for **10 min** at 65ºC. Centrifuge for **10 min** at 11,000 x *g* to elute the DNA. Repeat this step with another 50 µL Buffer PE (65ºC) and elute into the same tube.

A2. Provasoli Enriched Seawater recipe (adapted from Provasoli's [1968] ES medium)

For 1 L of Stock Solution, combine the following amounts of each solution:

Solution 1: Base	599 mL
Solution II: Fe	200 mL
Solution III: PII metals	200 mL
Solution IV: Vitamins	1 mL

For 1 L of PES natural seawater, add 20 mL of Stock Solution to 980 mL of sterile natural seawater. Store at 4°C with no light.

Solution 1: Base

599 mL DI water

4 g Tris Buffer

2.8 g $NaNO_3$

0.4 g Na_2 glycerophosphate

0.004 g Thiamine HCl (Vitamin B_1)

Solution II: Fe

1 L DI water

0.700 g $Fe(NH_4)_2 (SO_4)_2 6H_2O$

0.600 g Na_2 EDTA

Solution III: PII metals

1 L DI water

1 g Na_2 EDTA

1.140 g H_3BO_3 (Boric acid)

0.049 g $FeCl_3$ $6H_2O$

0.130 g $MnSO_4H_2O$

0.005 g $CoSO_4$ $7H_2O$

0.022 g $ZnSO_4$ $7H_2O$

Solution IV: Vitamins

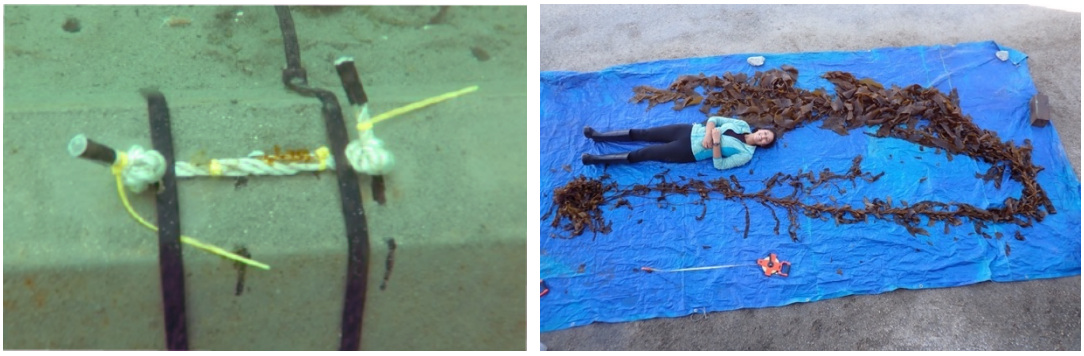
25 mL DI water

0.002 g Vitamin B_{12}

0.001 g Biotin

A3. Photos of kelp sporophyte growth from lab to field for Chapter 2.

Panel 1. Kelp sporophytes growing on nylon strings (6 cm long) in a Petri dish, viewed with the unaided eye (left) and under a dissecting microscope (right), approximately 1 week prior to outplanting.



Panel 2. Left: Kelp string (3.2 mm diameter) threaded into the outplanting line (6.4 mm diameter) and attached with nylon zipties to a concrete parking bumper in the ocean. Right: Harvested kelp outplant from experiment after approximately 6 months in the ocean, measuring 9 m in length (measuring tape is pulled out to 1 m for reference).

References

Aguilar-Rosas, L. E., Aguilar-Rosas, R., Marcos-Ramírez, R., Caceres- Rubio, C. F. & McPeak, R. H. (2003). New record of *Macrocystis laevis* Hay (Laminariales, Phaeophyta) on the Pacific coast of Chile. *Proc. Int. Seaweed Symp*., *17*, 337–40.

