KELP IN HOT WATER: DIRECT AND INDIRECT EFFECTS OF WARMING SEAWATER TEMPERATURE ON KELP IN NOVA SCOTIA

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
May 2015

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Abstract

Recent declines and losses of highly productive and diverse kelp beds have been observed globally and linked to increases in ocean temperature. Warming seawater temperatures may impact kelp populations directly or indirectly, by altering the interactions of kelp with other species. I investigated the direct impacts of 4 temperature treatments (11, 14, 18 and 21 ˚C) on the dominant kelp species in Nova Scotia: *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum*. Exposure to 21 ˚C led to at least twice the tissue loss observed at 11 ˚C and mortality within the first 2 wk of exposure for all species. Temperature-induced damaged to the blade tissue was evident after 1-wk exposure to temperatures above 11 ˚C in *S. latissima* and *L. digitata*, and resulted in reduced tissue strength and extensibility. *Agarum clathratum* suffered little tissue damage and was less susceptible to temperature-induced tissue weakening and loss. *Saccharina latissima* increased in strength over summer, suggesting possible acclimation to changing temperatures. I also investigated the indirect impacts of warming temperatures on Nova Scotian kelps by examining the potential for temperature to modify their interactions with the gastropod mesograzer *Lacuna vincta* and the invasive bryozoan *Membranipora membranacea*. The nutritional quality (C/N) of kelps were unaffected by temperature, and chemical defenses (phlorotannins) were reduced only in *A. clathratum* after 1-wk exposure to 21 ˚C. In feeding experiments *L. vincta* consumed more *S. latissima* pretreated at 21 ˚C than that pretreated at 11 ˚C only when grazing rate was high. The quality of *S. latissima* as a food source for *L. vincta* was not affected by temperature, as diets of kelp pretreated at 11 and 21 ˚C supported similar growth, reproduction, and survival of snails. Temperature did not affect the quality of kelp as a substrate for *M. membranacea*, as settlement rates were not different between *S. latissima* pretreated at ambient temperature (9 – 14 ˚C) or 21 ˚C. The absence of temperature-induced changes in kelp quality suggests that the effects of *L. vincta* and *M. membranacea* will act additively with the direct effects of temperature to increase biomass loss from Nova Scotian kelp beds. This thesis suggests a mechanism by which rising temperatures could contribute to observed population declines of kelp species.

Lists of Abbreviations and Symbols Used

Abbreviation/Symbol	Definition
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
B	Estimated slope
C	Cortex
C	Carbon
C/N	Carbon-nitrogen ratio
CO_2	Carbon dioxide
D	Loss of differentiation among cell layers
DF	Degrees of freedom
DM	Damage to the meristoderm
dw	Dry weight
E	Effect size
Exp	Exposure time
F	F-value
H	Holes in tissue
HSD	Honestly Significant Difference
L:D	Light-dark ratio
M	Meristoderm
ME	Medulla
n	Sample size
N	Nitrogen
p	p-value
Phl	Phlorotannin
R^2	Coefficient of determination
RE	Relative effect size
SCUBA	Self Contained Underwater Breathing Apparatus
SD	Standard deviation of the mean
SE	Standard error of the mean

Abbreviation/Symbol	Definition
SM	Splitting of the medulla
t	t-value
T	Number of trials
Temp	Temperature
v_{11}	Value of variable in 11 ˚C treatment
v_{18}	Value of variable in 18 ˚C treatment

Acknowledgements

I would like to first acknowledge and thank my supervisors, Anna Metaxas and Robert Scheibling, for their support and guidance at every step of this thesis. Thank you Bob and Anna, I could not have accomplished this work without your help. Thank you as well to my committee members, Sandra Walde and Heike Lotze, for your suggestions and advice.

I would like to thank John Lindley, Karen Filbee-Dexter, Colette Feehan, John O'Brien, Fiona Francis, Danielle Denley, and Kevin Sorochan for their help with field work; and Michaela Harris, Delphine Durette Morin, Elizabeth Nagel, and John O'Brien for help in the laboratory. I would like to acknowledge Pat Colp for her expertise and assistance with histology. I would not have accomplished my chemical analyses without the support of Shannah Rastin and Jessica Miller, as well as Hugh MacIntyre and Marcus Kienast, who provided access to their labs and equipment.

Thank you to both the Metaxas and Scheibling labs (Myriam, Kevin, Danielle, Swaantje, Andrea, Colette, Karen, and John), as well as the larger Oceanography and Biology departments. I was blessed to have had the resources, support, and friendship from two labs and two departments. To my family and friends, thank you for all of your love, support and encouragement.

My research was funded by a NSERC CGS-M and a fellowship from the Dalhousie Faculty of Graduate Studies, as well as NSERC Discovery grants to Anna Metaxas and Robert Scheibling.

CHAPTER 1

Introduction

In marine ecosystems, the observed effects of climate change on communities are broad ranging. Range shifts and local extirpation, changes in community structure and declines in ecosystem services such as productivity (Harley et al. 2006, Müller et al. 2009, Smale et al. 2013) result from impacts on individual species and on the ecological interactions among species. Changes to biotic interactions may enhance or mitigate the direct effects of climate change (Harley et al. 2006, Zarnetske et al. 2012, HilleRisLambers et al. 2013). Biotic interactions are expected to change when interacting species are lost or gained, due to changes in phenology or range, whereas changes in interaction strength are expected when interacting species undergo behavioural or physiological changes (Zarnetske et al. 2012, HilleRisLambers et al. 2013, Vergés et al. 2014). Habitat-forming species, such as kelp, are predicted to have an increasingly important role in maintaining ecosystem function with changing climate, as they can mitigate the effects of stresses on their associated communities (Bruno et al. 2003, Halpern et al. 2007, Wernberg et al. 2010).

Kelp species (large brown algae of the order Laminariales) form important biogenic habitat in the rocky subtidal zone of coastal temperate waters (Steneck et al. 2002), supporting highly diverse and productive communities that include many species of commercial importance (Wharton & Mann 1981, Dayton 1985, Steneck et al. 2002). The global distribution of kelp is determined primarily by seawater temperature (van den

Hoek 1982, Lüning 1984, Breeman 1988), with climate-driven ocean warming being linked to range contractions and declines in kelp populations worldwide (Anderson et al. 2011, Fernández 2011, Tuya et al. 2012, Wernberg et al. 2013). These declines are associated with shifts towards communities dominated by ephemeral turf algae (Connell & Russell 2010, Anderson et al. 2011, Moy & Christie 2012, Wernberg et al. 2013), a transition considered to be a degradation of the ecosystem state (Christie et al. 2009, Wernberg et al. 2013). In Nova Scotia, declines in kelp populations and increased dominance of turf algae have been observed in sheltered embayments, with changes being correlated to warming seawater temperature (K. Filbee-Dexter & R.E. Scheibling, unpublished manuscript). Changes in temperature may contribute to the observed declines in kelp populations by impacting kelp directly, or indirectly by modifying the interactions between kelp and other species.

Direct impacts of increasing seawater temperature on kelp populations occur via changes to physiological processes such as photosynthesis and respiration (Bolton & Lüning 1982, Gerard & Du Bois 1988, Andersen et al. 2013). Kelp species generally exhibit temperature-dependent growth, with reduced growth at temperatures above optimum due to energy limitation imposed by increases in respiration rate (Staerh & Wernberg 2009), and decreased photosynthetic efficiency above the temperature optimum for photosynthesis (Davison 1991). At the cellular level, thermal stress induces the formation of reactive oxygen species that damage cell membranes (Wang et al. 2009, Zhou et al. 2009, Cruces et al. 2012) that may weaken kelp tissue, leading to greater tissue loss from erosion and breakage. Reductions in growth rate will hinder the ability of kelp to recover from a loss of tissue (Röthausler et al. 2009, Wernberg et al. 2010), and a

shift in the balance between growth and loss of tissue will impact kelp survival. However, little is known about the effects of warming seawater temperature on the material properties of kelp tissue, such as strength and extensibility.

Indirect temperature-induced impacts on kelp populations can also occur if changing temperature alters interspecific interactions, such as those between kelp and herbivores or epibionts (Harley et al. 2012, Vergés et al. 2014). Herbivores can regulate the populations of their algal food sources (Lubchenko & Gaines 1981) and the kelp-herbivore interaction could be altered by changes in consumption rates of herbivores (O'Connor 2009) or the palatability of kelp (Harley et al. 2012) caused by warming temperature. Changes in the palatability of kelp may result from temperature-induced changes in either the nutritional content or the chemical or mechanical defenses of kelp (Staehr & Wernberg, 2009, Weinberger et al. 2011). Increased temperature can prevent the induction of phlorotannins (Cruces et al. 2012, Cruces et al. 2013), which act as a chemical defense against herbivory (Steinburg 1984, Targett & Arnold 1998) and can deter settlement of epibionts (Wilkström & Pavia 2004). Temperature-induced changes in phlorotannin content could then affect the success of herbivores and epibionts, altering their impacts on kelp populations.

Although warming temperatures have been frequently correlated to declines in kelp populations, the mechanisms by which temperature is contributing to the observed declines are unknown, both in Nova Scotia and in kelp systems worldwide. In this thesis, I experimentally test the direct and indirect effects of temperature on kelp to examine the mechanistic link between warming temperatures and declining kelp populations. In Chapter 2, I examine the effect of temperature on the growth, tissue loss, material

properties and tissue structure of the kelps *Agarum clathratum*, *Laminaria digitata* and *Saccharina latissima*. Through these comparisons, I assess how increasing temperature modifies the balance between growth and loss of kelp tissue and the survival of these kelps. In Chapter 3, I examine the effect of temperature on the quality of kelp tissue as a food for the gastropod mesograzer *Lacuna vincta*, and as a settlement substrate for the encrusting bryozoan *Membranipora membranacea*, both species that damage kelp tissue and increase erosion and breakage, causing extensive biomass loss from Nova Scotian kelp beds (Johnson & Mann 1986, Duggins et al. 2001, Saunders & Metaxas 2008, Scheibling & Gagnon 2009, Krumhansl & Scheibling 2011, Krumhansl et al. 2011). I assess the potential for changes in kelp tissue quality that alter the interaction of kelp with these species, leading to indirect temperature-induced loss of kelp biomass. Chapters 2 and 3 are intended as standalone manuscripts for publication in the primary literature, and as such contain some repetition of information, particularly in the methods. Lastly, in Chapter 4, I summarize the findings of Chapters 2 and 3, provide recommendations for future research, and provide context for the relevance of my findings to understanding observed declines in kelp populations.

CHAPTER 2

Warming seawater temperature induces weakening and loss of kelp tissue

2.1 Abstract

Recent declines and losses of highly productive and diverse kelp beds have been observed worldwide and linked to increases in ocean temperature. We investigated the impacts of 4 temperature treatments (11, 14, 18 and 21 ˚C) on growth, net length change and mortality of the dominant kelp species in Nova Scotia, *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum*. Growth rates of *A. clathratum* were reduced at 18 ˚C over 3 wk of exposure, and all species experienced negative net changes in length at this temperature. Exposure to 21 ˚C led to tissue loss at least twice that observed at 11 ˚C and mortality within the first 2 wk of exposure. 1-wk exposure to 21 ˚C reduced blade tissue strength (breaking stress) and extensibility (breaking strain) by 40 – 70% in *S. latissima* and *L. digitata*, and all 3 species exhibited reduced strength after 3-wk exposure to 18 ˚C. Histological examination of the blade tissue showed temperature-induced damage to the cellular structure of blades of *S. latissima* and *L. digitata*. *Agarum clathratum* displayed limited tissue damage and was less susceptible to temperature-induced tissue weakening and loss. Breaking stress and strain of *S. latissima* collected biweekly in summer 2013 and 2014 indicated an increase in strength over summer, suggesting that acclimation of material properties to changing temperatures may be possible. Our findings provide a mechanism by which rising temperatures could contribute to observed population declines of kelp species.

2.2 Introduction

Climate change is having significant and diverse impacts on the structure and function of marine ecosystems worldwide. Climate impacts on habitat-forming species are of special concern, as changes in populations of these species can have drastic effects on their associated communities (Jones et al. 1994). Environmental stresses imposed by climate change are predicted to increase the importance of habitat-forming species in maintaining ecosystem function, as they can mitigate the effect of stresses on the community (Bruno et al. 2003, Halpern et al. 2007, Wernberg et al. 2010).

In coastal temperate waters, the shallow rocky subtidal zone is often dominated by kelp species (large brown algae of the order Laminariales) that form structurally complex beds or forests (Steneck et al. 2002). These beds and forests form some of the ocean's largest biogenic habitats and support high levels of productivity and species diversity, including many commercially important species (Wharton & Mann 1981, Dayton 1985, Steneck et al. 2002). The predicted effects of climate change on kelp habitats are wide ranging and include range shifts and local extirpation, changes in community structure and species interactions, and declines in ecosystem services such as productivity, nutrient cycling, and coastal defense (Müller et al. 2009, Harley et al. 2012, Smale et al. 2013).

The global distribution of kelp is primarily determined by water temperature (van den Hoek 1982, Lüning 1984, Breeman 1988), and pole-ward range shifts are predicted for many kelp species as a result of climate-driven ocean warming (Müller et al. 2009). Increasing sea surface temperature has also been implicated in population declines of perennial algae such as kelp, and shifts towards communities dominated by ephemeral

turf algae (Connell & Russell 2010, Andersen et al. 2011, Moy & Christie 2012, Wernberg et al. 2013). These turf communities represent a degradation of the habitat, and are associated with changes in community structure (Wernberg et al. 2013) and reductions in density and diversity of associated invertebrate species (Christie et al. 2009). A recent extreme warming event in southern Australia resulted in the range contraction of one habitat-forming alga, and shifts in the composition of associated algal and fish assemblages (Smale & Wernberg 2013, Wernberg et al. 2013). Gradual warming has been implicated in population declines and range contractions of kelp species in the eastern Atlantic, including populations in Norway (Moy & Christie 2012, Andersen et al. 2013), Spain (Fernández 2011) and Portugal (Tuya et al. 2012).

Increasing temperature can impact kelp populations by various, potentially interacting mechanisms. Reduced growth due to physiological changes to photosynthesis is a direct effect of warming temperature (Bolton & Lüning 1982, Gerard & Du Bois 1988, Davison 1991, Andersen et al. 2013). Kelp species generally exhibit temperature-dependent growth, with a thermal optimum above and below which growth rate is reduced (Fortes & Lüning 1980, Bolton & Lüning 1982, Gerard & Du Bois 1988, Davison 1991). As temperature rises, the rate of respiration can increase more rapidly than that of photosynthesis, resulting in a reduction in energy available for growth (Staerh & Wernberg 2009). Growth is further reduced at temperatures above the photosynthetic optimum due to decreased photosynthetic efficiency (Davison, 1991). These reductions in individual growth rate reduce the ability of both individuals and populations to recover from a loss of biomass (Rosthäusler et al. 2009, Wernberg et al. 2010).

Kelp tissue is continuously lost through the erosion of the distal end of the blade, and through breakage during large wave events (Filbee-Dexter & Scheibling 2012, Krumhansl & Scheibling 2012). Rates of erosion and breakage depend on the material properties of the kelp tissue, such as strength and extensibility. Predictions of breakage based on the measured breaking strengths of undamaged algae often underestimate fracture rates observed the environment (Friedland & Denny 1995, Utter & Denny 1996, Kitzes & Denny 2005, Mach et al. 2011). High rates of blade breakage in the field are attributed to reductions in strength due to physical or physiological damage (Krumhansl et al. 2011, de Bettignies et al. 2013). Temperature stress induces the formation of reactive oxygen species that cause lipid peroxidation and damage to cell membranes (Wang et al. 2009, Zhou et al. 2010, Cruces et al. 2012), in turn weakening tissues and leading to tissue loss. Although the effect of temperature on the material properties of kelp tissue is largely unknown, statistical models provide evidence that temperature directly affects blade erosion rate in *Saccharina latissima* (Krumhansl & Scheibling 2011a, Krumhansl et al. 2014). The balance between tissue loss and growth will determine survival of kelp.

Although there has been no evidence of reduction in the ranges of kelp species in the western Atlantic in the past 100 years (Merzouk & Johnson 2011), declines in kelp populations and increasing dominance of turf algae have been observed at some sites along the Atlantic coast of Nova Scotia (K. Filbee-Dexter & R.E. Scheibling, unpublished manuscript). The coastal Northwest Atlantic, including Nova Scotia, is an area of active warming, with observed increases in sea-surface temperature of 0.8 – 1.6˚C over the past 3 decades (Baumann & Doherty, 2013) and continued warming by an

additional 4˚C predicted by 2100 (Müller et al. 2009).

We examined the effects of temperature on the 3 dominant kelp species *Agarum clathratum, Laminaria digitata* and *Saccharina latissima* in Nova Scotia. In the laboratory, we subjected kelp to temperature treatments to determine the effect on growth, tissue loss and survival. We used histological techniques to examine temperature-induced changes to the tissue structure. We also measured the material properties of the kelp tissue including breaking stress (tensile strength) and maximum strain (extensibility of the tissue) after exposure to the temperature treatments in the laboratory and with seasonal changes in temperature in the field. We predict that increasing temperature will both reduce kelp growth and damage kelp tissue, compromising both the tissue strength and extensibility, and resulting in greater tissue loss at higher temperatures.

2.3 Materials and Methods

2.3.1 Experimental design

Mature individuals of *Saccharina latissima* (blade length: 1 – 1.5 m), and *Laminaria digitata* and *Agarum clathratum* (0.5 – 1 m) were collected using SCUBA in June/July 2013 at 12 m depth from Splitnose Point (44˚28'38.45" N, 63˚32'48.21" W), 20 km south of Halifax, Nova Scotia. Kelp were transported in coolers to the laboratory, where they were fixed by their holdfasts to plastic racks using elastic bands and suspended in a 3000-L circular tank (1.87 m diameter, 1.08 m height) with continuously flowing ambient seawater (flow rate: 430 Lh^{-1}). Within 24 h of collection, 9 replicate

individuals of each of the 3 species were transferred and hung similarly in each of 4 additional experimental tanks of similar size and flow rate for a total of 27 individual kelps per tank. Each experimental tank was maintained at one of 4 temperature treatments: 11, 14, 18 or 21 ˚C. These levels represent a growth optimum for *S. latissima* and *L. digitata* (11 ˚) (Bolton & Lüning 1982), a commonly experienced summer temperature (14 ˚) (Scheibling et al. 2013), a likely maximum average temperature experienced over 1 – 2 weeks (18 ˚) (Scheibling et al. 2013), and an anticipated maximum temperature based on climate change predictions (21 ˚) (Müller et al. 2009). 3 trials of the experiment were conducted in June and July 2013. To assess potential tank effects, a single trial was conducted in June 2014 with all 4 tanks at 11.5 ˚C. No differences were detected among tanks, except in 1 case out of 12 (growth of *L. digitata* in week 2, Appendix A).

