Evolutionary Ecology of Seaweed Strength and Flexibility

by

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Abstract

An organism’s success is largely dependent upon its ability to function and survive within the physical constraints of its environment. The wave-swept shoreline is one of the most mechanically challenging environments on Earth, with passing waves imposing hydrodynamic forces comparable to those in a hurricane every 5-10 seconds. Nonetheless, the wave-swept intertidal hosts an exquisite diversity of plants and animals. I used field and laboratory techniques to examine morphological and material adaptations of seaweeds to life in high-energy environments. In Chapter 2, I experimentally manipulated size and shape of 16 species of foliose red algae and showed that variation in tissue material and structural properties explain differences in hydrodynamic performance among species—thinner, more flexible tissues allow blades to reconfigure in flow and reduce drag. Because material and structural properties may shift through an organism’s lifespan, I then investigated how reproduction and aging impact seaweed mechanical traits. In Chapter 3, I documented the mechanical costs associated with reproduction in the winged kelp, Alaria marginata, and found that shifts in other structural and material traits compensated for the increased drag associated with reproductive blades. In Chapter 4, a survey of 27 species of foliose algae showed that aging affected material properties of all species similarly.
However, the implications of aging tissues on mechanical design varied with the growth form of the species: apically growing red algae have their oldest (stiffest) tissue attaching their blades to the substrate, while kelps, whose blades grow basally, are supported by their newest (most flexible) tissue. Finally, in Chapter 5 I tested for intraspecific variation in mechanical traits and found that variation among individuals (Egregia menziesii) at an exposed site accurately predicted survivorship during winter storms. Individuals with weaker fronds were more likely to survive because their increased propensity to self-prune in smaller waves reduces their risk of dislodgement in larger waves. Taken together these results support the notion that the material and structural properties of organisms have important functional consequences and highlight how mechanical traits can impact ecological and evolutionary processes.
Preface

Much of the work included in this dissertation has been published or submitted for publication to peer-reviewed scientific journals:

Chapter 2 I designed the experiment, collected and analyzed the data, and wrote the first version of the manuscript. Emily Carrington and John Gosline provided equipment, guided the research, and edited the manuscript. Patrick Martone edited the manuscript.


Chapter 3 I designed the experiment, collected and analyzed the data and wrote the first version of the manuscript. Laura Anderson provided the illustration for Figure 3.1 and edited the manuscript. Chris Harley edited the manuscript. Emily Carrington provided equipment and edited the manuscript.


Chapter 5 With Jonathan Pruitt’s guidance, I designed the experiment, collected and analyzed the data, and wrote the first version of the
manuscript. Jonathan Pruitt and Chris Harley edited the manuscript. Emily Carrington provided equipment and edited the manuscript.


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I owe special thanks to two colleagues: William Iles- whose sassy and clever cynicism towards ecological work strengthened the scope and quality of my thesis work while toughening my skin and deflating my ego over hundreds of tea breaks, lunches, and jogs & Jonathan Pruitt- whose creativity and dedication to science has continued to inspire me and influence my work since my academic naissance.

Finally, I thank my family for unconditional support through two decades of education. I especially thank my grandparents Mary Lou and Joseph Demes for encouraging me to follow my dreams and pursue higher education and my mother, Helen Demes, for instilling in me a sense of wonder and respect for the natural world that inspired this work and drove me to complete it.
Chapter 1

Introduction

Since the naissance of ecology, researchers have attempted to explain underlying mechanisms dictating the success and distribution of species. Ecologists commonly take the approach that species presence and abundance are governed by non-living environmental stresses. Menge & Sutherland (1987) classically described abiotic environmental stresses as the sum of physiological and physical stresses in an environment. Physiological stresses (e.g., temperature, salinity, nutrient levels) influence an organism’s success in an environment by altering enzymatic processes, which scale up to dictate growth, reproduction, and survival (Hochachka & Somero, 2002; Kordas et al., 2011). Because physiological stresses impact organisms at such a fundamental level, knowledge of only a few abiotic factors can often accurately predict the distribution of species (e.g. Graham et al., 2007; Pearson & Dawson, 2003; Verbruggen et al., 2009). Physical stresses (i.e. mechanical stress from forces applied on the organism by its external environment) are also important in controlling species abundance patterns because they can impair functional performance of organisms (e.g., inability of barnacles to feed at high water velocities) and can be a major source of mortality (e.g., dislodgement from wind or waves) in mechanically stressful environments.
Depending upon the environment and organisms in question, the relative roles of physical vs. physiological stresses in controlling community dynamics will vary (Menge & Sutherland 1976), but neither can be ignored in any system. In other words, a thorough integration of both physiological and physical stresses and their impacts on organisms is necessary to understand patterns observed in nature. However, compared to physiological stresses, physical stresses have received much less attention historically and are only recently beginning to be thoroughly integrated into ecological research (Denny & Gaylord 2010). The field of biomechanics uses mechanical and engineering approaches to address how organisms meet the physical challenges of their environments. This physical interplay of organisms and their environment includes both the generation of forces by organisms, e.g. for moving (Fish 1993; Mann & Hagy 1980; Tobalske 2007) or feeding (Deban & Olson 2002; Westneat 1995), and the resistance of the effects of external forces such as gravity (Spatz & Bruchert 2000; Vogel 2006) or drag (Koehl 1982; Niklas 1998). Most sessile organisms do not actively generate movement so the external forces in their environment dictate their success in a given area.

In marine environments, the amount of flow at a given site is a principle determinant of the community it supports. Indeed, variation in hydrodynamic regime can impact presence or absence of a species, individual growth (Hepburn et al. 2007; Leonard et al. 1998; Steffani & Branch 2003), and species interactions (Gabel et al. 2011; Leonard et al. 1998). A general parabolic effect of water motion on diversity has been described: at very
low water velocities, metabolism and growth of organisms is limited by nutrient and/or gas exchange (Mass et al., 2010; Thomas et al., 2000), such that increases in water velocity in otherwise calm areas can have positive effects on growth and diversity (Demes et al., 2012). However, as gas and nutrient diffusion become saturated by water motion, further increases in flow begin to negatively impact diversity (Nishihara & Terada, 2010) through dislodgement by the hydrodynamic forces accompanied by large waves.

The shores along wave-exposed coastlines are hydrodynamic hell. All objects in a flowing medium (e.g. wind, water, etc.) experience drag proportional to the size and shape of the object and the speed and density of the flowing fluid (Eq. 1). Wind velocities in a category 5 hurricane can exceed 80m/s (Bell & Montgomery, 2008) while water velocities associated with large waves do not exceed 30m/s (Denny et al., 2003). However, because the density of seawater is \( \sim 800 \) times greater than that of air, large waves can exert almost 100 times the force as hurricane winds on the same object (Denny & Gaylord, 2002). Even common waves along wave swept shorelines would carry forces great enough to dislodge terrestrial plants and animals that are not adapted to such an intense physical environment.

Equation 1: \( F = \frac{1}{2} \cdot SA \cdot v^2 \cdot \rho \cdot C_d \), where \( F = \) Drag, \( SA = \) object’s surface area, \( v = \) fluid’s velocity, \( \rho = \) fluid’s density, and \( C_d = \) object’s drag coefficient.

Despite its mechanical hostility, the wave-swept intertidal hosts an incredible diversity of plants and animals adapted to thriving in this environment. From a mechanical perspective, adaptations to success in this environment would arise through drag reduction and/or drag resistance.
Drag resistance is achieved through mechanical strength, i.e., increasing
the attachment strength of organisms to their rocky substratum and/or the
strength of tissues. From a material performance perspective, increasing the
force required to break a seaweed could be achieved through increasing the
breaking stress of the tissue (force per cross-sectional area, a material prop-
erty) or increasing cross-sectional area (a structural or anatomical property).
A general negative association has been described between the thickness of
seaweed tissues and the breaking stress of their tissues (Martone, 2007),
suggesting that species strengthen either by increasing tissue strength or by
increasing tissue thickness. If drag resistance were the sole mechanism ex-
plaining seaweeds’ success in the wave-swept intertidal, one might expect to
find seaweeds with either incredibly thick or strong tissues. In fact, seaweed
tissues are actually much thinner and weaker than terrestrial plant tissues
(Vogel, 2003), suggesting that drag reduction may play a key role in their
adaptation to the mechanically stressful wave-swept shore.

The only factors influencing drag experienced in flow that seaweeds can
alter are their surface area and their coefficient of drag, a dimensionless
coefficient related to the shape of the object in flow (Martone et al., 2012;
Vogel, 2003). Likewise, much biomechanical research has suggested that
the hydrodynamic forces imposed by passing waves constrain the size of
intertidal organisms (Blanchette et al., 2002; Carrington, 1990; Denny et al.,
1985; Martone & Denny, 2008). However, many large species (e.g., kelps) do
inhabit and thrive in the most exposed sites, suggesting that remaining small
may not be the only drag reducing strategy among intertidal organisms.
Indeed, in flow it’s not your surface area, per se, that dictates drag, but
rather the amount of surface area that you project perpendicular to flow. Intuitively flattened surface area and surface area perpendicular to a flowing medium are perfectly correlated for rigid objects, but flexible organisms may be able to reduce surface area exposed to flow without reducing size through a process termed reconfiguration. When exposed to a flowing fluid, the force exerted on flexible organisms will result in deflection in the direction of flow and the organisms will reconfigure into more streamlined shapes and sizes (e.g. Boller & Carrington 2006, Carrington 1990, Koehl 1982, Martone et al. 2012).

Reconfiguration is considered a paramount adaptation to surviving in the wave-swept intertidal (Harder et al. 2006). Because wave-induced dislodgement is thought to be a principle contributor to mortality in the wave-swept intertidal, traits responsible for reconfiguration are likely to be under strong selective pressure. Nonetheless, the underlying processes regulating interspecific variation in reconfiguration potential remain unresolved. Intuitively, the flexibility of seaweed tissues will play a role in the reconfiguration process with more flexible tissues being able to reconfigure more easily. This notion is supported by a study that linked stiffness with drag (Boller & Carrington 2007); however it is difficult from this study to definitively state that material and structural traits dictate hydrodynamic performance because of confounding morphological and phylogenetic factors. Nonetheless, sources of variation in material and structural properties among individuals and species, as well as across an individual’s lifespan, remain unclear.
1.1 Structure of this thesis

The goal of my thesis is to explicitly explore how the material and structural properties of seaweeds impact their hydrodynamic performance. Although the drag reducing reconfiguration process in seaweeds has previously been associated with differences in material properties (Boller & Carrington, 2007), we are unable to disentangle the effects of material properties on drag from other confounding factors such as habitat, shape, size, and phylogeny. Furthermore, the effects of ontogenetic processes (e.g. reproduction and aging) on tissue material properties remain unclear. Finally, the literature is devoid of evidence supporting the notion that intraspecific variation in mechanical traits influences survivorship, constraining evolutionary discussion of material properties to mere speculation.

In Chapter 2, I experimentally control for differences in shape and size among 16 species of foliose red algae (Rhodophyta) collected from hydrodynamically similar sites to isolate the effects of tissue mechanical properties on hydrodynamic performance. In addition to characterizing differences in tissue mechanical traits among species and testing for correlations between mechanical traits and functional performance, I also address anatomical variation among species responsible for the observed mechanical traits. The associations between anatomical properties and mechanical traits observed in Chapter 2 inspired the work presented in Chapters 3 and 4, which detail anatomical and mechanical shifts associated with ontogenetic processes and their consequences for seaweeds.

In Chapter 3, I aimed to address whether and how spore production
(i.e. reproductive investment) in the winged kelp, *Alaria marginata*, impacts blade mechanical traits and hydrodynamic performance. In kelps, the production of spores occurs at the blade’s surface, resulting in additional blade thickness ([Fritsch 1959](#)) and may occur across 75% of the blade’s surface ([Klinger 1985](#)). I investigated material and morphological shifts in reproductive tissue and experimentally assessed the contribution of each trait shift to hydrodynamic performance. Finally, I calculated risk of dislodgement of reproductive and vegetative blades to address whether or not observed morphological and material shifts in reproductive tissues represent a mechanical cost of reproduction.

In addition to maximum wave forces threatening the ability of seaweeds to remain attached to rocks, recent work has shown that repetitive hydrodynamic loading from smaller waves throughout an individual’s life can result in tissue failure by fatigue ([Mach et al. 2007](#) [Mach 2009](#)), suggesting a role of aging in tissue mechanical performance. Susceptibility to fatigue failure is thought to be greater in kelps due to the decreased resilience of their tissues to multiple strains ([Hale 2001](#)), yet fatigue failure of kelps has yet to be documented. In Chapter 4, I attempted to shed light on how kelps might be able to thrive in wave-swept intertidal zones despite their tissues’ decreased resilience. I investigated the effects of tissue aging on the material properties of 27 species of kelps, red algae, and green algae to test (1) whether the effects of aging on material properties are conserved across all major groups of algae and (2) if differences in growth form (basal vs. apical vs. diffuse) among species resulted in divergent spatial patterns of material properties across blades.
In Chapter 5 I address the ecological and evolutionary importance of mechanical traits by testing their effects on whole-plant survivorship in the field. In 40 feather boa kelp, *Egregia menziesii*, I measured holdfast size and tissue material properties of multiple fronds to test for significant differences among individuals in mechanical traits. I then tracked survivorship of individuals over the winter storm season and tested for associations between mechanical traits and survivorship. Model selection procedures were then used to assess how holdfast size and frond mechanical traits jointly influence an individual’s ability to survive winter waves.

Finally, in Chapter 6 I make some concluding remarks about work presented in the previous chapters and go on to describe future research priorities that will enhance our ability to describe and predict biological patterns with mechanical data. I will then conclude with a discussion on future work that, along with the work presented here, will help more thoroughly integrate biomechanics into an ecological and evolutionary framework.

Before continuing on, I first need to briefly define a few key terms commonly used throughout my thesis to enhance the clarity of the subject matter and its accessibility to ecologists, who may not be familiar with these terms commonly used in biomechanics and functional morphology. In the context of this thesis, **material properties** refer to physical properties of seaweed tissues that are independent of their dimensions, including: *extensibility* or *breaking strain* (the percent change in length before a specimen breaks), *tissue strength* or *breaking stress* (the amount of force a specimen can withstand before breaking per cross-sectional area), and *tensile stiffness* (the slope of the stress vs. strain curve). **Structural properties** refer to
mechanical traits that are composites of both material properties and the dimensions of the specimen, including: breaking force (the amount of force required to break a specimen, which is a function of breaking stress and cross-sectional area) and flexural stiffness (the resistance of a specimen to bending, which is a function of tensile stiffness and second moment of area). I refer to material and structural properties together as an individual’s mechanical traits.

I also need to briefly define my use of morphological vs. anatomical traits, which take on a variety of definitions in ecological and biomechanical literature. In this thesis, morphological traits refer to characteristics of an individual that describe its size and/or shape. This includes a large range of scales (blade shape and size to cross-sectional thickness), but is distinct from material and structural properties (although structural properties are a combination of material and morphological properties). Finally, anatomical traits refer specifically to morphological traits that are relevant at the level of cells and tissues (e.g., width of cortical tissue, etc).
Chapter 2

Tissue mechanical traits dictate hydrodynamic performance

2.1 Synopsis

Over the last two decades, many studies on functional morphology have suggested that mechanical properties of seaweed tissues may influence their fitness. Because hydrodynamic forces are likely the largest source of mortality for seaweeds in high wave energy environments, tissues with mechanical properties that behave favorably in these environments are likely to be selected for. However, it is very difficult to disentangle the effects of tissue mechanical properties on seaweed performance because size, shape, and habitat also influence hydrodynamic performance. In this study, anatomical and mechanical properties of 16 species of foliose red macroalgae were determined and their effects on hydrodynamic performance were measured in laboratory experiments holding size and shape constant. We found that increased blade thickness (primarily caused by the addition of medullary
tissue) results in higher flexural stiffness which inhibits seaweeds’ ability to reconfigure in flowing water, and thereby increases drag. However, this increase is concurrent with an increase in the force required to break tissue, possibly offsetting any risk of failure. Additionally, while increased non-pigmented medullary cells may pose a higher metabolic cost to the seaweed, decreased reconfiguration causes thicker tissues to expose more photosynthetic surface area incident to ambient light in flowing water, potentially ameliorating the metabolic cost of producing these cells. Tissue mechanical properties can result in differential performance of morphologically similar species. Future studies on ecomechanics of seaweeds in wave-swept coastal habitats should consider the interaction of multiple trade-offs.