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Arvizu-Higuera, D. L., Hernández-Carmona, G., & Rodríguez-Montesinos, Y. E. (2002). Parámetros que afectan la conversión del ácido algínico en alginato de sodio. *Ciencias Marinas*, *28*(1), 27–36. https://doi.org/10.7773/cm.v28i1.208

Astorga, M. P., Hernández, C. E., Valenzuela, C. P., Avaria-Llautureo, J., & Westermeier, R. (2012). Origin, diversification, and historical biogeography of the giant kelps genus Macrocystis: Evidences from Bayesian phylogenetic analysis. *Revista de Biología Marina y Oceanografía*, *47*(3), 573–579. https://doi.org/10.4067/S0718-19572012000300019

Augyte, S., Lewis, L., Lin, S., Neefus, C. D., & Yarish, C. (2018). Speciation in the exposed intertidal zone: the case of Saccharina angustissima comb. nov. & stat. nov. (Laminariales, Phaeophyceae). *Phycologia*, *57*(1), 100–112. https://doi.org/10.2216/17-40.1

Brostoff, W. N. (1988). Taxonomic studies of Macrocystis pyrifera (L.) C. Agardh (Phaeophyta) in Southern California: Holdfasts and basal stipes. *Aquatic Botany*, *31*(3–4), 289–305. https://doi.org/10.1016/0304-3770(88)90018-6

Buschmann, A. H., Vásquez, J. A., Osorio, P., Reyes, E., Filún, L., Hernández-González, M. C., & Vega, A. (2004). The effect of water movement , temperature and salinity on abundance and reproductive patterns of *Macrocystis* spp. (Phaeophyta) at different latitudes in Chile. *Marine Biology*, *45*, 849–862. https://doi.org/10.1007/s00227-004-1393-8

Camus, C., & Buschmann, A. H. (2017). *Macrocystis pyrifera* aquafarming : Production optimization of rope-seeded juvenile sporophytes. *Aquaculture*, *468*, 107–114. https://doi.org/10.1016/j.aquaculture.2016.10.010

Camus, C., Faugeron, S., & Buschmann, A. H. (2018a). Assessment of genetic and

phenotypic diversity of the giant kelp, *Macrocystis pyrifera*, to support breeding programs. *Algal Research*, *30*, 101–112. https://doi.org/10.1016/j.algal.2018.01.004

Camus, C., Infante, J., & Buschmann, A. H. (2018b). Overview of 3 year precommercial seafarming of *Macrocystis pyrifera* along the Chilean coast. *Reviews in Aquaculture*, *10*(3), 543–559. https://doi.org/10.1111/raq.12185

Camus, P. A. (2001). Biogeografía marina de Chile continental. *Revista Chilena de Historia Natural*, *74*(3), 587–617. https://doi.org/10.4067/s0716-078x2001000300008

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Cheshire, A. C., & Hallam, N. D. (1985). The environmental role of alginates in *Durvillaea potatorum* (Fucales, Phaeophyta). *Phycologia*, *24*(2), 147–153. https://doi.org/10.2216/i0031-8884-24-2-147.1

Chin, N. K. M., Brown, M. T., & Heads, M. J. (1991). The biogeography of Lessoniaceae, with special reference to *Macrocystis* C. Agardh (Phaeophyta: Laminariales). *Hydrobiologia*, *215*(1), 1–11. https://doi.org/10.1007/BF00005895

Coyer, J. a, Smith, G. J., & Andersen, R. a. (2001). Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. *Journal of Phycology*, *37*, 574–585. https://doi.org/10.1046/j.1529-8817.2001.037001574.x

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2), 1–4. https://doi.org/10.1093/gigascience/giab008

Demes, K. W., Graham, M. H., & Suskiewicz, T. S. (2009). Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: The giant kelp, *Macrocystis* (Laminariales, Phaeophyceae), is a monospecific genus. *Journal of Phycology*, *45*(6), 1266–1269. https://doi.org/10.1111/j.1529-8817.2009.00752.x

Donati, I., Holtan, S., Mørch, Y. A., Borgogna, M., Dentini, M., & Skjåk-Bræk, G. (2005). New hypothesis on the role of alternating sequences in calcium-alginate gels. *Biomacromolecules*, *6*, 1031–1040.

Draget, K., Smidsrød, O., & Skjåk-Bræk, G. (2005). Alginates from algae. In: A. Steinbüchel and S. K. Rhee (Eds.), *Polysaccharides and Polyamides in the Food Iindustry: Properties, Production and Patents.* pp 1–30. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/3527600035.bpol6008/full

Druehl, L. D., & Kemp, L. (1982). Morphological and growth responses of geographically isolated *Macrocystis integrifolia* populations when grown in a common environment. *Canadian Journal of Botany*, *60*, 1409–1413. https://doi.org/10.1139/b82-179

Ertesvåg, H., & Skjåk-Bræk, G. (1999). *Modification of Alginate Using Mannuronan C-5-Epimerases*. Totowa, NJ: Humana Press Inc. https://doi.org/10.1007/978-1-59259-261-6_6

Frei, E., & Preston, R. D. (1962). Configuration of alginic acid in marine brown algae. *Nature*, *196*, 130–134.