Mean temperature (± 1 SD) in each treatment tank, recorded with data loggers (Maxim Integrated Thermochron iButtons) throughout the experiment, was 11.6 ± 0.9, 14.4 ± 0.8, 18.2 ± 1.6, and 20.8 ± 1.1 ˚C, respectively. Light was provided at a 12 L: 12 D cycle by overhead fluorescent fixtures. Light levels in the tanks at the attachment point of the kelp, approximately 30 cm from the water surface, were 9.3 ± 0.2 μmol m^{-2} s^{-1} (mean ± SE, n=88). Average summer daylight light levels at 8 m within a Nova Scotia kelp bed were measured as 50 – 75 μmol m^{-2} s^{-1} (Gagné et al. 1982).

2.3.2 Growth, tissue loss and mortality

To quantify growth, each individual was labeled and holes were punched 10 cm from the blade-stipe interface before placement in the temperature treatments. 3

individuals of each species from each temperature treatment were sampled without replacement weekly for 3 wk. The distance of the punched hole from the blade-stipe interface was re-measured upon sampling and growth rate (mm d^{-1}) was calculated as the change in the position of the hole punch (mm) divided by the length of exposure to the temperature treatments (d). Tissue loss was determined by measuring the blade length of each individual before exposure to the temperature treatments and upon removal from the treatments, and then calculating the change in length (cm). If kelp in a given temperature treatment showed signs of massive tissue loss and imminent mortality, it was sampled prior to the set sampling interval and deemed not to have survived to the next interval. As trials of the experiment overlapped, the total number of individuals per tank varied between 18 and 36. Light levels remained constant when density was reduced from 27 to 18 individuals per tank (ANOVA, $F_{1,3} = 4.04$, $p = 0.14$).

2.3.3 Mechanical properties

Mechanical properties of kelp tissue were measured using pull-to-break tensile tests with a digital force meter (Pasco PS-2104; range: ± 50 N, resolution: 0.03 N). Initial mechanical properties before temperature treatment were assessed for 3 individuals of each species within 24 h of collection. Effects of temperature on mechanical properties were examined after 1, 2 and 3 wk of exposure to temperature treatments. Upon removal of an individual from a temperature treatment, a 3 x 10 cm tissue sample was excised in a longitudinal direction from the blade centre, using a “dog bone”-shaped template, 25 cm (*L. digitata* and *A. clathratum*) or 40 cm (*S. latissima*) from the blade-stipe interface (Fig. 2.1). *Saccharina latissima* has a greater growth rate than the other 2 species (Bolton &

Lüning 1982); therefore samples were excised further from the blade-stipe interface in *S. latissima* to insure they were taken from tissue that had not grown during the experiment. Tissue samples were excised at a constant distance from the blade-stipe interface to control for decreasing strength with increasing distance from the meristem (Krumhansl et al. 2011). The thickness of each sample was measured at 3 points along its length using digital calipers (resolution 0.01 mm). Samples were held between clamps lined with neoprene and fine sandpaper, attached at either end to the force meter and a water receptacle. The force meter was fixed in place while a constant force rate (mean ± SD: 8.80 ± 0.18 N min^{-1}) was being applied to the sample by the continuous delivery of water to the receptacle, controlled by a hose-clamp valve (Fig. 2.1). Force was recorded at 0.04-s intervals. The kelp tissue was marked by painting 2 white dots ~10 mm apart on the sample using solvent-based paint. Extension of the tissue between these 2 marks was recorded using a video camera (GoPro Hero 2) at 25 frames s^{-1}. Initial distance between dots and distance at breakage, as well as width of the sample, were obtained from video using image analysis software (Image J, National Institute of Health). Breaking stress (MPa) was calculated as applied force (N) at breaking divided by cross-sectional area (mm^2) of the sample. Maximum strain was calculated as the difference between initial length and length at breaking, divided by initial length.

To track changes in mechanical properties in the field as seawater temperature increases over summer, 10 – 15 individuals of *Saccharina latissima* (1.0 – 1.5 m blade length) were collected bi-weekly from 8 m depth at Splitnose Point, from 18 June to 10 September 2013 and from 3 June to 26 September 2014. Kelp were transported to the laboratory where tissue samples were immediately excised and mechanical properties

tested as above. Temperature at the collection site was continuously monitored over sampling period using data loggers (Onset HOBO Pendant) anchored to the seabed.

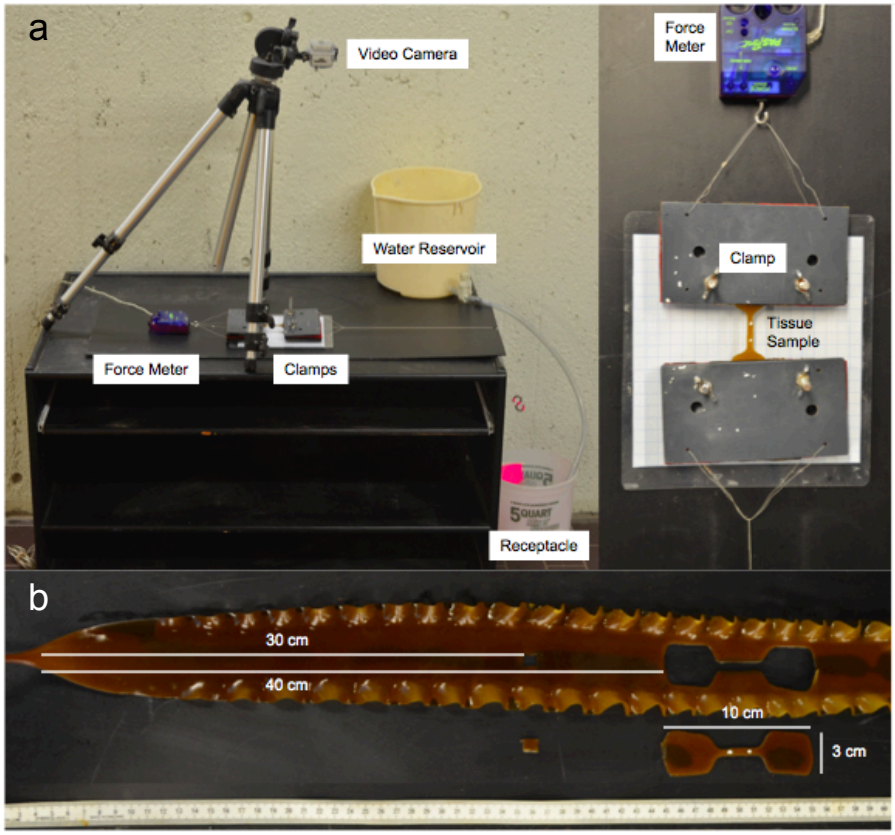

Figure 2.1 (a) Equipment used to measure breaking strength and maximum strain of kelp tissue samples. See text for full description. (b) Location and size of tissue samples used for histology and mechanical properties testing on *Saccharina latissima*.

2.3.4 Histology

Histological techniques were used to examine effects of temperature on the cellular structure of the kelp blade tissue. Tissue samples (~ 1 cm^2) from the same 3 individuals of each species in each temperature treatment as used for the determination of mechanical properties were taken 15 cm (*Laminaria digitata* and *Agarum clathratum*) or

30 cm (*Saccharina latissima*) from the blade-stipe interface at each sampling interval, in 1 trial of the experiment (Fig. 2.1). Samples were also taken from 3 individuals of each species upon collection, before exposure to the laboratory temperature treatments. Samples were fixed in 7% formalin in seawater for 24 h, washed twice in 70% ethanol and stored in 70% ethanol for up to 1 month before further processing. An ASP300 Leica Tissue Processor was used to dehydrate and clear samples and then infiltrate them with paraffin. Embedded samples were cut into 5 µm-transverse sections using a semi-automatic Leica microtome (RM 2255) and mounted on positively charged slides (Fisherbrand). Slides were oven dried for 24 h at 37 ˚C, de-paraffinized using xylene and rehydrated with graded alcohols, then stained in 0.1% Saffranin for 6 min. Stained slides were then dehydrated in graded alcohols and mounted to cover slips with Cytoseal (Richard-Allan Scientific). Blade tissue sections were examined at 100x magnification using a light microscope (Nikon Eclipse E600) and photographed with a Nikon Coolpix 955 digital camera.

2.3.5 Statistical analyses

As kelp did not survive more than 1-wk exposures to 21 ˚C, we examined the effect of temperature (11, 14, 18 and 21 ˚C; fixed factor) and trial (random factor) on growth rate, length change, breaking stress and maximum strain separately for the first week using analysis of covariance (ANCOVA) with initial blade length as a covariate, for each of the 3 kelp species. Because there was no effect of initial blade length and no interaction of initial blade length with temperature for any response variables or species, data were reanalyzed using 2-way ANOVA. If there was no significant interaction of trial

x temperature treatment ($p > 0.2$), data from the 3 trials were pooled and analyzed with a -way ANOVA. Significant interactions between temperature treatment and trial were found for growth rate of *Saccharina latissima* and breaking strain of *Laminaria digitata* ($F_{6, 30} = 4.86$, $p = 0.001$ and $F_{6, 30} = 3.94$, $p = 0.005$, respectively). Examination of individual trials for these response variables showed differences in magnitude but not direction of effects; therefore, trials were also pooled for these variables and species and effects of temperature analyzed using 1-way ANOVA. Effects of temperature after the first week (11, 14 and 18 ˚C; fixed factor), exposure time (1, 2 or 3 wk; fixed factor) and trial (random factor) on the growth rate, length change, and breaking stress and strain for each kelp species were examined using 3-way ANOVA. Data from the 3 trials were pooled when the trial x temperature treatment x exposure time interaction was non-significant ($p > 0.2$), and analyzed using 2-way ANOVA.

Data were transformed when necessary to meet the assumption of homogeneity of variance (Levene's test, $p > 0.05$) as follows: $\log (x + 2)$ for length change for *Saccharina latissima*, $\log (x + 1.5)$ for length change for *Agarum clathratum*, and square root $(x + 0.001)$ for growth rate for *A. clathratum*. Pair wise comparisons between temperature treatments and exposure times were conducted using Tukey's HSD tests. ANOVAs were performed using the car package (Fox & Weisberg 2011) in R (R Core Team 2013).

For each species, relative effect size of each variable at each exposure time was calculated as follows. Effect size (E) for each trial was calculated as the difference in means between the 11 and 18 ˚C treatments according to the equation:

$$E_{Trial} = \frac{\sum_{i=1}^{n} v_{11_i}}{n} - \frac{\sum_{i=1}^{n} v_{18_i}}{n} \quad (1)$$

where v_{11} is the value of the variable in the 11 ˚C treatment, v_{18} is the value of the variable in the 18 ˚C treatment, and n is the number of replicates in the trial. The mean effect size was then calculated using the equation:

$$E_{Mean} = \frac{\sum_{i=1}^{T} E_{Trial_i}}{T} \quad (2)$$

where T is the number of trials. Standard deviation of effect size was calculated using the equation:

$$E_{StdDev} = \sqrt{\frac{\sum_{i=1}^{T} \left(E_{Trial_i} - E_{Mean}\right)^2}{T-1}} \quad (3)$$

A relative effect size (RE) for each trial was then calculated by standardizing by the mean and standard deviation of effect size, according to the equation:

$$RE_{Trial} = \frac{E_{Trial} - E_{Mean}}{E_{StdDev}} \quad (4)$$

Lastly, the mean relative effect size was calculated as:

$$RE_{Mean} = \frac{\sum_{i=1}^{T} RE_{Trial_i}}{T} \quad (5)$$

Mean relative effect sizes were calculated for 1- and 2-wk exposures, and the values for the 3-wk exposure reflect the relative effect size in the single trial where individuals survived the full 3-wk at 18 ˚C.

Changes in material properties of *Saccharina latissima* over the summer were examined using simple linear regression of the ordinal date of collection, or the average temperature in the 2 weeks preceding collection, on the tissue's breaking stress and

maximum strain. Examination of the model residuals revealed no violations of the model assumptions.

2.4 Results

2.4.1 Growth, tissue loss and mortality

None of the 3 kelp species survived to the 2-wk sampling interval in the 21 ˚C treatment; *Laminaria digitata* was the most strongly affected and did not even survive the first week of exposure (Fig. 2.2). Kelp also suffered mortality in the 18 ˚C treatment, each species surviving the full 3-wk exposure in only 1 trial (Fig. 2.2). There was no mortality observed in the 11 or 14 ˚C treatments (Fig. 2.2).

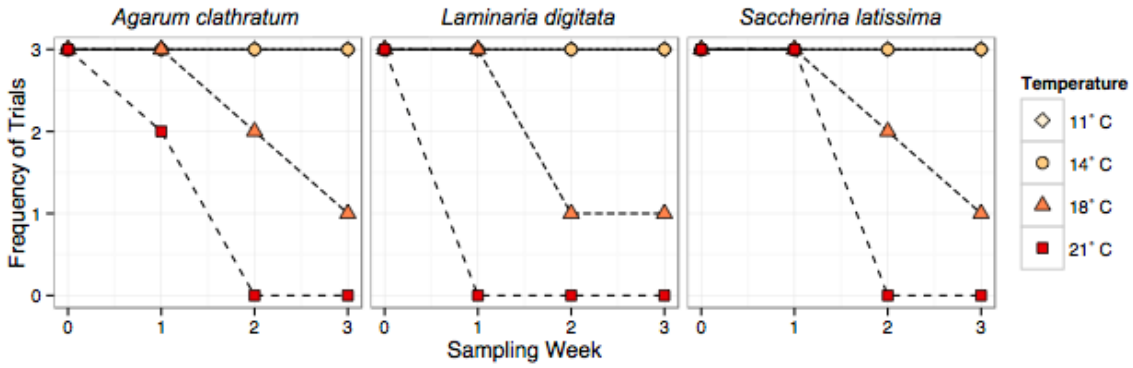

Figure 2.2 Frequency of trials with surviving individuals of *A. clathratum, L. digitata*, and *S. latissima* after 1-, 2- or 3-wk exposure to 11, 14, 18 and 21 ˚C temperature treatments (n = 3 trials).

Growth of all 3 kelp species was observed at all temperatures (Fig. 2.3). There was no effect of temperature on growth rate after the first week of exposure (Table 2.1), but within each sampling week *Agarum clathratum* and *S. latissima* exhibited a trend of

decreased growth rate at temperatures above 11 ˚C (Fig. 2.3). Growth rate of *A. clathratum* was significantly reduced in both the 14 and 18 ˚C treatments compared to the 11 ˚C treatment (Table 2.2).

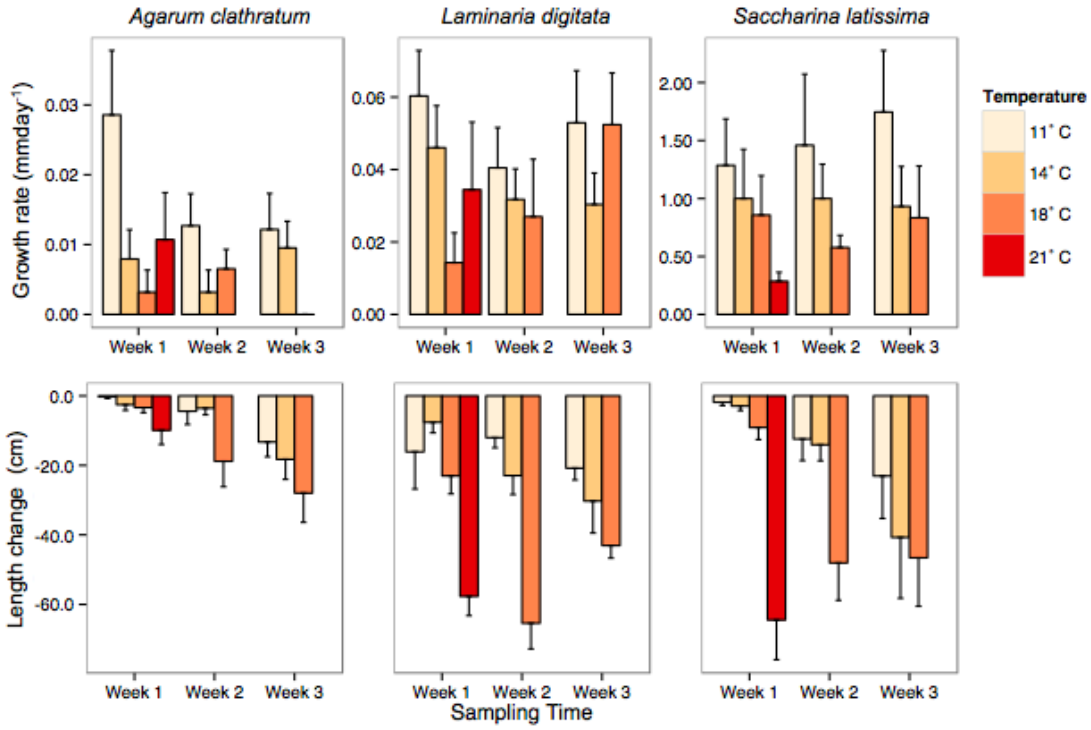

Figure 2.3 Mean (± 1 SE) growth rate or net length change of *A. clathratum*, *L. digitata*, and *S. latissima* individuals after 1-, 2- or 3-wk exposure to 11, 14, 18 and 21 ˚C temperature treatments (data from 3 trials pooled, n = 3 – 9).

Table 2.1 Results of ANOVA to examine differences in growth rate or length change among temperature treatments (11, 14, 18 and 21 ˚C) after 1-wk exposure for each kelp species. Significant results given in bold.

Species	Variable	DF	F	p	Tukey's HSD
A. clathratum	Growth rate	3, 39	2.29	0.09	
	Length change	3, 43	1.63	0.20	
L. digitata	Growth rate	3, 33	1.42	0.25	
	Length change	3, 51	13.04	**<0.001**	11=14=18<21
S. latissima	Growth rate	3, 33	1.67	0.19	
	Length change	3, 38	19.11	**<0.001**	11=14=18<21

Table 2.2 Results of ANOVA comparing growth rate or length change among temperature treatments (11, 14, 18 ˚C) and exposure times (1-, 2- and 3-wk exposure) for each kelp species. Significant results given in bold.