2.2 Introduction

Seaweeds have an intricate relationship with water velocity. While submerged, increased flow facilitates acquisition of CO$_2$ and nutrients for growth and productivity (e.g. Cornelisen & Thomas, 2006; Hurd et al., 1996). However, moving water also imposes drag on seaweeds $F = \frac{1}{2} \rho v^2 AC_d$ (F = Drag, $\rho$ = density, $v$ = velocity, A = surface area, $C_d$ = Drag Coefficient) and seaweed tissues must be stronger than the hydrodynamic forces they experience to avoid dislodgement and subsequent mortality. Seaweeds are able to resist hydrodynamic forces by strengthening their tissues (Lowell et al., 1991; Martone, 2007) or by reducing area exposed to flowing water by remaining small or by reconfiguring (Armstrong, 1989; Boller & Carrington, 2006; Carrington, 1990; Koehl & Alberte, 1988; Vogel, 1984) to become more
hydrodynamically streamlined. While seaweed strength and reconfiguration potential (indexed by flexibility) are both dependent on tissue mechanical properties, little is known about how seaweeds produce tissues of varying properties.

Unlike terrestrial plants, which produce multiple tissue types for different structural roles (Kokubo et al., 1989; Vincent, 1991), macroalgae are usually limited to pigmented cortical cells at the tissue surface and non-pigmented medullary cells in the tissue center. The cortical cell layer is comparable among most species, comprising one to several layers of tightly packed photosynthetic cells. Medullary tissue, on the other hand, varies greatly among taxa in terms of thickness, degree of compaction, and cell shape (Fritsch, 1959). Although medullary cells are responsible for the translocation of photosynthate in kelps (Schmitz & Lobban, 1976), in red macroalgae (Rhodophyta), connections between medullary cells are blocked by proteinaceous pit plugs (Pueschel, 1989), leaving their function unclear.

Despite the wide-spread prevalence of non-pigmented medullary tissue among red macroalgae, little is known about its function. However, the addition of medullary cells may have significant biomechanical consequences for seaweeds. Most importantly, increasing tissue thickness should increase breaking force and flexural stiffness (Vogel, 2003), both of which likely affect seaweed fitness. Flexural stiffness is the resistance of an object to bending and is defined as the product of a material’s stiffness, or modulus of elasticity (E), and its second moment of area (I). Increasing tissue thickness should not affect E, but will greatly affect I, which increases with thickness cubed (Vogel, 2003). All else being equal, thicker blades will be stiffer and...
therefore less easily reconfigured. Likewise, the addition of medullary cells may decrease the ability of seaweeds to reconfigure thereby exposing them to higher hydrodynamic drag, which may in turn have negative effects on fitness.

The ability of macroalgae to reconfigure in flowing water and thereby reduce drag is known to be ecologically important (Carrington, 1990; Koehl & Alberte, 1988). However, only recently have researchers attempted to quantify differences in reconfiguration potential among species and found that stipe bendiness, (EI)$^{-1}$, is largely responsible for this process in turf forming seaweeds (Boller & Carrington, 2006). Most species converge on similar maximally reconfigured shapes at high velocities that are representative of exposed intertidal sites, (Martone et al., 2012), however large differences in reconfiguration potential occur among species at lower velocities (<2 m/s) that are representative of protected, high current-exposed, or subtidal sites. Reconfiguration may come at the metabolic cost of self-shading, presenting an interesting trade-off between maximizing photosynthetic surface area incident to light (productivity) and minimizing hydrodynamic drag through reconfiguration (hydrodynamic forces) (Koehl & Alberte, 1988; Koehl et al., 2008).

Hydrodynamic forces imposed by strong currents and breaking waves have been proposed to be a strong selective pressure in shaping the evolution of intertidal organisms (e.g. Denny, 1988). Likewise a plethora of literature exists on hydrodynamic performance of numerous species, largely focusing on mechanical limits to size (e.g. Carrington, 1990; Denny, 1988; Koehl, 1986; Koehl et al., 2008; Martone & Denny, 2008). However, other
seaweed biomechanical studies have highlighted the importance of tissue material and structural properties (i.e. strength, extensibility, bendiness, etc.) on hydrodynamic performance (Boller & Carrington, 2007; Gaylord & Denny, 1997; Harder et al., 2006; Johnson & Koehl, 1994; Lowell et al., 1991). Despite recent advances in this field, a paucity of data exists describing (1) variation in mechanical properties among species (although see Koehl, 2000) (2) how variation in mechanical properties is produced by the individual (however see Martone, 2007) and (3) how differences in mechanical properties affects seaweed fitness. While previous comparative studies have provided important insight to how differences in biomechanical properties (strength, flexibility, and flexural stiffness or bendiness) may affect the fitness of marine organisms, it is very difficult to disentangle the relative effects of species identity, size, shape, and environment. Furthermore, how internal growth form and thallus construction affect tissue mechanical properties is almost entirely unexplored in these studies (however, see Koehl, 1999; Martone, 2007).

Foliose red macroalgae (Rhodophyta) provide an excellent way to tease apart the aforementioned confounding factors and explore effects of tissue construction on mechanical properties because of the abundance of taxonomically distinct blades which can be found growing next to one another. While all red blades are often lumped into the functional group foliose macroalgae (Steneck & Dethier, 1994), it is possible that many different thallus constructions and tissue types could produce a bladed morphology. In this study, we quantify material properties of 16 species of foliose red macroalgae. We controlled size and shape in the laboratory to test the effects of mechanical
properties on hydrodynamic performance. Specifically, we ask: (1) Do differences in mechanical properties exist among foliose red macroalgae? (2) If variation in mechanical properties exists among species, are anatomical differences responsible for such variation? (3) Does variation in mechanical properties explain differences in hydrodynamic performance?

2.3 Methods

2.3.1 Specimen collection and processing

Table 2.1 lists the taxonomic placement and collection locality of species used in this study (identifications by and authorities available through [Gabrielson et al. 2006]). Because variation in material properties within a species has been shown to result from environmental factors [Armstrong 1987, Kitzes & Denny 2005, Kraemer & Chapman 1991], care was taken to collect specimens from locations of similar exposure. All collections were made within the San Juan Islands (Washington, USA) at moderately protected sites where largest maximum water velocities are from currents. Thirteen species were collected from the Friday Harbor Laboratories dock lines or adjacent rocks. *Prionitis* and *Smithora* were collected from docks in Roche Harbor and from eelgrass blades in False Bay, respectively. *Holmesia*, which is a less common species, was collected opportunistically by divers at approximately 10m near Stuart Island.

Specimens were kept alive in the laboratory in seawater tables before being processed. Material property tests (described below) took place no longer than 24 hours after collection and hydrodynamic performance tests
(described below) were run 2–3 days after collection. For each species, voucher specimens were pressed and deposited in the Friday Harbor Laboratories Herbarium. Corresponding samples were preserved in silica gel for future molecular analyses.
Table 2.1: Taxonomic placement (Order and Family) and collection site of specimens used in this study. Voucher specimens are archived in the Friday Harbor Laboratory herbarium.

<table>
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<th>Species</th>
<th>Order</th>
<th>Family</th>
<th>Collection site</th>
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<tr>
<td><em>Porphyra</em> sp.</td>
<td>Bangiales</td>
<td>Bangiaceae</td>
<td>FHL Intertidal</td>
</tr>
<tr>
<td><em>Polynea latissima</em></td>
<td>Ceramiales</td>
<td>Delessariaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Holmesia californica</em></td>
<td>Ceramiales</td>
<td>Delessariaceae</td>
<td>Turn Point, Stuart Is. (10m)</td>
</tr>
<tr>
<td><em>Haralidiophyllum nottii</em></td>
<td>Ceramiales</td>
<td>Delessariaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Smithora naiadum</em></td>
<td>Erythropeltidales</td>
<td>Erythrotichiaceae</td>
<td>False Bay</td>
</tr>
<tr>
<td><em>Neodilsea borealis</em></td>
<td>Gigartinales</td>
<td>Dumontaceae</td>
<td>FHL Shallow subtidal</td>
</tr>
<tr>
<td><em>Constantinea subulifera</em></td>
<td>Gigartinales</td>
<td>Dumontaceae</td>
<td>FHL Shallow subtidal</td>
</tr>
<tr>
<td><em>Opuntiella californica</em></td>
<td>Gigartinales</td>
<td>Furcellariaceae</td>
<td>FHL Shallow subtidal</td>
</tr>
<tr>
<td><em>Chondracanthus exasperatus</em></td>
<td>Gigartinales</td>
<td>Gigartinae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Mazzaella splendens</em></td>
<td>Gigartinales</td>
<td>Gigartinae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Cryptonemia obovata</em></td>
<td>Halymeniales</td>
<td>Halymeniaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Prionitis lanceolata</em></td>
<td>Halymeniales</td>
<td>Halymeniaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Halymenia californica</em></td>
<td>Halymeniales</td>
<td>Halymeniaceae</td>
<td>Roche Harbor</td>
</tr>
<tr>
<td><em>Schizymenia pacifica</em></td>
<td>Nemastomatales</td>
<td>Schizymeniaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Sparlingia pertusa</em></td>
<td>Rhodymeniales</td>
<td>Rhodymeniaceae</td>
<td>FHL Dock</td>
</tr>
<tr>
<td><em>Fryeella gardneri</em></td>
<td>Rhodymeniales</td>
<td>Rhodymeniaceae</td>
<td>FHL Dock</td>
</tr>
</tbody>
</table>
2.3.2 Variation in anatomy and mechanical properties

In order to determine anatomical variation among species, cortex thickness, medulla thickness, and total blade thickness (Figure 2.1) were determined through microscopy. Measurements on blades thinner than 200 \( \mu m \) were to nearest 10 \( \mu m \) while those above 200 \( \mu m \) were measured to the nearest 50 \( \mu m \). This represents a substantial fraction of blade thickness. However, rounding effects should be distributed randomly, adding increased error to statistical analyses and making statistical analyses more conservative. In order to control strain localization and accurately describe tissue properties of the different species, longitudinal test shapes (Fig. 2.2A) were cut from specimens after Mach (2009).

Because mechanical properties may vary along the length of blades (Armstrong, 1987; Koehl, 2000), especially near the stipe/blade junction (apophysis), all test shapes were cut ~5 cm above the apophysis (or stipe, when apophysis absent), oriented from the center of the blade to the distal end. Tissue breaking force, breaking stress (force per initial cross-sectional area), breaking strain (change in length divided by initial length), and modulus of elasticity (slope of terminal linear portion of stress vs. strain curve) were analyzed using the tensile test setting on an Instron tensometer (model 5565 Norwood, MA). Test shapes were attached to the tensometer via pneumatic clamps at 60psi lined with sand paper. For several species, soft paper was added as padding to prevent tissue breakage from the clamping apparatus. The tensometer strained the seaweeds at a constant rate of 10 mm·min\(^{-1}\) and measured the resisting force (N) at 1 Hz until the tissues broke. All
samples were run wet, but out of water. Specimens were periodically and carefully rewetted with a paintbrush if needed. Specimens which broke at or near the clamps were not included.

Beam theory (Figure 2.3) was used to measure flexural stiffness (resistance of an object to bending) as described in Vogel (2003). Longitudinal rectangular shapes cut from blades (Fig. 2.2B) were sandwiched between a glass microscope slide and a flat edge along a 90° edge, demarcated with a mm scale. Test shapes (cantilever beams) were then incrementally pulled from the slides until deflection (measured to the nearest 0.5 mm of deflection) reached approximately 10% of the beam length (species for which less than 10% deflection was not achieved were left out of analyses). Flexural stiffness was determined for three replicates within each species using the following equation: \[ EI = \frac{FL^3}{3y} \] whereby \( EI \) = flexural stiffness, \( F \) = weight of unsupported beam, \( L \) = length of unsupported beam, \( y \) = deflection (Vogel 2003). Beam mass was measured to the nearest 1 mg using an analytical scale. Weight was then calculated by multiplying the mass by gravity and was assumed to be uniformly loaded across the beam.

2.3.3 Hydrodynamic performance

In order to test the effects of mechanical properties on hydrodynamic performance, without the confounding effects of shape and size, blades were cut longitudinally into standardized shapes (Figure 2.2C). The hydrodynamic test shape was chosen as a morphologically non-descript foliose rhodophyte; size (approximately 12 cm\(^2\)) was constrained by the smallest blade used (Smithora naiadum). The hypothetical stipe portion of the sections was
Figure 2.1: Anatomy of red blades. Model cross-sectional view of a red blade showing anatomical measurements (blade thickness, cortex thickness, and medulla thickness). Shaded areas represent pigmentation.
Figure 2.2: Standardized test shapes cut out of specimens for (A) tensile tests, (B) flexural stiffness measurements, and (C) hydrodynamic performance measurements in the flume. Cut outs are drawn to scale.
Figure 2.3: Empirical measurement of tissue flexural stiffness (EI) through beam theory deflection. All samples were of constant width so that deflection (y) varied only as a result of beam length and the force applied on that beam by its weight.

attached directly to a force transducer using super glue and placed in a high speed recirculating flume developed by Boller & Carrington (2006). Drag was measured directly by the force transducer for five replicate test shapes of each species at 1.71 m/s. In order to determine the effect of flow-induced re-configuration on planform surface area (perpendicular to flow), which would not affect form drag (although would likely influence skin friction drag) but would theoretically be exposed to sunlight and be capable of photosynthesizing, photos were taken from below the seaweeds using a mirror secured at a 45° angle to the flume. Surface area was measured using ImageJ photo analysis software.

2.3.4 Statistical analyses

In order to determine if variation among species existed in breaking force, breaking stress, breaking strain, modulus of elasticity, flexural stiffness, and planform area at 1.71 m/s, one-way analysis of variance (ANOVA) was per-
formed on species identity. To test the relative contribution of increasing cortex vs. medulla tissue in thicker blades, analysis of covariance (ANCOVA) homogeneity of slopes test was used with tissue type as a fixed factor, blade thickness as the covariate and tissue thickness as the response variable. Significantly different slopes would suggest that one tissue type over the other contributes more to blade thickening. Linear regression analysis was used to determine if hydrodynamic performance (drag) could be explained by anatomical and mechanical properties. Non-linear regression analysis [exponential rise to maximum, \( y = a(1-\exp(-bx)) \)] was used instead for planform area in flowing water because values were constrained by the surface area of the test shape, which they could not exceed. Finally, power curve fitting was used to test the effects of blade thickness on flexural stiffness, given the a priori expectation that stiffness will increase with thickness cubed. Levene and Shapiro-Wilk tests were used to test assumptions of homogeneity of variance and normality, respectively. If assumptions could not be met after data transformations, non-parametric analyses were used instead. A result was considered to be significant with \( \alpha = 0.05 \) and \( p < 0.05 \). All statistical analyses were performed in R Statistical Package 2.10.1.