Gaylord, B., Reed, D. C., Raimondi, P. T., & Washburn, L. (2006). Macroalgal spore dispersal in coastal environments: Mechanistic insights revealed by theory and experiment. *Ecological Monographs*, *76*(4), 481–502. https://doi.org/10.1890/0012-9615(2006)076[0481:MSDICE]2.0.CO;2

Gaylord, B., Reed, D. C., Raimondi, P. T., Washburn, L., & McLean, S. R. (2002). A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. *Ecology*, *83*(5), 1239–1251. https://doi.org/10.1890/0012-9658(2002)083[1239:APBMOM]2.0.CO;2

Gomez, C. G., Pérez Lambrecht, M. V., Lozano, J. E., Rinaudo, M., & Villar, M. A. (2009). Influence of the extraction-purification conditions on final properties of alginates obtained from brown algae (*Macrocystis pyrifera*). *International Journal of Biological Macromolecules*, *44*(4), 365–371. https://doi.org/10.1016/j.ijbiomac.2009.02.005

Graham, M. H., Vásquez, J. A., & Buschmann, A. H. (2007). Global ecology of the giant kelp *Macrocystis*: From ecotypes to ecosystems. *Oceanography and Marine Biology: An Annual Review*, *45*, 39–88.

Grand View Research. (2021). *Alginate Market Size, Share & Trends Analysis Report by Type (High M, High G), by Product (Sodium, Propylene Glycol), by Application (Pharmaceutical, Industrial), by Region, and Segment Forecasts, 2021-2028.* Available at: https://www.grandviewresearch.com/industry-analysis/alginate-market

Grasdalen, H. (1983). High-field, 1H-n.m.r. spectroscopy of alginate: Sequential structure and linkage conformations. *Carbohydrate Research*, *118*(C), 255–260. https://doi.org/10.1016/0008-6215(83)88053-7

Gregor, J. W. (1944). The ecotype. *Biological Reviews*, *19*(1), 20–30.

Gutierrez, A., Correa, T., Muñoz, V., Santibañez, A., Marcos, R., Cáceres, C., & Buschmann, A. H. (2006). Farming of the giant kelp *Macrocystis pyrifera* in southern Chile for development of novel food products. *Journal of Applied Phycology*, *18*(3–5), 259–267. https://doi.org/10.1007/s10811-006-9025-y

Hargarten, H. (2015). A tale of two morphs: Genetic and genotypic structure between *Macrocystis pyrifera* and *Macrocystis integrifolia*. Master's thesis. University of Wisconsin, Milwaukee, 133 pp.

Haug, A., Larsen, B., & Smidsrød, O. (1966). A study of the constitution of alginic acid by partial hydrolysis. *Acta Chemica Scandinavica*, *20*, 183–190. https://doi.org/10.1007/978-0-387-30160-0_400

Hay, C. H. 1986. A new species of *Macrocystis* C. Ag. (Phaeophyta) from Marion Island, southern Indian Ocean. *Phycologia* 25:241–252.

Haye, P. A., Segovia, N. I., Muñoz-Herrera, N. C., Gálvez, F. E., Martínez, A., Meynard, A., Pardo-Gandarillas, M. C., Poulin, E., & Faugeron, S. (2014). Phylogeographic structure in benthic marine invertebrates of the southeast pacific coast of Chile with differing dispersal potential. *PLoS ONE*, *9*(2), 1–15. https://doi.org/10.1371/journal.pone.0088613

Hernandez-Carmona, G., Mchugh, D. J., Arvizu-Higuera, D. L., & Rodriguez-Montesinos, E. (1999). Pilot plant scale extraction of alginate from *Macrocystis pyrifera*. 1. Effect of pre-extraction treatments on yield and quality of alginate. *Journal of Applied Phycology*, (10), 507–513.

