

Growth, survival  
and reproduction  
in the kelp

*Saccharina latissima*

Seasonal patterns  
and the impact of epibionts

Dissertation presented for the degree of  
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Yet if in any country a forest was destroyed, I do not believe nearly so many species of animals would perish as would here, from the destruction of the kelp.

– Charles Darwin  
(Voyage of the Beagle, June 1<sup>st</sup>, 1834)



# Preface

Compiling years of work into this synthesis has been the most rewarding part of my period as a PhD-student. It has been exciting to see how the pieces finally fit together, how the story grew and how what seemed hopeless for frustratingly long periods actually made sense in the end. I have learned a lot, sworn a lot, cried a lot, laughed a lot, gotten a couple of years older and a couple of pints wiser, and although it's been rough at times, I am glad I followed through. But, I wouldn't have gotten this far without co-workers, friends and family.

First, I would like to thank my supervisors, professor Stein Fredriksen, Hartvig Christie and Frithjof Moy for trusting me with the fellowship and involving me in the project and for all your advice. Frithjof, Hartvig and Lise Tveiten have all been instrumental in doing hard labor in the field whether it was sunny and warm or snowy and freezing cold (without *ever* complaining), and for that I am very grateful. Hartvig, for your constant support and your kindheartedness I will always be particularly indebted. I would also like to thank Morten Foldager Pedersen, Søren Nielsen and the people I came in contact with in Roskilde for welcoming me into their lab, for all the discussions and for teaching me a bunch of stuff. It was great fun to work with you. Thanks to Henning Steen and Kjersti Sjøtun for willingly answering all my questions and offering great advice. A special thanks also to Søren Larsen for “ruthless” and insightful comments on a previous draft of this thesis - you were mostly right. Kristina, your help was also much appreciated.

Marianne, Ragnhild, Benedicte and Ingvild - Thank you for your warmth and patience, your support, your jokes, the dinners, the drinks and all the laughs and hugs that dragged me out of my frustrating bubble and kept my head in a reasonably sane place most of the time.

Thanks to mum and dad who have always offered a safe harbor. Thanks to my siblings, Marte, Mari and Even, my partner in life, Lars, and my dog, Birk, for cheering me on and making me feel stronger every day. Lars - I would not have gotten through this without our long discussion, your quirkiness and your awesomeness.

And to my grandmother, mormor; your strength is inspiring - I dedicate my thesis to you.

# List of papers

This PhD thesis is based on the following list of publications and manuscripts (I–IV). They are later referred to by their Roman numerals.

- I:** Sogn Andersen, G., Steen, H., Christie, H., Fredriksen, S. & Moy, F.E. (2011)  
**Seasonal Patterns of Sporophyte Growth, Fertility, Fouling, and Mortality of *Saccharina latissima* in Skagerrak, Norway: Implications for Forest Recovery.**  
*Journal of Marine Biology*, **2011**, 1–8.
- II:** Sogn Andersen, G., Christie, H. & Moy, F.E.  
**In a squeeze: Epibionts may affect the distribution of *Saccharina latissima*.**  
*Ready for submission.*
- III:** Sogn Andersen, G.  
**Patterns of *Saccharina latissima* recruitment.**  
*Accepted for publication in PLoS ONE.*
- IV:** Sogn Andersen, G., Foldager Pedersen, M. & Nielsen, S.L. (2013)  
**Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae).**  
*Journal of Phycology*, **49** (4), 689–700.



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# Abstract

Recent large-scale loss of the kelp *Saccharina latissima* from the south and south west coast of Norway has raised considerable concerns. Kelp forests are high-productive areas that provide ecosystem services on which many coastal communities depend. Both the ecological and economical consequences of kelp forest loss is therefore likely to be negative, – and substantial.

*S. latissima* is a cold temperate water species, and events of extreme high sea water temperatures in the late 1990's and early 2000's have been considered the most plausible driver of the initial losses. Small populations remained as scattered patches along the impacted coastline, and on the west coast, these populations were able to recolonize some areas within a couple of years. Less forest recovery was observed along the south coast, and in Skagerrak most areas are still devoid of kelp. The lack of recolonization in Skagerrak can not be explained by temperature effects alone, since the sea water temperature has remained within the tolerance range of *S. latissima* for several years.

This synthesis investigates important aspects of the biology of Norwegian *S. latissima*. In doing so I hope to provide a basis for identification and further investigations of mechanisms that prevent kelp forest recovery in Skagerrak at present. Firstly, the seasonal pattern of growth, reproduction and mortality in a sample population was documented in order to pinpoint the periods in which to look for potential vulnerabilities, i.e. periods of high mortality or failure to reproduce (Paper I). Secondly, the seasonal pattern was investigated at several depths and including both extremities of a recovery gradient spanning from the south west to the Skagerrak coast of Norway (Paper II). In both studies (Paper I and II), high mortality of kelp coincided with the settlement and growth of epibiotic organisms covering the kelps' tissue. The epibionts may impact the kelp particularly through shading, reducing the light available for photosynthesis, and thereby the kelps' ability to harvest energy. The shading caused by epibiont layers was therefore documented (Paper II), and the impact was shown likely to be substantial, especially in summer and fall (Paper II in relation to Paper IV). Thirdly, the seasonal patterns in *Saccharina latissima* reproduction was investigated in order to assess the recruitment potential in Skagerrak (Paper III). Lastly, temperature acclimation of photosynthesis and the level of tolerance in relation to high temperature stress was investigated in *S. latissima*

from the south west and Skagerrak coast of Norway (Paper IV). All specimens experienced high levels of stress at temperatures reaching 20 °C, supporting the notion that high temperature events harm *S. latissima* populations.

The sampled kelp populations followed a normal seasonal pattern, with reproduction in winter, and high rates of growth in spring and early summer. In fall however, heavy loads of epibionts on kelp plants in the depth range from 1 to 9 m coincided with high mortality in Skagerrak. The probability of survival was highest (close to 60 %) between 10 and 15 m depth, while most kelp at 24 m depth died. These results suggest that a range contraction may have occurred in Skagerrak, rendering kelp recolonization and forest recovery difficult. Studies over greater spatial and temporal scales are however needed in order to fully validate this hypothesis. Finally, I briefly summarize my results in the context of kelp forest management and measures aimed at forest regeneration.

# Chapter 1

## Introduction

Kelps, seaweeds and sea grasses provide the foundation for important ecosystem services, like food-web production processes that results in making goods available for exploitation. The macrophyte communities have therefore been, and are still, of considerable economic and cultural value for many societies. Recently, large-scale loss of these marine communities has become a widespread concern, and the contemporary scientific debate has received contributions from several corners of the world [see e.g. 38, 59, 61, 39, 25].

The fundamental ecological niches of macrophytes largely depend on the species' requirements for light, nutrients and substrate, and its tolerance-limits in relation to temperature and salinity. Within these boundaries, the distributions are also limited by interspecific competition and grazing. For decades, sea urchin grazing was considered the strongest driver of kelp deforestation [51]. Although grazing remains influential, the recent focus has shifted towards environmental change and pollution, as the impacts of these factors on the rest of the biosphere (including grazers) have become increasingly evident. The list of drivers responsible for kelp losses now includes ocean warming, eutrophication, overfishing, competitive exclusion and other biotic interactions [8, 18, 29, 60, 62, 36, 16, 39, 25]. Some authors worryingly emphasize that the effect of any stressor on the kelp forest ecosystems will depend upon the presence and magnitude of other limiting or disruptive stressors [see 25]. Anthropogenically forced changes in the environment tend to occur simultaneously, and synergism may therefore reinforce their impacts. The web of interacting mechanisms that reduce macrophyte communities may look different in different areas, and the holistic picture that emerges from around the globe is increasingly complex.

### 1.1 Drivers causing loss of kelp forests

Due to climate change, the average sea surface temperature has increased [40]. The geographical distribution of most marine algae is determined by water temperature [56, 33, 58], and ocean warming is expected to cause changes in the global distribution and

abundance of macrophytes [57, 1, 38, 5]. Temperature driven distribution changes have already been documented for inter-tidal seaweeds [61, 13], and to some extent also for kelps [49, 29, 62]. In addition to a general temperature increase, another important consequence of climate change is the higher frequency of extreme temperatures [40, 54], and these events can be detrimental to kelp forests [62].

Climate change has also caused increases in precipitation and in the number freeze-thaw cycles, ultimately causing fresh-water run-off from land masses into coastal waters. The increased input of fresh-water, loaded with organic material, particles and nutrients, has reduced the transparency of the sea water in coastal areas (referred to as coastal darkening) [2, 3]. The bursts of nutrients and organic matter from land masses stimulate production and algal blooms [e.g. 52] which add to the darkening of the sea water and result in increased accumulation of sediments on the sea floor [11]. The combined effects of high water temperature and reductions in water transparency may reduce the abundance and incidence of kelp [34]. Further, thick layers of sediments may hinder recovery by impairing spore attachment, increasing sand scour which detaches settled recruits, by blocking light, and by causing anoxic conditions that reduce the survival of recruits [23, 48, 12].