2.4 Results

2.4.1 Variation in mechanical properties

Tissue mechanical property data are summarized in Table 2.2. Tissue breaking stress ranged from 0.6 MPa in *Halymenia* to 4.1 MPa in *Opuntiella* and was significantly different among species (\( F_{15,65} = 11.140, p < 0.001 \)). Break-
ing strain was also different across species ($F_{15,65} = 34.513$, $p < 0.001$) and ranged from 0.07 in *Polyneura* to 0.8 in *Mazzaella*. Because residuals were not normally distributed for modulus of elasticity, data were analyzed with ANOVA on ranks. Modulus of elasticity varied from 0.9 MPa in *Halymenia* to 17.5 MPa in *Polyneura* and was significantly different among species ($p < 0.001$). Log transformation was required to meet the homogeneity of variance assumption of ANOVA for flexural stiffness. Flexural stiffness was highly variable within a species, likely because, to maintain 10% deflection of beam length, 0.5 mm resolution proved somewhat crude given the flexibility of the specimens. Nonetheless, a significant effect ($F_{11,24} = 6.03$, $p < 0.001$) of species identity was still detected with mean values ranging from $6.0 \times 10^{-6}$ in *Holmesia* to $7.5 \times 10^{-4}$ Nm$^2$ in *Neodilsea*. Deflections under 10% could not be achieved for *Smithora*, *Porphyra*, *Haraldiophyllum*, and *Sparlingia* and therefore these species were not included in analyses.
Table 2.2: Summary of red algal blade mechanical properties (N=3 for all flexural stiffness tests). ND = Not determined. Values are mean ± SE.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Breaking Strain (MPa)</th>
<th>Breaking Stress (MPa)</th>
<th>Modulus of elasticity (MPa)</th>
<th>Flexural stiffness (µNm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondracanthus</td>
<td>3</td>
<td>.44 ± .03</td>
<td>1.7 ± .2</td>
<td>4.1 ± .2</td>
<td>493 ± 248</td>
</tr>
<tr>
<td>Constantinea</td>
<td>5</td>
<td>.20 ± .02</td>
<td>1.5 ± .1</td>
<td>7.9 ± .6</td>
<td>285 ± 136</td>
</tr>
<tr>
<td>Cryptonemia</td>
<td>5</td>
<td>.39 ± .05</td>
<td>2.1 ± .3</td>
<td>6.1 ± .6</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Fryeella</td>
<td>3</td>
<td>.07 ± .02</td>
<td>0.4 ± .1</td>
<td>4.7 ± .6</td>
<td>129 ± 63</td>
</tr>
<tr>
<td>Halymenia</td>
<td>3</td>
<td>.35 ± .03</td>
<td>0.3 ± .03</td>
<td>0.9 ± .1</td>
<td>64 ± 19</td>
</tr>
<tr>
<td>Haraldiophyllum</td>
<td>4</td>
<td>.15 ± .03</td>
<td>2.0 ± .4</td>
<td>14.6 ± .5</td>
<td>ND</td>
</tr>
<tr>
<td>Holmesia</td>
<td>5</td>
<td>.15 ± .02</td>
<td>1.6 ± .4</td>
<td>11.6 ± 2.2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Mazzaella</td>
<td>5</td>
<td>.8 ± .03</td>
<td>1.9 ± .1</td>
<td>3.1 ± 0.1</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>Neodilsea</td>
<td>5</td>
<td>.41 ± .02</td>
<td>1.9 ± .3</td>
<td>4.7 ± .4</td>
<td>749 ± 329</td>
</tr>
<tr>
<td>Opunticella</td>
<td>5</td>
<td>.43 ± .05</td>
<td>3.5 ± .4</td>
<td>6.9 ± .3</td>
<td>299 ± 210</td>
</tr>
<tr>
<td>Polyneura</td>
<td>5</td>
<td>.07 ± .01</td>
<td>1.2 ± .3</td>
<td>3.1 ± .1</td>
<td>558 ± 230</td>
</tr>
<tr>
<td>Porphya</td>
<td>4</td>
<td>.40 ± .07</td>
<td>1.3 ± .2</td>
<td>4.4 ± .3</td>
<td>ND</td>
</tr>
<tr>
<td>Prionitis</td>
<td>3</td>
<td>.37 ± .03</td>
<td>2.6 ± 0.1</td>
<td>7.2 ± .2</td>
<td>282 ± 216</td>
</tr>
<tr>
<td>Schizymenia</td>
<td>5</td>
<td>.38 ± .03</td>
<td>0.40 ± .1</td>
<td>1.5 ± .1</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Smithora</td>
<td>3</td>
<td>.11 ± .03</td>
<td>0.49 ± .2</td>
<td>6.4 ± 1.2</td>
<td>ND</td>
</tr>
<tr>
<td>Sparlingia</td>
<td>4</td>
<td>.17 ± .04</td>
<td>1.2 ± 0.3</td>
<td>7.4 ± .3</td>
<td>ND</td>
</tr>
</tbody>
</table>
2.4.2 Anatomical sources of variation in mechanical properties

Large variation was present in internal anatomy across species with monostromatic blades (composed of a single cell layer) 25\(\mu\)m thick (Smithora) to leathery blades 500 \(\mu\)m thick (Neodilsea). Furthermore, increasing cross-sectional thickness was found to be driven primarily by increasing thickness of medullary tissue rather than cortical tissue (Figure 2.4), such that thicker seaweeds had disproportionately thicker medulla (\(F_{3,28}= 65.99, p <0.001\)). In other words, medulla thickness increased faster with increasing cross-sectional area than did cortex thickness. Breaking force was positively correlated with blade thickness (Figure 2.5; \(p <0.001\)). In an analysis containing all species, we were unable to detect an effect of increasing blade thickness on flexural stiffness (\(R^2 = 0.171, p = 0.063\)). Evident in Figure 2.6 Polyneura and Holmesia are outliers, being much stiffer than expected by just blade thickness alone. Analysis without these two species found a significant relationship between blade thickness and flexural stiffness (\(R^2 = 0.777, p <0.001\)). Furthermore, the cubic exponent predicted by increases in flexural stiffness due to second moment of area, was not significantly different than the exponent observed in the partial model (3.37 \(\pm\) 0.76). None of the anatomical characters measured were found to predict tensile modulus of elasticity, breaking stress, or breaking strain (\(p \gg 0.05\)).
Figure 2.4: Contributions of cortex & medulla to blade thickness. As blade thickness increases, thickness of medullary tissue increases at a faster rate than cortex tissue. In other words, thicker blades exhibit disproportionately larger medulla.
Figure 2.5: Breaking force vs. thickness. Blade thickness is positively associated with force (N) to break tissue (from standardized test sections).
Figure 2.6: Flexural stiffness as a function of blade thickness. Line represents partial model (filled circles only) excluding (1) *Polyneura* and (2) *Holmesia*. Equation and statistics derived from partial model (solid line).
2.4.3 Hydrodynamic performance

Flexural stiffness was the best predictor of drag and explained 68% of the variation at 1.71 m/s (Figure 2.7; \( p < 0.001 \)). However, seaweeds which experienced higher drag required more force to break (Figure 2.8; \( R^2 = 0.326, p = 0.021 \)). Because flexural stiffness could not be determined for all species (particularly the thinner, more flexible species that are likely more susceptible to folding), the effect of blade thickness (which was shown to be positively associated with flexural stiffness) on planform surface area was analyzed instead. Significant variation in planform area at 1.71 m/s was detected (\( F_{13,41} = 21.830, p < 0.0001 \)) among species. This variation was partly explained by blade thickness, such that thinner blades reconfigured more readily in flow and exhibited decreased planform area (Figure 2.9; \( R^2 = 0.391, p = 0.017 \)).

2.5 Discussion

Reconfiguration significantly reduces hydrodynamic forces on seaweeds (Boller & Carrington, 2006; Carrington, 1990) and is essential for life in the intertidal zone (Harder et al., 2004). However, reconfiguration may also carry with it the added cost of self-shading when thallus portions fold on top of one another (Koehl & Alberte, 1988). Our results show that thinner blades reconfigure more readily in flowing water than thicker blades and consequently experience lower drag but higher self-shading (lower planform surface area). On the other hand, thicker blades can withstand larger forces before breaking and are able to resist mechanical failure as they expose more surface
Figure 2.7: Stiffer blades experience more drag. Flexural stiffness values are in $10^{-3}$ N·m² to increase axis clarity.
Figure 2.8: Association between drag experienced & breaking force. Blades which experience higher drag are also stronger and can withstand larger forces before tissue failure.
Figure 2.9: Planform area of standardized test sections (same shape and size) at 1.71 m/s as a function of blade thickness. Thicker blades have higher surface area exposed to sunlight while waves pass.
area for photosynthesis and reconfigure less.

Why then are not all red blades thick? One possible explanation is a metabolic cost associated with increased medullary thickness. Thicker blades can absorb more than 90% of ambient light and likely reflect the rest (Beach et al. 2006). Light penetrance into tissue may therefore limit the thickness of the cortex (pigmented cells). Since medullary cells are non-pigmented, it is reasonable to assume that they rely metabolically on cortical cells. This scenario suggests a maximum medulla to cortex ratio and may set an upper limit to blade thickness. The integration of metabolic costs of tissue production into ecomechanics of seaweeds presents a fascinating and unexplored area of future research.

Although flexural stiffness depends on both tensile stiffness (E) and blade thickness (I, second moment of area), both of which varied in this study, ~80% of the variation in flexural stiffness was explained by blade thickness alone for most species. However, two species in this study displayed substantially higher flexural stiffness than expected given their thickness (see Figure 2.6), suggesting other mechanisms for increasing stiffness. *Polyneura latissima* is unique among the species in this study in that it possesses thickened veins running through its blade. Differentially thickened thallus portions, such as veins or midribs, may also result in higher flexural stiffness. Thickness of blade tissue between veins was used in our analyses. Using the thickness of the veins instead may have provided a more accurate prediction of flexural stiffness in this species. While the other disproportionately stiff species, *Holmesia californica*, does not possess veins, it stands out from the other species in regards to medullary construction. Medullary
tissue in most of the species used is comprised of loosely packed spherical or filamentous cells. To the contrary, medullary tissue of *H. californica* is composed of tightly compacted cuboid cells, which may better resist bending. Contributions of venation and medullary cell type to mechanical traits and hydrodynamic performance require data from many more species and remain unresolved.

While many of the species used in this study are not easily morphologically discerned from one another without the use of microscopy, some are very easily distinguished by anecdotal tactile tests. Phycologists are often seen in the field tugging, tearing, or rubbing foliose red macroalgae for easy field identification. This practice is supported here by differences in tissue mechanical properties found across species. One structural property in particular, flexural stiffness, has a significant effect on hydrodynamic performance such that increases in flexural stiffness result in increased drag. Previously, [Boller & Carrington] (2007) found that variation in reconfiguration potential in, and therefore drag experienced by, 10 species of taxonomically, morphologically, and ecologically dissimilar species was related to flexural stiffness of stipe tissue. The current study further supports their findings that increased flexural stiffness of thallus tissue results in increased drag among much more closely related species, while controlling for differences in size and shape.

The novelty of this study is that we controlled size and shape of seaweeds and showed how tissue mechanical properties alone can affect hydrodynamic performance. However, it is important to note that the species here naturally vary in size and shape to various degrees. For instance, two species of
drastically different tissue mechanical properties may experience comparable
drag by altering their morphology (shape and/or size). Therefore, whether
or not tissue mechanical properties of seaweeds actually affect fitness (pro-
ductivity or survivorship) of individuals in the field is difficult to discern
from this study. Experimental studies measuring selection on intraspecific
variation in tissue mechanical properties and phylogenetic analyses of tis-
sue mechanical properties are lacking, but could shed light on whether or
not effects of tissue mechanical properties on performance are evolutionarily
significant.
Chapter 3

Dealing with mechanical costs of reproduction

3.1 Synopsis

In addition to metabolic costs associated with reproduction, morphological and mechanical changes accompanying reproductive effort can affect an organism’s performance. We investigated mechanical and morphological changes associated with reproduction in the winged kelp, *Alaria marginata*. Compared to vegetative sporophylls, reproductive sporophylls were longer, were similar in width, and had greater surface area. Reproductive sporophylls were also thicker and less ruffled. Tissue extensibility and breaking stress were not different in reproductive vs. vegetative sporophylls. However, reproductive tissue exhibited greater tensile stiffness, flexural stiffness, and force to break. Reproductive sporophylls experienced greater drag (despite decreased flapping) than did vegetative sporophylls, but did not experience greater size-specific drag. Tissues cut into experimental blades of the same size and shape experienced greater drag when cut from reproductive tissue suggesting that the decreased ruffliness associated with
the onset of reproduction ameliorates the cost of increased tissue stiffness. Nonetheless, increased breaking force in reproductive individuals resulted in elevated sporophyll safety factors (breaking force/drag experienced) in reproductive compared to non-reproductive sporophylls. In sum, changes in blade ruffliness and strength associated with the onset of reproduction in *Alaria marginata* ameliorate the concomitant mechanical costs of decreased flexibility and increased size.

### 3.2 Introduction

Understanding how reproductive processes influence an organism’s performance has been a perennial goal in the field of evolutionary ecology: What limits reproductive output? What factors dictate reproductive timing? These and other questions have been approached through the framework that reproductive effort poses some cost to the individual and that reproductive strategies develop as a means to optimize trade-offs between growth, reproduction, and mortality ([Bell](#) [1980]). These trade-offs stem from interacting metabolic and performance costs. The production of offspring requires energetic investment, which may be siphoned away from investment in growth and/or defenses. Such metabolic costs of reproduction are well documented in diverse taxa including plants ([Calow](#) [1979]; [Obeso](#) [2002]), invertebrates ([Taylor & Leelapiyanart](#) [2001]), squamates ([Angilletta & Sears](#) [2000]), and mammals ([Randolph et al.](#) [1977]).

Performance costs to reproduction arise from decreased performance of reproductive organisms and usually result from morphological and/or me-
chanical consequences of reproductive effort. The most intuitive examples of performance costs to reproduction are the decreased speed and mobility of gravid animals, notably mammals (e.g. Noren et al. 2011), squamates (e.g. Brown & Shine 2004, Shine 2003), crustaceans (Cromarty et al. 1998) and spiders (Pruitt 2010). Decreased speed and agility pose a cost to the individual because they adversely affect the individual’s ability to capture prey and/or escape predation. In order to compensate for such performance costs to reproduction, gravid animals often alter their anti-predator behaviour, e.g., by increasing their sensitivity to predator cues or shifting their behaviour away from anti-predator strategies that rely on escape velocity (e.g. Pruitt 2010, Schwarzkopf & Shine 1992).

Reproduction may impose performance costs, even in non-animal taxa. For instance, the survival and functioning of plants and seaweeds is commonly dependent on the ability of their supporting structures to resist the forces they experience (via drag or self-loading weight), e.g., the production of fruits and large inflorescences by terrestrial plants adds non-trivial loads, which branches and stems must resist to avoid breaking (Etnier & Vogel 2000, Peters et al. 1988). For some plant species, an even greater cost is incurred when the fruit serves as an invitation to grazing by large animals, which in turn damage or break the plant (Hemborg & Bond 2006). In addition to tissues needing to bear increased weights associated with reproduction, plants and seaweeds may experience threatening increases in drag in areas of high wind and water disturbance.

The wave-swept intertidal zone is often referred to as the most mechanically hostile environment on the planet, with crashing waves posing a lethal
threat to intertidal seaweeds (Denny, 1988). In this environment, survival of seaweeds is dependent upon their ability to resist the hydrodynamic forces they experience (Chapter 5). Drag experienced by seaweeds attached to rocks is dictated by the density and velocity of the water, the amount of surface area of the seaweed perpendicular to the direction of flow, and the drag coefficient of the seaweed (related to its shape) (Denny, 1988). However, because seaweeds are composed of non-rigid tissues, they do not maintain their shape in flowing water (e.g. Chapter 2, Boller & Carrington, 2007). Indeed, the extent to which seaweeds can reduce drag by reconfiguring into smaller and/or more streamlined shapes (Martone et al., 2012) is dependent upon the flexibility of their tissues; more flexible tissues reconfigure into smaller and/or more streamlined shapes in flow. Likewise, flexibility is important to the survival of individuals on the wave-swept shore (Harder et al., 2006).

Variation in tissue flexibility within and among species of seaweeds is dependent upon both tissue thickness and material properties (see Chapters 2 & 5), both of which can potentially change during the onset of reproduction. For instance, spore production in the kelps (Laminariales, Phaeophyceae) occurs with the addition of a layer of sterile protective cells (Fritsch, 1959). This layer of cells increases the overall thickness of the tissue and, because of its anatomical distinction from the other tissue layers, likely changes the material properties of the tissue as well. Kelps are of particular mechanical interest because they thrive in wave-swept environments despite their enormous size. Thus, obstruction of drag reducing/resisting strategies of kelp by reproductive efforts could have drastic consequences. Nonetheless, kelps survive and reproduce in the most wave-exposed of environments, suggesting
adaptations to ameliorate the mechanical costs to reproduction.

Previous studies have reported mechanical adaptations and strategies among kelps that are responsible for their success in hydrodynamically stressful environments. For instance, high extensibility of kelp tissues allows them to *go with the flow* and minimize drag ([Koehl & Wainwright] 1977), allometric growth (change in shape with increases in size) has been reported to reduce drag in *Nereocystis leutkeana*, *Eisenia arborea* and *Pterygophora californica* ([Denny et al. 1997] [Gaylord & Denny 1997] [Johnson & Koehl 1994]), and conspecifics can exhibit variable mechanical traits that are presumed adaptive in their respective hydrodynamic regimes ([Armstrong 1987] [Kraemer & Chapman 1991]). Furthermore, some species adapt to hydrodynamic regimes by changing the ruffliness of their blades: individuals are ruffled in low flow environments, and flat in high flow environments, thereby reducing drag, via a reduction in blade flapping ([Koehl et al. 2008]).

Despite considerable attention to kelp mechanical design, whether and how reproduction might influence their mechanical traits and/or hydrodynamic performance remains unclear. Previously, the only attempt to address mechanical costs of reproduction in a kelp occurred as an ancillary test in a series of experiments investigating physiological costs of reproduction ([Pfister 1992]). In this study, the presence of reproductive blades (hereafter referred to as sporophylls) did not significantly increase drag on *Alaria marginata* plants: controls with sporophylls experienced similar drag to plants whose sporophylls had been experimentally removed. This attempt to characterize mechanical costs of reproduction was limited by the researcher’s ability to account for differences in size and shape of experimental
plants and to control and replicate hydrodynamic treatment among plants. Furthermore, because *Alaria marginata* always possesses sporophylls, irrespective of its reproductive status, we argue that a more appropriate metric of mechanical costs of reproduction would be to measure how reproductive state of sporophylls impacts mechanical performance. Additionally, while only a few kelp species possess sporophylls, all kelp species produce spores on blades, so understanding how spore production impacts mechanical performance is more broadly applicable to other kelp species. Likewise, we sought to investigate whether and how the drag reducing/resisting strategies of kelps are changed during spore production.