Species	Variable		DF	F	p	Tukey's HSD
A. clathratum	Growth rate	Temperature	2, 65	5.40	**0.007**	11>14=18
		Exposure	2, 65	0.61	0.55	
		Temp x Exp	4, 65	1.35	0.26	
	Length change	Temperature	2, 70	3.17	**0.05**	11<18
		Exposure	2, 70	8.55	**<0.001**	W1=W2<W3
		Temp x Exp	2, 70	0.75	0.56	
L. digitata	Growth rate	Temperature	2, 55	1.96	0.15	
		Exposure	2, 55	0.44	0.65	
		Temp x Exp	4, 55	0.96	0.44	
	Length change	Temperature	2, 70	10.99	**<0.001**	W2:
		Exposure	2, 70	6.40	**0.002**	11=14<18
		Temp x Exp	4, 70	3.79	**0.008**	18: W1<W2
S. latissima	Growth rate	Temperature	2, 61	2.23	0.12	
		Exposure	2, 61	0.08	0.92	
		Temp x Exp	4, 61	0.21	0.93	
	Length change	Temperature	2, 64	6.26	**0.003**	11=14<18
		Exposure	2, 64	17.53	**<0.001**	W1<W2=W3
		Temp x Exp	2, 64	0.09	0.99	

Despite positive growth rates, the negative length changes observed for each species indicate a net tissue loss in all temperature treatments, although tissue loss was minimal after 1-wk exposure at 11 or 14 ˚C, particularly for *Agarum clathratum* and *Saccharina latissima* (Fig. 2.3). Kelps exposed to 21 ˚C for 1 wk lost at least twice as much tissue as those exposed to 11 ˚C (Fig. 2.3). Loss of tissue by *Laminaria digitata* and *S. latissima* was significantly greater in kelps exposed to 21 ˚C compared to all other temperature treatments (Table 2.1). Across the 3-wk exposure period tissue loss increased with increasing temperature for all species (Fig. 2.3), and tissue loss was significantly greater for kelp exposed to 18 compared to 11 ˚C in both *A. clathratum* and *S. latissima* (Table 2.2). Tissue loss of *L. digitata* was significantly greater at 18 ˚C than the other 2

temperatures only after 2-wk exposure (Table 2.2). Length of exposure also increased tissue loss in *A. clathratum* and *S. latissima* independently of temperature (Fig. 2.3, Table 2.2). As the length of exposure increased, the relative magnitude of the effect of the 18 ˚C treatment (compared to the 11 ˚C treatment) on both growth rate and tissue loss increased in *S. latissima*, where as in *A. clathratum* and *L. digitata* the strongest effects were observed after 1- or 2-wk exposures (Fig. 2.4). The range of relative effects of the 18 ˚C treatment was greater for length loss than for growth rate in both *L. digitata* and *S. latissima*, but in *A. clathratum* the range of relative effect of temperature for length was less than that for growth (Fig. 2.4).

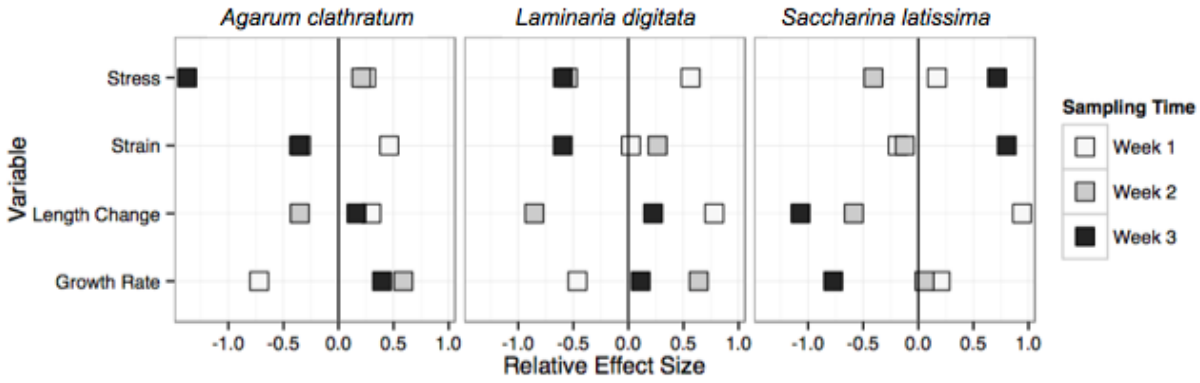

Figure 2.4 Mean relative effect size of 18 ˚C treatment (compared to 11 ˚C treatment) on breaking stress, breaking strain, net length change, or growth rate of *S. latisima*, *L. digitata*, and *A. clathratum* (n = 1 – 3 trials). Zero line indicates the mean effect of each variable for that species. See text for a description of effect size calculations.

2.4.2 Material Properties

The stress and strain at breaking of *Laminaria digitata* and *Saccharina latissima* blade tissue were reduced significantly (by 60 – 70% and 40 – 70%, respectively) after 1-wk exposure to 21 ˚C, when compared to 1-wk exposure to 11 ˚C (Fig. 2.5, Table 2.3). In

contrast, there was no effect of temperature on breaking stress or strain of *Agarum clathratum* after 1 wk (Fig. 2.5, Table 2.3). Over the 3-wk period, the breaking stress of *A. clathratum* and *L. digitata* exposed to 18 ˚C was significantly reduced compared to both the 11 and 14 ˚C treatments, but breaking strain was not affected (Table 2.4). Breaking stress and strain of *S. latissima* tissue were reduced at 14 and 18 ˚C, compared to 11 ˚C, across the 3-wks of exposure (Table 2.4). The relative effect of the 18 ˚C treatment on breaking stress and strain was similar within *S. latissima* and *L. digitata* (Fig. 2.4). Breaking stress was the variable most affected by temperature in *A. clathratum* (Fig. 2.4).

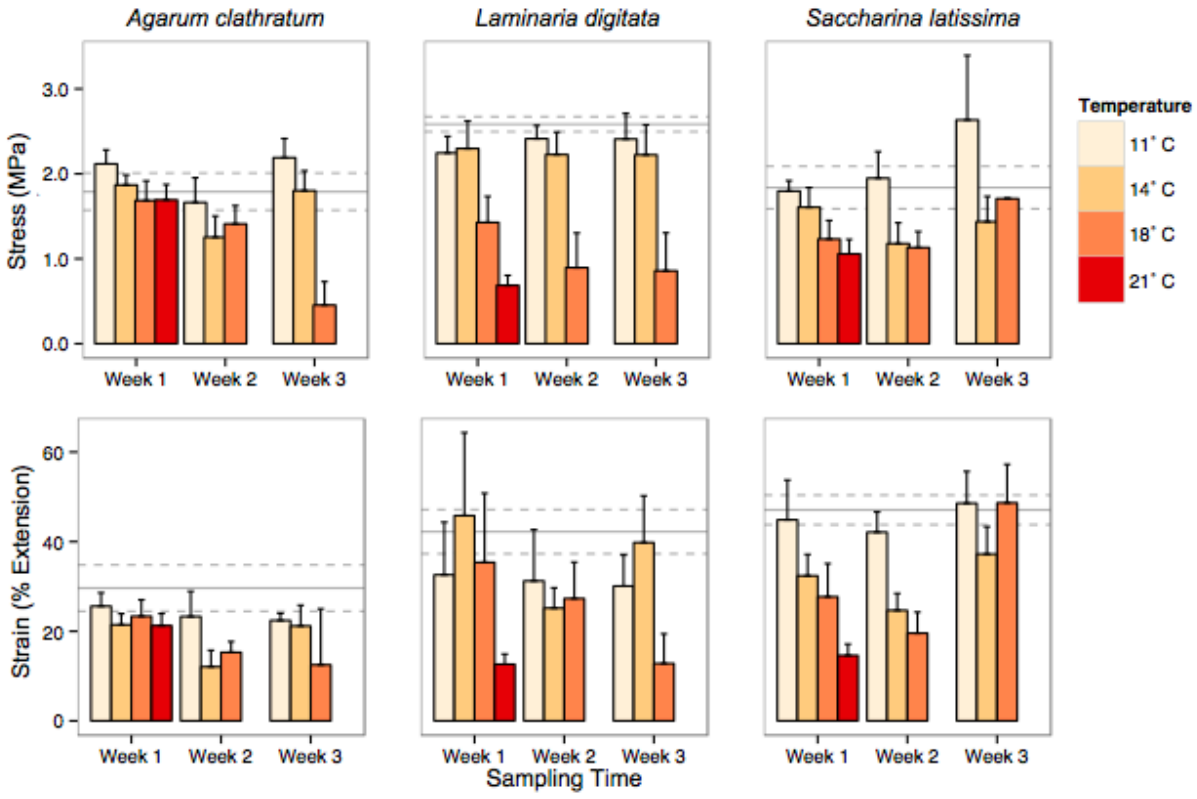

Figure 2.5 Mean (± 1 SE) stress or strain at breaking of *A. clathratum*, *L. digitata*, and *S. latissima* tissue samples taken after 1-, 2- or 3-wk exposure to 11, 14, 18 and 21 ˚C temperature treatments (data from 3 trials pooled, n = 3 – 9). Solid and dashed lines represent mean ± 1 SE stress or strain at breaking of initial tissue samples taken upon collection (n = 3).

Table 2.3 Results of ANOVA to examine differences among temperature treatments (11, 14, 18 and 21 ˚C) after 1-wk exposure for breaking stress or breaking strain of each kelp species. Significant results given in bold.

Species	Variable	DF	F	p	Tukey's HSD
A. clathratum	Stress	3, 39	0.92	0.44	
	Strain	3, 40	0.41	0.75	
L. digitata	Stress	3, 38	15.28	**<0.001**	11=14>21
	Strain	3, 39	5.53	**0.003**	11=14>21
S. latissima	Stress	3, 33	3.32	**0.03**	11>21
	Strain	3, 33	4.14	**0.01**	11>21

Table 2.4 Results of ANOVA comparing breaking stress or breaking strain among temperature treatments (11, 14, 18 ˚C) and exposure times (1-, 2- and 3-wk exposure) for each kelp species. Significant results given in bold.

Species	Variable		DF	F	p	Tukey's HSD
A. clathratum	Stress	Temperature	2, 62	6.35	**0.003**	11=14>18
		Exposure	2, 62	4.57	**0.01**	W1>W2
		Temp x Exp	4, 62	1.89	0.12	
	Strain	Temperature	2, 62	2.34	0.10	
		Exposure	2, 62	2.67	0.08	
		Temp x Exp	4, 62	0.74	0.57	
L. digitata	Stress	Temperature	2, 60	12.86	**<0.001**	11=14>18
		Exposure	2, 60	0.25	0.78	
		Temp x Exp	2, 60	0.45	0.77	
	Strain	Temperature	2, 62	2.22	0.12	
		Exposure	2, 62	0.60	0.55	
		Temp x Exp	4, 62	0.45	0.78	
S. latissima	Stress	Temperature	2, 61	5.31	**0.007**	11>14=18
		Exposure	2, 61	1.45	0.24	
		Temp x Exp	4, 61	0.73	0.57	
	Strain	Temperature	2, 58	3.61	**0.03**	11>14=18
		Exposure	2, 58	2.98	0.06	
		Temp x Exp	4, 58	0.43	0.78	

Within the 14 ˚C treatment, a consistent pattern emerged in breaking stress or strain across all 3 species. Breaking stress and strain decreased from the first to the second sampling interval, followed by a partial or full recovery in week 3 (Fig. 2.5). This

pattern was repeated in the relative effect sizes of the 18 ˚C treatment on both breaking stress and strain of *Saccharina latissima,* with the greatest effects seen in week 2 followed by recovery in week 3 (Fig. 2.4). Both *Agarum clathratum* and *Laminaria digitata* exhibited the greatest effect of the 18 ˚C temperature treatment after 3-wk exposure (Fig. 2.4). In *A. clathratum*, breaking stress was significantly reduced in week 2 across all temperature treatments (Table 2.4). Throughout the experiment, mean breaking stress and strain of kelp in the 11 ˚C treatment were similar to those measured in initial samples taken upon collection (Fig. 2.5).

Temperature at Splitnose Point in 2013 remained below 12 ˚C for most of the sampling period (Fig. 2.6). Maximum temperature was 17.6 ˚C and temperatures above 14 ˚C persisted for only 10 d in early September. Summer 2014 was warmer, with a maximum temperature of 19.2 ˚C and 2 periods when temperature exceeded 14 ˚C for at least 10 d. Breaking stress of kelp collected at Splitnose Point increased slightly over the summer in both 2013 and 2014. There was a positive relationship between breaking stress and ordinal date in 2013, although the effect was small and explained little of the observed variance in breaking stress, and a stronger relationship with both ordinal date and mean temperature in the 2-wk period preceding sampling in 2014 (Table 2.5). Maximum strain decreased with ordinal date in 2013, and increased with mean temperature in the preceding 2-wk period in 2014 (Table 2.5).

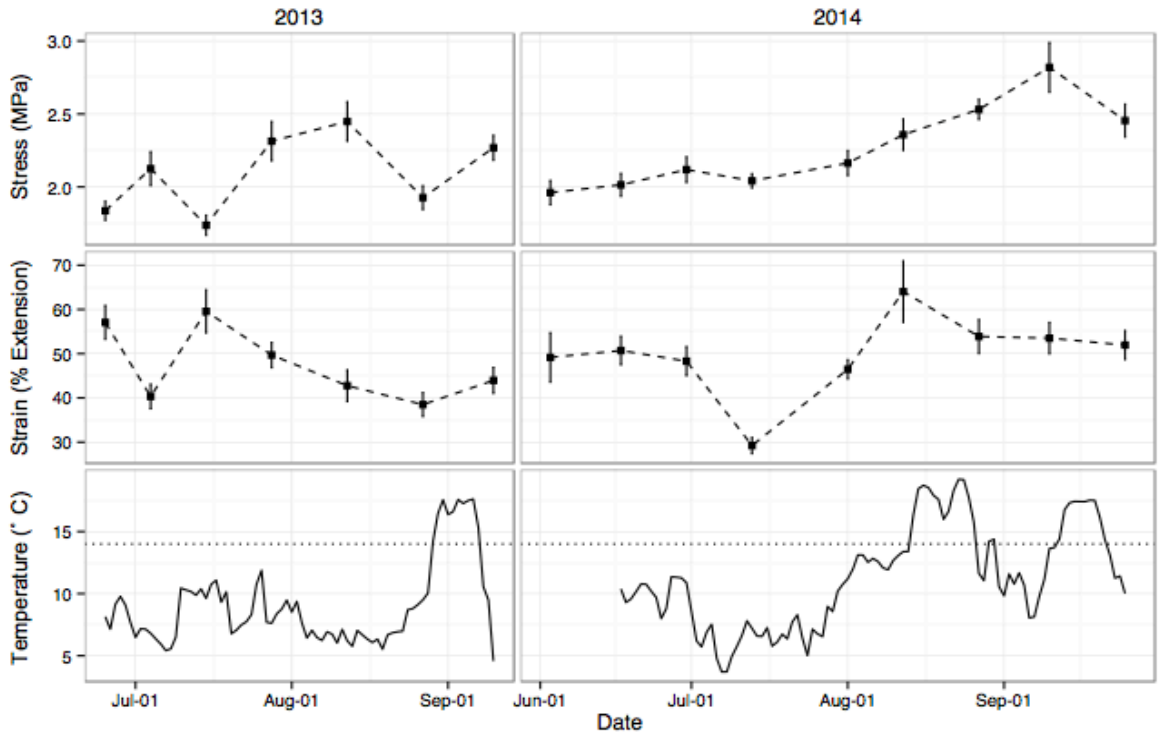

Figure 2.6 Mean (± 1 SE) breaking stress or breaking strain of *Saccharina latissima* collected bi-weekly in summer 2013 and 2014 from 8 m depth at Splitnose Point (n = 10 – 15), and mean daily temperature at the collection site. Dotted horizontal line is 14˚ C.

Table 2.5 Estimates of relationships between breaking stress or strain of *Saccharina latissima* collected biweekly from Splitnose Point and the ordinal date and mean temperature in the 2-wk period preceding a sampling day. Significant results given in bold.

Year	Response	Predictor	B	p	R^2
2013	Stress	Date	0.004	**0.01**	0.07
		Mean Temp	0.03	0.08	0.04
	Strain	Date	-0.16	**0.003**	0.09
		Mean Temp	0.06	0.93	<0.01
2014	Stress	Date	0.006	**<0.001**	0.34
		Mean Temp	0.04	**<0.001**	0.15
	Strain	Date	0.08	0.07	0.04
		Mean Temp	1.8	**<0.001**	0.18

2.4.3 Histology

The tissue of *Agarum clathratum, Laminaria digitata* and *Saccharina latissima* is divided into 3 distinct cell layers (Fig. 2.7). The outer meristoderm consists of several layers of small epidermal cells that gradually increase in size towards the underlying cortex. The cortex is a layer of large round cells surrounding a central medulla of densely packed elongate cells. The tissue structure of all species after 1-wk at 11 ˚C was similar to that of individuals sampled upon collection (not shown), indicating that this temperature treatment did not affect tissue structure. Changes appear in the tissues of both *L. digitata* and *S. latissima* in the higher temperature treatments. After 1 wk at 14 ˚C, these species exhibit some degradation of the cell layers, including the formation of holes in the medulla and a thinning of the meristoderm (Fig. 2.7). *Laminaria digitata* and *S. latissima* exposed to 18 ˚C show further degradation and splitting of the medulla, as well as a loss in the differentiation between cell layers in some areas (Fig. 2.7). The greatest damage was observed in the 21 ˚C treatment, where both *L. digitata* and *S. latissima* displayed deformation and breaks in the meristoderm, a loss of both differentiation and connectivity among cell layers, and large holes throughout the tissue (Fig. 2.7). In contrast, *A. clathratum* exhibits few effects of temperature on the tissue structure: blade tissue shows some degradation and splitting of the cortex only at 21 ˚C (Fig. 2.7). The structure of *A. clathratum* midrib tissue was unaffected by the temperature treatments (not shown).