An alternative ecological system can become permanent, even if the pressure from the driver of the ecological shift was to be released (e.g. when the temperature goes from extreme high and back to normal), because new mechanisms that reinforce the change may be established [39]. On several coasts where humans have altered the chemical, physical and biological conditions in the ocean through harvesting, land-use and pollution, canopies of kelp and seaweeds have been replaced by mats of turf-forming algae [15, 10, 36]. While kelp canopies inhibit turfs [28, 44, 16], reduced water quality resulting in more nutrients and less light may give the turf communities a competitive advantage and enable them to expand [23]. Subsequently, the cover of turf algae may hamper the recruitment of kelp and regeneration of kelp forests. Gorman and Connell [23] demonstrated a positive correlation between established turf carpets and sediment accumulation, that ultimately seemed to inhibit canopy recovery in kelp forest areas. Deforested coastal areas may thus be the result of many different factors and cascading events.

## 1.2 Kelp loss in Norway: *Saccharina latissima*

*Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders (formerly known as *Laminaria saccharina*) is a common forest founding kelp species in the North Atlantic. The species used to dominate most of the sub-tidal vegetation in sheltered areas along the Norwegian coast, but reports of *S. latissima* forests in decline started to

emerge in the late 1990's and early 2000's. Later surveys [37, 35] showed that the forest deterioration had been extensive somewhere in between 1996 and 2002, but the lack of historic baseline data has rendered accurate assessments difficult. Estimates of losses range from 51 to 80% [6, 36] along the Norwegian Skagerrak coast (approx. 7 900 km), while a loss of 40% was estimated from the west coast up to Møre and Romsdal county (approx. 26 000 km) [36].

Moy and Christie [36] offered multiple explanations for the onset and continuance of the forest deterioration, but the lack of empirical data unfortunately left proper testing of their hypotheses impossible. That being said, *S. latissima* is considered a cold-temperate water species [63], and a couple of summers with extraordinarily high temperatures were considered the most likely initiator [36]. The Norwegian surveys also showed that the kelp beds were replaced by turfs of ephemeral algae, suggesting that inhibition of forest recovery through competitive exclusion may have reinforced the demise.

While large kelp forest areas along the west coast of Norway recovered in cooler periods, most areas in Skagerrak remained devoid of kelp [36]. The mechanisms responsible for the lack of regrowth in Skagerrak have been unknown. However, a substantial change in the vertical distribution of many macroalgae has occurred in Skagerrak, and reduced water transparency seems to have been an important driver [43, 41, 15]. The range of mechanisms and cascading events identified as drivers of increased loss and reduced kelp fitness elsewhere on the globe, thus, seem highly relevant in Norway as well.

### 1.3 Fitness and ecotypes

Fitness is a central idea in evolutionary theory, and can be defined as a phenotypes ability to survive and reproduce in a given environment. As the environment change, the combination of traits that are most likely to ensure the reproductive success and the survival of a species may also change.

Genetic differentiation on a geographic scale typically occurs as populations adapt to a locally specific set of environmental conditions, and a geographically distinct variety of a species is often defined as an ecotype (*sensu* Turesson [55]). Ecotypic differentiation is therefore expected in broadly distributed seaweed species [14]. *Saccharina latissima* is a widely distributed species, known to comprise populations that differ both morphologically and physiologically [4], and ecotypic differentiation has been documented in relation to both thermal stress and light related responses within the species [19, 22, 20, 21, 7, 32]. The ability of *S. latissima* to acclimate/acclimatize and tolerate environmental stress

is therefore likely to depend on adaptations that vary geographically. Still, few investigations and comparisons of physiological traits in Norwegian populations have been published previous to the works included in this synthesis.

## 1.4 The scope of the synthesis

The main objectives of the present thesis is to 1) evaluate the ability of *Saccharina latissima* to grow, reproduce and survive in the water column in Skagerrak and 2) identify factors that reduce the fitness of Norwegian *S. latissima*, and therefore are likely to obstruct recovery. More specifically, for each paper the aim is to:

### **Paper I:**

Document the seasonal patterns of *S. latissima* growth, sporophyte fertility, fouling by epibionts and mortality in Skagerrak.

### **Paper II:**

Investigate the seasonal and depth related patterns in growth responses, survival and fouling on kelp from both Skagerrak and the west coast regions. Secondly, to investigate the shading properties of the epibionts most commonly found on kelp fronds in Skagerrak.

### **Paper III:**

Investigate the seasonality in *S. latissima* recruitment and the extent of coupling to sori development in the parent population. Secondly, to contribute with an evaluation of dispersal potentials and the potential for connectivity between *S. latissima* populations in Skagerrak.

### **Paper IV:**

Examine the ability in *S. latissima* for temperature acclimation of photosynthesis and the level of tolerance in relation to high temperature. Secondly, we investigate whether kelp from different parts of a temperature gradient along the Norwegian coast may represent different temperature ecotypes.

# Chapter 2

## Results and Discussion

### 2.1 Seasonal patterns

The seasonal variations in abiotic conditions affect the growth performance in most photosynthesizing organisms. Because the seasonality of temperature, irradiance and photoperiod is most pronounced at high latitudes, algae in these areas are particularly affected. The growth in N Atlantic species of *Laminaria* (including *S. latissima*) is generally rapid in winter and spring, and slows down in summer [4, and references therein]. As expected, we found the highest rates of growth in spring, and the lowest in summer and fall (Paper I and II, see Figure 2.1). These results are also in concurrence with a previous study from the west coast area [50], indicating a consistency in the seasonality of *S. latissima* growth along the entire south to south west coast of Norway.

The formation of sori (meiospore producing tissue) in adult kelp was initiated in fall and continued throughout the winter in Skagerrak (Paper I), and successful recruitment on clean, artificial substrate was observed all through the fertile period (Paper III). Recruitment was highest in winter, and highly connected to the development of sori on the parental kelp plants. The pattern of development seems synchronized along the south coast of Norway with the main season of reproduction in winter [Paper I, Dr.scient thesis of Kjersti Sjøtun, NIVA-reports (Sukkertareprosjektet), Unpublished data from Flødevigen (Arendal) and Espegrend (Bergen)], indicating that the potential for connectivity between the *S. latissima* populations is high (see Paper III). Paper I and III show that sori development, sporulation, gametophyte germination, gametogenesis, fertilisation and sporophyte germination was possible in the physical environment in the water column. However, the deforested areas in Skagerrak are now predominantly covered by turf algae and sediments [36], and these conditions may obstruct settlement and germination [12, 48]. Successful recruitment may therefore not have occurred on natural substrate.

The loss of monitored individuals was greatest in summer and fall (Paper I and II, see Figure 2.1). Sjøtun [50] reported extensive loss of *S. latissima* (at 5 m depth) in the same period in 1981 and 1982, but did not identify any particular cause. In the

works presented in this thesis, however, a link between loss of kelp plants and epibiont fouling was identified in Skagerrak (Paper I and II, see Figure 2.1). The epibionts started to settle in early summer (around June), and as the layers grew denser, the condition of the kelp individuals worsened. Sjøtun observed scattered occurrences of epiphytes (algal epibionts) on the west coast in her studies, but the epiphyte communities (mostly filamentous red algae) did not grow to the extent presently observed in Skagerrak [Sjøtun, personal communication]. Recent observations suggest that epibionts may have become more common, but they are still scarcer along the west coast compared to in Skagerrak [36, and own observations].

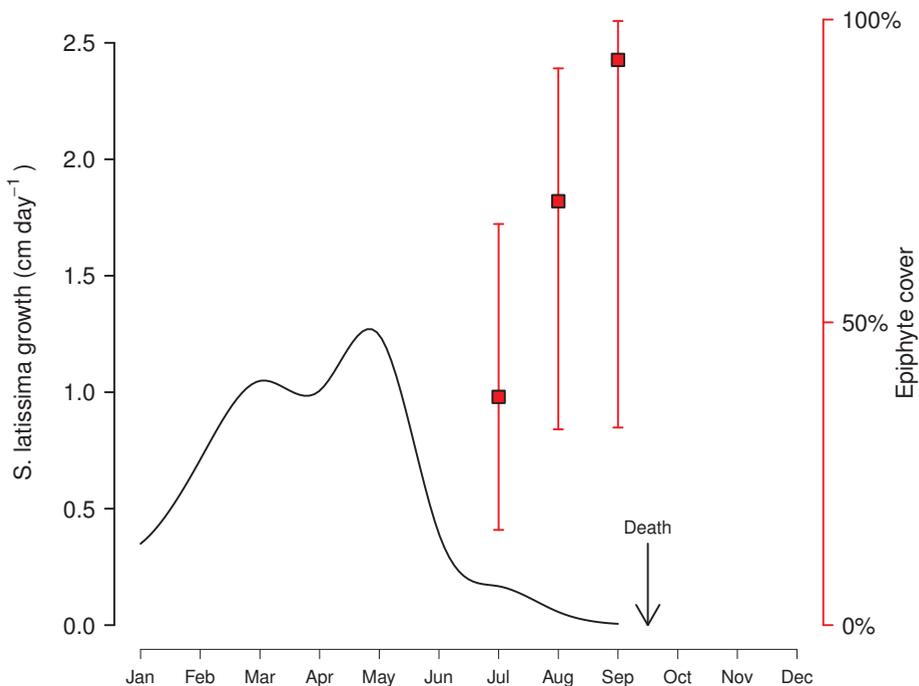


Figure 2.1: **Seasonal patterns** of growth, epibiont fouling and mortality of adult *Saccharina latissima* as observed in Skagerrak. The error bars represent the 95% confidence intervals of the average epibiont covers based on a binomial distribution. (Paper I gives a more detailed description.)