In this study we measured performance traits and hydrodynamic performance of reproductive and non-reproductive *Alaria marginata* (Laminariales, Phaeophyceae) sporophylls to assess mechanical costs associated with spore production. Specifically, we compared vegetative and reproductive sporophylls with respect to (1) morphological traits: size, shape, thickness and degree of ruffliness, (2) tissue mechanical traits: breaking stress, breaking force, and tensile and flexural stiffness, (3) hydrodynamic performance: drag on entire sporophylls, size-specific drag, drag on sporophylls of artificially constant shape and size, and degree of flapping at six different velocities (0.18–2.73 m/s). Because of the addition of sterile protective cells during reproduction, we predicted that reproductive sporophylls would be thicker and stiffer than non-reproductive fronds, resulting in decreased flexibility among reproductive fronds. We hypothesized increased size and stiffness would increase drag on reproductive fronds, but that changes in morphological traits may, to some degree, mediate these biomechanical costs of
reproduction.

3.3 Methods

3.3.1 Study species and sample collection

*Alaria marginata* is abundant in a variety of intertidal and shallow subtidal habitats from Alaska to central California. This species consists of a single, main blade, which may grow to >4 m in length, and multiple smaller (<30 cm) reproductive blades, here termed sporophylls, located near the plant’s holdfast (Fig. 3.1). The successful completion of the *A. marginata* life cycle requires the production and subsequent release of competent spores from sporophylls. Theoretically, dislodgement of a ripe sporophyll could serve as a dispersal vector. However, because most drift accumulates at or above the high tide mark (where recruits could not survive) as beach wrack, sporophyll dislodgement is probably not a common dispersal method (although no evidence currently exists in favour or against this hypothesis). Although sporophylls are present year-round, sori (reproductive patches on sporophylls where spores are produced) are most abundant May–July (McCinnico & Foster, 2005). Reproductive and non-reproductive sporophylls were collected randomly from *A. marginata* individuals growing on docks at the Friday Harbor Laboratories on San Juan Island, WA (48°32’42.62N, 123°0’43.83W) between May and July, 2011 to assess the effects of reproductive status on mechanical traits and hydrodynamic performance. Specimens were placed in a flow through water table until biomechanical analyses, which were performed within 48 hours of collection.
Figure 3.1: Mature, adult *Alaria marginata* sporophyte showing main vegetative blade and sporophylls: dark patches within sporophylls are soral (reproductive) patches which, when mature, take up more than 75% of sporophyll surface area. Artwork created by Laura M. Anderson
3.3.2 Morphological properties

Because sporophylls are roughly ovular (Fig. 3.1), length and width describe most of the variability in shape. Length and width were measured to the nearest 0.1 cm and the length:width ratio was used to describe sporophyll shape. Size of each sporophyll was measured from digital scans of specimens in ImageJ photo analysis software (U.S. National Institutes of Health, Bethesda, MD, USA) as the total surface area of a sporophyll (cm$^2$). Sporophyll thickness was measured to the nearest 0.1 mm using digital calipers 5 cm from above the petiole (point of attachment between the seaweed stipe and sporophyll). Sporophyll ruffliness index (detailed in Johnson & Koehl [1994]) was measured as the ratio of actual sporophyll surface area (after deruffling) to projected sporophyll blade area (while still ruffled).

3.3.3 Mechanical properties

Dumbbell shaped working sections were cut from 5 cm above the petiole to determine tissue mechanical properties of samples (detailed in Mach [2009]). Working sections were then attached to an Instron tensometer (model 5565 Norwood, MA) via pneumatic clamps (90 psi). Strain was applied to tissues at a rate of 10 mm·min$^{-1}$ until failure occurred and the resisting force was measured at a frequency of 5 Hz. Tissues which failed at or near the clamps were discarded from analyses. The working section constrained sample length and width to 55 mm and 5 mm, respectively. Tissue thickness of each sample was measured to the nearest 0.1 mm using digital calipers. From each tensile test, breaking strain (change in length over initial length)
and breaking force (N) were extracted. These variables, along with tissue thickness, were then used to calculate breaking stress (Force per initial cross-sectional area, MPa), tensile stiffness (initial linear slope of stress vs. strain curve, MPa), and flexural stiffness (product of tensile stiffness and second moment of area, \(\mu N\cdot m^2\)).

3.3.4 Hydrodynamic performance

Drag was measured on entire sporophylls in a recirculating flume (Boller & Carrington, 2006) at six water velocities: 0.18, 0.75, 1.00, 1.71, 2.00, and 2.73 m/s. Although A. marginata can occur in areas with much greater maximum velocities, the population from which experimental specimens were collected is geographically sheltered from waves and the hydrodynamic regime is dominated by tidal currents that do not likely exceed 3 m/s. Reproductive (n = 45) and non-reproductive (n = 43) sporophylls were attached directly to a pre-calibrated force transducer (detailed in Boller & Carrington, 2006), which measured force at 10 Hz for 10 s at each velocity (the average was used for all drag measurements). Drag is influenced by size, shape (Carrington, 1990), and flexibility (Chapter 5), all of which could conceivably vary with reproductive state. In addition to measuring differences in drag between reproductive and vegetative sporophylls, we attempted to disentangle the contributions of variation in each to differences in drag. First, we measured drag on entire sporophylls. Next, we calculated size-specific drag (drag per surface area, N\cdot m^{-2}) of reproductive sporophylls to correct for differences in size (shape and mechanical properties still considered potentially different). Finally, we cut vegetative and reproductive sporophylls
into standardized working sections (detailed in Chapter 2) of the same size and shape (differing only in tissue mechanical traits).

Sporophyll flapping will increase the overall drag experienced in flow as well as the variance in drag. Because we measured drag at 10 Hz for 10 s, we were able to measure flapping as a dimensionless coefficient of variation \( \left( \frac{\text{st.deviation}}{\text{mean}} \cdot 100 \right) \) in drag for each sporophyll at each speed. We used this coefficient of variation as a measure of sporophyll flapping.

A subset (n = 46) of the sporophylls used in hydrodynamic performance measurements (from July collections only) were also used in materials testing to allow the calculation of sporophyll safety factors (breaking force/drag experienced) and compared among reproductive status groups at the six velocities tested.

### 3.3.5 Statistical analyses

One-way analysis of variance was used to test for differences in morphological and mechanical properties in reproductive vs. non-reproductive sporophyll tissues. Drag, flapping, and sporophyll safety factors were measured on the same sporophylls at multiple velocities, requiring the use of repeated measures ANOVA to account for the non-independence of samples, while testing for differences in drag, flapping, and sporophyll safety factor among individual samples with respect to reproductive status. Repeated measures ANOVA was also used to test the effects of sporophyll L:W ratio on size-specific drag in vegetative vs. reproductive sporophylls. The assumption of sphericity was violated in all analyses, necessitating the use of the Greenhouse-Geisser test statistic. Sporophyll safety factor and drag per sur-
face area data was log-transformed before analyses to meet the assumption of a normal distribution. All statistical analyses were implemented in SPSS 17 with $\alpha = 0.05$.

### 3.4 Results

#### 3.4.1 Morphological properties

Results from statistical comparisons of differences in morphological traits between reproductive and vegetative sporophylls are graphically depicted in Figure 3.2a-d. Reproductive sporophylls had significantly more surface area than vegetative sporophylls ($F_{1,84} = 10.70$, $p = 0.002$). Sporophyll width was not significantly different among reproductive status groups ($F_{1,84} = 1.17$, $p = 0.283$), while reproductive sporophylls were significantly longer ($F_{1,84} = 12.50$, $p = 0.001$), resulting in greater length:width ratios ($F_{1,84} = 18.95$, $p < 0.001$) in reproductive vs. vegetative sporophylls. Reproductive sporophylls were also significantly thicker ($F_{1,84} = 324.49$, $p < 0.001$) than vegetative sporophylls. Ruffliness indices were marginally significantly ($F_{1,45} = 4.022$, $p = 0.051$) lower in reproductive vs. vegetative sporophylls.
Figure 3.2: Comparison of (a-d) morphological and (e-h) mechanical properties between vegetative, i.e. non-reproductive, (empty circles) and reproductive (filled circles) A. marginata sporophylls. Values are mean ± S.E.
3.4.2 Mechanical properties

Associations of tissue mechanical traits with reproductive state are shown in Figure 3.2e-h. Although we detected no differences associated with reproduction in breaking stress ($F_{1,63} = 2.08, p = 0.154$), reproductive tissue was more likely than not to have a lower mean breaking stress. We did, however, detect higher tensile stiffness ($F_{1,85} = 24.25, p < 0.001$) in reproductive sporophylls. Furthermore, tissue from reproductive sporophylls exhibited significantly greater force required to break sporophylls ($F_{1,63} = 74.26, p < 0.001$) and flexural stiffness ($F_{1,85} = 177.33, p < 0.001$) than vegetative sporophylls.

3.4.3 Hydrodynamic performance

Statistical results from repeated measures ANOVA testing the effects of reproductive state, water velocity, and their interaction on sporophyll drag (N) and drag per surface area (N·cm$^{-2}$) are presented in Table 3.1. Entire reproductive sporophylls experienced greater drag than did vegetative sporophylls; to an increasing extent with increasing water velocity (Figure 3.3a). However, vegetative and reproductive sporophylls experienced similar size-specific drag (N·cm$^{-2}$) (Figure 3.3b). When sporophylls were cut into hydrodynamic working sections of the same shape and size (only differing in material), drag increased with increasing velocity ($F_{1,17} = 367.24, p < 0.001$), was higher in working sections cut from reproductive materials ($F_{1,17} = 8.54, p = 0.009$), and the rate of increase in drag with increasing water velocity was marginally higher among reproductive working sections.
Sporophyll shape (L:W ratio) and interactions with water velocity and reproductive state were not significant predictors of size specific drag in Repeated Measures ANOVA analyses (Table 3.2).

Repeated Measures ANOVA revealed that flapping decreased with water velocity ($F_{1,93,77.01} = 167.10, p < 0.001$), flapping was generally lower in reproductive sporophylls ($F_{1,40} = 6.318, p = 0.016$), and the difference between reproductive status groups was dependent upon water velocity (Fig 3.4 $F_{1,93,77.01} = 14.277, p < 0.001$). Post-hoc tests between reproductive groups at each velocity revealed that vegetative sporophylls experienced greater flapping at 0.18 and 1.71 m/s; there were not significant differences in vegetative vs. reproductive sporophylls at the other velocities tested.

Sporophyll safety factor decreased with increasing velocity for all sporophylls ($F_{1,33} = 2751.54, p < 0.001$), but was significantly higher ($F_{1,33} = 4.787, p = 0.036$) for reproductive sporophylls (Fig. 3.5). We did not detect a significant interaction between water velocity and reproductive state on sporophyll safety factor ($F_{1,33} = 2.593, p = 0.117$).
Table 3.1: Effects of reproductive state on drag. Results from Repeated Measures ANOVA testing the effects of reproductive state, water speed, and their interaction on whole sporophyll drag and drag per surface area. Results are visually presented in Figure 3.3a-b.

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>p</th>
<th>Source</th>
<th>F</th>
<th>p</th>
<th>Source</th>
<th>F</th>
<th>p</th>
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<td>152.6</td>
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<td>Speed</td>
<td>2380.44</td>
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<td>Speed</td>
<td>264.89</td>
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<tr>
<td>State</td>
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<td>State</td>
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<td>State</td>
<td>8.54</td>
<td>0.009</td>
</tr>
<tr>
<td>Sp * St</td>
<td>10.62</td>
<td>0.001</td>
<td>Sp * St</td>
<td>1.41</td>
<td>0.246</td>
<td>Sp * St</td>
<td>3.027</td>
<td>0.015</td>
</tr>
</tbody>
</table>
Table 3.2: Effects of shape and reproductive state on drag. Statistical output from 3-way Repeated Measures ANOVA testing how reproductive state, water speed, and sporophyll shape (i.e. length:width ratio) affect log transformed drag per surface area of *A. marginata* sporophylls.

<table>
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<th>df</th>
<th>F</th>
<th>p</th>
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</thead>
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<td>State (St)</td>
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<td>0.497</td>
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<td>St*L:W</td>
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<td>St<em>Sp</em>L:W</td>
<td>1.4, 101.1</td>
<td>2.051</td>
<td>0.149</td>
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</tbody>
</table>

3.5 Discussion

Reproduction is essential for population and species persistence, especially in environments characterized by high individual mortality, such as the wave-swept intertidal zone. However, reproductive investment is usually non-trivial for individuals and often involves metabolic and performance costs. Likewise, the optimization of reproductive strategies is an important component of an organism’s overall fitness. Metabolic costs of reproduction have received much attention in phylogenetically disparate taxa (e.g. Bell, 1980; Calow, 1979; Obeso, 2002; Rose & Bradley, 1998); but mechanical costs to reproduction are much less well characterized and are described almost exclusively in animal taxa. Nonetheless, mechanical traits important to survival in most plants and algae have the potential to be affected by the onset of reproduction.

Variation in hydrodynamic performance of seaweeds has been associated
Figure 3.3: Comparison of hydrodynamic performance between reproductive and vegetative sporophylls: (a) Drag on entire sporophylls, (b) Drag per surface area of entire sporophylls, and (c) Drag on working sections (same shape and size) cut out of reproductive and non-reproductive sporophyll tissue. In all graphs, vegetative, i.e. non-reproductive, samples are represented by empty circles and dashed lines while reproductive samples are denoted with filled circles and solid lines. Values are mean ± S.E.
Figure 3.4: Flapping, measured as the coefficient of variation in drag, in reproductive (filled circles) vs. vegetative (empty circles) sporophylls. Values are mean ± S.E.
Figure 3.5: Safety factor (breaking force/drag experienced, on a log scale) of reproductive and vegetative sporophylls at six water velocities. Values for reproductive and vegetative sporophylls (represented by filled and empty circles, respectively) are mean ± S.E. Dotted line represents the value at (and below) which sporophylls break.
with tissue mechanical (Boller & Carrington, 2007; Gaylord & Denny, 1997; Koehl, 1986; Koehl et al., 2008, Chapter 2) and morphological (e.g. Koehl et al. 2008, Carrington 1990) traits. We documented shifts in morphological, mechanical, and performance traits within a species of kelp during reproduction. Our study shows that seaweed mechanical and morphological traits interact to determine their hydrodynamic performance, but also highlights that the onset of reproduction adds further complexity to these interactions. Reproductive sporophylls of *A. marginata* experienced greater drag than non-reproductive sporophylls. However, after correcting for differences in size among sporophylls, there was no difference in drag (size-specific) between reproductive states, suggesting that increased size is an important component of the added drag experienced by reproductive *A. marginata* sporophylls. This lack of difference in size-specific drag exists in spite of differences in mechanical and morphological traits known to influence drag: flexural stiffness and ruffliness. When sporophylls were cut into the same size and shape (varying only in flexural stiffness), they experienced greater drag when cut from reproductive tissue than from vegetative tissue, reconfirming that increased flexural stiffness increases drag (e.g. Chapter 2) and suggests that some morphological aspect compensates for it in entire sporophylls.

Given their marked differences in flexural stiffness, how then did size-specific drag not vary between reproductive states? Perhaps the most straightforward interpretation is that any increased drag arising from increased flexural stiffness was compensated for by another mechanism, two of which are readily apparent: (1) a non-linear relationship between surface-area and
drag and/or (2) variation in morphology between reproductive states. Opposing the former hypothesis, relationships between surface area and drag at each velocity tested were strongly linear ($p<0.001$, $r^2 = 0.50–0.65$). Consistent with the latter hypothesis, reproductive sporophylls displayed decreased ruffliness and were significantly longer than vegetative sporophylls.

Assessing the contribution of differences in sporophyll shape (i.e. length vs width) is complicated by a correlation between shape and size variables and an interaction between shape and tissue mechanical traits in determining drag. Width was not different among reproductive states, but length and surface area were larger in reproductive sporophylls. Because size is positively related to drag, relating changes in length, or length to width ratios, to differences in drag does not disentangle size and shape. Likewise, we tested the effect of sporophyll shape on drag by relating how the length:width ratio of sporophylls influenced size-specific drag. Differences in shape among reproductive groups did not explain variation in size-specific drag (Table 3.2).

Decreased sporophyll ruffliness seems more likely to be the factor offsetting the cost of increased flexural stiffness among reproductive sporophylls. Increased ruffling is thought to increase drag [Koehl & Alberte 1988] by increasing sporophyll flapping (thereby increasing both area perpendicular to flow and turbulence downstream of the sporophyll), and could have offset the cost of decreased flexibility in reproductive *A. marginata* tissues. Likewise, we found decreased ruffliness and a concomitant decrease in flapping among reproductive sporophylls. Variation in ruffliness has been observed among conspecifics at different sites [Koehl et al. 2008], but this is the first
report of ruffliness varying among developmental states at the same site.