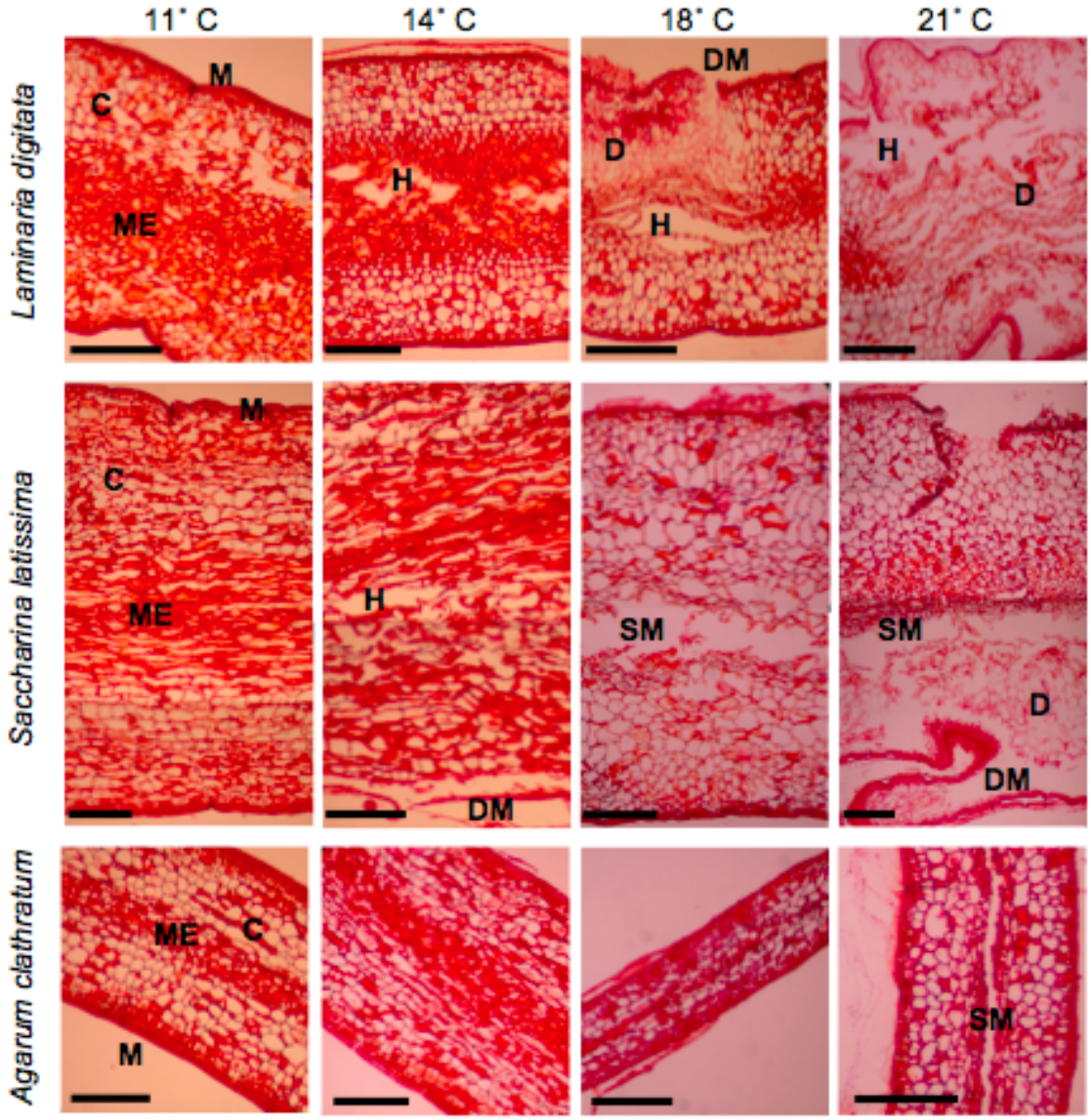

Figure 2.7 Transverse sections of *L. digitata*, *S. latissima* and *A. clathratum* blade tissue after 1-wk exposure to 4 temperature treatments (11, 14, 18 and 21˚ C). Scale bars are 200 µm. Tissue is composed of 3 layers: the outer meristoderm (**M**), the cortex (**C**) and the central medulla (**ME**). Observed damage to the tissue structure includes holes (**H**), splitting of the medulla (**SM**), damage to the meristoderm (**DM**), and loss of differentiation between tissue layers (**D**).

2.5 Discussion

2.5.1 Effects of temperature on growth, tissue loss and mortality of kelp

Growth of kelp was not affected by temperature in the first week of exposure, and only in *Agarum clathratum* was growth rate significantly reduced in treatments above 11 ˚C after 3 weeks of exposure. Vadas (1968) reported that growth rates of *A. clathratum* were highest in late fall, winter and early spring, when water temperatures are lowest. In *Laminaria digitata* and *Saccharina latissima* mean growth rates at 18 ˚C were lower than those at 11 ˚C, although the difference was not statistically significant. The growth optimum for both *S. latissima* and *L. digitata* is 10 – 15 ˚C, and previous studies show reduction in the growth rate of these species after 1-wk or 3-wk exposure to temperatures above 15 ˚C (Fortes & Lüning 1980, Bolton & Lüning 1982, Gerard & Du Bois 1988).

Reductions in kelp growth rate occur when rising temperatures increase the rate of respiration to exceed that of photosynthesis (Staerh & Wernberg 2009). Above the temperature optimum for photosynthesis, growth is further affected by reductions in photosynthetic efficiency caused by increased photorespiration and the destabilization of photosystem II (Davison 1991). Increases in respiration and declines in the photosynthetic rate increase the light compensation point, so that higher light levels are needed to maintain positive growth (Davison 1991, Andersen et al. 2013). Greater light requirements leave kelp vulnerable to other stressors that reduce light availability, such as eutrophication and increased turbidity. Temperature-induced reductions of growth rate both reduce productivity and hinder the ability of kelp to recover from biomass loss (Rothäusler et al. 2009).

Mean growth rates (over all exposure times) of *Saccharina latissima* (1.5 mm d^{-1}) and *Laminaria digitata* (0.05 mm d^{-1}) in the 11 ˚C treatment were lower than those previously recorded during summer at 4 – 6 m depth in Nova Scotia (3.1 – 9.1 and 1.5 – 3.7 mm d^{-1}, respectively; Appendix B). Low light in the laboratory could have limited our ability to detect an effect of temperature on growth rate, particularly for *L. digitata*. However, the light level in experimental tanks (9.3 µmol m^{-2} s^{-1}) was above the compensation point for each species at 11 ˚C (2 – 5 µmol m^{-2} s^{-1} for *S. latissima*, 3 µmol m^{-2} s^{-1} for *L. digitata*) (Rodriges et al. 2000, Anderson et al. 2013). Also, mean growth rates (4.9 mm d^{-1} for *S. latissima*, 1.0 mm d^{-1} for *L. digitata*) in the trial to examine tank effects at the same temperature and light levels were within the range observed in the field, suggesting that growth was not light limited in the latter experiment.

Temperatures of 20 – 25 ˚C have been shown to cause mortality in both *Saccharina latissima* and *Laminaria digitata* (Fortes & Lüning 1980, Bolton and Lüning 1982, Lüning 1984), consistent with the observed mortality at 21 ˚C in our experiment. Mortality in our experiment was caused by massive tissue loss, including disintegration of the whole blade and meristematic region. All 3 kelp species also experienced significant sub-lethal tissue loss at 18 ˚C, although *L. digitata* and *S. latissima* were more strongly affected. In kelp, net tissue gain or loss depends on the balance between the production of new tissue (growth) and the loss of tissue from the distal end of the blade through erosion or breakage (Krumhansl & Scheibling 2011a). Both reduced growth rates and increased erosion could have contributed to the greater net tissue loss observed at higher temperatures. Tissue loss was an order of magnitude greater than the observed

reduction in growth rate, indicating that erosion and breakage were the dominant factors driving the net loss of tissue.

Currents recorded during July and August 2012 in an urchin barren adjacent to the kelp bed at Splitnose Point ranged from 4.5 to 7.3 cm s^{-1} (Harding & Scheibling 2015). Although currents within the kelp bed would be somewhat dampened (Konar & Estes 2003), they would still be much higher than the flow we are able to provide in the lab (~7 L min^{-1}). Despite differences in water motion between the field and the laboratory, mean rates of tissue loss measured for *Saccharina latissima* and *Laminaria digitata* in the 11 and 14 ˚C treatments were comparable to erosion rates recorded for these species in the field (Appendix B), and tissue loss in *S. latissima* at 18 ˚C was at the upper limit of documented erosion rates (Appendix B). Tissue loss in *S. latissima* and *L. digitata* in the 21 ˚C treatment was 4 to 8 times the maximum erosion rates reported for these species in subtidal habitats in Nova Scotia (Appendix B). Erosion rates in *S. latissima,* but not *L. digitata*, have been positively related to temperature in the field (Krumhansl & Scheibling 2011a, Krumhansl et al. 2014). Tissue loss in *Agarum clathratum*, measured as a reduction in blade length, was less than that recorded in the other 2 species. Although *A. clathratum* was vulnerable to temperature-mediated reduction in growth, its tissue may be more resistant to increased erosion or breakage at higher temperatures. Tissue loss also may have been underestimated in *A. clathratum* because, unlike the other species that erode from the distal end, *A. clathratum* also tends to lose tissue from the margins of the blade (E.J. Simonson, personal observation).

Increased tissue loss, due to either erosion or breakage, will reduce photosynthetic area and can lead to mortality if the rate of loss exceeds that of growth for a sufficiently

long time. In our experiment, we observed rapid disintegration and loss of tissues in the 21 ˚C treatment, and for *L. digitata* mortality after as little as 3 days of exposure. None of the species survived longer than 9 days of exposure to 21 ˚C. Similarly, Bolton and Lüning (1982) report disintegration and mortality of both *L. digitata* and *S. latissima* after 7 days at 23 ˚C and Lüning (1984) observed complete mortality of these species after 7 days at 23 ˚C, and mortality of some individuals at 20 ˚C. Tissue lost from kelp is an important source of detritus that subsidizes communities in other less productive habitats (Krumhansl & Scheibling 2012). The measured increase in tissue loss at high temperatures supports predictions of a pulse of initial increased detrital production with rising temperature (Krumhansl et al. 2014). Continued reductions in biomass will eventually decrease the amount of kelp available as detrital subsidies.

2.5.2 Effects of temperature on material properties and structure of kelp tissue

Increased erosion and breakage of kelp blades at higher temperatures can be explained by changes in the material properties and structure of the kelp tissue. The tensile strength (breaking stress) of blade tissue of *Saccharina latissima* and *Laminaria digitata* in the 11 ˚C treatment falls within the range previously recorded for undamaged tissue of these species (Harder et al. 2006, Krumhansl et al. 2011). Tensile strength of blade tissue was reduced by exposure to 18 ˚C in all 3 kelp species; *S. latissima* was most sensitive to warmer temperature, experiencing a reduction in strength at 14 ˚C. In the red algae *Mazzaella,* breakage occurs when small cracks form and then quickly propagate through the tissue leading to failure (Mach 2009). Damage to the blade concentrates the applied force, resulting in the formation and propagation of cracks at lower forces (Mach

2009). Histological examination of blade tissue of *S. latissima* and *L. digitata* showed damage to the cellular structure and breaks in the meristoderm that would concentrate forces at the damaged area, leading to a reduction in strength. Damage to the cellular structure may result from the production of reactive oxygen species, which can damage cell membranes. Temperature stress can lead to formation of reactive oxygen species and lipid peroxidation of membranes in *L. japonica* (Wang et al. 2009, Zhou et al. 2010). *Agarum clathratum* did not exhibit temperature-induced damage to the midrib tissue after 1 week, and neither stress nor strain were reduced after the first week of exposure, suggesting it may be more resistant to temperature-induced damage and subsequent weakening and loss of tissue.

The observed reductions in breaking strength, which range from 0.7 – 1.7 MPa, represent a reduction in force that an average blade can withstand without breakage of approximately 5 – 15 N. Drag forces experienced by *Saccharina latissima* in the North Sea range from 2 N at low current velocities to 35 N during storm events (Buck & Buchholz 2005). Therefore, the observed strength reductions could have significant impacts on the amount of blade breakage that occurs, given a similar force regime. Kelp blade tissues have low tensile strength compared to many other biomaterials, but are able to survive in forceful environments by being highly flexible and extensible (Johnson & Koelh 1994, Harder et al. 2006). The main force exerted on kelp by water movement is drag, and the flexibility of kelp stipes and blades allows them to reconfigure to the direction of flow allowing the drag forces to be distributed along the length of the blade (Mach et al. 2007). High extensibility (breaking strain) allows blades to absorb strain energy as elastic or plastic deformation (Johnson & Koehl 1994). This deformation may

blunt the tips of cracks, broadening the area over which force is concentrated and slowing their propagation (Mach et al. 2007). We documented reductions in breaking strain in both *S. latissima* and *Laminaria digitata* at 21˚ C and in *S. latissima* at 18˚ C, indicating that these species would be less extensible and more vulnerable to crack propagation. Reductions in the forces kelp can withstand will render kelp more vulnerable to breakage, especially during large wave events.

In contrast to the decreased strength of *Saccharina latissima* in the laboratory at 14 ˚C, *S. latissima* collected over the summer from Splitnose Point was not negatively affected by periods of temperature above 14 ˚C, and became slightly stronger over the course of the summer in both 2013 and 2014. Although the slopes of the relationships between ordinal date and breaking stress are small, they represent an increase of strength 0.5 – 0.75 MPa over the 4-month period, which corresponds to a 4 – 6 N increase in the drag force the average *S. latissima* can withstand without breaking. This increased resistance to force is potentially significant given the range of drag forces measured for *S. latissima*. There is some evidence of increased breaking or attachment strength in other seaweeds between summer and late fall or winter that is attributed to the removal of weaker individuals by storms, leaving only stronger individuals to be sampled (Milligan & DeWreede 2000, Pratt & Johnson 2002). Although the removal of stronger individuals could contribute the pattern of increasing strength that we observed, it is unlikely, given the relatively calmer waters during summer, and we noticed no major losses of kelp due to storms.

Increasing strength of *Saccharina latissima* over the summer could result from increased grazing pressure. Grazing damage by the gastropod mesograzer *Lacuna vincta*

increases over summer to a peak in September (Krumhansl & Scheibling 2011a). Increases in tensile strength in the brown alga *Ascophyllum nodosum* have been induced by simulated grazing excavations, similar to those inflicted by *L. vincta* (Lowell et al. 1991). Carbohydrates (C content) and secondary metabolites (phlorotannins) also increase during summer, while growth is N limited (Steinburg 1995, Bartsch et al 2008, Neilsen et al. 2014, Chapter 3). The allocation of energy to production of storage products and secondary metabolites may increase tissue strength; for example, phlorotannins are important cell wall components (Schoenwaelder & Clayton 1999).

The observed increase in strength with warming temperatures may also be evidence of thermal acclimation. While there are no other reports of warming sea temperature increasing kelp tissue strength, environmentally induced strengthening of tissue can occur in response to high flow (Kraemer & Chapman 1991) or damage (Lowell et al. 1991). These results suggest that if damage to the tissue does not result in immediate breakage, kelp may be able to compensate for damage by strengthening tissue. There is also evidence of thermal acclimation of material properties in our laboratory experiment, where the effect of temperature treatments was sometimes mitigated by longer exposure times. This pattern is observed in breaking stress and strain of all 3 species at 14 ˚C, and in *Saccharina latissima* at 18 ˚C, which all exhibit stronger effects of temperature after 2 than 3 weeks of exposure. *Saccharina latissima* can tolerate a broad range of temperature across its geographic range (Müller et al. 2009). This ability to cope with varying temperature likely depends on a combination of local adaptation and thermal acclimation. The potential for kelp to acclimate to warming seawater

temperatures suggests that rapid short-term changes in temperature (heat waves) could have a much greater effect on kelp populations than gradual warming.

2.5.3 Ecological consequences of ocean warming on kelp beds in the Northwest Atlantic

Although kelp growing on exposed headlands, such as Splitnose Point, are not often exposed to the higher temperature levels used in our experiment, nearby sheltered bays regularly reach 18 ˚C or higher during July and August, and these temperatures can persist for up to 3 weeks. The damage to kelp tissue, reductions in strength and extensive tissue loss at temperatures above 18 ˚C in our experiment suggests that warming water temperature may play a role in the observed declines in some Nova Scotian kelp beds. *Agarum clathratum* was less damaged by warm temperatures than the other 2 kelp species, resulting in a lower degree of tissue weakening and loss. The resistance of *A. clathratum* to warmer temperatures may confer a competitive advantage that will allow it to move into shallower habitats where previously it was excluded by other kelp species (Vadas 1968, Gagnon et al. 2005), resulting in changes in kelp bed composition. Harris & Tyrrell (2001) observed replacement of *Saccharina latissima* with *A. clathratum* over a 25-year period at some shallow sites in the Gulf of Maine, concurrent with a 1 – 2 ˚C increase in sea-surface temperature. Replacement of *S. latissima* and *Laminaria digitata* by *A. clathratum* would maintain many of the ecological functions of kelp beds in Nova Scotia. Efird & Konar (2014) found *A. clathratum* and *S. latissima* to be functionally equivalent understory kelp species in structuring fish assemblages in Alaskan kelp forests. However, this transition has potential consequences for assemblages of associated

invertebrates, as *A. clathratum* can support assemblages distinct from those observed on other seaweeds (Bégin et al. 2004). Increased dominance of *A. clathratum* may also impact energy transfer to higher trophic levels, as *A. clathratum* is highly chemically defended, making it a lower quality food source, both fresh and as detritus (Blain & Gagnon 2014, Dethier et al. 2014). Despite the potential for some effects on the associated communities, increasing dominance of *A. clathratum* is of less concern than transitions from kelp-dominated to turf-dominated systems. Transitions from kelp to turf have been observed in sheltered embayments in Nova Scotia (O'Brien et al. 2015, K. Filbee-Dexter & R.E. Scheibling, unpublished manuscript), and also in Norway (Andersen et al. 2011, Moy & Christie 2012) and Australia (Connell & Russell 2010, Wernberg et al. 2013). These turf communities do not provide the same kind of 3-dimensional habitat structure as kelp beds and thus support lower species diversity (Christie et al. 2009).

Temperature-induced damage to the cellular structure, followed by reduced strength and a subsequent increased vulnerability to waves, could provide a mechanistic link between rising seawater temperature and declines in kelp populations globally. Although the effects of temperature on kelp tissue reported here are sublethal at temperatures below 21 ˚C, increasing temperature may increase the vulnerability of kelp to other stressors (Wernberg, 2010) and act synergistically with factors such as eutrophication, increasing levels of dissolved CO_2, herbivory, and fouling by epiphytes to further impact kelp populations (Andersen et al. 2011, Connell & Russell 2012, Moy & Christie 2012, O'Connor 2009). With continuing climate change, predicted increases in the frequency and intensity of storms will impose greater forces on kelp, and exacerbate

tissue loss from kelp already weakened by warm temperatures. Declines in kelp populations or biomass will potentially decrease the amount of available 3-dimensional habitat and impact productivity, community composition and diversity. Changes in detrital subsidies and energy export resulting from reduced kelp populations will have broad-ranging effects on adjacent coastal and deep-water ecosystems.

CHAPTER 3

Effects of warming seawater temperature on kelp quality as a food source and settlement substrate

3.1 Abstract

Predicting the effect of climate change on communities requires an understanding of the effects of environmental conditions on species and their interactions. We investigated the potential for warming seawater temperature to modify the interactions of the gastropod mesograzer *Lacuna vincta* and the invasive bryozoan *Membranipora membranacea* with Nova Scotia kelps. The nutritional content (C/N) of the kelps *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum* were unaffected by temperature (11, 18 and 21 ˚C), and chemical defenses (phlorotannins) were reduced only in *A. clathratum* after 1-wk exposure to 21 ˚C. C/N and phlorotannin content increased over the season in *S. latissima* collected monthly in summer 2013 and 2014. The effect of potential temperature-induced changes in kelp on the grazing of *L. vincta* was assessed using feeding experiments with *S. latissima* pretreated at 11 or 21 ˚C. Snails consumed more kelp pretreated at 21 ˚C only when grazing rate was high. The quality of *S. latissima* as a food source for *L. vincta* was not affected by temperature, as diets of kelp pretreated at 11 and 21 ˚C supported similar growth, reproduction, and survival of snails. Temperature also did not affect the quality of kelp as a substrate for *M. membranacea*, as settlement rates were not different between *S. latissima* pretreated at ambient temperature (9 – 14 ˚C) or 21 ˚C. The absence of temperature-induced changes in kelp quality suggests that the effects of *L. vincta* and *M. membranacea* will act additively with the

direct effects of temperature to increase biomass loss from Nova Scotia kelp beds.