The field studies (Paper I and II) showed that the mortality of adult *S. latissima* sporophytes was high in fall in Skagerrak. Because fall is also the period in which reproduction is initiated, these results reveal a severe loss of fitness. The loss of fitness coincided with increasing covers of epibionts, indicating that fouling may have been an important driver.

## 2.2 Depth related patterns – kelp in a squeeze

Kelps, like all photosynthetic organisms, need light to sustain metabolic processes and life functions, and the depth to which a kelp can grow is limited by light availability [24].

A general decrease in *S. latissima* growth with increasing depth (1, 3, 6, 9, 15, 24 m) was observed, and this pattern was expected as a consequence of light attenuation in the water column (Paper II, see Figure 2.2). The pattern was, surprisingly, not consistent throughout the experiment. In fall, better growth and greater survival was observed at 15 m compared to at 3 m depth (Figure 2.2 and Figure 2.3) and this pattern seemed linked to the extent of epibiont covers (see e.g. Figure 2.6).

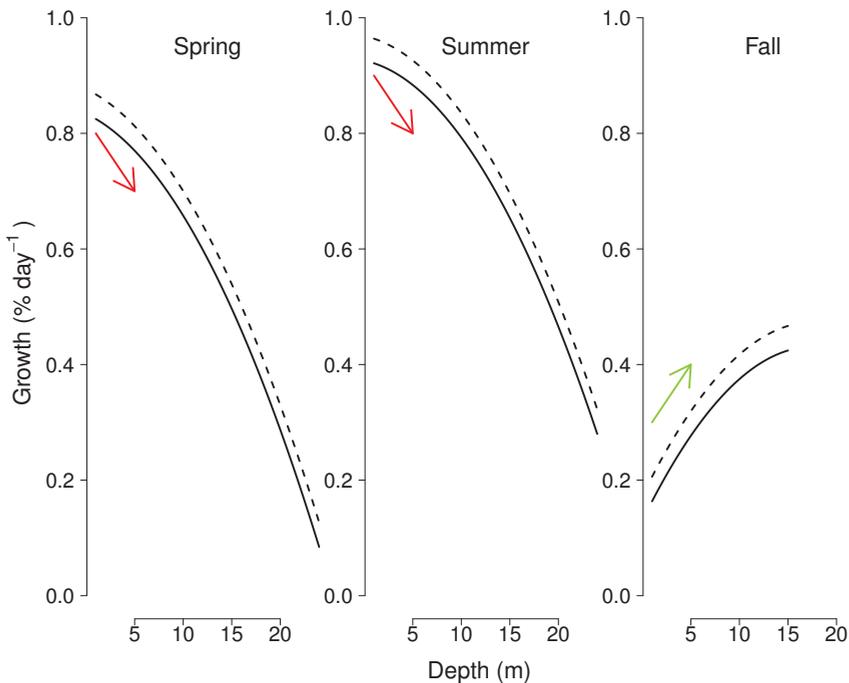


Figure 2.2: **Depth related patterns of growth** in *Saccharina latissima* from both the Skagerrak (solid line) and the west coast (dashed line) populations in summer, spring and fall. (Paper II gives a more detailed description.)

The survival of kelp plants was low at 24 m depth in Skagerrak. Although a large proportion of the individuals at 15 m depth in our study survived until fall, the light conditions may at some point become too low in winter (see Paper II and IV). Greater survival at 24 m depth was observed in the west coast area, which supports investigations indicating that *S. latissima* has a greater vertical range and grow deeper on the west coast

[53]. Both the depth range changes of macroalgae in Skagerrak and comparatively deeper ranges in the west coast area have been linked to light availability (see section 1.2). The darkening of coastal waters is considered a consequence of climate change, and if this is a progressing phenomenon (which is highly likely [see e.g. 40]), the depth range of *S. latissima* is likely to contract.

Survival of kelp plants was also low in the range from 1 to 9 m, and higher temperatures close to the surface in combination with the impacts of epibiont covers may underlie this pattern (shown in Figure 2.3).

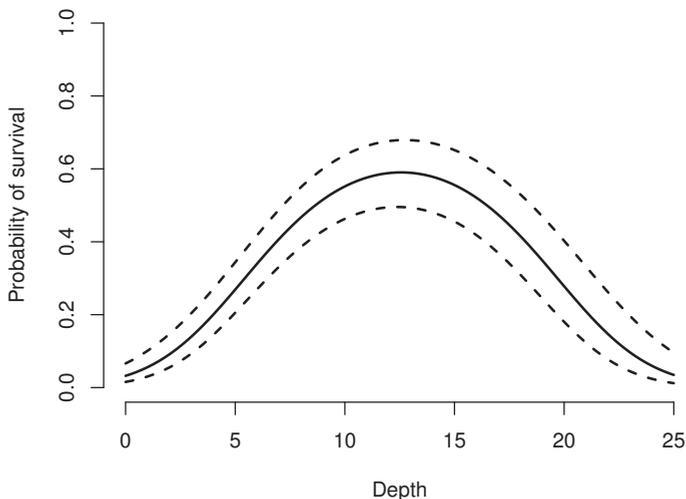


Figure 2.3: **Probability of survival with depth** predicted from fall data in Skagerrak. The dashed lines represent the standard error of the predictions. (Paper II gives a more detailed description.)

## 2.3 Temperature and fitness

Temperature affects both the performance (e.g. photosynthesis, growth and reproduction) and the survival of an algae, and therefore its fitness. The responses to temperature change involve three different types of mechanisms: (1) Genetic adaptation to new conditions (ecotypic differentiation); (2) phenotypic acclimatization in response to variation of environmental conditions (e.g. seasonal variations); or (3) short-term physiological regulation in response to environmental fluctuations (e.g. from day to night). While the regulation may take place on a timescale of seconds or minutes, acclimatization usually

takes days, and adaptation several generations [14]. The temperature responses within a species may therefore vary seasonally, among life stages and between ecotypes.

Easily measured parameters (e.g. photosynthetic efficiency) are often used as proxies for fitness parameters that may be more ecologically relevant (e.g. growth, survival and reproduction) [25]. Photosynthetic performance is considered a useful and practical measure of fitness in photosynthetic organisms, because it provides the foundation for success. The conversion of energy through photosynthesis has to be sufficient to fuel cell maintenance, growth and reproduction in order to sustain life functions in the organism and ensure recruitment to the population. The optimum temperature for *S. latissima* growth, reproduction and germination seems to be between 10 and 15 °C [see e.g. 38], and the results presented in Paper IV suggests that this is valid for the photosynthetic performance in individuals from southern and western Norway as well. The upper temperature limit has been determined at temperatures between 20 and 25 °C depending on life stage and geographical origin [see 38, for an overview], and the kelp plants we exposed to 20°C for several weeks also showed clear signs of reduced fitness (Paper IV). The respiration in kelp plants exposed to 20 °C increased considerably, and the maximum rate of gross photosynthesis ( $P_{\max}$ ) was reduced. The severe reductions in photosynthesis was caused by impaired functionality of Photosystem II as well as prominent pigment reductions. The low  $P_{\max}$  further indicated impairment of the activity of Rubisco, which would also have reduced the photosynthetic capacity of the specimen (see Paper IV). Much more light was therefore required for the kelp to sustain a positive carbon budget in high temperatures (>20°C) as compared to in optimal temperatures (between 10 and 15°C) (see Figure 2.4).

Even though light conditions were good in shallow waters, the growth was low and the mortality was high from 1 m and down to 9 m depth (Paper II), suggesting the kelps' ability to utilize the light may have been too poor in summer and fall (see Paper II). The temperatures were higher in shallow compared to deeper waters in late summer and fall, and slower growth and reduced fitness was expected at higher temperatures (Paper IV). However, poor growth and loss of kelp plants was also observed in years where temperatures remained within the limits *S. latissima* has been able handle in the past (Paper I and II). Hence, other factors may be involved in the continued lack of forest recovery in Skagerrak, and the studies included in this thesis suggest that the impact of epibionts may be important.