Howe, M.A. 1914. The marine algae of Perú. *Memoirs of the Torrey Botanical Club* 15:1–185.

Imeson, A. (2010). *Food Stabilisers, Thickeners and Gelling Agents*. Chichester: Wiley-Blackwell.

Jeffries, S. (2015). Impact of individual and population-scale dynamics on growth and reproduction of two morphologies of *Macrocystis* in central California. Master's thesis. California State University Monterey Bay, Seaside, 69 pp.

Johansson, M. L., Alberto, F., Reed, D. C., Raimondi, P. T., Coelho, N. C., Young,

M. A., Drake, P. T., Edwards, C. A., Cavanaugh, K., Assis, J., Ladah, L. B., Bell, T. W., Coyer, J. A., Siegel, D. A., & Serrão, E. A. (2015). Seascape drivers of *Macrocystis pyrifera* population genetic structure in the northeast Pacific. *Molecular Ecology*, *24*(19), 4866–4885. https://doi.org/10.1111/mec.13371

KBV Research. (2021). *Global Alginate Market by Type (High G, High M), by Product (Sodium Alginate, Calcium Alginate, Potassium Alginate, Propylene Glycol Alginate and Others), by Application (Industrial, Pharmaceutical, Food & Beverages and Others), by Region, Industry Analysis and Forecast, 2020-2026.* Available at: https://www.kbvresearch.com/alginate-market/

Kelly, B. J., & Brown, M. T. (2000). Variations in the alginate content and composition of *Durvillaea antarctica* and *D. willana* from southern New Zealand. *Journal of Applied Phycology*, *12*, 317–324. https://doi.org/10.1023/A

Kopczak, C. D., Zimmerman, R. C., & Kremer, J. N. (1991). Variation in nitrogen physiology and growth among geographically isolated populations of the giant kelp, *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology*, *27*(2), 149–158.

Lewis, R. J., & Neushul, M. (1994). Northern and Southern Hemisphere hybrids of *Macrocystis* (Phaeophyceae). *Journal of Phycology*, *30*, 346–353.

Lewis, R. J., Neushul, M., & Harger, B. W. W. (1986). Interspecific hybridization of the species of *Macrocystis* in California. *Aquaculture*, *57*(1–4), 203–210. https://doi.org/10.1016/0044-8486(86)90198-5

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lindberg, D. R. (1991). Marine biotic interchange between the Northern and Southern Hemispheres. *Society*, *17*(3), 308–324.

Macaya, E. C., & Zuccarello, G. C. (2010a). Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern Pacific. *Marine Ecology Progress Series*, *420*, 103–112. https://doi.org/10.3354/meps08893

Macaya, Erasmo C., & Zuccarello, G. C. (2010b). DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (laminariales). *Journal of Phycology*,

46(4), 736–742. https://doi.org/10.1111/j.1529-8817.2010.00845.x

Mackenzie, I. M. (1993). Genetic and morphological variation in *Macrocystis* C. Agardh (Laminariales, Phaeophyceae) of the North American coast. Master's thesis. Simon Fraser University, 78 pp.

Manns, D., Nielsen, M. M., Bruhn, A., Saake, B., & Meyer, A. S. (2017). Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. *Journal of Applied Phycology*, *29*(3), 1493–1506. https://doi.org/10.1007/s10811-017-1056-z

Martinsen, A., Skjåk-Bræk, G., & Smidsrød, O. (1989). Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnology and Bioengineering*, *33*(1), 79–89. https://doi.org/10.1002/bit.260330111

McKee, J. W. A., Kavalieris, L., Brasch, D. J., Brown, M. T., & Melton, L. (1992). Alginate content and composition of Macrocystis pyrifera from New Zealand. *Journal of Applied Phycology*, (4), 357–359. https://doi.org/10.1007/BF02185794

Meneses, I., & Santelices, B. (2000). Patrones y puntos de quiebre en la distribución de algas bentónicas a lo largo de la costa Pacífica de Sudamérica. *Revista Chilena de Historia Natural*, *73*(4), 615–623.

Million Insights. (2017). *Alginate Market Size & Forecast Report, 2014-2025*. Available at: https://www.millioninsights.com/industry-reports/alginate-market

Moe, S. T., Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1995). Ch. 9 Alginates. In: A. M. Stephen (Ed.), *Food polysaccharides and their applications*. pp. 245–286. New York, Basel, Hong Kong: Marcel Dekker, Inc.