3.2 Introduction

Effects of climate change on community structure and function will integrate environmental impacts on individual species with changes in interactions among species (Harley et al. 2006). Alteration of biotic interactions can have significant effects on community composition and ecosystem function that can enhance or diminish the effects of climate change (Zarnetske et al. 2012, HilleRisLambers et al. 2013). Climate driven changes in biotic interactions can occur through introduction or loss of interacting species due to changes in distributional range or phenology, or through changes to the strength of interactions due to alteration of behavior or physiology (Zarnetske et al. 2012, HilleRisLambers et al. 2013, Vergés et al. 2014). Changing climate also can facilitate the invasion of non-native species or exacerbate their effects on native communities (Cockrell & Sorte 2013, Floerl et al. 2013). Alteration of biotic interactions can combine with the direct effects of climate change on a species, resulting in impacts that may not have been expected when considering the effect of climate alone (HilleRisLambers et al., 2013).

Globally, increasing seawater temperature has been linked to range contractions and declines in kelp populations (Andersen et al. 2011, Fernández 2011, Tuya et al. 2012, Wernberg et al. 2013). Kelp (large brown algae of the order Laminariales) are important foundation species on temperate rocky reefs, supporting high community productivity and biodiversity (Dayton 1985, Steneck et al. 2002). Direct effects of increasing

temperature on kelp include reduced growth (Bolton & Lüning 1982, Gerard & Du Bois 1988, Davison 1991, Andersen et al. 2013), weakening and loss of kelp tissue (Chapter 2), or mortality when physiological limits are exceeded (Wernberg et al. 2013). Temperature-mediated changes in kelp bed communities also may occur indirectly through the alteration of interspecific interactions (Harley et al. 2012, Vergés et al. 2014). The interaction between herbivores and their algal food sources can regulate kelp communities through the consumption of algal biomass (Lubchenco and Gaines, 1981), and may be altered by temperature stress due to changes in either algal growth rate, herbivore consumption (O'Connor 2009), or changes in algal palatability (Harley et al. 2012). For example, warming seawater temperatures in southern Japan have both impacted kelp directly and enhanced feeding rates of herbivorous fish, triggering shifts from beds of *Ecklonia cava* to barrens (Vergés et al. 2014).

Changes in the palatability of kelp, and thus its vulnerability to herbivores, could be driven by temperature-induced changes to the morphological or chemical characteristics of the kelp tissue. Herbivores alter both rate of consumption and feeding preference based on the palatability of their food sources, selecting foods that have fewer mechanical or chemical defenses, or greater nutritional content (Duffy & Paul 1992, Pansch et al. 2008, Steneck & Watling 1982). Warmer temperatures decreased palatability of *Ecklonia radiata* by increasing the C:N ratio (Staehr & Wernberg 2009), as herbivores tend to select food sources enriched in nitrogen (Duffy & Paul 1992). In contrast, increases in temperature prevent the induction of anti-herbivore defenses in the brown alga *Fucus vesiculosus*, increasing consumption (Weinberger et al. 2011).

Kelp (and other brown algae) produce phlorotannins (polymers of phloroglucinol)

as a chemical defense against herbivory (Steinburg 1984, Targett & Arnold 1998). Phlorotannins also act as antioxidant compounds, and can be induced by UV stress (Cruces et al. 2012). Kelp may defend against cellular damage caused by thermal stress by upregulating antioxidant compounds, such as phlorotannins, to combat the effects of reactive oxygen species. However, in the three species examined to date, thermal stress was not observed to induce phlorotannins, and at high levels may even inhibit phlorotannin production (Cruces et al. 2012, Cruces et al. 2013). Phlorotannins have also been implicated as chemical defenses against epibionts (Iken et al. 2009, Wikström & Pavia 2004), suggesting that temperature-induced changes in phlorotannin content could affect the vulnerability of kelp to both grazing and fouling.

In kelp beds in Nova Scotia, the gastropod mesograzer *Lacuna vincta* is a dominant herbivore that feeds by creating surface excavations and perforations on kelp blades (Johnson & Mann 1986). Grazing by *L. vincta* removes kelp tissue not only directly, but also indirectly by increasing erosion and breakage of blades (Johnson & Mann 1986, Duggins et al. 2001, Krumhansl & Scheibling 2011a, Krumhansl et al. 2011), which can result in large-scale loss of kelp biomass (Fralick et al. 1974, O'Brien et al. 2015). Although a generalist herbivore, *L. vincta* exhibits dietary selectivity both among algal species and among tissue types within species, with a strong preference for *Saccharina latissima* (Johnson & Mann 1986, Chavanich & Harris 2001, Toth & Pavia 2002, Dubois & Iken 2012). Temperature-mediated changes in defensive chemicals, tissue toughness, or nutritional value of *S. latissima* could affect feeding rate of *L. vincta* and the impact of these snails on kelp beds.

The invasive bryozoan *Membranipora membranacea* also causes extensive

biomass loss from Nova Scotian kelp beds, by encrusting kelp blades, weakening the tissue and leading to blade breakage (Krumhansl et al. 2011). Outbreaks of *M. membranacea*, combined with large wave events, result in the large-scale defoliation of kelp beds (Saunders & Metaxas 2008, Scheibling & Gagnon 2009). Since the introduction of *M. membranacea* to the region in the early 1990s, the extent of bryozoan outbreaks and consequent damage to kelp beds have been closely tied to warming temperatures (Scheibling & Gagnon 2009, Saunders et al. 2010), which result in earlier settlement (Saunders & Metaxas 2007) and increased colony growth (Saunders & Metaxas 2009). The damage inflicted on kelp beds by *M. membranacea* is an indirect effect of increasing temperature, which may act synergistically with the direct effects of increased temperature on kelp. Increased temperature also may limit population growth of *M. membranacea* on kelp blades if it causes a reduction in tissue quality that limits larval settlement or the persistence of newly established colonies. In the laboratory, larvae of *M. membranacea* exhibit settlement preferences, both for algal species and for specific areas of kelp blade (Matson et al. 2010), suggesting that they can detect differences in substrate quality. Increases in temperature that degrade the structural integrity of blade tissue, or increase phlorotannin content, could prevent settling larvae from attaching, resulting in an antagonism between the direct effects of temperature on the kelp and the effects of temperature on the interaction between kelp and *M. membranacea.*

We examined the potential for synergistic or antagonistic effects of *Lacuna vincta*, *Membranipora membranacea* and warming seawater temperature on biomass loss from Nova Scotia kelp beds. Specifically, we investigated the impact of warming

temperatures on the phlorotannnin content and C/N ratio of tissue of *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum* in a laboratory experiment, as changes in these chemical properties could potentially alter interactions of the kelp with *L. vincta* or *M. membranacea.* We used feeding experiments to determine whether temperature-induced changes in kelp tissue quality altered feeding rate of *L. vincta*. We predicted that changes in the kelp tissue at warmer temperatures would make kelp easier to consume or more palatable, leading to increased feeding rate. Increases in feeding rate would indicate a synergism where warming temperature both directly and indirectly (through herbivory) increases kelp tissue loss. We also examined the effect of temperature-induced changes in kelp tissue quality on settlement of *M. membranacea*, predicting that settlement would be reduced on degraded kelp exposed to warmer temperatures. Reduced settlement rate would indicate an antagonism whereby increasing temperature directly results in increased loss of kelp biomass, but at the same time mitigates loss caused through encrustation by the bryozoan.

3.3 Materials and Methods

3.3.1 Chemical composition of kelp tissue

Experimental design. Mature individuals of *Saccharina latissima* (1 – 1.5 m blade length), *Laminaria digitata* and *Agarum clathratum* (0.5 – 1.0 m), were collected by SCUBA from 12-m depth at Splitnose Point (44˚28’38.45” N, 63˚32’48.21” W) in June/July 2013. Kelp were transported to the laboratory in coolers, and stored upon arrival in a 3000-L circular tank (1.87 m diameter, 1.08 m height) with continuously

flowing ambient seawater (flow rate: 430 Lh^{-1}) by fixing the kelp holdfasts with elastic bands to plastic racks suspended within the tank. Within 24 h of collection, 9 individuals of each species were suspended in experimental tanks of similar size and flow rate (total: 27 individuals per tank) and exposed to 1 of 3 temperature treatments: 11, 18 or 21 ˚C. These temperatures represent a growth optimum for *S. latissima* and *L. digitata* (11 ˚C), a typical maximum average temperature experienced over 1 – 2 weeks (18 ˚C) (Scheibling et al. 2013), and an anticipated maximum temperature based on climate change predictions (21 ˚C) (Müller et al. 2009).

Tissue samples (5.5 cm diameter) were collected for chemical analysis from 3 individuals of each species within 24 h of field collection (before temperature treatment), from 3 individuals of each species after 1-wk exposure to 11 and 21 ˚C, and from another 3 individuals of each species after 2-wk exposure to 11 and 18 ˚C (for phlorotannin content) and 3-wk exposure to 11 and 18 ˚C (for C/N) (Fig. 3.1). Tissue samples were excised 30 cm from the blade-stipe interface in *S. latissima*, and 15 cm from the blade-stipe interface in *L. digitata* and *A. clathratum*, to control for any variation in chemical properties along the length of a blade. 3 trials of the experiment were conducted in June/July 2013 and all tissue samples were stored at -80 ˚C until further processing. Tissue samples for determination of C/N were collected only in the first trial (n = 3 individuals) and were dried at 60˚ C for 48 h until constant weight. Tissue samples for phlorotannin content were collected in all 3 trials (n = 9 individuals). During the trials temperature was recorded in each tank with data loggers (Maxim Integrated Thermochron iButtons) as 11.6 ± 0.9, 18.2 ± 1.6, and 20.8 ± 1.1 ˚C (mean ± 1 SD, n = 58).

To track changes in the chemical composition of kelp blades over the summer, 10 – 15 mature *Saccharina latissima* (1.0 – 1.5 m blade length) were collected monthly from 8-m depth at Splitnose Point, from 25 June to 27 August 2013 and from 30 June to 25 September 2014. Kelp were transported to the laboratory in coolers and, immediately on arrival, 5.5 cm diameter tissue samples were excised 30 cm from the blade-stipe interface. Tissue samples were stored at -80 ˚C until further processing. Temperature at the collection site was continuously monitored over the collection periods using data loggers (Onset HOBO Pendant) anchored to the seabed.

C/N analysis. Dried algal tissue was ground to a fine homogeneous powder using a mortar and pestle and packaged in known weights into tin capsules. C and N content were determined using a Costec ECS 4010 CHNSO analyzer with acetanilide as a standard (detection limit = 0.001 mg).

Phlorotannin analysis. To extract phlorotannins, tissue samples were first freeze-dried and ground to a fine powder with a mortar and pestle. For each sample, 100 mg dried tissue was placed in 5 ml 70% acetone and extracted overnight with continuous shaking (Koivikko et al. 2005). Samples were then centrifuged for 10 min at 3200 g and 0.05 mL supernatant was withdrawn for phlorotannin quantification. Phlorotannin content was determined using the Folin-Ciocalteu assay (Van Alstyne 1995) with phloroglucinol (1,3,5-Trihodroxybenzene, Sigma-Aldrich) as a standard. The 0.05 mL aliquot was mixed with 1.0 ml distilled water and 1.0 mL 40% Folin-Ciocalteu reagent. After standing 5 min, 1.0 ml $NaC0_3$ was added and samples were incubated for 30 min at 50 ˚C. Absorbance was read at 765 nm using a Cary WinUV 4000 spectrophotometer (Agilent Technologies, detection limit = 0.23 mg).

Statistical analyses. The effect of temperature treatment after 1 wk (11 and 21 ˚C) or after 3 wks (11 and 18 ˚C) on C/N ratio was analyzed using 2-tailed independent samples t-tests, for each kelp species. Variance was heterogeneous for C/N ratio data for *Agarum clathratum* and *Laminaria digitata* after 1 wk, so the Welch-Satterthwaite modification of degrees of freedom was used. The effect of temperature treatment (1 wk at 11 or 21 ˚C, or 2 wk at 11 or 18 ˚C; fixed factors) and trial (random factor) on phlorotannin content was examined using 2-way ANOVA. If the trial x treatment interaction was not significant ($p > 0.25$), data from the 3 trials were pooled and analyzed using t-tests. Seasonal differences in phlorotannin content or C/N ratio among sampling dates were examined using 1-way ANOVA. Post hoc comparisons of sampling dates were performed using Tukey's HSD test.

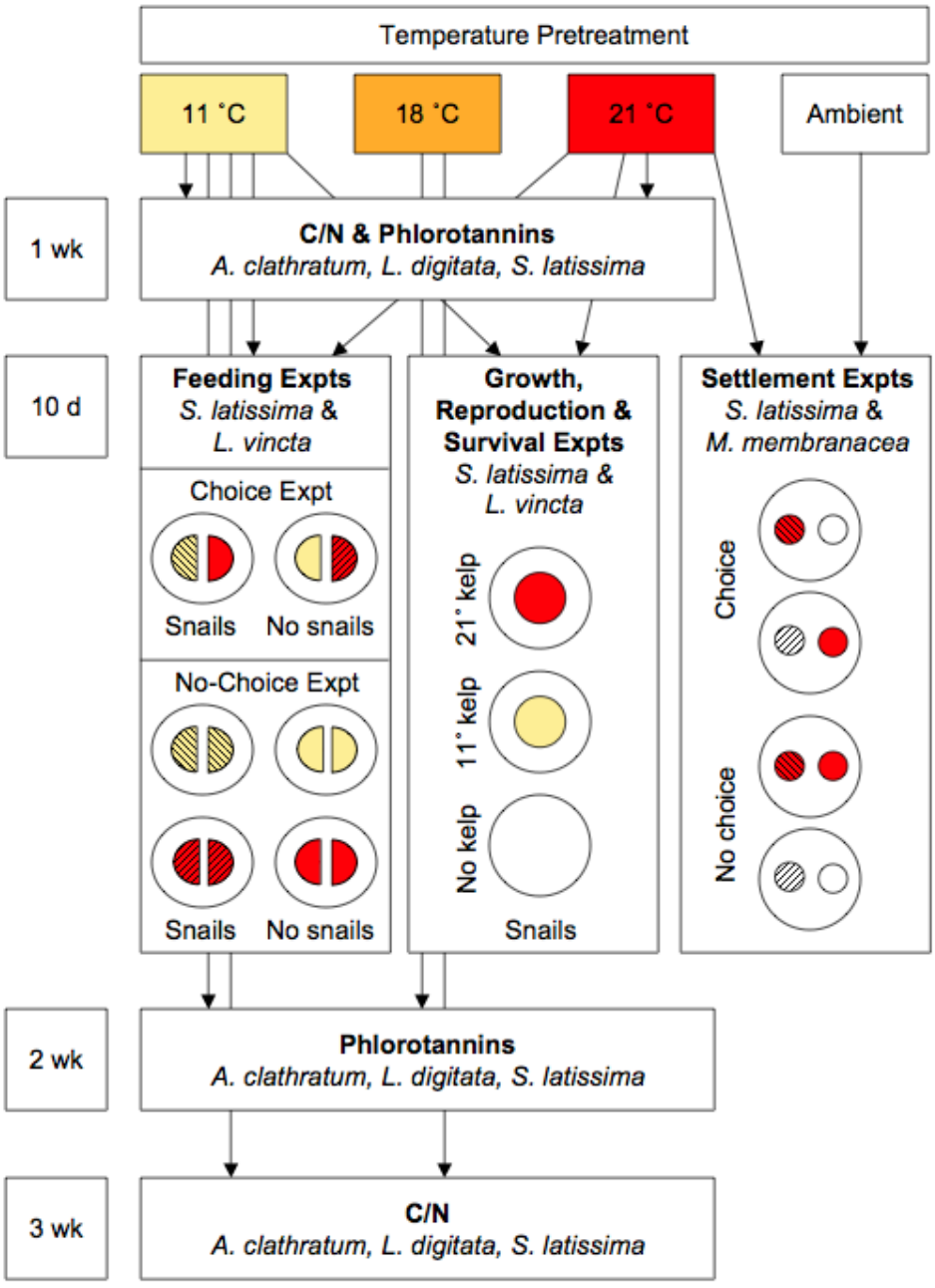

Figure 3.1 Schematic of temperature pretreatments and experimental design for quantification of C/N and phlorotannins, feeding experiments, growth, reproduction and survival experiments, and settlement experiments. In feeding, growth, reproduction and survival, and settlement experiments, temperature pretreatment of disks or half-disks is indicated by colour. Pairs of disks or half-disks sharing the same texture (hatched or open) come from one individual of *Saccharina latissima.* Pairs with one hatched and one open disk or half-disk are made up of two individuals. See text for full description of experimental design.

3.3.2 Temperature-induced changes in kelp as a food source

Material collection and preparation. Lacuna vincta were collected along with associated *Saccharina latissima* from 8 to 12-m depth at Splitnose Point in June/July 2013. Snails and kelp were transported to the laboratory in coolers. Within 24 h of collection, *L. vincta* were removed from the kelp blades and placed within fine mesh bags in a tank with flow-through ambient seawater. Once cleaned of snails, thalli of *S. latissima* were suspended in the temperature treatment tanks (as in temperature experiment) and pretreated for 10 d at either 11 or 21 ˚C before use in feeding or survival, growth and reproduction experiments. *Saccharina latissima* were placed in temperature pretreatments within 24 h of collection for use in feeding experiments, and within 2 wk of collection for the survival, growth and reproduction experiment. Before the start of the feeding experiments, *L. vincta* were fed kelp ad libitum for 7 d, and then starved for 3 d.

Feeding experiments. The effect of temperature-induced changes in kelp tissue quality on *Lacuna vincta* feeding rate and preference were examined by conducting choice and no-choice feeding experiments with 2 diet treatments: *Saccharina latissima* pretreated at 11 or 21 ˚C. 2 disks of kelp (5.5-cm diameter) were excised 30 cm from the blade-stipe interface from each of 8 individuals of *S. latissima* (maintained for 10 d in the experimental temperatures), and then cut in half. In the choice experiment, the initial blotted wet weight of each half-disk was recorded, and half-disks from the 2 diet treatments were paired by weight (Fig. 3.1). Each set of paired half-disks was placed in a flow-through cylindrical container (10-cm diameter, 8-cm height, with 0.2 cm holes) with a mesh top (n = 8). 4 of the containers were randomly designated as autogenic controls to record changes in kelp mass in the absence of snails. Groups of 8 snails (shell length 4–7

mm) were individually measured (0.1-mm precision) and added to each of the other 4 containers. In the no-choice experiment, half-disks from the same individual of *S. latissima* were paired, weighed and placed together in 16 flow-through containers. 8 containers (4 with *S. latissima* pretreated at 11 ˚C, 4 with *S. latissima* pretreated at 21 ˚C) were randomly designated as autogenic controls and the remaining 4 containers for each diet treatment received groups of 8 snails from the same population and size range as those in the choice experiment (Fig. 3.1).