Changes in shape through development (allometric growth) have been previously associated with drag reduction in kelps (Denny et al., 1997; Johnson & Koehl, 1994). In this study, the increased size (surface area) associated with reproductive sporophylls was a consequence of greater length, but not width, resulting in increased length:width ratios among reproductive sporophylls. However, changes in L:W ratios were not associated with a reduction in size-specific drag in reproductive sporophylls. For flexible bladed seaweeds, like the sporophylls of *A. marginata*, drag reduction is thought to occur primarily by tissue flexibility allowing blades to change into more hydrodynamically streamlined shapes (Martone et al., 2012). Because initial shape was not associated with drag in our analyses, it seems likely that initial size and flexibility were more important in determining reconfigured shape/size than initial size.

Although changes in morphology appear to have compensated for the increased flexural stiffness among reproductive sporophylls, whole reproductive sporophylls were larger and consequently experienced proportionately greater drag. Despite the observed increase in drag, sporophyll safety factor was not decreased in reproductive sporophylls, and was even significantly higher at two velocities, owing to the increased breaking force associated with the thicker, reproductive sporophylls. Shape was not associated with drag in our study; it seems that the shape and size into which sporophylls reconfigured in flow was more dependent upon tissue flexibility and size than unreconfigured shape.

Our results show mechanical costs to reproduction in a marine seaweed
via greater drag caused by increased size and decreased flexibility. These costs are mediated by concomitant increases in mechanical strength and changes in morphological traits, respectively. Because seaweeds are firmly attached to rocks, they cannot behaviorally modify their interactions with their environment in the same way that mobile animals can (Bradshaw 1972, Huey 2002). Instead, seaweeds often modify growth form as a sort of behavior to optimize function in wave (Blanchette 1997, Koehl et al. 2008) environments. In this sense, our results parallel studies from terrestrial and marine animals, which have reported that animals rely on behavioral modifications to compensate for the mechanical costs of decreased agility and speed associated with gravidity (e.g., Pruitt 2010, Schwarzkopf & Shine 1992).
Chapter 4

Contrasting implications of tissue aging in red algae vs. kelps

4.1 Synopsis

The mechanical design of an organism (i.e., the composite value of its morphological and mechanical traits) determines its functional performance and therefore influences fitness. Because developmental processes inevitably shift morphological and material traits, shifts in ancillary traits are necessary to continue optimizing functional performance throughout an individual’s lifespan. Previous studies have noted that older tissues have compromised material properties and that repeated loading of even small forces on the same tissue over time can have fatal consequences (fatigue failure). In spite of the observation that kelp tissues are more susceptible to fatigue failure than red algae, large kelps dominate in high energy zones. Using 10 species of kelps, 16 species of red algae, and 1 green alga, we show that the effects of aging on tissue extensibility, strength, and stiffness are comparable among
all species. However, differences in growth form among species resulted in divergent consequences of tissue aging for the mechanical design of kelps vs. red algal blades. Blades of red algae, which grow out from their apices, are supported by their oldest tissue. The bladed green alga, *Ulva lactuca*, has diffuse growth and lacked a relationship between position on blade and material properties. Kelp blades on the other hand grow basally allowing new tissue to support the hydrodynamic loading of the blade while older tissue is moved towards blade extremities until it is eroded off. We argue that the basal growth strategy, unique among kelp blades, may help to explain how kelps persist in high energy environments in spite of their enormous size and environmental susceptibility to fatigue failure.

### 4.2 Introduction

Mechanical design is woven together by interacting material properties and morphology. For example, as trees grow they must resist greater forces from self-loading due to their greater weight, (Peters et al. 1988; Spatz & Bruechert 2000) and increased aerodynamic drag. Ultimately, the loads they can withstand, and therefore, the sizes they can reach depend on their underlying material properties. Less rigid plants can reduce the amount of drag they experience per unit surface area in wind or flowing water by going with the flow more than resisting it (Puijalon et al. 2011; Vogel 1984). Seaweeds and flexible plants reduce drag by reconfiguring into more streamlined shapes and sizes in moving water or wind (Harder et al. 2004; Martone et al. 2012); generally speaking, the floppier the individual, the
less drag it experiences in flow (Chapter 2). Likewise, floppier tissues are thought to confer a strong fitness advantage in hydrodynamically stressful environments, such as the wave-swept intertidal (Harder et al., 2006), where water velocities can reach 25m/s. In at least some seaweed species, the ability to survive in the mechanically hostile wave-swept intertidal is largely dependent upon drag reducing and/or resisting mechanisms (Chapter 5). These mechanisms, however, ultimately arise from interacting tissue material properties and morphology.

Tissue material properties and morphological traits both change throughout the course of an organism’s life (e.g. Johnson & Koehl 1994; Chapter 3). To continue optimizing mechanical performance throughout an individual’s lifetime, shifts in material properties or morphology throughout ontogeny must be compensated for by shifts in other material or morphological traits. Indeed, numerous studies have shown that some seaweeds can ameliorate the drag associated with increased size during development by growing into more streamlined shapes as they develop, i.e. allometric growth (e.g. Denny et al., 1997; Gaylord & Denny, 1997; Johnson & Koehl, 1994). However, growth is only one ontogenetic process that has the potential to influence mechanical traits (and consequently hydrodynamic performance) of seaweeds. For instance, during reproduction, tissue stiffening occurs in the winged kelp, Alaria marginata (Chapter 3). Although tissue stiffening decreases reconfiguration potential, and therefore increases drag, blades also become less ruffled. Decreased ruffliness results in decreased flapping, which compensates for the added drag associated with their decreased reconfiguration potential (Chapter 3). Thus, shifts in morphological traits ameliorate shifts
in material properties associated with reproduction that would, otherwise, decrease the functional performance of *A. marginata*.

Material properties may also change as the tissue ages. A weakening and/or stiffening of older tissues has been reported in red [Koehl 2000; Martone 2007] and brown [Armstrong 1987; Stewart 2006] algae. Stiffening of tissues with age may result from thickening of cell walls [Martone 2007]. The causes of tissue weakening with age in seaweeds have not been explicitly explored, but repetitive mechanical loading of tissues is a likely culprit. Seaweeds along wave-swept shorelines must withstand waves every 5–10 seconds. Recent research has shown that failure from repeated loading of smaller forces may threaten seaweeds more than forces imposed by maximum wave velocities, because it weakens tissue gradually [Mach et al. 2007; Mach 2009].

Kelps (brown algae of the order Laminariales) thrive in the wave-swept intertidal for multiple years [Klinger & DeWreede 1988] in spite of the reported menace of fatigue failure in their tissues as they resist the large forces generated by each passing wave as a consequence of their enormous size. Fatigue failure has been experimentally assessed in only a handful of species, all of which are small flexible red algae with annual blades [Mach et al. 2007; Mach 2009; Mach et al. 2011]. If the perils of material fatigue failure are indeed greater in kelps, we might expect their tissues to be adapted to reduce fatigue. However, data from tissue cycling tests suggest otherwise. Hale (2001) performed tensile cycle tests on several species of red algae and kelps and measured the amount of strain energy dissipated during each cycle (hysteresis). He found that kelp tissues gave off more energy (were less re-
silient) than those of red algae and speculated that accordingly, kelps should be more susceptible to failure by fatigue than red algae.

If kelp tissues are more susceptible to fatigue failure than red algal tissues, and failure by fatigue is a major source of red algal mortality (Mach et al., 2007; Mach, 2009), then how do kelps thrive, and even perenniate, in the wave-swept intertidal? We propose that basal growth (Figure 4.1), a derived trait unique to blades of kelps and their sister groups (Kogame & Kawai, 1996), provides them with an escape from fatigue failure. In contrast, most red macroalgae grow apically, whereby new growth occurs via meristems located at their distal tips. Apical growth (also found in some brown algae) results in the newest tissue occurring at blade extremities and oldest tissue at the base, nearest to where the seaweed attaches to the rock. Therefore, the tissue holding the seaweed to the rock is not renewed throughout the blade’s lifetime. In kelps, blade growth occurs at the bottom of blades, with the result that the newest tissue always supports blade attachment while older tissue migrates to the blade apex, where it eventually erodes off the tips (Mann, 1973), analogous to a conveyor belt. Through basal growth, kelp blades may therefore escape fatigue failure by sloughing off older tissue and relying on newer tissue for continued mechanical support.

In this study, we investigated whether shifts in material properties associated with aging were similar across large foliose seaweeds, and whether and how species’ growth forms constrained longitudinal distributions of material properties along blades. We used 16 species of foliose red seaweeds, 1 green algal species, and 10 species of kelps to ask: (1) Are shifts in material properties of wave-swept macroalgae associated with tissue aging? (2) Is the
longitudinal distribution of material properties along seaweed blades associated with their growth form (i.e., diffuse vs. apical vs. basal)? The green algal species, *Ulva lactuca*, possesses diffuse growth (where growth occurs throughout the blade in every direction), and so including it allowed us to see if longitudinal patterns of material properties were achieved in absence of a clear relationship between blade position and tissue age. We predicted that aging should consistently decrease breaking strain and breaking stress and increase stiffness of tissues of all species. However, because the relationship between distance from the base of the blade and age of tissue is different between red macroalgae and kelps, we hypothesized that the two groups would display opposite trends in the longitudinal distribution of material properties.
Figure 4.1: Schematic demonstrating differences in growth forms among morphologically similar species of different taxonomic groups. From left to right: *Mazzaella splendens* is a red alga (Rhodophyta) with apical growth, *Saccharina sessilis* is a kelp (Laminariales) which has basal growth and *Ulva lactuca* is a green alga (Chlorophyta) that has diffuse growth. In each species, the dashed circle(s) indicate the location of a growth meristem. *Ulva lactuca* lacks one localized meristem, and instead growth occurs in every direction from all parts of the blade. Artwork created by Laura M. Anderson
4.3 Methods

4.3.1 Sample collection

Specimens were collected in May 2011 from Port Renfrew on Vancouver Island, BC (48°31’33.46N, 124°26’50.22W) or Friday Harbor Laboratories on San Juan Island, WA (48°32’42.62N, 123°0’43.83W). A total of 27 species were collected: 10 species of kelps, 16 species of red algae, and a green alga (collection details summarized in Table 4.1). Specimens were collected at multiple sites to increase the number of species sampled. The diversity of kelps large enough for analyses was higher in Port Renfrew (likely due to its exposed nature). For foliose red algae (large enough for this study), the diversity was much great at Friday Harbor Laboratories (a much more protected site). After collection, specimens were placed in a flow-through water table until analyses, all of which were performed within 48 hours of collection.

4.3.2 Determination of tissue material properties

Tissue material properties were determined along a longitudinal series from the point of blade attachment (the stipe if present, or holdfast when the stipe was absent) to the blade apex. Dumbbell-shaped working sections (Chapter 2 Mach 2009) were cut from seaweed tissues longitudinally at regular intervals to ensure that 4–8 working sections were obtained from each specimen. Sampling at regular intervals (with the intervals scaled to the specimen’s length) across the whole specimen allowed for the same scale of youngest to oldest tissue to be sampled in all individuals, in spite of
Table 4.1: Summary of specimens used and collection site, sorted by taxonomic grouping.

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<th>Species</th>
<th>Collection site</th>
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<td>Rhodophyta</td>
<td><em>Polyneura latissima</em></td>
<td>Friday Harbor, WA</td>
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<td></td>
<td><em>Sparlingia pertusa</em></td>
<td>Friday Harbor, WA</td>
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<tr>
<td></td>
<td><em>Cryptonemia borealis</em></td>
<td>Friday Harbor, WA</td>
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<td></td>
<td><em>Mazzaella splendens</em></td>
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<td></td>
<td><em>Palmaria hecatensis</em></td>
<td>Port Renfrew, BC</td>
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<td></td>
<td><em>Chondracanthus exaspertus</em></td>
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<td></td>
<td><em>Palmaria mollis</em></td>
<td>Friday Harbor, WA</td>
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<td><em>Opuntiella californica</em></td>
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<td><em>Prionitis lanceolata</em></td>
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<td><em>Neodilsea borealis</em></td>
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<td><em>Cryptopleura ruprechtiana</em></td>
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<td><em>Weeksia reticulata</em></td>
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<tr>
<td></td>
<td><em>Schizymenia pacifica</em></td>
<td>Friday Harbor, WA</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td><em>Ulva lactuca</em></td>
<td>Friday Harbor, WA</td>
</tr>
</tbody>
</table>
differences in their size. The position along the blade of each test-section was recorded as the distance (nearest 0.5 cm) from the center of the working section to the blade’s point of attachment.

Pneumatic grips (90 psi) were used to attach the tissue working sections to an Instron tensometer (model 5565 Norwood, MA) which strained the seaweeds at a constant rate of 10 mm-min⁻¹. The resisting force (N) of tissue was measured at 10 Hz until tissue failure occurred. Width and cross-sectional area of working sections were measured before tensile tests to the nearest 0.1 mm using digital calipers. All tensile tests were performed on tissues while submerged in 10°C seawater from Friday Harbor Laboratories.

Tissue displacement (0.1 mm) and the resisting force (N) of tissues were extracted from the Instron program. Strain was calculated as the change in length of the tissue divided by the initial length. Extensibility (breaking strain) and the force required to break samples were recorded as the final strain and force values. Force values were divided by initial tissue cross-sectional area to calculate dimension-independent characteristics of tissue properties: tissue breaking strength (stress required to break tissue) and tensile stiffness (strain experienced by tissue bearing a given load). Because algal tissues do not display linear stress vs. strain curves (Hale, 2001), tensile stiffness was determined at the initial and terminal portions of the individual stress vs. strain curves and defined hereafter as initial and final stiffness, respectively.
4.3.3 Statistical analyses

Because only one species of green algae (Chlorophyta) was large enough to obtain material property data at multiple positions along the blade, this species (*Ulva lactuca*) was analyzed separately from the red and brown algae. Position along blade and tissue material property data were standardized for each specimen (with zero representing the mean value, negative values representing values below the mean, and positive values representing values greater than the mean) because tissue material properties (as well as length of specimens) vary among species within (Chapter 2) and among phyla (Martone, 2007). Linear mixed effects models were then performed on standardized data to test the hypothesis that variation in growth patterns between rhodophyte and kelp species results in differential trends in material properties along blades. In the model, group (red algae vs. kelps) and position along blade were included as fixed factors, while species was included as a random factor. A group × distance interaction term was included as an explicit test of the differences in the relationship between position along blade and the given mechanical property of kelps and red algae. All statistical analyses were performed in JMP Pro 9 and considered significant with $\alpha = 0.05$ and $p < 0.05$.

4.4 Results

In all kelp species tested, extensibility (breaking strain) decreased along the length of the blade, whereas the strength (MPa) and initial and final tensile stiffness (E, MPa) increased (Figure 4.2 gray lines). For the red algae,
the opposite trend was found for each tissue material property, as extensibility increased with distance from the blade’s base, whereas the breaking stress and initial and final tensile stiffness decreased (Figure 4.2, black lines). Thus, in all specimens of kelp and red algae, older tissue (basal vs. apical) showed decreased extensibility and elevated strength and stiffness. For the green alga tested, *Ulva lactuca*, the position on the blade (n = 5) was not significantly associated with any material properties: extensibility (R² = 0.04, p = 0.745), strength (R² = 0.07, p = 0.658), initial stiffness (R² = 0.341, p = 0.224), or final stiffness (R² = 0.001, p = 0.961).

In the overall mixed effects analysis, taking species into account as a random factor nested within taxonomic group, a significant group*position on blade interaction term was detected for all tissue mechanical properties tested (Table 4.2). The sign of the relationship between distance from the base of the blade of the tissue to its material properties (i.e., extensibility, tissue breaking stress, and initial and final tensile stiffness) was opposite for kelps and red algae in all material properties (Fig. 4.2).
Table 4.2: Effects of aging on material properties in kelps vs. red algae. Variation in material properties (first standardized by specimen) explained by group (kelps vs. foliose red algae), position on blade (distance from blade’s attachment point), and the interaction between the two. Values are F-ratios. * denotes p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001.