## 2.4 The impact of epibiont covers

The relationship between the macroalgal hosts and their epibionts range from mutualistic to parasitic in a continuous manner [42, and references therein]. Epibionts may decrease the hosts susceptibility to herbivores [see 31], facilitate nutrition in times of nitrogen

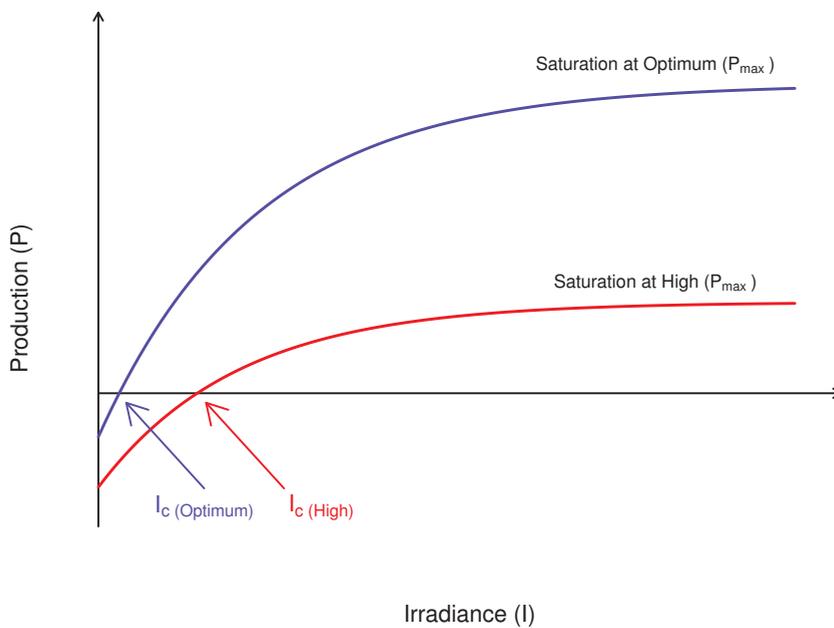


Figure 2.4: **Photosynthesis vs irradiance** measured in *Saccharina latissima* at optimum (10 and 15°C) and high (20°C) temperatures. The compensation points ( $I_c$ ) indicates the light levels where production equals consumption at the respective temperatures. (Paper IV gives a more detailed description.)

depletion [26, 27], and shield the host from damaging effects of excessive light. On the other hand, fouling exerts a wide range of adverse effects because epibionts may damage the host tissue, reduce light availability, increase the impact of mechanical stress, increase the probability of being eaten, reduce inflow of and compete for nutrients and carbon, reduce exchange of excretates and reduce the hosts reproductive output [17, 30, 46, 31, 47]. In Norwegian areas where the status of the forest is considered poor, *S. latissima* is usually covered by heavy loads of epibiontic organisms [see e.g. 36], and the relationship is probably more parasitic than mutualistic.

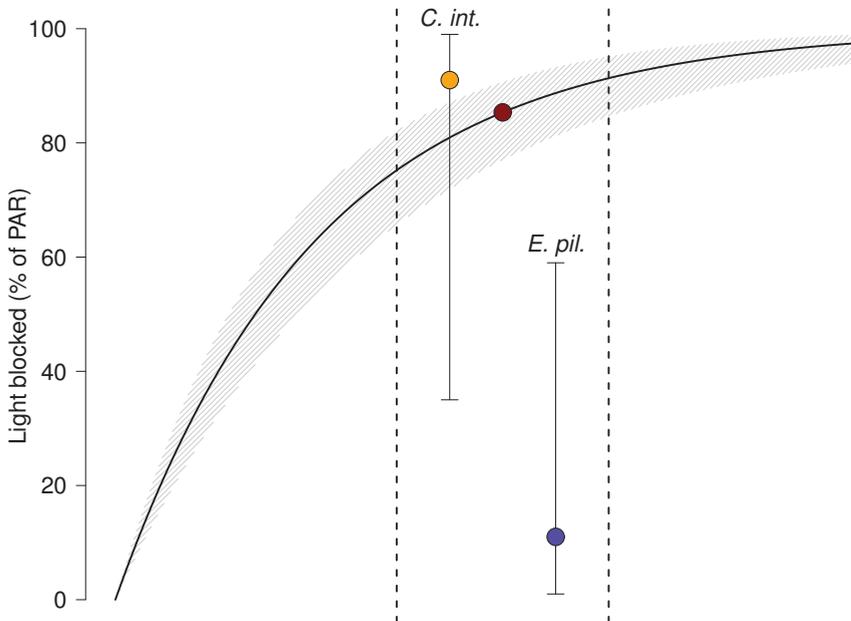


Figure 2.5: **Shading by epibionts** occurring on *Saccharina latissima* *in situ*. The error bars represent the 95% confidence interval for the average shading caused by covers of *Ciona intestinalis* (vase tunicate) and *Electra pilosa* (bryozo) (based on a binomial distribution). The dot without error bars marks the average density of algal epibionts (dry weight in  $\text{g cm}^{-2}$ ) found in October, while the vertical (dashed) lines indicates the 95% confidence interval for this average ( $0.02\text{--}0.04 \text{ g cm}^{-2}$ ). The curved (solid) line represents the modelled shading effect of increased algal densities, and the shaded area indicates the 95% confidence interval for this model. The x-axis is not printed because it relates only to the algal epibionts. (Paper II gives a more detailed description.)

In our studies, epibionts started to settle on the kelp fronds in Skagerrak in summer, and the covers grew progressively through summer and fall (Paper I and II). A depth

related pattern in epibiont growth was identified (Paper II), and the covers were much more extensive on shallow grown individuals (see Figure 2.6). The covers found on *S. latissima in situ* was further shown to absorb considerable amounts of light (Figure 2.5). In comparing the depth versus light profiles to light reductions expected from epibiont covers, we suggested that fouled kelp plants grown at 3 m depth experienced light conditions very similar to the clean kelp at 15 m depth (Figure 2.7). This indicates that growth conditions should have been approximately the same. However, better growth was found at 15 m compared to at 3 m depth. Explanations for this apparent discrepancy may be 1) that additional adverse effects of epibiont covers was at play (e.g. reduced inflow of nutrients and carbon), 2) that the epibionts deprived the kelp of more light than expected, 3) that higher temperature close to the surface made the kelp less efficient in utilizing light at 3 m as compared to at 15 m depth or 4) a combination of the above. Regardless, epibionts seem likely to have negative effects on the fitness of *S. latissima*.

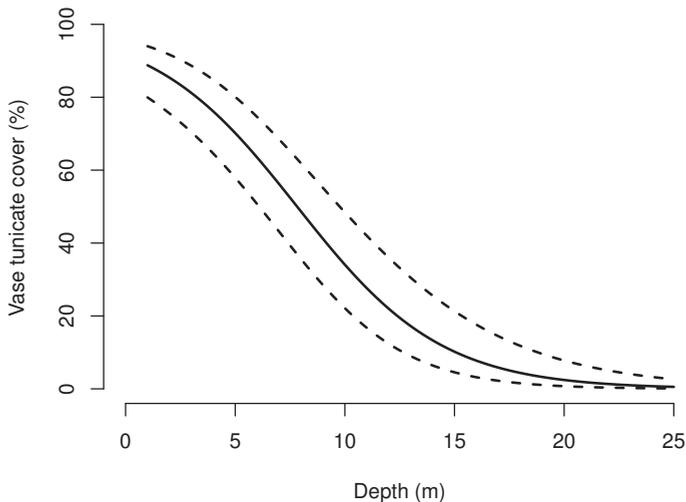


Figure 2.6: The cover of a light depriving epibiont (*Ciona intestinalis*) in relation to depth. The solid line represents model predictions, while the dashed lines represent the standard error of the predictions. (Paper II gives a more detailed description)

The higher presence of fouling organisms in shallow areas may constrain the distribution of *S. latissima* by pushing the kelps upper growth limit into deeper waters. In an area like Skagerrak, where survival seems limited by light availability, the downward push may be particularly critical. If the upper limit is pushed all the way down to the critical depth limit, the consequence will become total exclusion of the species. However, different adaptations (ecotypic differentiation) may render certain populations of the species

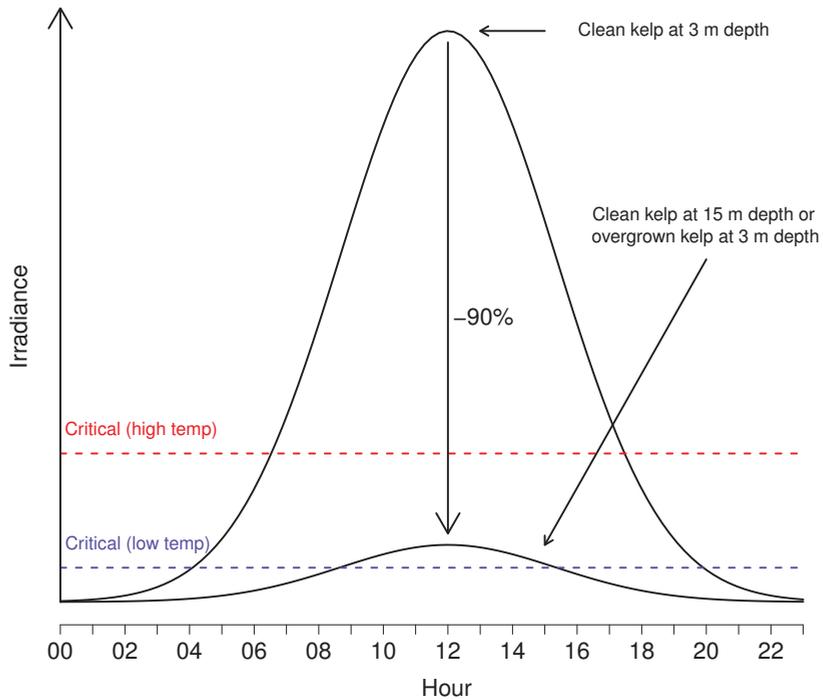


Figure 2.7: **Light received** by clean and fouled kelp at a 3 m depth and clean kelp at a 15 m depth in fall. The dashed line represent the minimum amount of light required to maintain a positive carbon budget at high (20 °C) and optimal (10 and 15 °C) temperatures respectively. (The concept is illustrated here, while Paper II and IV give more detailed descriptions.)

more tolerant towards both temperature and light stresses. Ecotypic differences between Norwegian populations may therefore be important to identify.