The containers were weighted and submerged, and randomly interspersed in the same flow-through seawater table, where they were maintained for 5 d. The mass of algal tissue grazed in each container was calculated as the change in blotted wet weight (0.001-g precision) of the half-disk(s) from the beginning to the end of the 5-d period. For both the choice and no-choice experiments, 3 trials with 3 – 4 replicate containers were conducted in June/July 2013. Water temperature during each trial was (˚C, mean ± SD, n = 6): trial 1, 7.89 ± 1.27; trial 2, 10.17 ± 0.68; trial 3, 8.96 ± 1.58. Because of a recording failure within the Aquatron facility at Dalhousie University, temperature during each trial was obtained from average daily seawater temperature at 8-m depth at Splitnose Point (~15 km from the Aquatron seawater intake, and at the same depth). Temperatures in Aquatron during August 2013 were generally within 1 ˚C of those at Splitnose Point (mean ± SD difference in temperature: 0.11 ± 0.86 ˚C; n = 28).

Survival, growth and egg production. The effect of temperature-induced changes to kelp tissue quality on the survival, growth and reproduction of *Lacuna vincta* was determined in an 8-wk laboratory experiment (13 July – 5 September 2013). Groups of 8 snails were placed in flow-through containers (as in feeding experiments) and randomly

assigned 1 of 3 diet treatments: *Saccharina latissima* pretreated at 11 ˚C, *S. latissima* pretreated at 21˚C, or starved controls (n = 15 containers for each treatment) (Fig. 3.1). All containers were randomly placed in a flow-through seawater table. Fed treatments were provided 5.5-cm diameter disks of kelp pretreated for 10 d in the 11 or 21 ˚C temperature treatments, as in feeding experiments. Kelp disks were replaced weekly. Snails were marked with nail polish and their individual growth rates recorded by measuring changes in shell length from digital photographs, taken initially and then at biweekly intervals for 8 weeks. Shell length was measured using image analysis software (ImageJ, National Institutes of Health, USA). Initial shell lengths were 3 – 8 mm, and did not differ among treatments (1-way ANOVA: $F_{2,357} = 0.33$, $p = 0.72$). Growth was calculated as the change in shell length over each 2-wk sampling interval. Dead snails and egg masses were counted and removed at each measurement interval. Reproductive output per snail for each container was calculated as the number of egg masses produced divided by the number of snails. Upon termination of the experiment, a subset of surviving snails (from 30 containers) was sexed. Sex ratio in the sampled containers did not differ among treatments (1-way ANOVA, $F_{2,27} = 0.11$, $p = 0.90$) and the mean (± SD) proportion of females was 0.51 ± 0.29 (n = 123). The sex ratio in each container was therefore assumed to be 1:1.

Statistical analyses. Analysis of feeding experiments incorporated the controls for autogenic changes in kelp mass as described in Peterson and Renaud (1989). In the no-choice experiment, mass changes (mg d^{-1}) of half-disks were analyzed using 3-way ANOVA with diet treatment (pretreatment at 11 or 21 ˚C) and herbivore (presence, absence) as fixed factors, and trial as a random factor. There was a significant trial x

herbivore x diet interaction ($F_{2,32} = 11.42$, $p < 0.001$), so trials were analyzed separately with 2-way ANOVA. Data in trial 3 were log transformed to meet the assumption of homogeneity of variance (Levene's test, $p > 0.05$). In the choice experiment, the difference in mass change between diet treatments was calculated for each replicate container, and the differences for containers with and without snails were compared using a 2-way ANOVA with herbivore (presence, absence) as a fixed factor and trial as a random factor. Although the interaction between trial and herbivore was not significant ($F_{2,16} = 13.03$, $p = 0.11$), the trials were analyzed separately using 1-way ANOVA for consistency with the no-choice feeding experiment. Data in trial 3 did not meet the assumption of homogeneity of variance even after transformation, and results from untransformed data are presented.

Differences in growth rate of *Lacuna vincta* between diet treatments (*Saccharina latissima* pretreated at 11 or 21 ˚C; fixed factor) and among weeks (random factor), with repeated measures within weeks, were examined using repeated-measures ANOVA on the average length change (mm) of snails within each container over each 2-wk interval. Similarly, differences in the effect of diet (kelp pretreated at 11 or 21 ˚C; fixed factor) on the number of egg masses $snail^{-1}$ and proportion snails surviving over each 2-wk interval (random factor) in each container were analyzed using repeated measures ANOVA, with repeated measures within weeks. The proportion of snails surviving violated the assumption of sphericity (Mauchly's test, $p < 0.05$), and the Greenhouse-Geisser adjustment was used for p-values.

3.3.3 Temperature-induced changes in kelp as a substrate

The effect of the temperature-induced changes in kelp tissue on settlement of *Membranipora membranacea* was examined with a settlement choice experiment. Larvae of *M. membranacea* were isolated from plankton samples collected from St. Margaret's Bay (~ 35 km W of Splitnose Point) in October/November 2013 and July/August 2014. *Saccharina latissima* was collected over the same periods from 8 to 12-m depth at Splitnose Point, and pretreated for 10 d in experimental tanks with either ambient (see below) or 21 ˚C seawater (tank set up as in temperature experiment). Tissue samples (trials 1, 2: 11-cm^2 half-disks; trials 3 – 5: 8-cm^2 disks) were excised from pretreated kelp, 30 cm from the blade-stipe interface, paired and placed in 250-mL beakers with filtered seawater. Pairs consisted either of 2 samples pretreated at ambient temperature, 2 samples pretreated at 21 ˚C, or 1 sample pretreated at ambient temperature and 1 at 21 ˚C (Fig. 3.1). 30 competent larvae of *M. membranacea* were then added to each beaker and allowed 72 h to settle on the kelp substrates. During this time, beakers were placed in a continuous-flow seawater table to maintain the beakers at ambient seawater temperature. After 72 h, the kelp was examined microscopically and the number of larvae that settled on each sample was counted. 3 trials were conducted in October – November 2013 (mean ± SD ambient water temperature: 14.4 ± 2.2 ˚C, n = 30) and 2 trials in July – August 2014 (9.3 ± 2.1 ˚C, n = 20). Ambient water temperature was recorded in the pretreatment tank at 1 h intervals with data loggers (Maxim Integrated Thermochron iButtons). A total 28 of larvae were scored, and all were observed to settle on 1 of the 2 kelp substrates. Although area of tissue varied among trials, total settlement within beakers did not differ with total area available for settlement (1-way ANOVA, $F_{1,18} = 0.02$, $p = 0.40$). The

number of settlers on each sample was divided by the area of the sample, giving settlers cm^{-2}.

Statistical analyses. The effect of the temperature-mediated quality of the kelp tissue on settlement of *Membranipora membranacea* was determined by comparing the number of larvae that settled on a substrate (kelp pretreated at ambient temperature or 21 ˚C) when larvae were given a choice of substrates to the number of larvae that settled on that substrate when there was no choice. Beakers in which no settlement occurred were not included in the analyses. For the comparisons using kelp pretreated at ambient temperature, all 5 trials were included in the analysis. However, for the comparisons using kelp pretreated at 21 ˚C, only 3 trials (those with settlement in both choice and no-choice beakers) were included. In the no-choice beakers, settlement did not differ between the 2 tissue samples within each beaker (paired t-tests, ambient beakers: $t_5 = 0.31$, $p = 0.77$; 21 ˚C beakers: $t_4 = -0.93$, $p = 0.41$); to maintain independence of replicates 1 sample was randomly selected from each no-choice beaker for the analysis. The effect of treatment (choice, no-choice; fixed factor) and trial (random factor) on the number of settlers cm^{-2} was examined using 2-way ANOVA for each temperature pretreatment (ambient, 21 ˚C). Because there was no effect of trial, and no treatment by trial interaction ($p > 0.2$) at either temperature, trials were pooled and re-analyzed with 2-tailed independent samples t-tests.

3.4 Results

3.4.1 Chemical composition of kelp tissue

There was no effect of temperature on C/N ratio for any of the 3 kelp species after 1-wk exposure between 11 and 21 ˚C, or after 3-wk exposure between 11 and 18 ˚C (Fig. 3.2, Table 3.1). There was a trend of increasing C/N ratio over the summer in both 2013 and 2014 (Fig. 3.3), although differences among sampling dates were not significant (Table 3.2).

Phlorotannin content of *Agarum clathratum* significantly decreased by 1 % dw after 1-wk exposure to 21 ˚C, compared to 11 ˚C (Fig. 3.2, Table 3.1), but there was no difference in phlorotannin content in *A. clathratum* after 2-wk exposure between 11 and 18 ˚C (Fig. 3.2, Table 3.1). Phlorotannin content in *Laminaria digitata* and *Saccharina latissima* was approximately 10 % and 25 % that of *A. clathratum*, respectively (Fig. 3.2). There was no difference in phlorotannin content for either species after 1-wk exposure between 11 and 21 ˚C, or after 2-wk exposure between 11 and 18 ˚C (Fig. 3.2, Table 3.1). Phlorotannin content of *S. latissima* collected from Splitnose Point was higher in 2013 than in 2014 across all sampling dates (Fig. 3.3). During 2013, phlorotannin content increased throughout the summer (Fig. 3.3), although there were no significant differences among sampling dates (Table 3.2). In 2014, phlorotannin content of *S. latissima* increased slightly from June to August and then decreased by significantly by 0.6 % dw in September (Fig. 3.3, Table 3.2).

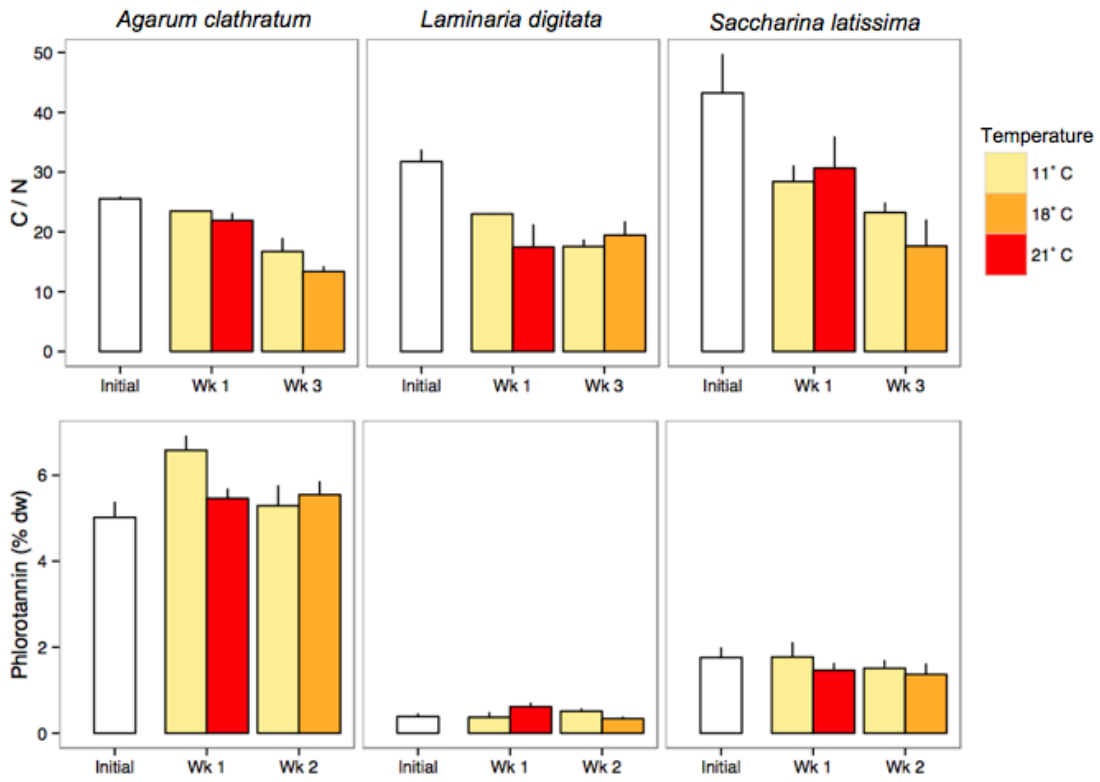

Figure 3.2 Mean (± 1 SE) C/N (n = 3) and phlorotannin content (% dw; n = 9) and of *A. clathratum, L. digitata,* and *S. latissima* immediately after collection from the field (Initial) and after 1-wk exposure to 11 and 21 ˚C, and 2-wk or 3-wk exposure to 11 and 18 ˚C in the laboratory.

Table 3.1 Results of independent samples t-test to examine differences in C/N ratio and phlorotannin content (Phl; % dw) between 11 and 21 ˚C temperature treatments after 1-wk exposure, or between 11 and 18 ˚C treatments after 2- or 3-wk exposures, for each kelp species.

Variable	Species	Exposure (wk)	Temperature (˚C)	t	DF	p
C/N	*Agarum clathratum*	1	11 vs 21	1.24	2.1*	0.34
		3	11 vs 18	1.37	4	0.24
	Laminaria digitata	1	11 vs 21	1.45	2.0*	0.28
		3	11 vs 18	-0.73	4	0.51
	Saccharina latissima	1	11 vs 21	-3.76	4	0.73
		3	11 vs 18	1.17	4	0.30
Phl	*Agarum clathratum*	1	11 vs 21	2.81	14	**0.01**
		2	11 vs 18	-0.45	16	0.66
	Laminaria digitata	1	11 vs 21	-1.59	16	0.13
		2	11 vs 18	1.88	15	0.08
	Saccharina latissima	1	11 vs 21	0.80	16	0.43
		2	11 vs 18	0.45	16	0.66

*Welch-Satterthwaite adjustment due to unequal variance (F-test, $p < 0.05$)

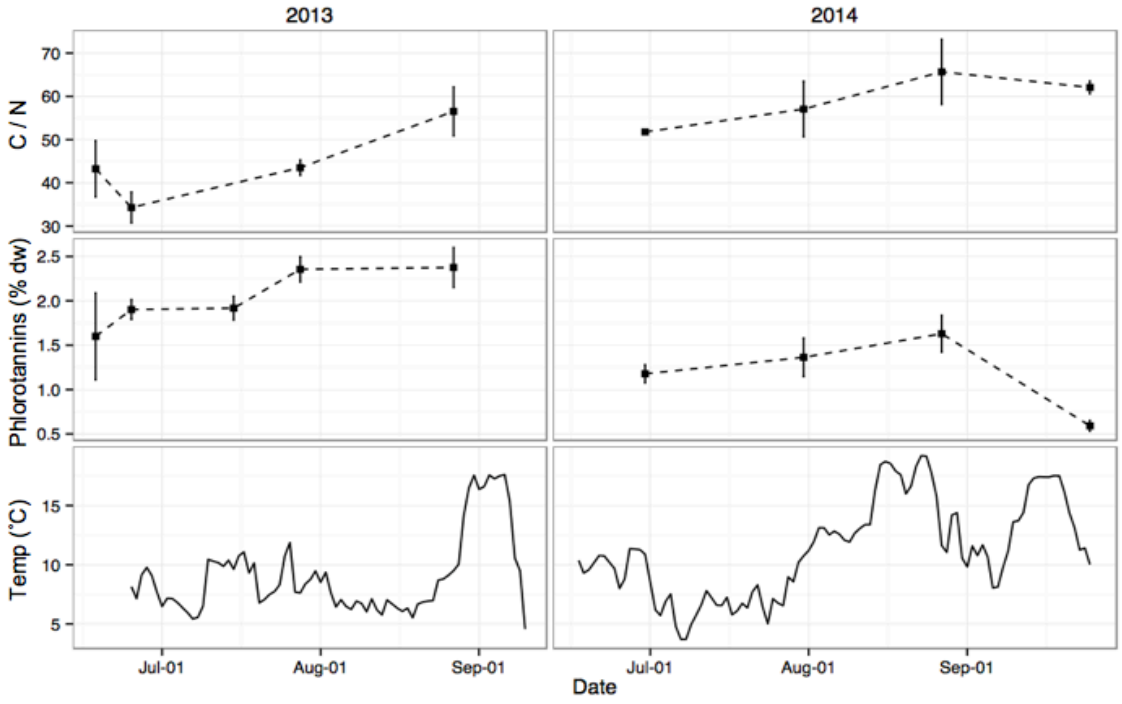

Figure 3.3 Mean (± 1 SE) C/N (n = 3) and phlorotannin content (% dw; n = 10) of blade tissue of *Saccharina latissima* collected monthly in summer 2013 and 2014 from 8-m depth at Splitnose Point, and mean daily temperature (˚C) at collection site.

Table 3.2 Results of ANOVA comparing C/N ratio or phlorotannin content (Phl) of blade tissue of *Saccharina latissima* among sampling dates (2013: 18 and 25 June, 15 July [Phl only], 28 July, 27 August; 2014: 30 June, 31 July, 27 August, 25 September).

Year	Variable	F	DF	p	Tukey HSD
2013	C/N	3.70	3, 8	0.06	
	Phl	2.61	4, 31	0.09	
2014	C/N	1.46	3, 8	0.30	
	Phl	4.08	3, 24	**0.001**	Sep < Aug = Jul

3.4.2 Temperature-induced changes in kelp as a food source

In the no-choice feeding experiment kelp loss was greater in the presence of *Lacuna vincta*, and autogenic loss of kelp pretreated at 21 ˚C was greater than kelp pretreated at 11 ˚C (Fig. 3.4, Table 3.3). In trial 1, a significant diet by herbivore interaction indicated that grazing rates of *L. vincta* were greater on kelp pretreated at 21 ˚C than at 11 ˚C (Fig. 3.4, Table 3.3). There was no difference in grazing rates between diets in trials 2 and 3 (Fig. 3.4, Table 3.3). In the choice experiment, the difference in mass change between half-disks of kelp pretreated at 11 and 21 ˚C was significantly greater in treatments with than without *L. vincta* in trial 1, indicating that snails fed preferentially on kelp pretreated at 21 ˚C (Fig. 3.4, Table 3.4). This was not the case in trials 2 and 3, although the direction of differences was consistent (Fig. 3.4, Table 3.4). As in the no-choice experiment, autogenic mass loss was greater for kelp pretreated at 21 ˚C in the choice experiment (Fig. 3.4).