<table>
<thead>
<tr>
<th></th>
<th>Extensibility</th>
<th>Breaking stress (MPa)</th>
<th>Initial stiffness (MPa)</th>
<th>Final stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Group</td>
<td>2.656</td>
<td>1.328</td>
<td>0.4877</td>
<td>3.005</td>
</tr>
<tr>
<td>Position on blade</td>
<td>* 6.413</td>
<td>2.138</td>
<td>* 5.019</td>
<td>0.623</td>
</tr>
<tr>
<td>Group-position on blade</td>
<td>*** 27.911</td>
<td>** 10.006</td>
<td>** 11.397</td>
<td>*** 25.91</td>
</tr>
</tbody>
</table>
Figure 4.2: How tissue material properties vary along the lengths of blades of kelps (grey lines) and foliose red algae (black lines): (A) Breaking strain (B) Breaking stress (C) Initial tensile stiffness and (D) Final tensile stiffness. All values are standardized to allow comparison among species and taxonomic groups. The x-axis is standardized position on blade with zero representing the center of the blade, negative values representing areas closer to the blade’s base, and positive values representing distal portions of the blades. Slopes for the green alga, *Ulva lactuca*, were not significantly different from zero and are excluded from graphs. Solid lines represent estimated linear fit by groups and dashed lines represent 95% confidence intervals.
4.5 Discussion

In blades of both red algae and kelps, extensibility was lower in older tissue. We observed higher stiffness in older tissues of kelp and red algae. These results mirror previous studies (Armstrong 1987, Koehl 2000, Stewart 2006) and corroborate the notion that aging compromises the desired extensibility and flexibility of seaweed tissues. Because high extensibility (Koehl & Wainwright 1977) and low stiffness (Chapter 2, Boller & Carrington 2007) are thought to be important components of mechanical adaptation of seaweeds to life in the wave-swept intertidal the finding of diminished mechanical qualities within tissues supporting the blade suggests that basal growth among kelps enhances functional performance. We also found significant increases in tissue strength (as breaking stress) with age. Initially, this finding seems to be at odds with the hypothesis that continued stress on tissues over time results in a weakening of constituent material properties. However, further attention to the complexity of seaweed tissues when discussing their strength may resolve this discrepancy.

Breaking stress is calculated as the breaking force (N) of the sample divided by the cross-sectional area. Therefore, knowing the breaking stress of a material (e.g. steel), allows calculation of the force required to break a beam of that material at any size. Seaweeds, and indeed, most other biological tissues are complex, nuanced structures that are composed of a diversity of biomaterials. Seaweeds are composed of boxes and/or spheres (cells) of varying sizes, filled to varying degrees with water, cell walls, and various cellular constituents. The breaking strength of a seaweed is related to the
amount and quality of the underlying material properties of structural materials (i.e. the cell wall) and can therefore be affected by both. Martone (2007) demonstrated that the increased breaking stress of older segments of the red alga *Calliarthron tuberculatum* was due to an increase in the amount of cell wall structural materials, which disproportionately accounted for the decrease in the breaking stress of these aging materials. Although this has only been investigated directly in one species (Martone 2007), the observation that older tissues have a disproportionately greater amount of cell wall tissue in cross-section could help explain why we detected higher tissue breaking stress in these tissues, even if the structural materials composing the cell walls have been weakened by repeated exposure to waves throughout their lives.

We were unable to determine associations between age and tissue material properties in *Ulva lactuca* because its diffuse growth form does not allow blade position to serve as a proxy for tissue age. Not surprisingly, then, the position along the blade was not significantly associated with variation in any of the tested material properties. Our inability to detect such relationships may have resulted from the lack of a spatial trend in tissue age and/or from an inherent lack of aging effects on tissue mechanical properties. Further studies are required to investigate whether the effects of aging on green algal tissues are comparable to those reported in kelps and foliose red algae.

Although the effects of aging on seaweed tissues were shared among kelps and red algae, they have divergent implications for their mechanical design, owing to differences in their growth form. Because the majority of foliose red algae (and all of the species tested) grow from blade apices, the
oldest tissue attaches the blade to the substrate and this likely increases the susceptibility of blade loss due to tissue fatigue failure (Mach, 2009). Susceptibility to fatigue failure as a result of older tissue occurring at the base of the blade may be partially mitigated by greater strength in red algal tissues (Martone, 2007) and/or an adaptation of red algal tissues to retain increased energy after each cycle of stress applied to the tissue (Hale, 2001), although the mechanisms governing interspecific variation in susceptibility to tissue fatigue remain completely unexplored. Even though kelp tissues retain less energy in repetitive loading (Hale, 2001), they may be able to mitigate the threat of fatigue failure simply by ensuring that the newest (most extensible and least stiff) tissue is always bearing the hydrodynamic loading, achieved through basal growth of blades.

This study provides evidence that ontogenetic shifts in material properties of seaweed tissues can be compensated for by differences in growth form. Specifically, the high susceptibility of kelp tissues to fatigue failure is likely mitigated by their basal growth form, which means that the newest tissue is consistently at the blade’s base, where the largest hydrodynamic loads occur. Nonetheless, there are thousands of foliose species of red algae living along wave-swept shores. Their ability to thrive in these mechanically hostile environments may be related to other growth form and/or life history traits. For instance, many other red algal species can proliferate from crustose holdfasts- these species may sacrifice blades in particularly hydrodynamically stressful times to allow the holdfast to remain attached and regenerate blades (D’Antonio, 1986; Marshall, 1949). Other red algae are annual and may rely on having one large reproductive event before the onset
of large winter swells dislodges them as a strategy for persistence. Clearly, it is important to consider morphological, material, and life history traits when addressing adaptations of seaweeds to wave-exposed environments.
Chapter 5

Mechanical traits explain differences in survival

5.1 Synopsis

Organisms’ ability to withstand the physical forces of their environment is a key determinant of their success. Mechanical performance of organisms is often associated with the properties of the tissues that compose them. In mechanically stressful habitats, intraspecific variation in tissue properties may result in differential survivorship and enable natural selection to act on mechanical traits. We tested the hypothesis that tissue mechanical properties affect survivorship (a fitness component) of the perennial kelp, *Egregia menziesii*, in a mechanically stressful, wave-swept intertidal habitat. We measured intraspecific variation in strength and flexibility in 38 *E. menziesii* and tracked their survivorship in the field over the winter storm season to determine if variation in mechanical properties led to differential survivorship. Significant inter-individual variation was found in most mechanical properties, including breaking force and flexibility. Individuals with increased flexibility and decreased breaking force were more likely to
survive the duration of our study, although this effect was more pronounced in individuals with smaller holdfasts. Increased frond breaking force was also associated with a reduction in self-thinning, potentially explaining the observed increase in whole plant mortality with increasing frond breaking force. Results from this study demonstrate that variation in tissue mechanical properties among conspecifics can influence survivorship and this may have important evolutionary implications.

5.2 Introduction

Species distributional patterns are determined by their ability to thrive under a range of biotic and abiotic conditions. One often overlooked, albeit crucial, element of environmental variation is the physical forces organisms must endure, and organisms exhibit an exquisite diversity of form to meet these challenges. For example, many trees must withstand the violent forces of seasonal storms (Spatz & Bruechert, 2000), vertebrate tendons must withstand stresses associated with creating movement, and spider silk must absorb the energy of rapidly moving prey, while adhering to the prey’s surface (Agnarsson & Blackledge, 2009). Of all the mechanically challenging environments on earth, the wave-swept shore may be one of the most inhospitable owing to extreme mechanical stressors. Organisms within this zone must endure the forces of crashing waves, which can be 100 times greater, for the same organism, than in any reported wind storm, every 5–10 seconds (Denny & Gaylord, 2002). Nonetheless, the wave-swept intertidal hosts a tremendous diversity of plants and animals that exhibit highly adapted
forms to meet these challenges.

In order for intertidal organisms to prevent becoming beach wrack, their tissues must remain stronger than the forces they experience. Some species accomplish this by strengthening their tissues (Lowell et al., 1991; Martone, 2007) while others create flexible tissues that can reconfigure in flow to generate more streamlined shapes (Boller & Carrington, 2006; Koehl, 1984). Flexibility is often thought of as a prerequisite for attaining large body size along wave-swept shorelines (Harder et al., 2004) because tissue flexibility dramatically decreases drag experienced and most intertidal plants are markedly more flexible than terrestrial plants. Whether individuals create strong and/or flexible tissues, their hydrodynamic performance is largely dependent upon the properties of their tissues (Chapter 2; Boller & Carrington, 2007). Therefore, tissue mechanical properties are thought to be under strong selective pressure along high-energy shorelines, where wave-induced damage may be the largest cause of mortality of sessile organisms.

Despite the wealth of research suggesting that tissue mechanical properties dictate performance of organisms in the wave-swept intertidal (e.g. Denny, 1988; Koehl, 1984; Koehl & Wainwright, 1977), there is a paucity of data on how tissues’ mechanical properties impact whole-organism survival and reproductive success. Many studies have compared mechanical properties among species (e.g. Chapter 2; Boller & Carrington, 2007; Harder et al., 2004) and speculated about the fitness consequences of between-species variation in mechanical design. For instance, Dudgeon & Johnson (1992) related differences in mechanical properties to differential survivorship in two competing species of macroalgae, suggesting that tissues’ components can shape
differential survival among species. However, whether or how variation in tissue mechanical traits among individuals within a site affects whole plant survivorship has not been considered explicitly. This deficit persists despite the broadly accepted view that evolution shapes traits via selection on intraspecific trait variants (Darwin 1859).

In order for evolution to shape organisms’ mechanical properties, there must first be heritable within-species variation on which selection can act. Intraspecific variation has been documented in many species of intertidal macroalgae in two contexts: differences among populations and differences within populations. Studies on local adaptation of tissue mechanical properties have demonstrated differences in mechanical strength and stiffness among sites (Blanchette et al. 2002; Johnson & Koehl 1994). Such variation, however, may either be due to genetic divergence between populations or simply plasticity. Within-site variation in mechanical properties has been documented along exposure gradients (Kitzes & Denny 2005) and through laboratory culture studies of individuals from the same site under different conditions (Kraemer & Chapman 1991). Such within-site variation has often been attributed to plasticity, where individuals produce different tissue mechanical properties based on the hydrodynamic cues they receive during development. However, within-site variation in mechanical properties can also arise as the result of underlying genetic variation among individuals. If such variation results in differential performance, it is possible for selection to act on tissue mechanical properties. Studies considering whether or how contemporary selection acts on standing intraspecific variation in mechanical properties are absent.
In this study, we used *Egregia menziesii*, a common kelp species along exposed coastlines, to test (1) whether intraspecific variation in frond mechanical traits was repeatable and (2) whether variation in mechanical properties among conspecifics resulted in differential survivorship. Because this species can live for several years, its holdfast must withstand the largest hydrodynamic forces imposed by winter swells to avoid dislodgement. Black (1976) showed that grazing by the limpet *Acmaea incessa* increased survival of *E. menziesii* individuals by pruning fronds (likely as a consequence of decreasing frond breaking strength), resulting in smaller plants during periods of winter swell. Because pruning seems to be related to frond breaking strength, it is also possible that intraspecific variation in mechanical strength, in the absence of herbivores, results in differential pruning and therefore survivorship. Specifically, we predicted that plants with lower frond breaking strength would be more likely to self-prune, and thereby decrease the probability of whole-plant dislodgement (i.e., holdfast failure) during winter storms.

5.3 Methods

5.3.1 Study species and site

*Egregia menziesii* (Turner) JE Areschoug (Fig. 5.1) is a perennial kelp (Laminariales, Phaeophyceae) common at exposed sites from Alaska to Baja California. Adult *E. menziesii* sporophytes typically consist of ~25 fronds emerging from a single holdfast. In September 2010, 38 *E. menziesii* individuals with at least 20 fronds were tagged (non-destructively by attaching
a foam label through holdfast haptera with a cable tie) at Botanical Beach (48°31’33.46N, 124°26’50.22W), an exposed rocky promontory located near Port Renfrew, British Columbia, Canada. Each month, the number of fronds was recorded as well as whether or not the holdfast had become dislodged. Because spatial variation in hydrodynamic forces could affect survivorship, this study was performed within a small area (~150 m²) along a homogeneous, flat intertidal bench. Maximum wave velocities were characterized to determine if there were spatial trends in maximum wave forces by randomly placing 10 spring scale dynamometers (Carrington Bell & Denny, 1994) throughout the study site. The relative positions of all plants and dynamometers were measured to the nearest cm to test for spatial autocorrelation.

5.3.2 Sample collection and processing

In October 2010, five randomly selected fronds from each of the 38 tagged individuals were harvested and transported in seawater from the collection site to Friday Harbor Laboratories for biomechanical analyses. Upon arrival, fronds were placed in flow-through seawater tables until tested. All specimens were tested within 48 hours of collection. To ensure that all tissues tested were roughly the same age, the distal most 11 cm of each rachis, just below the terminal lamina, (growth in this species occurs at the rachis apices) was used in mechanical tests. Tissues were then cut into standardized working sections to control localized strain while reducing the likelihood of biases from clamp-induced damage (Chapter 2 Mach, 2009). Sample width and length were held constant by the standardized working section, but
Figure 5.1: *Egregia menziesii*. Starting at left, then clockwise- close-up photo of a holdfast firmly attached to rock (photo courtesy of Tess Grainger), a mature individual (thinned for photographic purposes) splayed out on a dock to show size (scale = 50cm), and a close up of fronds exposed at low tide showing frond structure: blades and pneumatocysts (air bladders) are born on both sides of a central frond axis (rachis).
thickness of each sample was measured to the nearest 0.1mm using digital calipers before tensile tests. Working sections were then secured, via pneumatic clamps at 90 psi, to a tensometer machine (5565, Instron, Massachusetts, USA). The Instron strained samples at a rate of 10mm·min\(^{-1}\) and recorded the resisting force (N) every second until tissue failure. Tensile tests were performed in air on wet tissue. Any tissue samples which failed near the attachment clamps were discarded from the analysis.

5.3.3 Calculation of mechanical properties

Strain was calculated as the change in length over the initial length (Engineer’s strain). Tissue stress was calculated as force (N) per initial cross-sectional area (m\(^2\)) and reported in MPa. Tensile stiffness (Modulus of elasticity, E) was calculated as the slope of stress vs. strain curves. Because the nature of such curves for kelp tissues is r-shaped, stiffness was calculated from the initial linear phase. Breaking stress and breaking stain were taken as the last values before failure occurred. Whole frond breaking force was determined by multiplying breaking stress of a test section by the cross-sectional area of the rachis from which it was taken. Bending (flexural) stiffness was calculated as the product of initial tensile stiffness (E) and the second moment of area (I), which was estimated for rachis tissue using the equation for a beam, I = \(\frac{1}{12}\)·width·thickness\(^3\) (Vogel, 2003). Holdfast attachment area of individuals was calculated with the equation for area of a circle using the diameter of the holdfast, which was measured to the nearest cm.
5.3.4 Statistical analyses

Because none of the mechanical properties met the assumption of homogeneity of variances for ANOVA, Welch’s ANOVA (Welch 1951) was used to test for repeatability in mechanical properties among individuals. For individuals that did survive the winter, linear regression analysis was used to determine if mean frond breaking force (N) significantly influenced self-pruning (proportion of fronds retained). Binary logistic regression was used to determine if mean mechanical properties and holdfast attachment area were associated with whole plant survivorship. Reverse stepwise (log likelihood ratio) multiple logistic regression was then used to assess the relative importance of holdfast attachment area and mechanical properties in predicting survivorship. Spatial autocorrelation of maximum wave velocity and whole plant survivorship was tested using SAM (Spatial Analysis in Macroecology) v4.0. Other statistical analyses were implemented in SYSTAT 13 and were considered significant with $\alpha = 0.05$ and $p < 0.05$.

5.4 Results

Significant differences among individuals were detected in all tissue mechanical properties except breaking strain (Table 5.1). Linear regression analysis revealed that proportion of initial fronds retained throughout the winter was significantly ($n = 25$, $R^2 = 0.218$, $p = 0.033$) positively related to mean rachis breaking force (Fig. 5.2).

Binary logistic regression revealed that holdfast attachment area, mean frond breaking force, and mean frond flexural stiffness were significantly as-
sociated with whole plant survival (Fig. 5.3). We failed to detect significant associations with other tissue mechanical properties and survivorship (Table 5.1). Pearson’s correlation analysis failed to detect relationships between holdfast attachment area and frond breaking force \( (p > 0.4) \) but did find a positive correlation between flexural stiffness and mean frond breaking force \( (n = 38, r = 0.353, p = 0.027) \). Because flexural stiffness and frond breaking force were correlated, only frond breaking force, holdfast attachment area, and their interaction were included in the overall model predicting survivorship; frond breaking force was chosen over flexural stiffness because of its higher \( R^2 \) value (Table 5.1).

The full model including frond breaking force, holdfast attachment area, and their interaction was significant \( (p < 0.001) \) and explained 60% of the variability in whole plant survivorship. However, removal of the holdfast attachment area term did not significantly change the model’s predictive power, resulting in a reduced model containing only mean frond breaking force \( (p = 0.008) \) and the interaction between mean frond breaking force and holdfast attachment area \( (p = 0.018) \). This reduced model was significant \( (p < 0.001) \) and also explained 60% of the variability in whole plant survivorship. The nature of the significant interaction was such that individuals with larger holdfasts could survive even with unrealistically high frond breaking forces, while individuals with smaller holdfasts survive only with weaker fronds (i.e. more susceptible to self-pruning) (Fig. 5.4). Further removal of either term from the reduced model resulted in significantly diminished predictive power \( (p < 0.001) \). We were unable to detect spatial trends in either maximum wave velocities or whole plant survivorship (Fig.
at any of the spatial scales analyzed (p ≫ 0.05).
Table 5.1: Summary of frond mechanical properties and their effects on whole plant survivorship. ANOVA values represent results from Welch’s ANOVA with plant ID as the independent factor. Logistic regression values are resultant from binary logistic regression analyses on each factor individually.