## 2.5 Ecotypic differentiation

Temperature acclimatization is common in kelps, and previous studies have documented a high potential in certain populations. Gerard and Du Bois [22] found that populations of *S. latissima* growing near their southern boundary in the NW Atlantic (New York State) endured higher temperatures than kelp from regions with lower water temperatures (Maine). The heat tolerance among the southern-most population was attributed to adaptations causing an improved ability to maintain high nitrogen (N) reserves. A high N reserve was thought to aid the production of heat shock proteins (HSPs) and to conserve photosynthetic performance under environmental stress [21]. Similar results have been reported for *Saccharina japonica* [32]. We found no clear evidence of ecotypic differentiation in relation to nitrogen reserves or temperature stress among the studied populations (Paper IV). Although kelp plants from the south and south west coast of Norway responded to the temperature treatments in the photo-physiological studies with slight differences (Paper IV), the magnitudes were not statistically significant.

Ecotypic differentiation in relation to light responses has also been reported in *S. latissima* [19], and if epibionts affect the kelp mainly through light deprivation, *S. latissima* from different populations could have different tolerance limits in relation to both depth and fouling. However, the similar patterns of growth and mortality in Skagerrak, where kelp from both sample populations were equally fouled by epibionts and equally affected by depth (Paper II), suggest that this is not the case.

## 2.6 A broader perspective

There is no doubt that extreme high temperatures reduce the fitness and cause high mortality in *S. latissima* populations. A couple of summers with extreme high temperatures in the late 90's and early 2000's may very well have caused massive population declines, as suggested by Moy and Christie [36]. As substrate previously occupied by *S. latissima* was made available, turf algae communities may have found a window of opportunity in which they could become established. Gorman and Connell [23] found a negative correlation between turf algae and canopy recovery in South Australia, and the shift from kelp to turf algae dominated communities may suggest that competitive exclusion has impaired kelp forest recovery in Norway as well.

On the west coast, the covers of turf algae and sediments are often reduced in winter [36]. Kelp spores are released and settle in the same period, and the availability of clean

substrate may explain why forests in this area seem able to recover. In Skagerrak, where turf algae dominate the sea-floor all year round [36], the lack of clean substrate may to a greater extent obstruct recruitment. Several studies indicate that ongoing climate change and human activities causing pollution, increased run-off and eutrophication reinforce the growth of turf algae communities [8, 14, 45, 64]. If competitive exclusion is limiting *S. latissima* forest recovery, the ongoing change in their environment is likely to make matters worse.

Falkenberg et al [16] suggested that regeneration and maintenance of canopy layers inhibit the establishment of turf communities, and enable the kelp forest habitats to persist. Physical abrasion caused by kelp fronds sweeping the sea-floor may prevent recruitment of sessile invertebrates and algae [9, 28], which otherwise dominate under lower light and high sedimentation. Opportunistic algae and sessile invertebrates are found both in turf algae communities and as epibionts on kelps (Paper I and II), and the presence of intact kelp forests may therefore control both the amount of turf algae and the fouling loads.



# Chapter 3

## Overall assessment

### 3.1 Conclusions

Clean and healthy *Saccharina latissima* is evidently able to grow and reproduce in the physical environment of the water column in Skagerrak (Paper I, II and III). However, the combination of heavy fouling by epibionts and high temperature in shallow waters may restrict the upper limit of the kelps' depth distribution, especially in fall (Paper I, II and IV). The lower limit of the depth distribution is, on the other hand, determined by the transparency of the water, which several lines of evidence [presented in section 1.1 and 1.2] suggest has been reduced. Paper II strongly suggests that the combination of several drivers has resulted in a contraction of the depth range of *S. latissima* in Skagerrak. If true, this range contraction is likely to hamper kelp forest recovery.

### 3.2 Is natural recovery in Skagerrak still possible?

This thesis suggest that recovery of Skagerrak kelp forests may be possible through the facilitation of natural recruitment. The results presented in Paper III suggest that the potential for connectivity between kelp populations along the south coast of Norway is high, and that the present day water environment does not impair recruitment. If this is true, then remnant populations should be able to recolonize the deforested areas. However, studies from other corners of the world indicate that both sediment and turf algae covers may prevent recolonization [see section 1.1 and 2.6], and the present day bottom conditions in Skagerrak may thus render recruitment impossible. In spite of this, I remain optimistic and suggest that management efforts aimed at the control of sedimentation and turf blooms may help establish a pioneer population. If canopy stands are in fact able to control the amount of sedimentation, turf algae, and epibionts [as suggested in section 2.6], the effect of their establishment may be an expansion of fully restored and resilient kelp forests in this region. Kelp forest restoration may therefore be

possible despite forecasted climate changes and environmental factors that would appear to work against it [see also 16].

### 3.3 Where to go from here...

Competitive exclusion by turf algae, and negative impacts of sedimentation, are both plausible culprits when it comes to the obstruction of kelp forest recovery. The relationships have however not been studied with regard to *Saccharina latissima* in Norway. International scientific literature strongly suggest that this is a shortcoming in our understanding of the kelp forests losses, and therefore a topic in need of study.

This thesis suggest that epibiont communities are important drivers of kelp loss, but the studies in focus were geographically relatively restricted, and the methodology (the use of rigs) was questioned by several reviewers (especially in relation to Paper II). Large scale studies of kelp attached to natural substrates would provide stronger evidences for the extent and impact of epibiontism in natural populations, and especially so in relation to the geographical distribution of *S. latissima*. The following two working hypotheses may be worth exploring: 1) *Epibiontism vary geographically, and the success of kelp individuals is affected by this variation* and 2) *The success of kelp individuals determines the success of the kelp forest*.

In Paper III I state that the potential for connectivity between *S. latissima* populations along the Norwegian south coast may be large. This should be tested, and studies incorporating genetics as well as hydrographic models may prove fruitful in that respect.

On a general note, knowledge of how biotic interactions affect the kelp forest is needed in order to understand how the ecosystem actually works. To be able to predict the future of kelp forests, we need to know more about how these relationships are affected by climate change and other anthropogenic influences like different kinds of fisheries, pollution, eutrophication and land-use. This knowledge is important if we aim to protect and manage these systems.

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## Research Article

# Seasonal Patterns of Sporophyte Growth, Fertility, Fouling, and Mortality of *Saccharina latissima* in Skagerrak, Norway: Implications for Forest Recovery

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On the Skagerrak coast the kelp *Saccharina latissima* has suffered severe stand reductions over the last decade, resulting in loss of important habitats. In the present study, healthy kelp plants were transplanted into four deforested areas and their patterns of growth, reproduction, and survival were monitored through subsequent seasons. Our main objective was to establish whether the kelp plants were able to grow and mature in deforested areas. We observed normal patterns of growth and maturation at all study sites. However, heavy fouling by epiphytes occurred each summer, followed by high kelp mortality. The study shows that the seasonal variations and the life stage timing of *S. latissima* make formation of self-sustainable populations impossible in the present environment. Most noteworthy, we suggest that fouling by epiphytes is involved in the lack of kelp forest recovery in Skagerrak, Norway.

## 1. Introduction

*Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl, and G. W. Saunders is a large (1–3 m) brown alga in the order Laminariales (kelps). The species is common, and often dominates the subtidal vegetation on sheltered rocky shores in Norway. In 2002, a dramatic decline of *S. latissima* was observed along the south coast of Norway (Moy and Christie, submitted). Areas previously (in the mid 1990s and before) dominated by dense stands of kelps are now almost exclusively dominated by annual filamentous algae. A report published by The Norwegian Climate and Pollution Agency (Klif) in 2009 revealed the vegetational shift as a geographically widespread phenomenon along the entire south coast of Norway, from the Swedish border in the east to (and including) the Møre og Romsdal region in the west. When islands and fjords are included this stretch has approximately 34 500 km of coastline.

According to Moy and Christie (submitted) the wave sheltered fjords have been more affected than wave exposed coastline. Scattered *S. latissima* individuals were observed at several of the stations in poor condition, but mainly at very shallow waters (0–2 m depth) (Moy and Christie, submitted). The mechanisms that drove the shift and the mechanisms currently preventing recolonisation of kelp have not been identified. Increasing water temperatures in Skagerrak over the past decades and a couple of particularly warm summers (in 1997 and 2002) preceding observations of deforestation has pointed toward elevated temperature as a probable culprit (Moy and Christie, submitted).

Large-scale shift from perennial macrophytes to short lived ephemeral algae is a global problem [1–4]. Events likely to be involved are increased temperatures, increased eutrophication, reduced light availability, increased sedimentation, and changes in grazing pressure. Most of these events

are probably related and caused by the synergy of several agents, many of which are anthropogenically induced [5].