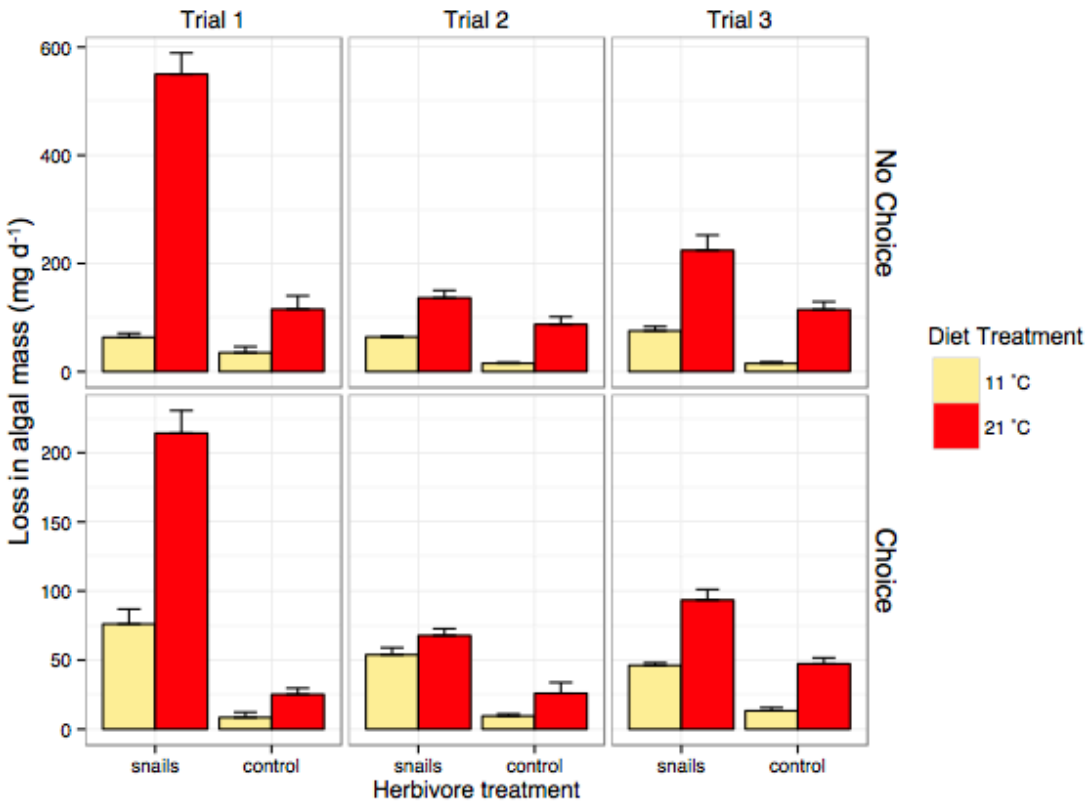

Figure 3.4 Mean (± 1 SE) rates of mass loss of *Saccharina latissima* pretreated for 10 d at 11 ˚C or 21 ˚C in the presence of *Lacuna vincta*, or in controls without *L. vincta* in 3 trials of choice and no-choice feeding experiments (trial 1: n = 3; trial 2 & 3: n = 4).

Table 3.3 Results of 2-way ANOVA examining effects of diet (*Saccharina latissima* pretreated for 10 d at 11 or 21 ˚C) and herbivore (presence or absence of *Lacuna vincta*) on rate of kelp mass loss (mg d^{-1}) in 3 trials of the no-choice feeding experiment. Significant results are given in bold.

Trial	Source	DF	F	p
1	Diet	1, 8	45.81	**<0.001**
	Herbivore	1, 8	30.55	**<0.001**
	Diet x Herbivore	1, 8	23.53	**0.001**
2	Diet	1, 12	13.25	**0.003**
	Herbivore	1, 12	6.08	**0.03**
	Diet x Herbivore	1, 12	0.00	0.99
3*	Diet	1, 12	33.89	**<0.001**
	Herbivore	1, 12	18.91	**<0.001**
	Diet x Herbivore	1, 12	3.87	0.073

*Mass change log transformed to meet assumption of homoscedasticity

Table 3.4 Results of 1-way ANOVA comparing difference in rate of mass change (mg d^{-1}) of *Saccharina latissima* pretreated for 10 d at 11 vs. 21 ˚C between herbivore treatments (presence or absence of *Lacuna vincta*) in 3 trials of the choice feeding experiment. Significant results are given in bold.

Trial	DF	F	p
1	1, 4	102.6	**<0.001**
2	1, 6	0.016	0.91
3*	1, 6	0.605	0.47

*Data heteroscedastic even after transformation. Untransformed data are presented.

At the end of the 8-wk growth experiment, mean growth (change in shell length) in containers of snails fed either of the 2 kelp diets was an order of magnitude higher than the mean growth of starved controls (Fig. 3.5a). There was no effect of kelp diet (*Saccharina latissima* pretreated at 11 or 21 ˚C) on the growth of snails over the 8-wk experiment, and no interaction between kelp diet and sampling week, although growth did differ among sampling weeks (Table 3.5). There was no production of egg masses in the starved controls after week 2, and egg production $snail^{-1}$ increased with sampling week in containers fed either kelp diet (Fig. 3.5b). There was no difference in the number of egg masses produced $snail^{-1}$ between containers fed kelp pretreated at 11 ˚C and those fed kelp pretreated at 21 ˚C (Table 3.5). Egg production did differ among weeks, but there was no interaction between kelp diet and sampling week (Table 3.5). Mean survival of snails in the experiment was high, exceeding 90% over the first 6 wk in all diet treatments (Fig. 3.5c). After 8 wk, mean survival of the starved controls declined to 75% (Fig. 3.5c). There was no difference in the survival between the 2 kelp diets, and no interaction between diet and sampling week, but there was a difference in survival among weeks (Table 3.5).

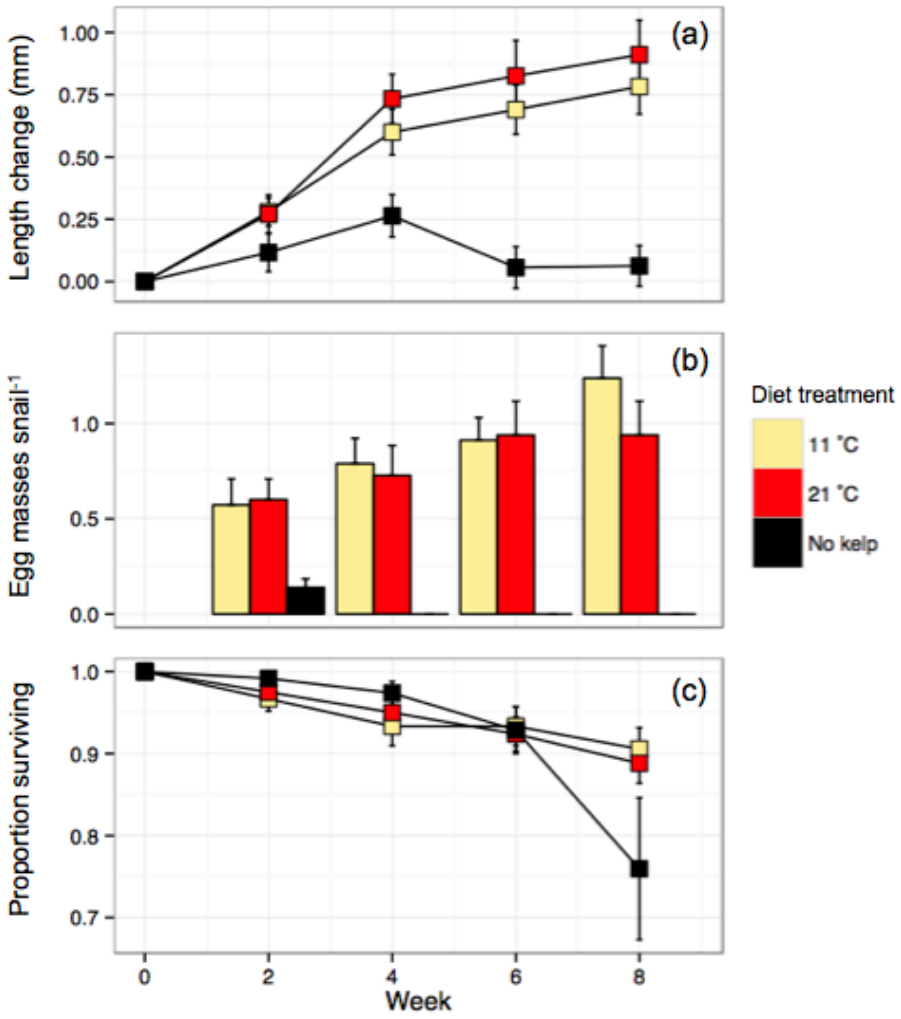

Figure 3.5 (a) Mean (± 1 SE) growth (length change) of *L. vincta* relative to initial shell length when fed 3 diets: *Saccharina latissima* pretreated 10 d at 11 ˚C, *S. latissima* pretreated 10 d at 21 ˚C, or no kelp. Standard error represents the variation among 15 containers. (b) Mean (± 1 SE) egg masses snail^{-1} produced by *L. vincta* when fed 3 diets (as above; n = 15 containers). (c) Mean (± 1 SE) proportion of *Lacuna vincta* surviving (relative to initial number of individuals) when fed 3 diets (as above; n = 15 containers).

Table 3.5 Results of repeated-measures ANOVA comparing proportion of *Lacuna vincta* surviving, average growth (length change; mm) and egg production (egg masses snail^{-1}) between 2 diets (*Saccharina latissima* pretreated at 11 or 21 ˚C; fixed factor). Repeated measures were taken at 2-week intervals (2, 4, 6 and 8 wk). Significant results are given in bold.

Variable	Source	DF	F	p
Proportion Surviving*	Between Subjects			
	Diet	1	0.0003	0.98
	Container(Diet)	28		
	Within Subjects			
	Week	3	13.73	**<0.001**
	Week x Diet	3	0.90	0.42
	Week x Container(Diet)	84		
Growth	Between Subjects			
	Diet	1	0.52	0.48
	Container(Diet)	28		
	Within Subjects			
	Week	3	11.28	**<0.001**
	Week x Diet	3	0.71	0.55
	Week x Container(Diet)	84		
Egg production	Between Subjects			
	Diet	1	0.21	0.65
	Container(Diet)	28		
	Within Subjects			
	Week	3	8.25	**<0.001**
	Week x Diet	3	1.05	0.37
	Week x Container(Diet)	84		

*Assumption of sphericity violated (Mauchly's test $p < 0.001$), and Greenhouse-Geisser adjustment applied.

3.4.3 Temperature-induced changes in kelp as a substrate

There was no difference in the number of larvae of *Membranipora membranacea* cm^{-2} that settled on *Saccharina latissima* pretreated at 21 ˚C or at ambient temperature when it was offered as a choice, compared to when it was offered without a choice (Fig. 3.6; independent samples t-test: pretreated at 21 ˚C, $t_{13} = -0.31$, $p = 0.76$; pretreated at ambient, $t_9 = -0.08$, $p = 0.93$). The lack of a difference between choice and no-choice

treatments indicates that larvae of *M. membranacea* have no preference for or avoidance of kelp pretreated at 21 ˚C compared to kelp pretreated at ambient temperature.

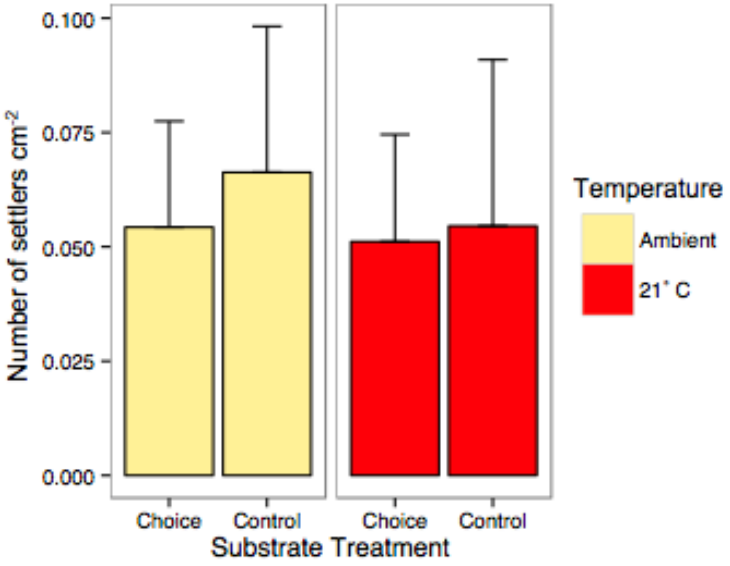

Figure 3.6 Mean (± 1 SE) number of settlers of *Membranipora membranacea* cm^{-2} on *Saccharina latissima* pretreated for 10 d at ambient temperature, or *S. latissima* pretreated 10 d at 21 ˚C, when a choice of substrates was offered and in a control with no choice of substrates (data from 5 trials pooled, n = 5 – 9). Ambient temperature (mean ± SD): 14.4 ± 2.2 ˚C for 3 trials in Oct/Nov 2013; 9.3 ± 2.1 ˚C for 2 trials in Jul/Aug 2014.

3.5 Discussion

3.5.1 Effects of temperature on chemical composition of kelp tissue

The increase in C/N ratio observed in *Saccharina latissima* over the summer follows the expected pattern of seasonal variation in this species (Gevaert et al. 2001, Nielsen et al. 2014). This pattern results from both a decrease in N and an increase in C over the summer (Gevaert et al. 2001, Nielsen et al. 2014). Over winter, when growth is light limited, N is stored and then used to support growth in spring and summer (Nielsen

et al. 2014). During N-limited growth in summer, C is stored as carbohydrates to allow for continued growth through the winter (Neilsen et al. 2014). It has been suggested that changes in temperature also may contribute to this pattern. A decrease in N content with increasing temperature has been observed for *S. latissima* from Helgoland (Olischläger et al. 2014) as well as in the kelps *Ecklonia radiata* (Staerh & Wernberg 2009) and *Eisenia arborea* (Matson & Edwards 2007). We found that neither 1-wk exposure to 21 ˚C nor 3-wk exposure to 18 ˚C changed C/N ratio in any of the species we examined, relative to 11 ˚C. N content (% dw) of field-collected kelps at the start our experiment (mean ± SE: *Agarum clathratum*, 1.29 ± 0.02; *Laminaria digitata*, 0.92 ± 0.05; *Saccharina latissima*, 0.67 ± 0.08) was less than the critical value of 1.8 %, below which the growth of *S. latissima* is N limited (Chapman et al. 1978). This suggests that upon collection any stores of N had already been consumed and the kelps were N limited throughout the experiment.

Phlorotannin content in *Agarum clathratum* was reduced after 1-wk exposure to 21 ˚C compared to 11 ˚C. Phlorotannins are a known antioxidant defense in brown algae, induced by exposure to ultraviolet radiation (Gómez & Huovinen 2010, Cruces et al. 2012, Cruces et al. 2013). In these studies, phlorotannins were not induced under thermal stress for periods of up to 72 h, despite increased lipid peroxidation indicative of a rise in activity of reactive oxygen species (Cruces et al. 2012, Cruces et al. 2013). Furthermore, temperature stress (20 and 28 ˚C) was found to inhibit induction of phlorotannin by ultraviolet radiation (Cruces et al. 2012, Cruces et al. 2013), suggesting that high temperatures may hinder the ability of kelp to produce phlorotannins, possibly by damaging the membranes of the Golgi-ER complex, where phlorotannins are produced

(Schoenwaelder & Clayton 2000). Phlorotannin content in *A. clathratum* was unchanged by 2-wk exposure to 18 ˚C, suggesting that there may be a threshold for temperature-induced reductions in phlorotannin content for this species.

In contrast to *Agarum clathratum*, there was no effect of temperature on phlorotannin content of either *Saccharina latissima* or *Laminaria digitata*. Phlorotannin content of *S. latissima* and *L. digitata* were low, although similar to previously reported levels in these species: 0.9 – 2.5 % dw and 0.15 – 0.3 % dw, respectively (Johnson & Mann 1986, Connan et al. 2006, Dubois & Iken 2012), reducing our ability to detect any effect of temperature. Measurement of non-phlorotannin compounds may have further hindered our ability to detect any effect of temperature (Van Alstyne 1995).

In the field, there was a pattern of increasing phlorotannin content over the summer in *Saccharina latissima*, followed by a decrease in September in 2014. Although phlorotannin content was similar in summer and winter in *S. latissima* in Alaska, and unrelated to irradiance or nutrient availability (Dubois & Iken 2012), in other brown algae (primarily the order Fucales) phlorotannin content peaks in the spring or summer (Steinberg 1995, Stiger et al. 2004, Kamiya et al. 2010). Higher phlorotannin content in summer has been attributed to increased grazer density, which can induce phlorotannin production (Van Alstyne 1988), or greater energy availability for phlorotannin production during periods of growth limitation (Steinburg 1995, Stiger et al. 2004).

3.5.2 Effects of temperature on kelp as a food source

Lacuna vincta consumed more kelp pretreated at 21 ˚C than at 11 ˚C in trial 1 of both the choice and no-choice feeding experiments, but not in the other 2 trials. The rate

of consumption of kelp in trial 1 was more than twice that in trial 2 or 3 in the choice experiment. The rate of consumption of kelp pretreated at 21 ˚C in trial 1was at least 2.5 times greater than that in trials 2 or 3 in the no-choice experiment. Differences in ambient temperature among trials could have caused these differences in feeding rate in response to metabolic demand, however temperature in trial 1 was lower than that in trial 2 or 3.

Greater feeding rate of *Lacuna vincta* on kelp pretreated at 21 ˚C in trial 1 could reflect a temperature-induced change in the palatability of kelp tissue that is apparent only at high feeding rates. Given that chemical quality of *Saccharina latissima* was unaffected by temperature, temperature-induced changes to the mechanical properties of kelp may account for the observed increased feeding rate. Higher grazing rates on kelp pretreated at 21 ˚C in both the choice and no-choice experiments indicate that *L. vincta* did not have an active preference for this tissue, but rather that kelp pretreated at 21 ˚C is easier to consume. *Lacuna vincta* has a taenioglossan radula that is more efficient on softer tissues (Steneck & Watling 1982), and prefers young tissue, even in the absence of any difference in C/N ratio or phlorotannin content, likely due to differences in tissue toughness (Toth & Pavia 2002, Chenelot & Konar 2007). Increasing temperature damages and weakens kelp tissue (Table 3.6), and this weaker tissue may be easier for *L. vincta* to consume.

Table 3.6 Effects of temperature treatments (11, 14, 18 and 21 ˚C) on chemical and mechanical metrics of kelp tissue quality (C/N, phlorotannin content [% dw], blade tissue strength [MPa] and blade tissue extensibility [% length change]) for *Agarum clathratum*, *Laminaria digitata* and *Saccharina latissima*. Quality metrics were significantly decreased by exposure to the listed temperatures.

Property	Species	Temperature	Reference
C/N	*A. clathratum* *L. digitata* *S. latissima*	No effect	1
Phlorotannin	*A. clathratum*	21 ˚C	
	L. digitata *S. latissima*	No effect	1
Strength	*A. clathratum*	18 ˚C	
	L. digitata	18, 21 ˚C	2
	S. latissima	14, 18, 21 ˚C	
Extensibility	*A. clathratum*	No effect	
	L. digitata	21 ˚C	2
	S. latissima	14, 18, 21 ˚C	

[1]This study. [2]Chapter 2.

The greater consumption of kelp pretreated at 21 ˚C in trial 1 indicates that the effect of temperature on the palatability of kelp tissue may depend on the feeding rate of *L. vincta*, and that the direct effects of temperature on kelp tissue loss and temperature-induced changes in herbivory of *L. vincta* can be synergistic when feeding rates are high. However, when feeding rates are lower (as in trials 2 and 3) there is no evidence for temperature-induced changes in palatability of *Saccharina latissima*, and the impacts of temperature and *L. vincta* on kelp tissue loss will likely be additive.