<table>
<thead>
<tr>
<th>Mechanical property</th>
<th>Range</th>
<th>r</th>
<th>df</th>
<th>F-ratio</th>
<th>p-value</th>
<th>R²</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Breaking strain</td>
<td>12.3 - 26.9</td>
<td>0.18</td>
<td>37, 44.1</td>
<td>1.416</td>
<td>0.134</td>
<td>0.002</td>
<td>0.827</td>
</tr>
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<td>Breaking stress (MPa)</td>
<td>1.4 - 3.9</td>
<td>0.48</td>
<td>37, 43.9</td>
<td>2.904</td>
<td>&lt;0.001</td>
<td>0.022</td>
<td>0.429</td>
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<td>Tensile stiffness (MPa)</td>
<td>4.1 - 24.6</td>
<td>0.39</td>
<td>37, 41.0</td>
<td>3.069</td>
<td>&lt;0.001</td>
<td>0.075</td>
<td>0.14</td>
</tr>
<tr>
<td>Flexural stiffness ($\mu$N*m²)</td>
<td>8.0-94.4</td>
<td>0.59</td>
<td>37, 40.7</td>
<td>2.78</td>
<td>&lt;0.001</td>
<td>0.177</td>
<td>0.022</td>
</tr>
<tr>
<td>Frond breaking force (N)</td>
<td>9.1 - 46.2</td>
<td>0.39</td>
<td>37, 42.7</td>
<td>56.36</td>
<td>&lt;0.001</td>
<td>0.212</td>
<td>0.011</td>
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<td>Holdfast attachment area (cm²)</td>
<td>19.6 - 188.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.384</td>
<td>&lt;0.001</td>
</tr>
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</table>
Figure 5.2: *Egregia* self-pruning. Proportion of initial fronds retained through the winter as a function of an individuals’ mean frond (rachis) breaking force (N) and fitted linear model. Individuals with stronger fronds kept a greater proportion of their fronds (pruned less).
Figure 5.3: Effects of mechanical traits on survival. Survivorship (1 = Survival, 0 = Dislodgement) as a function of A) holdfast attachment area, B) an individuals’ mean frond flexural stiffness, and C) an individuals’ mean frond (or rachis) breaking force (N) and fitted logistic models.
5.5 Discussion

Understanding how the traits of organisms are honed to meet the challenges of their environment is a perennial goal in organismal biology. Within the wave-swept intertidal, organisms must endure tremendous forces at regular intervals for the duration of their existence, and intuitively, these forces have imposed strong selection pressures on the form and function of these organisms. Although there is considerable interest in characterizing the performance consequences of mechanical properties in marine macrophytes (e.g., drag, flexibility), remarkably few studies have tested the effects of mechanical properties on more direct fitness metrics (e.g., survival, fecundity). In the present study, we document an association between individual *E. menziesii*’s material properties and their persistence. However, the relationship was not entirely intuitive. Rather than increased strength conferring a survival advantage, it was diminished strength that was positively associated with individuals’ survival probability. Our data suggest this advantage is conferred in the form of self-pruning, where weaker individuals lose more fronds, which reduces their drag, and diminishes their probability of being completely dislodged [Blanchette, 1997]. However, the importance of frond mechanical properties in predicting whole plant survivorship was diminished in individuals with larger holdfasts (Fig. 5.4). Our data represent some of the first evidence of variation in tissue mechanical properties among individuals within the same site explaining differences in survivorship of marine macrophytes.

Dislodgement has significant consequences for individuals’ fitness. In
Figure 5.4: Holdfast size and frond strength jointly determine survivorship. Empty circles represent individuals that were dislodged, while filled circles represent individuals that perenniated. The dashed line (discriminant function) represents the axis along which survivorship is differentiated; individuals in the gray area (below the line) are likely to survive, while individuals above this line are at high risk of dislodgement.
Figure 5.5: Spatial autocorrelation of wave velocity (black bars) and survivorship (gray bars). Reported values are Moran’s I values and were not significant ($p \gg 0.05$) for both variables at all distance classes.
perennial species such as *E. menziesii*, persistence confers a fitness advantage by increasing future reproductive opportunities. In contrast, individuals that are dislodged from the substrate are likely to quickly wash ashore (although, under certain conditions individuals may be able to remain in the water drifting for an unknown amount of time). Once ashore, individuals desiccate quickly and subsequently die. Thus, dislodgement has a tremendous impact on individuals’ survival, future reproductive success, and therefore, fitness.

Given the marked survival differences among individuals possessing different material properties, we ask- why does intraspecific variation in tissue mechanical properties persist within the population? Or alternatively, why do not all individuals possess weaker fronds? If survival is the key determinant of individuals’ fitness in this system, theory predicts that strong selection should rapidly eliminate inferior trait variants in mechanical properties. However, survival is not the only performance component in marine macrophytes. In fact, the advantage gained by weaker individuals in the form of self-pruning must inevitably come at a cost of reduced productivity. Or, in other words, individuals with a high propensity to self-prune must suffer reduced biomass and productivity as portions of the organism are torn off over time. Thus, we propose trait variation is maintained by a broadly applicable life history trade-off: individuals may either invest in their persistence (via self-pruning) at the cost of reduced fecundity, or adopt a high risk boom-and-bust strategy, where individuals enjoy high reproductive success but absorb the cost of reduced survivorship. It follows that the benefits of either strategy would vary spatially and temporally; variation among sites
and among years in maximum wave velocity could conceivably mediate this trade-off and maintain variation in mechanical properties within *E. menziesii* populations.

Another mechanism that might generate variation in tissue mechanical properties is condition-dependence. In the present study, the extent to which whole plant survivorship was dependent upon frond mechanical traits was dictated by the size of the individual’s holdfast (Fig. 5.4). The significance of this interaction term suggests a complex selection surface, which may be partially responsible for maintaining variation in frond mechanical traits. For instance, individuals with larger holdfasts may be able to sustain the costs of more (and therefore stronger) fronds. Larger holdfasts could arise from genetic underpinnings or could also result from desirable local environmental factors that enhance productivity and allow greater energy allocation to holdfasts.

Within population variation in mechanical traits could also arise through plasticity whereby individuals may adopt material properties based on the cues of their environment, or as a mere by-product of variation in resource acquisition. We attempted to minimize the potential for plasticity to have resulted in the observed trends by using a small, homogeneous area and testing for spatial trends in survivorship, mechanical traits, and maximum wave forces. However observed differences among individuals may still be due to either underlying genetic variation, environmental cues or some combination limiting our ability to make inferences about whether or not natural selection is occurring on material properties in this algae. A at present, we lack heritability estimates of material properties in *E. menziesii*, or indeed,
in any marine macrophyte and, instead, present repeatability, which is often used as a crude proxy for heritability in organisms that cannot be bred in laboratories (e.g. highly exposed kelp species) [Boake, 1989; Falconer & Mackay, 1996; Husak, 2006; Pruitt & Troupe, 2010].

Our data reveal significant, repeatable variation in tissue mechanical properties among conspecifics at the same site and that such differences are correlated with differential performance. Interestingly, the only mechanical properties that influenced survivorship (frond breaking force and flexural stiffness) are both composite properties dependent upon both tissue material properties and the amount of tissue present. Martone (2007) highlighted that intertidal seaweeds can increase breaking force by strengthening their tissues or increasing amount of tissue. Because kelps (Laminariales), are so large and happen to be a particularly morphological variable group (e.g. Demes et al., 2009; Koehl et al., 2008), much of their mechanical performance may be related to their morphology [Johnson & Koehl, 1994; Koehl & Alberte, 1988]. This highlights that while the materials intertidal organisms’ tissues are made of is important to their performance (Chapter 2; Boller & Carrington, 2007), anatomical and morphological strategies may also be important components of mechanical performance (e.g. Chapter 2; Harley & Bertness, 1996).

In this study we documented an association between intraspecific variation in mechanical properties and survivorship in *E. menziesii*, where decreased breaking force was associated with greater survivorship for individuals (especially for those with smaller holdfasts). This finding reaffirms the (often assumed) notion that organisms’ struggle with the physical forces of
their environments can shape ecological and evolutionary processes. Documenting links between intraspecific variation in mechanical traits and survivorship is an important piece in the bridge between biomechanical and evolutionary/ecological research, because it directly links trait performance with variation in an individual’s fitness. We argue that studies that explicitly consider the effects of mechanical traits on fitness are a vital, but largely missing, component in mechanical models, and that biomechanical research could benefit from a more thorough integration with evolutionary ecology research.
Chapter 6

Conclusion

The interaction between organisms and their non-living environment is a key component of ecological and evolutionary processes and has long inspired biologists. Often this interaction is conceptualized as how variation in abiotic factors such as nutrients, temperature, and light influence an individual’s growth, reproduction, and survival. However, in addition to dealing with the physiological constraints applied through these abiotic factors, organisms must also be able to withstand and function within the physical forces of their environment (Denny 1988, Vogel 2003). In this thesis, I investigated how the mechanical traits of seaweeds living in the wave-swept intertidal zone influences their hydrodynamic performance and survivorship. I found that material properties are heavily dependent upon anatomical characteristics and are responsive to anatomical changes associated with growth and development within an individual’s lifespan, among individuals within a species, and among species.

In chapter 2, I used 16 species of foliose red macroalgae (Rhodophyta) to test the effects of material properties on hydrodynamic performance. By using morphologically similar species and then controlling for differences in shape and size among specimens, I was able to directly assess the influ-
ence of tissue mechanical traits on drag in flowing water and the anatomical sources of variability among species in tissue strength and flexibility. Increasing cross-sectional thickness which arises via medullary, rather than cortical, tissue thickening results in increased strength and decreased flexibility. When size and shape were held constant, species with more flexible tissues experienced reduced drag due to their increased capacity for reconfiguration in flow.

In chapter 3, I examined the impact of reproductive investment on mechanical traits and hydrodynamic performance of the winged kelp, *Alaria marginata*. I showed that the thickening of blade tissue and increase in size associated with spore production in kelps has the potential to increase drag by reducing the reconfiguration potential of blades and increasing surface area exposed to flowing water. In *A. marginata*, however, the mechanical costs of increased size and flexural stiffness were mitigated by other morphological and mechanical shifts associated with reproductive onset: (1) Decreased blade ruffliness in reproductive blades decreased blade flapping and therefore drag, and (2) Increased breaking strength of reproductive tissues allowed them to safely experience more than the observed increases in drag without breaking. This work demonstrated that reproduction in seaweeds may have mechanical costs (in addition to the commonly discussed metabolic costs) but that undesirable shifts in morphological and material traits can be compensated by concomitant shifts in other mechanical traits.

In chapter 4, I tested the effects of aging on the material properties of 25 species of seaweeds’ tissues. Aging affected all species similarly: older tissue was stiffer, exhibited increased breaking strength, and was less extensible.
than younger tissue. However, differences among species in growth form resulted in the shared effects of aging having contrasting implications for red algae, kelps, and green algae. Because basal tissue attaches seaweeds to their substrate, it has to bear the greatest hydrodynamic loading. Basal tissue is the oldest tissue in red algae (which have apical growth), the youngest tissue in kelps (basal growth), and is a combination of new and old tissue in green algae (diffuse growth). This finding may explain how kelps dominate waveswept shores despite the reported increased susceptibility of their tissues to fatigue failure [Hale, 2001].

In chapter 5 I measured variation in tissue mechanical traits among conspecifics at the same site to address two previously unexplored hypotheses: (1) Individuals within a species possess significant variation in their tissue mechanical traits, even in the same hydrodynamic regime, and (2) Variation in mechanical traits is correlated with differential survivorship. I found significant variation in nearly all tissue mechanical properties among individuals, and also that mechanical traits explain 60% of the variation in survivorship of the feather boa kelp, _Egregia menziesii_. Individuals with weaker fronds were more likely to survive the winter as they self-pruned in smaller waves, thereby reducing drag on the holdfast in the largest swell events. However, the dependence of survival on frond material properties was reduced in individuals with larger holdfasts, presumably because of their ability to support the drag associated with non-pruned plants.

I will use the rest of this chapter to discuss some of the issues associated with the methods I used and how and whether they may have affected the results and conclusions presented in the previous chapters. Most of this
discussion will focus on specific issues arising from measuring and calculating material properties from biological tissues. I will then suggest research that could be done to improve upon these methods. Finally, in the last part of this chapter, I will propose future work on seaweed biomechanics that aims to integrate mechanical ideas more thoroughly into ecological and evolutionary frameworks.

6.1 Limitations and future suggestions

Multicellular organisms are inherently complex. Their tissues are composite materials made up of numerous different cells and compounds combined to promote countless biotic processes, in addition to structural support/functional performance. Looking at biological tissues strictly through the lens of an engineer building a structure to withstand the forces of their environment is not appropriate. Biological tissues perform differently than the rigid, synthetic materials used in most engineering projects because of their complex anatomical design, and it is worth taking time to consider some of the challenges inherent in measuring the material properties of seaweeds, and indeed all living tissues.

6.1.1 Strain rate

In all of the experiments above, and throughout the seaweed biomechanics literature, material properties are determined using tensile test set-ups which strain seaweeds on the order of $10 \text{ mm-minute}^{-1}$. In non-rigid tissues, such as those that comprise seaweed thalli, the rate at which the tissue is strained
may have a large impact on the determination of the material properties. Considering the strain-rate dependence of viscoelastic materials, researchers should strive to mimic realistic strain rates when measuring the material properties of biological tissues.

In situ tissue strain rates of seaweed tissues in the marine intertidal remain unknown, limiting our ability to replicate natural strain rates. Nonetheless, we can make some conjectures about the validity of using 10 mm-minute\(^{-1}\) to test the tensile properties of seaweeds living along wave-swept shores. For subtidal and intertidal species living in areas dominated by currents, constant unidirectional tensile loading may simulate the natural hydrodynamic loading imposed by slow-moving, unidirectional tidal currents, although we are still unable to compare the magnitude of strain rates in this environment to our testing strain rates. In intertidal zones exposed to high waves however, it seems safe to assume that 10 mm-minute\(^{-1}\) of unidirectional tensile loading does not even begin to approximate natural strains imposed by the chaotic, fast and furious nature of waves.

Given the strain-rate dependence of seaweed tissue material properties, it is important for comparative studies to standardize strain rate. In the experiments presented above, I chose a strain rate within the range of previous studies [Boller & Carrington 2007, Hale 2001, Harder et al. 2006] to enhance the comparability of my data. While the material property values I measured may be different from studies that use other strain rates, and so may not be representative of the actual performance of the tissues in situ, I feel confident that the values I obtained are still useful in the hypotheses tested. Although the estimated values of the material properties may dif-
fer from values obtained from realistically replicated strain rates, their rank order would likely be similar and the net result the same. For instance, in Chapters 2, 3, and 5, I found that variation among individuals or species in material properties was correlated with differences in hydrodynamic performance (i.e. drag, flapping, survivorship) in the direction expected by *a priori* hypotheses. Had the strain rate chosen affected the rank order of material properties, I ought not have been able to detect significant associations between material properties and hydrodynamic performance.

Lack of realistic replication of the forceful turbulence of crashing waves in a laboratory setting may be a caveat limiting the predictive ability of biomechanical experiments. Measuring material properties at a greatly reduced strain rate may still allow proof-of-concept relationship between material properties and hydrodynamic performance (Ch. 2, 3, and 5), but it likely impairs our ability to create models that accurately predict tissue failure and/or dislodgement of seaweeds in the field. Thorough studies are needed to first characterize the natural strain histories of seaweeds in the field so that we can begin to attempt replicating them in the laboratory.

### 6.1.2 Tensile stiffness

Young’s modulus of elasticity (E) is usually used as the measurement of tensile stiffness and is calculated as the slope of the line relating stress to strain from a tensile test ([Denny](#) 1988, [Vogel](#) 2003). The relationship between stress and strain in seaweed tissues displays an impressive diversity of forms, but only rarely approximates a linear relationship and usually displays a complex nonlinear curve. The stiffness value estimated from
tensile tests of seaweeds is therefore dependent upon at which strain the researcher draws the instantaneous slope measuring stiffness. Ideally, researchers should select the strain at which to measure tensile stiffness based on a strain selected a priori that is most appropriate for the hypothesis at hand. As highlighted above (section 6.1.1), we do not know the strain at which seaweeds are pulled to in situ and so do not know where along the curve stiffness should be calculated for any species, let alone whether and how that varies among species.