Murúa, P., Patiño, D. J., Müller, D. G., & Westermeier, R. (2021). Sexual compatibility in giant kelp gametophytes: Inter-cultivar hybridization is average between parents but excels under harsher conditions. *Journal of Applied Phycology*, *33*, 3261-3275. https://doi.org/10.1007/s10811-021-02506-z

Neushul, M., (1971). The species of Macrocystis with particular reference to those of North and South America. In: W.J. North (Ed.), *The Biology of Giant Kelp Beds (*Macrocystis*) in California*. *Nova Hedwigia*, 32: 211-222.

NOAA Office of Communications. (2020, September 28). *Seaweed Aquaculture.* Available at: https://www.fisheries.noaa.gov/national/aquaculture/seaweed-aquaculture

Nyvall, P., Corre, E., Boisset, C., Barbeyron, T., Rousvoal, S., Scornet, D., Kloareg, B., & Boyen, C. (2003). Characterization of mannuronan C-5-epimerase genes from the brown alga *Laminaria digitata. Plant Physiology*, *133*(2), 726–735. https://doi.org/10.1104/pp.103.025981

Onsøyen E. (1996). Commercial applications of alginates. *Carbohydr Eur, 14*, 26–31.

Perissinotto, R., & McQuaid, C. D. (1992). Deep occurrence of the giant kelp *Macrocystis laevis* in the Southern Ocean. *Marine Ec*, *81*, 89–95. https://doi.org/10.2514/6.1994-3835

Plá, P. C., & Alveal, K. (2012). Development of *Macrocystis pyrifera* from spores and gametes on artificial substrate: Algal production in a surface culture. *Lat. Am. J. Aquat. Res.*, *40*(2), 292–299. https://doi.org/10.3856/vol40-issue2-fulltext-5

Pucell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J. Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analysis. *The American Journal of Human Genetics*, *81*, 559–575.

Qin, Y. (2006). The characterization of alginate wound dressings with different fiber and textile structures. *Journal of Applied Polymer Science*, *100*(3), 2516–2520. https://doi.org/10.1002/app.23668

Raimondi, P. T., Reed, D. C., Gaylord, B., & Washburn, L. (2004). Effects of self-fertilization in the giant kelp, *Macrocystis pyrifera*. *Ecology*, *85*(12), 3267–3276.

Reed, D. C., Neushul, M., & Ebeling, A. W. (1991). Role of settlement density on gametophyte growth and reproduction in the kelps *Pterygophora californica* and *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology*, *27*, 361–366.

Reed, D. C., Schroeter, S. C., & Raimondi, P. T. (2004). Spore supply and habitat availability as sources of recruitment limitation in the giant kelp *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology*, *40*(2), 275–284. https://doi.org/10.1046/j.1529-8817.2004.03119.x

Rehm, B. H. A., & Moradali, M. F. (2018). *Alginates and Their Biomedical Applications*. Singapore: Springer Nature Singapore Pte Ltd.

Research and Markets. (2021). *Global Alginate Market by Type, by Product, by Application, by Region, Industry Analysis and Forecast, 2020-2026.* Available at: https://www.researchandmarkets.com/reports/5241452/global-alginate-

market-by-type-by-product-by?utm_source=BW&utm_medium=PressRelease&utm_code=5z3svq&utm_campaign=1507009+-+Global+Alginate+Market+(2020+to+2026)+-+by+Type%2c+Product%2c+Application+and+Region&utm_exec=jamu273prd

Rodriguez, G. E., Reed, D. C., & Holbrook, S. J. (2016). Blade life span, structural investment, and nutrient allocation in giant kelp. *Oecologia*, *182*(2), 397–404. https://doi.org/10.1007/s00442-016-3674-6

Rosell, K.-G., & Srivastava, L. M. (1984). Seasonal variation in the chemical constituents of the brown algae *Macrocystis integrifolia* and *Nereocystis luetkeana*. *Canadian Journal of Botany*, *62*(11), 2229–2236. https://doi.org/10.1139/b84-303

Salavarria, E., Macaya, E. C., & Gil-Kodaka, P. (2018). Haplotype diversity of *Macrocystis pyrifera* (Phaeophyceae: Laminariales) in the central and southern coast of Peru. *Pan-American Journal of Aquatic Sciences*, *13*(4), 311-319. https://doi.org/10.13140/RG.2.2.26986.57283

Schiel, D. R. and Foster, M. S. (2015). The Biology and Ecology of Giant Kelp Forests. University of California Press, Oakland, California. 395 pp.