In addition to being a widespread phenomenon, the shift from perennial (e.g., kelp) to annual foundation species appear to be persistent. Long-term ecosystem effects of kelp loss on higher trophic levels are poorly known. However, kelp forests serve key functions in the ecosystem as habitat builders and provide important feeding and nursery grounds for many invertebrate, mollusc, and fish species [6, 7]. Hence, substantial ecological effects across several trophic levels are expected (see e.g., [8]).

The kelp life cycle comprises multiple stages from the microscopic, gamet-producing gametophyte, to the macroscopic, spore-producing sporophyte [9]. The drivers of kelp death and preventives of recolonisation may act on any of these life stages. To be able to generate hypotheses addressing the deforestation and lack of recolonisation, we need knowledge of the viability and seasonality of the different life stages under the prevailing environmental conditions. In the present paper we focus on the sporophyte stage. Seasonality in *S. latissima* has been studied in other areas [10, 11], including the west coast of Norway [12]. However, the seasonal patterns in kelps in general are known to vary [9], even on local scales [13]. The aim of this study is firstly to establish whether adult kelp transplanted into a deforested area in Skagerrak (the south east coast of Norway) are able to survive. Secondly, it is to describe the timing and duration of frond elongation, meiospore formation and release, and fouling and longevity of the sporophytes. We will discuss the observed pattern of maturation and survival in relation to ambient temperature variations and fouling. The present study will offer plausible explanations for the lack of recovery of *S. latissima* beds in southern Norway.

## 2. Materials and Methods

The present project was undertaken from 2005 to 2009 and intensified in December 2007, expanding monitoring from one site in one area (Arendal) to four sites situated in two areas (Arendal and Grimstad). All monitored kelp individuals were transplanted from areas with healthy *S. latissima* forests, which were only found on moderately exposed sites. Transplantation is a widely used methodology in studies of kelp biology (see e.g., [14–19]). In total, 16 kelp individuals were monitored each year from 2005 until 2007, and 31 kelp individuals were monitored each year from 2007 until 2009.

**2.1. Arendal Area (2005–2009).** Field experiments were initiated in November 2005 and continued until October 2009. *S. latissima* sporophytes were transplanted from a moderately exposed into a sheltered site outside Arendal (the Institute of Marine Research Field Station at Flødevigen), where stand reductions for this species had been observed (Moy and Christie, submitted). The kelps were mounted on vertical ropes along the quay outside the field station. The collected sporophytes were classified into two age groups, adults (>1 year old sporophytes) and prereproductive juveniles (<1 year old sporophytes) based on size (stipes diameter and

blade area) and presence/absence of reproductive tissue. Two vertical ropes were used for each of the two age groups, and four sporophytes were mounted (ca 5 cm apart) at 3 m depth on each rope (16 kelps in total each year).

Elongation rate and reproductive status of the sporophytes were monitored monthly for approximately one year, before new series were started with freshly collected sporophytes in the fall. Backup sporophytes were kept on a separate rope each year. A backup sporophyte was brought into the series if an individual was lost (died), until the supply of backup plants ran out. To be able to measure frond elongation, a small hole was punched at the middle of each blade, 10 cm above the meristematic transition zone between blade and stipe. Elongation rates between sampling dates were estimated, following the methodology of Fortes and Lüning [20]. Reproductive status of sporophytes was monitored by visual inspections. If sori (meiospore containing compartments) were observed, samples of sori tissue were collected. The samples were rinsed in tap water and gently patted dry with paper towels to induce sporulation. The spore's ability to form gametophytes and sporelings was determined after 14 days of cultivation in IMR 1/2 medium at 16 : 8 light-dark cycle under  $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (PAR) at 8°C.

Sea temperatures have been measured daily at 1 m depth at Flødevigen Research Station since 1960. A complete temperature dataset was provided by the Institute of Marine Research. In the present study, annual average, maximum and minimum temperatures as well as monthly count of days with temperatures exceeding 20°C each year were extracted for analytic purposes, as the summer isotherm of 19–21°C is limiting its southern distribution according to Müller et al. [21].

**2.2. Grimstad Area (2007–2009).** In December 2007, 15 individuals of *S. latissima* were transplanted from moderately exposed natural kelp forest sites nearby Grimstad to 3 sheltered deforested localities (>300 m apart) in Groosefjord. Five kelp plants were transplanted into each locality. Each individual was mounted onto a separate rig (Figure 1). The kelp hapteron was attached to the rig at approximately 3 m depth. A plate was mounted onto the rig beneath the kelp (at 5 m depth). At each sampling event, which occurred monthly, four plexi glass pieces (3 × 3 cm) were mounted onto the plate acting as spore collecting devices. The following month, the pieces were collected for further cultivation (in IMR 1/2 medium at 16 : 8 light-dark cycle under  $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (PAR) at 12°C) and inspection of spore settling by the use of a light microscope.

To avoid any whiplash effect on the spore collecting devices, kelps were trimmed down to 1 m length if necessary. Elongation and reproductive status of the sporophytes were monitored as described in the previous section (Arendal 2005–2009). Epiphyte densities were noted as percentage frond cover judged by eye.

By February 2008 four of the fifteen rigs had been lost. These were replaced by new rigs and kelp that had been kept as a backup on separate ropes since the initiation in December 2007. In March 2008 two of the lost rigs

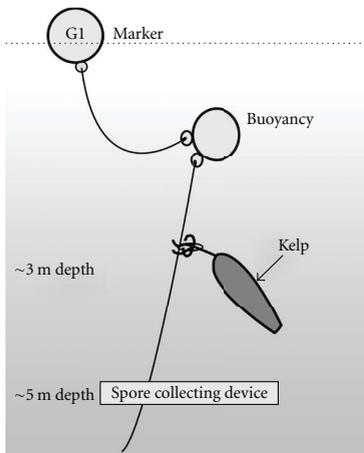


FIGURE 1: Grimstad rig with kelp and spore collecting device mounted at 3 m and 5 m depth, respectively.

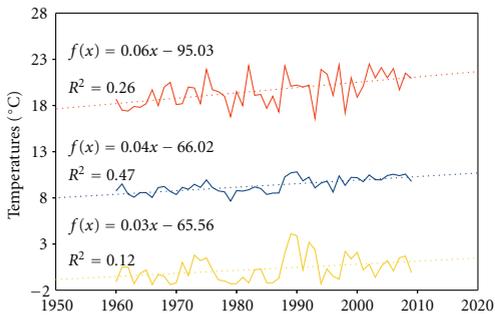


FIGURE 2: Minimum, mean, and maximum yearly temperatures measured at 1 m depth in Arendal from 1960 to 2009.  $f(x)$  are the linear regressions.

reappeared, and we continued to measure kelp growth on them. However, these were excluded from the monitoring of sporulation. In October 2008 the series from December 2007 was terminated. Monitoring of growth and sori formation in a new series of 15 newly harvested individuals was initiated at that time. Monitoring of this series continued until April 2009. Spore settling was not investigated in the latter series due to time limitations.

Positive controls were not performed. Monitoring kelp growing on natural substrate as well as on rigs in forested areas would have greatly improved the design. However, rigs located in the exposed areas of healthy kelp forests frequently disappeared after periods of bad weather, and diving in these areas to measure monthly growth on individuals still attached to substratum was considered too costly.

HOBO-loggers measuring temperature every 15 minutes were mounted onto each rig in March 2008. Temperatures

were logged until April 2009. The data from the 15 loggers were used to validate a temperature model for the area retrieved from the operational ocean forecast database at the Norwegian Meteorological Institute [22] (made available by Jon Albretsen), which provided a complete dataset for the monitored period.

In 2009 two additional, independent HOBO-loggers measuring light (lx) at 3 m depth every 30 minutes was placed in two of the study sites. The loggers were maintained by wiping them clean in late February (winter), late March (early spring), late May (early summer), and mid-August (early fall). Light data measured for one week after cleaning were retrieved and pooled giving intensities hour by hour through the average day.

### 3. Results

**3.1. Temperature and Light.** A linear regression of modelled temperatures at 5 m depth against the measured temperature (15 HOBO loggers) from the Grimstad area gave convincing results ( $r^2 = 0.92$  and  $P < 0.001$ ). Also, the modelled temperatures for Grimstad fitted significantly to measured temperatures at 1 m depth at Flødevigen Station of Marine Research in Arendal ( $r^2 = 0.93$  and  $P < 0.001$ ).

Annual maximum, mean, and minimum sea temperatures measured, at 1 m depth increased in the period from 1960 to 2009 (Figure 2). Years with sea temperatures above 20°C have increased in frequency and so has the duration of the warm periods. Particularly warm summers were recorded in 1997, 2002, and 2006 where the temperature at 1 m depth rose above 20°C for 39, 24, and 23 days, and maximum temperatures reached 22.4, 22.5, and 22°C, respectively.

Temperatures measured in Arendal in the period of the present study are presented in Figure 3. The warmest periods were recorded in the summer months from July to September. The number of days where recorded temperatures exceeded 20°C was 23 in 2006, none in 2007, four in 2008, and six in 2009. The coldest periods were recorded in late winter/early spring between February and April (except in 2008). In general, considerable year to year variation occurred (Figure 3).