The convention that has been established in the literature is to draw a line through the initial linear portion of the curve, which usually has the steepest slope, (Boller & Carrington, 2007; Harder et al., 2006; Martone & Denny, 2008) to calculate stiffness. As with strain rate (section 6.1.1), this procedure is likely to strongly influence predictive models, since the value of tensile stiffness will vary as a function of position along the stress vs. strain curve, but is not likely to impact comparative studies. A preliminary attempt to estimate at which strain we should be measuring tensile stiffness may be to simulate many stiffness values per stress vs. strain curve and see which value best predicts drag in a flume. Future studies would also greatly benefit from measuring the actual strains that seaweed tissues experience in the field.

6.1.3 Flexural stiffness

Although tensile stiffness (the ability of a material to resist strain in tension) is considered to be an effective predictor of drag (Boller & Carrington, 2007), it is perhaps not as relevant to the drag-reducing reconfiguration pro-
cess of seaweeds as flexural stiffness, the resistance of an object to bending. The best way to achieve estimates of flexural stiffness is through deflection tests (e.g. Chapter 2), where the degree to which an object bends is linked to the force applied to it. Flexural stiffness can also be calculated as the product of tensile stiffness (E) and the second moment of area of the object (I) (Chapters 3 and 5). Calculating flexural stiffness (EI) from tensile and morphological data is convenient because it does not require a complicated deflection set-up, and is more precise than visual self-loading deflection tests (Chapter 2). However, there are two major drawbacks associated with calculated vs. empirically measured estimates of flexural stiffness: uncertainty in measurement of tensile stiffness, E (due to the aforementioned nonlinearity of stress vs. strain curves), and disparity of tensile and compressive stiffness in non-rigid biological tissues.

When an object bends downward, it experiences equal forces in tension on its upper surface and in compression on its lower surface. If a material has approximately the same compressive and tensile stiffness, these two forces will result in similar tensile and compressive strains. However, seaweeds have markedly lower compressive stiffness than tensile stiffness (Biedka et al., 1987; Gaylord & Denny, 1997). Accordingly, the same force in compression and tension results in greater displacement along the surface being compressed, resulting in larger bending deflection that predicted simply by tensile stiffness alone. In seaweeds, this phenomenon likely occurs via the complex construction of tissues using spherical and cuboid cells; during compressive loading, the cell walls can rearrange and collapse in such a way that increases compressive strain without incurring elevated com-
pressive stress. Although calculated EI values diverge from actual flexural stiffness values, they accurately predict interindividual variation in drag and survivorship (Ch. 3 and 5) and likely do not impact the rank order of values, and therefore the relationships detected. Decreased compressive strain relative to tensile strain is likely an important component of the mechanical design of seaweeds, allowing them to be disproportionately floppy (a drag-reducing advantage), but it complicates our ability to obtain accurate predictions of flexural stiffness values from calculations that consider only tensile stiffness. Further considerations for predicting flexural stiffness from tensile stiffness in biological materials are reviewed in Vogel (2003).

6.1.4 Phylogenetic context

In Chapters 2 and 4, I tested for correlations among species traits without exploring the potential for shared evolutionary history to have biased results. This lack of phylogenetic context weakens the interpretations of these results because of the potential for the observed links to have resulted as a by-product of other co-inherited, unmeasured traits. In Chapter 2, I tested for correlations of material and morphological traits with hydrodynamic performance and interpreted significant associations as causal relationships. The concern is that other traits (that I did not measure) might be co-inherited with the mechanical traits I did measure— if these unmeasured traits cause differences in hydrodynamic performance, I might have erroneously concluded that the traits I did measure affect hydrodynamic performance. Incorporating phylogenetic data into analyses testing for mechanical-functional links would help reduce likelihood of such erro-
neous conclusions because it takes into account inter-relatedness among species, thereby reducing the probability of correlations with other unmeasured traits.

The molecular data necessary for a formal phylogenetic comparative analysis is lacking for most of the species tested in Chapter 2. However, examining the range of mechanical traits within and among families can give us a quick first look at whether or not shared phylogenetic history might be responsible for the observed trends. Comparing information in Tables 2.1 and 2.2, one can see that within families that have multiple representative species (e.g., Delessariaceae, Gigartinaceae, Halymeniaceae, Rhodymeniaceae), the range of values for material properties is large given the total range among all species, and the species with most similar material properties are not closely related. This lack of association between material properties and taxonomic placement strengthens my confidence in the observed trends; nonetheless future incorporations of phylogenetic data into mechanical-functional studies of seaweeds would be a great improvement.

In Chapter 4, I compared the longitudinal patterns of material properties across blades of multiple species and compared these trends with the growth form of each species. However, because all species with apical growth were red algae (Rhodophyta) and all species with basal growth were kelps (Laminariales, Phaeophyceae), the study lacked phylogenetic replication entirely. However, this lack of phylogenetic replication arose as a consequence of true evolutionary history and not biased sampling: basal blade growth seems to have only evolved once in foliose algae among the ancestors of kelps. The sister clades to kelps (the Desmarestiales and Sporochnales) posses intercalary
meristems that resemble those of kelps but do not result in basal growth (Kogame & Kawai 1996). Species in these clades occur in similar regions, but occupy areas with markedly lower wave energy (i.e., subtidal zones). This further suggests that the evolutionary novelty of basal growth among kelps enabled their ability to invade wave-exposed habitats and contributed to their success in these environments.

6.2 Trajectory of seaweed biomechanics

As the breadth of biological research grows, we are learning that organisms and communities are structured by complex interactions and trade-offs regulated by mechanisms at physiological, mechanical, ecological, and evolutionary scales. Studies investigating only a single factor or level of biological organization have little predictive power, calling for an increase in the interdisciplinarity of biological research. In the marine realm, one of the largest and most exciting mergers of disciplines currently underway is the incorporation of mechanical and functional performance data into ecological and evolutionary theory. Biomechanists are beginning to view their work in a framework that explores the consequences of an organism’s mechanical traits to how it interacts with other species (Bouma et al. 2005; Liffen et al. 2013) in addition to the organism’s fitness and evolutionary trajectory (Chapter 5; Swanson et al. 2007). Conversely, ecologists and evolutionary biologists have begun to incorporate the physical interactions of organisms with their environment into their work. However, many fundamental ecological and evolutionary questions about the role of mechanical constraints
on life remain unresolved.

6.2.1 Ecological costs and consequences of mechanical properties

Organisms are faced with a limited resource pool from which they must support growth, reproduction, and survival, resulting in trade-offs where increased investment in one pathway comes at the cost of decreased investment in another. Because the relative investments of an organism to growth, reproduction, and survival strongly impacts its fitness, understanding the basis of life-history trade-offs has been identified as one of the fundamental goals of ecology (Sutherland et al., 2013). I know of no studies that have explicitly explored the extent to which mechanical traits that enhance survival pose a physiological cost. However, investigating mechanical traits in a framework that incorporates physiological costs and life history trade-offs will greatly improve our understanding of the mechanical design of seaweeds.

Flexibility reduces drag, but may also reduce productivity

Flexibility is a key component of the drag-reducing strategy of seaweeds in the intertidal (e.g. Harder et al., 2004; Koehl, 1984, 1986) because it enables seaweeds to reconfigure in flowing water into smaller sizes and more streamlined shapes (Chapter 5; Boller & Carrington, 2007). Having flexible tissues may however come with the cost of decreased light interception. As photosynthetic organisms, the productivity potential (i.e., the upper limit of the energy budget) of seaweeds is dependent upon how much surface area is exposed to sunlight. The drag reduction associated with tissue flexibility,
however, works by minimizing surface area perpendicular to flow, and consequently also reduces surface area perpendicular to sunlight (Chapter 2). The ability to fold up on top of itself may reduce the drag that a seaweed experiences, but it may also reduce light penetrance to photosynthetic tissues, thereby reducing productivity. Future work should attempt to quantify the productivity costs of reconfiguration and whether (and how) seaweeds have adapted maximizing reconfiguration perpendicular to flow while minimizing reconfiguration perpendicular to light.

**Costs of strength**

Potential physiological costs to increasing breaking strength are abundant and straightforward to conceptualize. The two most obvious mechanisms by which increasing strength may come at a cost to seaweeds are through strengthening or increasing the size/thickness of tissues. Strengthening of tissues occurs through increasing the percentage of cross-sectional area of structural components, such as cell walls (Martone, 2007). The paucity of data on this topic limits our ability to infer much about the production costs and material properties of structural cell constituents. However, we can assume that because structural cellular constituents do not contribute to energy production directly, they represent an immediate net cost to produce.

In Chapter 2 I provided evidence that increased tissue breaking strength occurs through blade thickening. However, the upper limit of blade thickness (and therefore breaking force) may be constrained by metabolic demands. Seaweeds rely on light penetration into their tissues for photosynthesis, but light penetration decreases exponentially with increasing tissue thickness.
(Beach et al., 2006), and so the thickness of the pigmented cortical layer (i.e., the photosynthetic tissue layer) is likely limited by light penetration into tissues. Continued thickening occurs via increasing medullary tissue. Since photosynthesis does not occur in the medullary region, medullary cells are metabolically dependent upon the cortical region. Therefore, increased breaking strength via increased tissue thickening will inevitably come at a metabolic cost as the ratio of medullary to cortical tissue increases.

The constraint on strength via metabolic limitations to tissue size applies not only to blade tissue, but also to whole-plant survival. The survival of a seaweed is dependent upon the adherence strength of its holdfast to the substrate. In Chapter 5, I showed an association between increasing holdfast size and increasing probability of surviving winter storm events in the feather boa kelp, *Egregia menziesii*. Having a larger holdfast may increase substrate attachment strength, but it comes at the same price as increasing blade thickness: light can penetrate but a few millimeters into the surface of the holdfast, but production and maintenance of the holdfast consumes metabolic energy. The larger the size of the holdfast, the larger its metabolic demand. Future studies incorporating the metabolic demands of non-photosynthetic medullary and holdfast tissue may help us understand the trade-offs associated with strengthening associated with increasing tissue size and will further our understanding of the limitations of seaweed mechanical traits.
**Are mechanical traits correlated with life history and/or habitat traits?**

Whether and how physiological costs of increased flexibility and strength play a role in shaping the life histories of seaweeds remains poorly understood. Metabolic budgeting approaches (as described above) would greatly enhance our understanding of such physiological costs, but will require the development of specialized techniques. A first approach would be to test for correlations among mechanical and life history traits. If underlying costs associated with producing desirable mechanical traits constrain growth and reproduction among seaweeds, we might expect such trade-offs to be associated with contemporary species having annual vs. perennial life histories and their inhabiting intertidal vs. subtidal environments. Annual species likely invest disproportionately more energy into reproduction than mechanical strength and reproduce before the onset of winter storm seasons, while perennial species, which must withstand winter swells, must invest in mechanical strength to a greater degree. Similarly, we expect intertidal species to be stronger and/or more flexible than subtidal relatives because the wave forces are an order of magnitude greater in intertidal zones (Denny, 1988). Testing for such relationships would be relatively simply, given the large amount of mechanical data that is readily available for many species. Doing so in a way that accounts for phylogenetic history (Maddison, 2000) would be an important contribution to the field.
6.2.2 Evolutionary potential of mechanical traits

We need to begin to consider morphological and material traits in an evolutionary context. Currently, evolutionary inferences are made from measuring hydrodynamic performance (usually in the lab) of individual species, and then correlating this with morphological and/or material traits; arguments are then made about the influence of mechanical traits on an individual’s fitness. However, the contribution of mechanical traits to fitness has not yet been determined in any seaweed species: no comparative studies testing the relationship between mechanical traits and hydrodynamic performance or habitat have assessed the possibility of their shared evolutionary history confounding the results, and, perhaps most importantly, we still do not know if mechanical traits are heritable.

Heritability

Before any evolutionary argument pertaining to seaweed morphological and/or material adaptations can be invoked, variation among individuals must first be heritable, that is, variation in the trait should be passed down from parent to offspring. In Chapter 5 I showed that variation in frond tissue material properties was consistent (i.e., repeatable) among individuals, and that such differences resulted in differential survivorship. With this data, it is easy to jump to the conclusion that material property trait variants incur differential fitness and are therefore under natural selection. However, adopting this conclusion requires accepting the assumption that the repeatability of tissue material properties in fronds within individuals arises from genetic under-
pinnings. It is also important to note that repeatable variation in traits can also result from non-genetic processes, such as irreversible developmental plasticity.

External cues from the environment can elicit phenotypic responses through plasticity. In the marine realm variation in hydrodynamic regime among sites is a well known factor responsible for causing genotypically similar individuals to display variable morphological traits (e.g., Armstrong [1987], Blanchette et al. [2002], Kitzes & Denny [2005]). It is possible that the variation among individuals in Chapter 5 was the result of variation in an abiotic factor, such as wave force. Indeed, kelps are notoriously plastic species (e.g., Demes et al. [2009], Koehl et al. [2008]), and biomechanical traits have been linked with the amount of force that their tissues experience in the environment (Koehl & Alberte [1988], Kraemer & Chapman [1991]). I attempted to minimize the potential for spatial patterns in wave forces to have influenced the results by testing for spatial autocorrelation in wave forces, material properties, and survivorship. Although no such spatial autocorrelation was found, an irreversible development trigger at the time of settlement of each individual could conceivably have resulted in some phenotypic variation among individuals (e.g., Etter [1988]).

Heritability studies are lacking for mechanical properties in seaweeds and could help shed light on whether such material traits are, in fact, regulated by genetic processes. The perennial nature of many kelp species and the extreme forces associated with their habitat make it impossible to estimate heritability in lab-rearing studies. However, future studies would benefit from attempts to measure heritability in the lab or field of shorter lived
(and potentially more easily manageable) species, and could provide further insight into the unsolved question of the extend to which natural selection shapes material properties of seaweed tissues in populations.

Complexity of life histories

We also need to adopt an evolutionary framework that encompasses the complexity of seaweed life histories. Seaweeds aggravate countless undergraduate biology students every year with their frustratingly complex life histories. Species can have monophasic, biphasic, or triphasic life histories, haplontic or diplontic principle phases, monoecious or dioecious thalli, and alternating phases that are isomorphic or heteromorphic. This diversity of life history strategies poses the question: How does variation in life history strategy influence the evolution of mechanical traits?

In the simplest scenario of a monoecious monophasic life history, one can easily imagine that with a selective pressure like wave action rapidly eliminating inferior mechanical traits from the population, only individuals with desirable mechanical trait variants (for that time and place) will survive to reproduce. These individuals may fertilize other surviving individuals (or themselves) and create a new cohort of individuals with the desirable mechanical traits, resulting in a shift over time in the proportion of alleles for certain mechanical traits in the population over time (i.e., evolution through natural selection). Adding further levels of complexity in life history increases the complexity of the evolution of such traits. Adding separate male and female thalli or multiple phases complicates matters because underlying physiological differences among sexes or phases can result
in differing material properties, even in otherwise isomorphic thalli (Car-
tridge et al., 2001; Mach, 2009): these differences in mechanical traits may
result in differential selective pressures among sexes and life history phases.
Nonetheless, imagining the evolution of mechanical properties is still rela-
tively straightforward, even with multiple sexes and/or isomorphic phases.

I am left wondering how selection shapes mechanical traits in seaweeds
with heteromorphic life histories. In Chapter 5, I showed an association
between mechanical trait variants and survivorship of kelp sporophytes; in-
dividuals with weaker, more flexible fronds were able to survive the winter
storms and presumably have greater reproductive opportunities than indi-
viduals that were dislodged during large swell events. However, surviving
individuals will not necessarily produce novel sporophytes with the bene-
ficial mechanical traits. Instead they will produce microscopic filamentous
gametophytes. The production of novel kelp sporophytes will then be de-
pendent upon the survival and reproductive success of the gametophytes.
Envisioning how desirable mechanical traits of the large kelp sporophyte
could increase the survivorship and/or fertilization success of a microscopic
filament is far from intuitive. Two pathways for selection on mechanical
traits in such heteromorphic species are apparent: (1) the reproductive ben-
efit enjoyed by surviving sporophytes is so high that the gametophyte bank
is simply flooded with genes that will create mechanically superior kelps
and/or (2) correlated selection has led to a coupling of traits that enhance
survivorship and reproduction in both life history phases. Studies explicitly
addressing fitness of multiple life history phases exploring both of the afore-
mentioned hypotheses do not exist for seaweeds and would serve as a strong
contribution to our understanding of how selection acts on mechanical traits in seaweeds.

6.2.3 Conclusion

Mechanical approaches can profoundly enhance our understanding of how organisms interact with their non-living environment. However, there is a striking distance between mechanical and biological researchers that has prevented a proper integration of the two fields. A major contribution of this thesis is the integrative was I linked biomechanical data with ecological and evolutionary theory. I hope this work will serve as a stepping-stone in the creation of a larger, more unified bridge between researchers in biomechanics and evolutionary ecology.
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