Saccharina latissima is a nutritious food source for *L. vincta* that supports greater growth rates than other algal diets (Chavanich & Harris 2001). In our study, survival, growth and egg production of snails were unaffected by temperature-induced changes in kelp tissue, indicating that the nutritional quality of kelp did not change, as evidenced by

the lack of variation in C/N between temperature pretreatments. Mean growth rates of fed snails in our experiment (0.04 – 0.23 mm wk^{-1}) are similar to those previously recorded in the laboratory on a diet of *S. latissima* (0.04 – 0.12 mm wk^{-1}; Chavanich & Harris 2001). The similarity in the success of *L. vincta* (similar growth rates, survival, and reproductive output) fed kelp diets pretreated at 11 and 21 ˚C suggests that populations of *L. vincta* will likely remain stable with increasing temperature as long as kelp is available: i.e. there are no indirect effects of temperature that affect growth, survival or reproduction of snails.

3.5.3 Effects of temperature on kelp as a substrate

Temperature pretreatment of kelp tissue did not affect larval settlement of *Membranipora membranacea*. Abundance of settlers observed on kelp pretreated at ambient temperatures and that pretreated at 21 ˚C (mean: 0.05 – 0.07 settlers cm^{-2}) were comparable to the abundance of settlers on *Saccharina latissima* in the field during peak settlement (mean: 0.03 – 0.19 settlers cm^{-2}; Saunders & Metaxas 2007).

Larvae of *M. membranacea* demonstrate settlement preferences both among kelp species and among locations on the kelp thallus (Brumbaugh et al. 1994, Matson et al. 2010). They also exhibit fine-scale searching behaviour along the kelp substrate, which suggests they are able to detect differences in substrate quality (Matson et al. 2010). Across kelp species, larvae of *M. membranacea* show a preference for settlement on young tissue proximal to the blade meristem (Brumbaugh et al. 1994, Denley et al. 2014). The cues attracting larvae to young tissue are unknown, but the persistence of the preference when flow is reversed suggests that larvae use a physical or chemical

characteristic of the substrate to cue settlement (Brumbaugh et al. 1994). Phlorotannins have been suggested as a chemical cue preventing the settlement of fouling organisms (Wikström & Pavia 2004). Because the phlorotannin content of *Saccharina latissima* was unaffected by temperature, the kelp substrates in our experiment (pretreated at 21 ˚C and pretreated at ambient temperature, 9.3 – 14.4 ˚C) had similar levels of chemical deterrents. Brumbaugh et al. (1994) found that damage to the blade reduced settlement and postulated that larvae may avoid older sections of the blade due to greater levels of physical damage there. Temperature stress of 21 ˚C also damages and weakens kelp tissue (Table 3.6), however we saw no effect of this damage on settlement preference.

The lack of any temperature-induced changes in kelp quality as a substrate for settlement of *M. membranacea* suggests that increased population growth rate due to warming seawater temperatures (Saunders & Metaxas 2007, Saunders & Metaxas 2009) will not be retarded by lower settlement rates. The direct effects of temperature on kelp combined with temperature-mediated increases in *M. membranacea* populations are likely to cause large-scale biomass loss from kelp beds in Nova Scotia. However, this biomass loss could be mitigated if temperature-induced changes in substrate quality affect post-settlement mortality. Temperature-induced damage to the meristoderm could cause it break and peel away with the associated bryozoan colonies. Peeling of the outer layer of cells has been documented as a mechanical defense against fouling in several algal species (Sieberth & Tootle 1981, Nylund & Pavia 2005). Even in the absence of changes to post-settlement mortality, the loss of kelp biomass may in turn limit further outbreaks of *M. membranacea* by limiting the availability of preferred substrate.

3.5.4 Conclusions

An understanding of the simple and cumulative effects of environmental conditions on species interactions is imperative for predicting the effects of climate change on community function. We expected that warming seawater temperature would alter kelp tissue quality, which would in turn affect interactions between kelp and the mesograzer *Lacuna vincta* and encrusting bryozoan *Membranipora membranacea*. However, higher temperature did not affect the quality of *Saccharina latissima* as substrate for *M. membranacea,* and we observed temperature-induced changes in the consumption of kelp by *L. vincta* only when feeding rates were high. Nutrient content and chemical defense of both *S. latissima* and *Laminaria digitata* were unchanged by temperature, suggesting that the quality of these species as a food and a substrate would be similarly unaffected by increases in temperature. A temperature-induced reduction in the chemical defenses of *Agarum clathratum* at 21 ˚C suggests that this species might become more vulnerable to damage by *L. vincta* or *M. membranacea* at high temperatures.

We predict that the direct effects of temperature on kelp tissue, herbivory by *L. vincta* and encrustation by *M. membranacea* will act additively to increase biomass lost from kelp beds as seawater temperature increases. Warmer temperatures are expected to increase both outbreaks of *M. membranacea* (Scheibling & Gagnon 2009, Saunders & Metaxas 2007, Saunders & Metaxas 2009) and metabolic rates, and therefore feeding, of herbivores (O'Connor 2009). Encrustation by bryozoans, herbivory by *L. vincta,* and temperature-induced damage all weakened kelp tissue and increased vulnerability to wave forces (Krumhansl et al. 2001, Table 3.6). No antagonistic effects were manifested

through the inhibition of feeding by snails or settlement by *M. membranacea* that would mitigate other temperature effects on kelp. Using future climatic conditions (warming temperatures and larger waves), models of kelp detrital production have also predicted a loss of kelp biomass in Nova Scotia (Krumhansl et al. 2014). Reductions in standing kelp biomass could impact both habitat availability and community productivity (Dayton 1985, Steneck et al. 2002), while changes in the production and export of kelp detritus will impact adjacent coastal and deep-water ecosystems (Krumhansl & Scheibling 2012).

CHAPTER 4

Conclusion

This thesis documents the direct and indirect effects of warming seawater temperature on kelp species in Nova Scotia. Increasing temperature directly damaged kelp tissue, leading to decreased tissue strength and extensibility and rendering kelp more vulnerable to tissue loss through erosion and breakage. Of the three species examined, *Agarum clathratum* was more resistant to temperature-induced damage and less vulnerable to tissue loss than both *Laminaria digitata* and *Saccharina latissima*. The resistance of *A. clathratum* to increasing temperature may allow this species to expand into shallower habitats from which it is currently excluded by competitors *L. digitata* and *S. latissima* (Vadas 1968, Gagnon et al. 2005), thus changing the composition of Nova Scotia kelp beds. Loss of kelp biomass could also lead to the increasing dominance of ephemeral turf algae. Field surveys of changes in density of kelp and turf species over of time are needed to test these predictions of changing community composition. Observations of increasing kelp tissue strength over summer may be due to acclimation to warming temperature, suggesting that rapid short-term changes in temperature will have greater effects on kelp population than gradual warming. Further research is necessary to determine the ability of kelp species to acclimate their material properties to changing temperatures, and the time scales over which acclimation can occur.

This thesis found little evidence for indirect effects of temperature on Nova Scotian kelp species. Increasing temperature did not alter the quality of kelp tissue as a

food source for *Lacuna vincta* or as a settlement substrate for *Membranipora membranacea*. Grazing rate of *L. vincta* was greater on kelp pretreated at 21 ˚C than on kelp pretreated at 11 ˚C only when total consumption of kelp by snails was high. A lack of temperature-induced changes in kelp tissue quality suggests that the direct effects of temperature on kelp will act additively with the effects of *M. membranacea* and *L. vincta* to increase biomass loss from Nova Scotia kelp beds. Although this thesis found little evidence for increased tissue loss due to temperature-induced changes in kelp tissue quality, there was a potential synergistic interaction. Greater grazing rates on kelp pretreated at 21 ˚C when total kelp consumption by snails was high suggests warming temperature could increase the loss of kelp tissue both directly, and indirectly, by increasing the grazing rates of *Lacuna vincta*. Further investigation could establish the circumstances under which this synergism might arise and determine the effect warmer temperatures may have on snail grazing and population size. Further, to conclusively determine that temperature has no effect on the quality of kelp as a substrate for *Membranipora membranacea*, the effect of kelp tissue quality on post-settlement survival and growth of the bryozoan should be assessed.

This thesis provides a mechanistic link between warming seawater temperatures and observed declines of kelp populations. Temperature-induced damage resulting in the weakening and loss of kelp tissue could be impacting kelp populations in Nova Scotia and kelp systems worldwide. Declines in Nova Scotian kelp beds are likely due in part to peaks in seawater temperatures that act additively with other agents that damage kelp to increase biomass loss and slow recovery. Globally, sublethal warming seawater temperatures can increase the vulnerability of kelp populations to disturbances such as

large storms (Wernberg 2010), and can act synergistically other stressors, such as increases in dissolved CO_2 and eutrophication (Anderson et al. 2011, Connell & Russell 2012, Moy & Christie 2012), to cause declines in kelp populations. Predicted large-scale loss of biomass from kelp beds will have broad-ranging effects on coastal and adjacent deep-water communities due to changes in community composition and loss of habitat structure and productivity.

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APPENDIX A

Effects of tank on growth rate, length change, and breaking stress and strain

Table A.1 Results of 2-way ANOVA comparing breaking stress, maximum strain, length change and growth rate of *Agarum clathratum, Laminaria digitata* and *Saccharina latissima* among 3 exposure times (1-, 2- and 3-week exposure, fixed factor) and 4 tanks (random factor) with all tanks held at 11.5 ˚C. Significant results given in bold.

Species	Variable		DF	F	p
A. clathratum	Stress	Tank	3, 24	0.11	0.95
		Exposure	2, 6	10.31	**0.01**
		Tank x Exp	6, 24	0.58	0.75
	Strain	Tank	3, 23	0.35	0.79
		Exposure	2, 6	6.10	**0.04**
		Tank x Exp	6, 23	1.45	0.24
	Length change	Tank	3, 24	0.22	0.88
		Exposure	2, 6	23.14	**0.002**
		Tank x Exp	6, 24	0.17	0.98
	Growth rate	Tank	3, 24	0.38	0.77
		Exposure	2, 6	1.02	0.42
		Tank x Exp	6, 24	1.76	0.15
L. digitata	Stress	Tank	3, 24	1.92	0.15
		Exposure	2, 6	0.67	0.55
		Tank x Exp	6, 24	1.03	0.43
	Strain	Tank	3, 22	1.11	0.36
		Exposure	2, 6	2.48	0.16
		Tank x Exp	6, 22	0.12	0.99
	Length change	Tank	3, 24	0.95	0.43
		Exposure	2, 6	0.31	0.74
		Tank x Exp	6, 24	0.84	0.55
	Growth rate	Tank	3, 24	3.35	**0.04**
		Exposure	2, 6	0.54	0.61
		Tank x Exp	6, 24	4.71	**0.003***
S. latissima	Stress	Tank	3, 24	1.03	0.40
		Exposure	2, 6	3.63	0.09
		Tank x Exp	6, 24	1.53	0.21
	Strain	Tank	3, 24	1.13	0.36
		Exposure	2, 6	1.13	0.38
		Tank x Exp	6, 24	1.72	0.16
	Length change	Tank	3, 24	0.79	0.51
		Exposure	2, 6	1.20	0.36

Species	Variable		DF	F	p
S. latissima	Length Change	Tank x Exp	6, 24	1.48	0.23
	Growth rate	Tank	3, 24	2.42	0.09
		Exposure	2, 6	10.01	**0.01**
		Tank x Exp	6, 24	0.40	0.87

*Effects of tank within each exposure time:
Week1 $F_{3,8}$=0.16, p=0.92; Week 2 $F_{3,8}$=19.46, p<**0.001**; Week 3 $F_{3,8}$=1.22, p=0.36

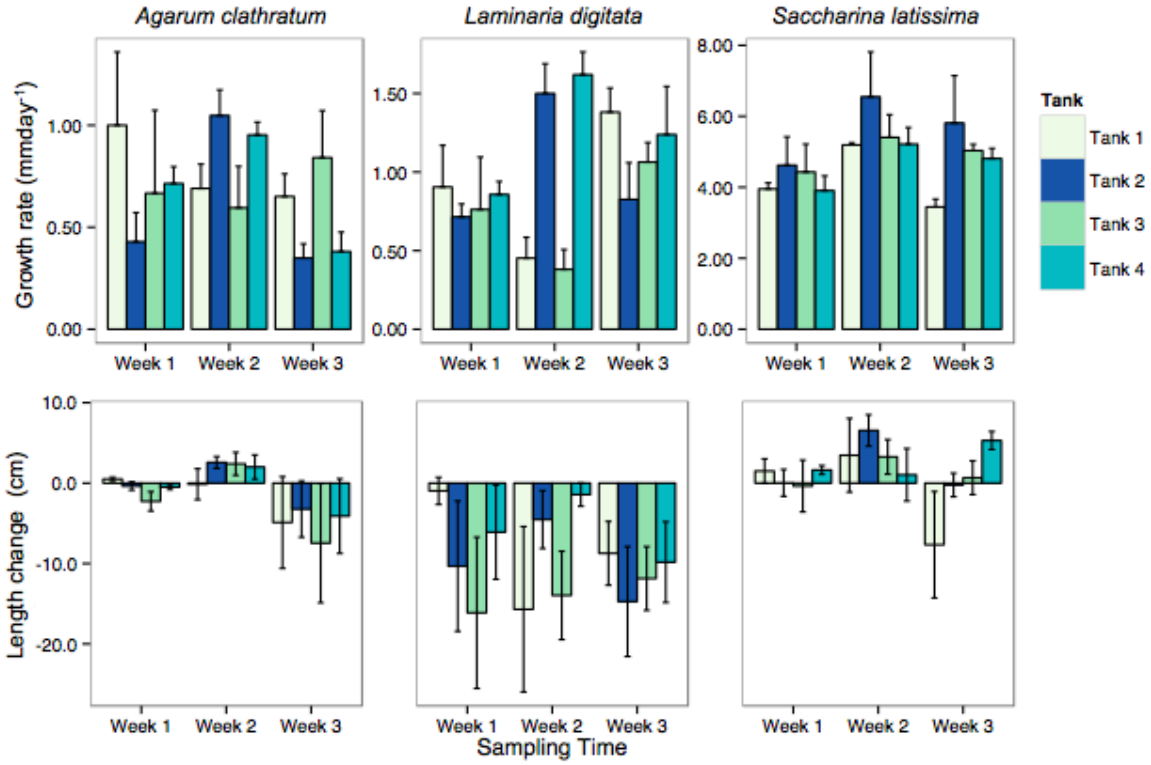

Figure A.1 Mean (± 1 SE, n = 3) growth rate or net length change of *A. clathratum*, *L. digitata*, and *S. latissima* individuals after 1-, 2- or 3-wk exposure at 11.5 ˚C in 4 tanks.

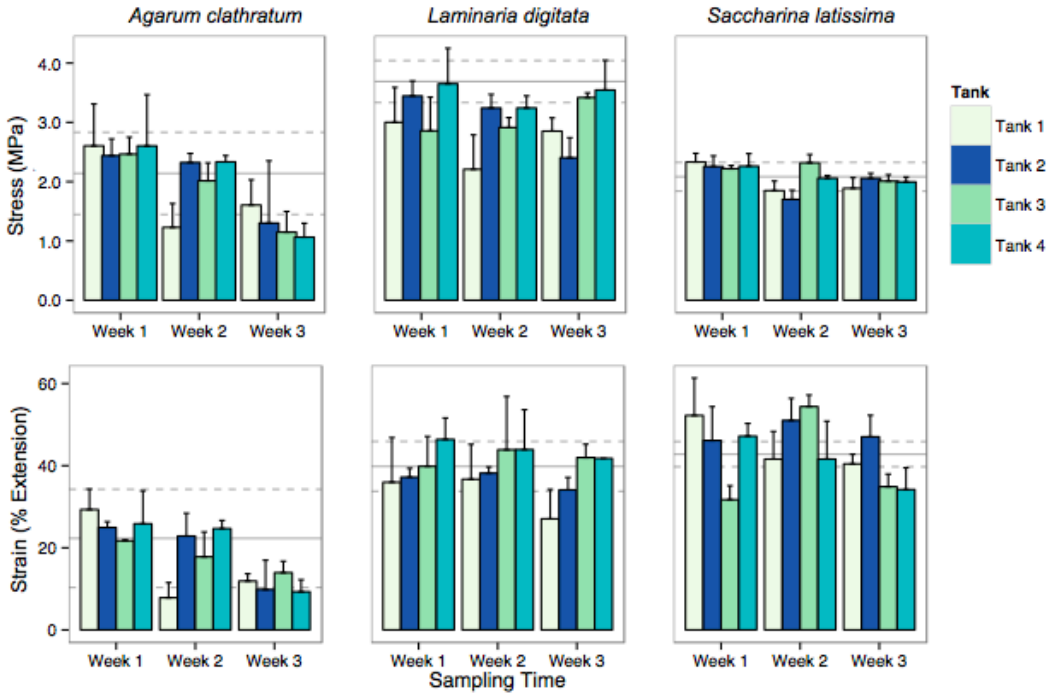

Figure A.2 Mean (± 1 SE, n = 3) stress or strain at breaking of *A. clathratum*, *L. digitata*, and *S. latissima* tissue samples taken after 1-, 2- or 3-wk exposure at 11.5 ˚C in 4 tanks. Solid and dashed lines represent mean ± 1 SE stress or strain at breaking of initial tissue samples taken upon collection (n = 3).

APPENDIX B

Growth and erosion rates of *Saccharina latissima* and *Laminaria digitata* in the field

Table B.1 Mean (± SE) growth rate (mm d^{-1}) and erosion rate (cm wk^{-1}) of *Saccharina latissima* and *Laminaria digitata* from 4 – 6 m depth at 3 – 5 sites (n) near Halifax, Nova Scotia in summer 2008 and 2009, (based on measurements of 10 – 30 individuals at each site; Krumhansl and Scheibling 2011b). For site locations and measurement methods, see Krumhansl and Scheibling 2011a.

Variable	Species	Date	Mean	SE	n
Growth rate (mm d^{-1})	*Saccharina latissima*	Jul 2008	5.44	0.85	5
		Sep 2008	4.42	0.47	5
		May 2009	9.09	0.69	5
		Sep 2009	3.06	0.03	5
	Laminaria digitata	Jul 2008	1.99	0.40	3
		Sep 2008	1.48	0.24	4
		May 2009	3.66	0.52	4
		Sep 2009	1.53	0.42	4
Erosion (cm wk^{-1})	*Saccharina latissima*	Jul 2008	5.22	0.84	5
		Sep 2008	5.38	0.98	5
		May 2009	6.21	1.34	5
		Sep 2009	9.39	1.88	5
	Laminaria digitata	Jul 2008	3.31	1.18	3
		Sep 2008	3.14	0.92	4
		May 2009	4.06	1.13	4
		Sep 2009	4.70	0.64	4