Daily hour-by-hour light intensities from HOBO-loggers indicating winter, spring, summer, and fall situations are presented graphically using the smooth spline function available in R [23] (Figure 4).

**3.2. Frond Elongation.** A distinct seasonal pattern of frond elongation was observed in the period from 2005 to 2009 in Arendal (Figure 5). The observations at all field sites in Grimstad from 2007 to 2009 also fit this pattern (Figure 6). Elongation in both young (~1 year) and older (>1 year) sporophytes in Skagerrak reached a peak in April/May and paused in June/July. The elongation rates remained very low from August to November/December.

**3.3. Mortality and Fouling.** Mortality rates seemed generally higher in fall (August–November) in both the adult and the juvenile group in Arendal, although some year-to-year

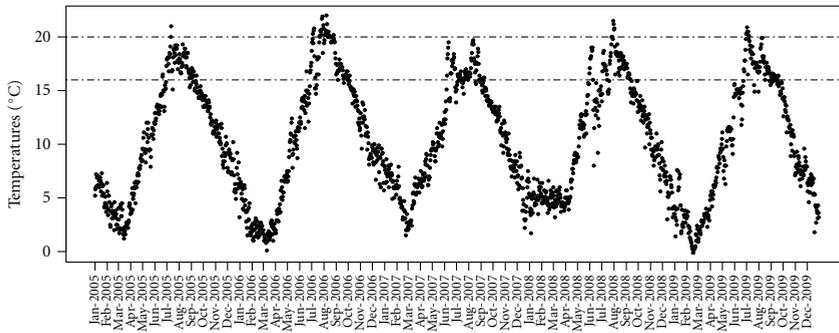


FIGURE 3: Temperatures measured at 1 m depth in Arendal from January 2005 to December 2009. Horizontal lines are drawn at 16°C and 20°C.

TABLE 1: Mortality of juvenile (J) and adult (Ad) kelp (% of population) in Arendal. (See text for results from Grimstad.) Values higher than 25% are marked in bold italic formatting. Blank fields means no data.

Year	Age	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Des
2006	Ad	12.5	12.5	0	0	0	0	0	0	0	<b>50</b>		
	J	<b>25</b>	0	0	0	0	0	0	12.5		<b>57.14</b>	<b>100</b>	0
2007	Ad		0	0	0	0	12.5	0	0	<b>25</b>	16.67	8.33	0
	J	0	0	0	0	0	0	0	0	0	0	12.5	7.14
2008	Ad	<b>50</b>	0	<b>25</b>		0	<b>25</b>	0	<b>50</b>	<b>33.33</b>	<b>25</b>	<b>25</b>	<b>25</b>
	J	0	12.5	0		0	0	0	<b>25</b>	<b>25</b>	20		
2009	Ad	0	0	12.5	<b>33.33</b>								
	J	0	0	25	0	0			12.5	12.5	22.5	0	

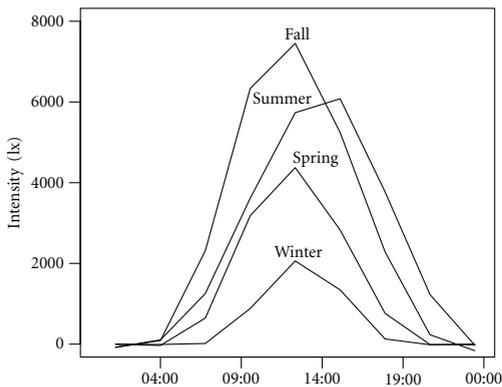


FIGURE 4: Light intensities at 3 m depth (in Grimstad) in winter (late February), spring (late March), early summer (late May), and fall (mid August). Time along the x-axis is displayed in a 24-hour format (HH:MM).

variation occurred (Table 1). Occasional high mortality at other times was most likely due to episodes of bad weather causing increased drag and loss of kelp plants. The specimens in Arendal and the specimens IN Grimstad showed similar

patterns of mortality. By September most kelps at the rigs in Grimstad had been lost or were considered deceased. The number of kelp individuals was reduced from 17 (15 original + 2 replacements) in March 2008 to 13 in July 2008 and to six in September 2008 (~65% reduction in all). The survivors at all sites (including Arendal) were heavily overgrown by floral and faunal epiphytes which had accumulated since June. Epiphytes were estimated to cover between 80 and 100% of the kelp fronds in Grimstad in September 2008 (Figure 7). The epiphyte communities consisted of various combinations of blue mussels (*Mytilus edulis*), sponges, bryozoans (mostly *Membranipora membranacea* and some *Electra pilosa*), filamentous algae, and an enormous invasion (especially in Grimstad) of the vase tunicate (*Ciona intestinalis*). The few kelp plants that survived the fouling period were observed to form healthy tissue free of epiphytes in the following elongation season. However, elongation was no longer measured on these individuals after the initiation of the new series.

**3.4. Fertility and Spore Production.** Spore producing tissue (sori) was generally present from October and until March. The timing and the duration of sori formation seem to vary somewhat but still, the pattern was largely the same at all sites. The sporophytes released viable spores that were able

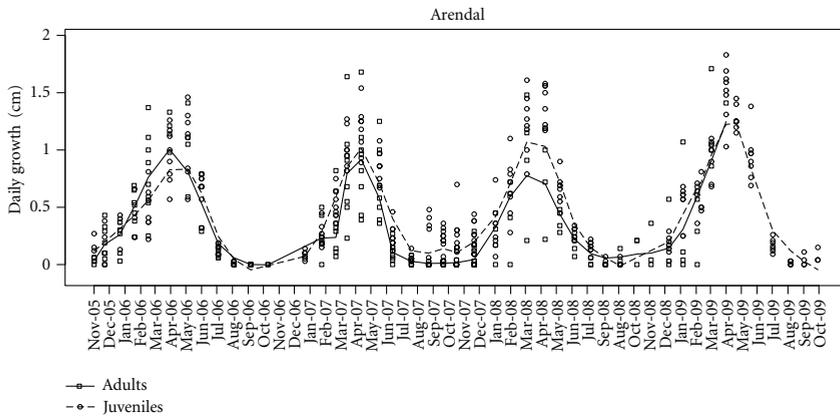


FIGURE 5: Frond elongation of adult and juvenile kelp at 3 m depth in Arendal from November 2005 to December 2009. Elongation rates are expressed as daily growth (cm).

TABLE 2: Adults able to provide viable spores (% of populations) in Arendal (A) and Grimstad (G). Values higher than 25% are marked in bold italic formatting. Blank fields means no data.

Year	Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	OCT	Nov	Dec
2005	A										75	100	100
2006	A	100	75	100	50		0	0	0		0	50	
2007	A	87.5	75	12.5	0	0	0	0	0	16.67	45.83	77.5	67.5
	G										0	100	100
2008	A	75	100	50	0	0	0	0	0	0	33.33	100	75
	G	100	100	13	0	0	0	0	0	0			

to settle and germinate all through the sori forming period (Table 2).

#### 4. Discussion

The present study showed that kelp translocated into deforested areas were able to grow and mature, and that high kelp mortality in summer coincided with heavy epiphytic fouling as well as high water temperatures.

The most popularly stated comments in media on why the *Saccharina latissima* kelp forests in Skagerrak struggle have put great emphasis on the effect of global warming and high summer temperatures. This emphasis is natural considering studies like that by Müller et al. [21]. The observed deforestation could be the consequence of particularly warm summers in 1997 and 2002. Indeed, negative effects of elevated sea temperatures on both growth and longevity of kelps are to be expected (see e.g., [24]). Bolton and Lüning [25] found the optimum growth temperatures of *S. latissima* sporophytes to span from 10 to 15°C. Growth was reduced by 50–70% at 20°C, and the specimens completely disintegrated after 7 days at 23°C [25]. Gerard and Du Bois [16] investigated two populations of *S. latissima* and found marked differences in their responses to temperatures. Specimens

that came from an area with ambient summer temperatures exceeding 20°C one and a half month (New York) seemed more temperature tolerant than specimens from an area rarely experiencing temperatures above 17°C (Maine). While >50% of the New York plants survived three weeks of temperatures above 20°C in field experiments, the Maine plants suffered 100% mortality. Both groups suffered 100% mortality after 3 days at 24°C in laboratory experiments [26]. *In situ* studies of *S. latissima* at its southern distribution in the Long Island sound, New York, showed decreased frond growth as the temperature exceeded 16°C and ceased growth when it exceeded 20°C [27]. Our results from the elongation studies in the deforested Skagerrak area are in concurrence with the patterns of growth described in the previously mentioned studies. The elongation rates were high in the cold periods (March to May), and reduced in June/July as the temperature rose (Figures 5 and 6). The concurring patterns indicate that for our interpretative purpose the data are reliable. Our main objective was to establish whether the kelp plants were able to grow and mature in deforested areas. In that respect, we do not consider the lack of positive controls a serious problem for the validity of our conclusion.