Schiener, P., Black, K. D., Stanley, M. S., & Green, D. H. (2015). The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *Journal of Applied Phycology*, *27*, 363–373. https://doi.org/10.1007/s10811-014-0327-1

Setchell, W. A., (1932). *Macrocystis* and its holdfasts. *Univ. Calif. Publ. Bot.*, *16* (13), 445-492.

Smidsrød, O., Haug, A., Lian, B., Huhtikangas, A., Pearson, W. B., & Meisalo, V. (1972). Properties of poly(1,4-hexuronates) in the gel state. II. Comparison of gels of different chemical composition. *Acta Chemica Scandinavica*, *26*, 79-88. https://doi.org/10.3891/acta.chem.scand.26-0079

Smidsrød, O.; Draget, K. I. (1996). Alginates: chemistry and physical properties. *Carbohydrates Eur.*, *14,* 6–13.

Stern, D. L. (2013). The genetic causes of convergent evolution. *Nature Reviews Genetics*, *14*(11), 751–764. https://doi.org/10.1038/nrg3483

Turesson, G. (1922). The species and the variety as ecological units. *Hereditas*, *3*(1), 100–113.

Vásquez, J. A. (2008). Production, use and fate of Chilean brown seaweeds: Resources for a sustainable fishery. *Journal of Applied Phycology*, *20*(5), 7–17. https://doi.org/10.1007/s10811-007-9285-1

Venegas, M., Edding, M. E., & Matsuhiro, B. (1993). Alginate composition of *Lessonia trabeculata* (Phaeophyta: Laminariales) growing in exposed and sheltered habitats. *Botanica Marina*, *36*, 47–51. https://doi.org/10.1515/botm.1993.36.1.47

Weber, X. A., Edgar, G. J., Banks, S. C., Waters, J. M., & Fraser, C. I. (2017). A morphological and phylogenetic investigation into divergence among sympatric Australian southern bull kelps (*Durvillaea potatorum* and *D. amatheiae* sp. nov.). *Molecular Phylogenetics and Evolution*, *107*, 630–643. https://doi.org/10.1016/j.ympev.2016.12.027

Westermeier, R., Murúa, P., Patiño, D. J., Muñoz, L., Ruiz, A., & Müller, D. G. (2012). Variations of chemical composition and energy content in natural and genetically defined cultivars of *Macrocystis* from Chile. *Journal of Applied Phycology*, *24*, 1191–1201. https://doi.org/10.1007/s10811-011-9752-6

Westermeier, R., Patin, D., Piel, M. I., Maier, I., & Mueller, D. G. (2006). A new approach to kelp mariculture in Chile: production of free-floating sporophyte seedlings from gametophyte cultures of *Lessonia trabeculata* and *Macrocystis pyrifera*. *Aquaculture Research*, *37*, 164–171. https://doi.org/10.1111/j.1365-2109.2005.01414.x

Westermeier, R., Patiño, D., & Müller, D. G. (2007). Sexual compatibility and hybrid formation between the giant kelp species *Macrocystis pyrifera* and *M. integrifolia* (Laminariales, Phaeophyceae) in Chile. *Journal of Applied Phycology*, *19*(3), 215–221. https://doi.org/10.1007/s10811-006-9126-7

Whyte, J. N. C., & Englar, J. R. (1978). Primary organic chemical composition of the marine alga *Macrocystis integrifolia* over the growing season. *Fisheries and Marine Services Technical Report*, (787), 1-22.

Womersley, H. B. S. 1954. The species of *Macrocystis* with special reference to those on southern Australian coasts. *University of California Publications in Botany, 27*, 109–132.

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

Zubia, M., Payri, C., & Deslandes, E. (2008). Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of Applied Phycology*, *20*, 1033–1043. https://doi.org/10.1007/s10811-007-9303-3