During the present project only July and August 2006 had particularly many days of high temperatures (23 days of temperatures above 20°C). Although kelp mortality was

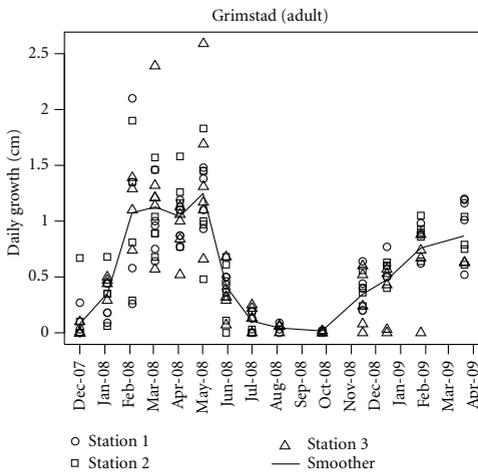


FIGURE 6: Frond elongation of adult kelp at 3 m depth in Grimstad. Elongation rates are expressed as daily growth (cm).

high after the warm summer of 2006, high mortality rates were also recorded in 2008, a year when the temperature exceeded 20°C for only four days (Table 1). Furthermore, high mortality occurred earlier in 2008 than in 2006, even though the maximum temperatures were recorded in the same period (Figure 3). The disappearance of *S. latissima* is by far most extensive in wave sheltered areas, where the water temperatures in summer are relatively high (Moy and Christie, submitted). However, near the surface where the water temperatures usually are the highest, the situation is far less severe (Moy and Christie, submitted). This fact along with our results suggests that even if a couple of warm summers in the late 1990s and early 2000s was the cause of the extensive deforestation observed in Skagerrak, it is less likely to be the preventer of kelp forest recovery at present.

Most organisms experience increased respiration as their surrounding temperature rises (see [28] for kelp example). One could therefore hypothesise that in low-light conditions of deeper waters, the kelp may not be able to photosynthesise sufficiently to support its respiration at elevated temperatures. This would explain why kelp populations in shallow waters, where light conditions are better, appear healthier. However, Davison et al. [29] found variation in respiratory rates of *S. latissima* grown at 15°C to be insignificant in the range from 10°C to 25°C. Daily water temperature as high as 25°C has not been recorded in Arendal in the period from 1960 and to this date. Furthermore, the respiratory rates were very similar to those measured for *S. latissima* grown and measured in cold water (5°C) [29]. Hence, variation in water temperatures in the range experienced in Skagerrak must have little effect on the kelps respiration. Irradiance required for compensation (5 and 25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  measured at 5 and 25°C, resp.) was also similar in the two groups. Light saturated photosynthesis for kelps grown at 15°C occurred approximately 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

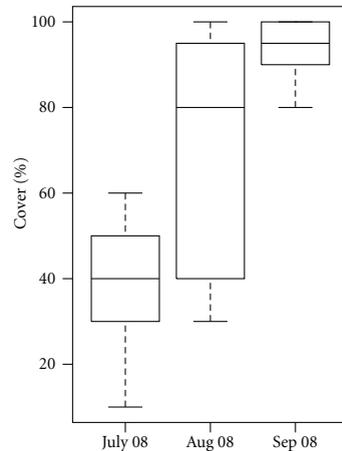


FIGURE 7: Estimated epiphyte cover on kelp fronds in Grimstad in 2008. Boxplots based on monitoring of 13 kelp individuals in July, 8 in August, and 6 individuals in September. The median value is marked by a horizontal line in the middle of each box. The boxes include the lower and the upper quartile. The whiskers extend to the extreme values.

when measured at 25°C [29]. Evidently, little light is necessary for the kelp to uphold a positive carbon budget. Considering all years, the present study documented a high mortality in summer/fall and generally low mortality in spring at 3 m depth. Since ambient temperatures seem to have little effect, the light conditions would have to be considerably worse in summer/fall than in spring to explain the seasonal kelp die-off by insufficient photosynthesis. Less light was available in spring than in summer/fall in 2009 (Figure 4), so we find such a scenario highly unlikely to be the case. Moreover, the light required for compensation and saturation of photosynthesis (as reported by Davison et al. [29]) roughly converts to 1320 and 2630 lx, respectively. Although the conversions are crude, the intensities were well above both levels for a longer period in summer/fall than in spring (Figure 4).

If not failure to compensate for respiration, what may explain the seeming correlation between deforestation and high sea temperatures? Increased sea temperatures appear to affect the growth of *S. latissima* negatively. Slow growth or elongation rate is not necessarily an indication of poor condition. However, reduced production of new thallus leaves the kelp more vulnerable to epiphytism and possibly to bacterial attacks and viral infections. In fact, along with elevated summer temperatures comes an increase in epiphytic load on the kelps. High mortality of kelp after heavy epiphytism has been reported from the northeastern coast of America [2, 10, 30]. Accumulating epiphytes cause increased brittleness resulting in defoliation, especially under increased mechanical disturbance at high energy events like storms [31]. Continuous exposure to moderate wave activity, however, may contribute in epiphyte control by washing

away new settlers [32, 33]. Such a mechanism could explain why kelps close to the surface and in relatively exposed areas are in better condition than deeper and more wave sheltered populations.

Observations of prominent epiphyte cover on kelps are very common in Skagerrak (Moy and Christie, submitted). In the present study, heavy fouling by epiphytes was observed at all sites by August. The epiphyte densities in Grimstad increased, covering 80 to 100% of each frond by September in 2008. Epiphyte densities were probably just as prominent in Arendal the other years (though actual cover was not estimated). Subjected to light limitations and increased drag disturbances caused by epiphytes, chances of survival seem drastically reduced. We do not know if translocation of kelp may affect the density of epiphytes growing on them. However, procedural control in another study has indicated that dislodgement and transplantation of kelps have no direct effect on the cover of epifauna [19]. Hence, we consider the cover of epiphytes likely to be the effect of the habitat rather than the methodology applied.

The present study showed that transplanted individuals free from epiphytes became fertile produced and released viable spores for five months in the deforested areas. It is evident that spores in the area are able to settle on clean substrate (spore collecting devices) and develop into small recruits. Still, we do not know if the spores are able to settle and germinate on the sea floor. Increasing amounts of sediments and a dense cover of filamentous algae may obstruct both settlement and germination [34]. Regardless, if recruits are formed, fouling may make them unlikely to survive until maturity (>1 year). Furthermore, if bryozoan cover cause reduced reproductive output, as reported by Saier and Chapman [35] the chances of reproductive success seems vanishingly small. Self-sustainable populations will not be able to form and full recovery of the kelp beds will not occur.

In conclusion, we consider the effects of heavy fouling in depriving the *S. latissima* sporophyte of light and in obstructing completion of the kelps life cycle likely to be the most important mechanism preventing recolonisation and recovery of *S. latissima* beds in the Skagerrak area.

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1     **In a squeeze: Epibionts may affect the**  
2     **distribution of *Saccharina latissima***

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6                     (August 2013)

7                     **Abstract**

8                     The lack of kelp forest recovery after a recent, large-scale loss of  
9                     the kelp *Saccharina latissima* from the south coast of Norway is an  
10                    ecological problem that is poorly studied. Previous investigations do  
11                    however suggest that the impact of biological interactions and reduced  
12                    water transparency may be important.

13                    We investigated the depth related pattern of growth, fouling by  
14                    epibionts and mortality in two sample populations of kelp, from the  
15                    south and the south west coast of Norway. The investigations were  
16                    performed over a period of seven months – in a crossed translocational  
17                    study – where kelps were mounted on rigs at six depths (1, 3, 6, 9, 15  
18                    and 24 m). In a second experiment, the shading caused by different

19 epibiont layers was determined, and the results were related to the  
20 effects observed in growth and mortality with depth.

21 Although the depth related results we present apply – in the strictest  
22 sense – only to kelp translocated on rigs, the relative patterns may be  
23 relevant for natural populations as well. While growth decreased with  
24 depth in spring and summer, better growth was observed at 15 m as  
25 compared to at shallower depths in fall. Epibionts covered the kelp  
26 individuals at depths from 1 to 9 m in fall, and the probability of sur-  
27 vival was greatest between 10 and 15 m. Our results indicate that a  
28 contraction of *S. latissimas*' depth range may have occurred in Skager-  
29 rak and – if true – this could render kelp forest recovery in this area  
30 very difficult. These suggestions should be further studied, because  
31 their implications may affect the management of coastal areas.

## 32 1 Introduction

33 Kelps, seaweeds and sea grasses provide important ecosystem services  
34 in coastal areas, and large scale losses of these macrophytes is a global  
35 concern [30, 48, 49, 31, 17]. Increased eutrophication in coastal areas  
36 [9, 48, 16], changes in keystone species and interactions across trophic  
37 levels [34, 43, 28, 1], climatic changes [20, 6, 50] or synergistic combi-  
38 nations of several of these factors [5, 26, 22, 49, 17, 1] have all been  
39 considered important drivers of recent losses.

40 *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl and  
41 G.W. Saunders is a relatively large kelp species. Its populations are  
42 often dense, and form underwater forest landscapes that provide habi-  
43 tats for myriads of species. *S. latissima* used to dominate in sub-tidal  
44 and sheltered areas along rocky parts of the Norwegian south coast,  
45 but disappeared sometime in the late 1990's [29]. Because this kelp  
46 is a cold-temperate water species [see 30], and unusually high sea  
47 water temperatures were recorded several summers during the late  
48 1990's and early 2000's, heat stress may have been the cause of the *S.*

49 *latissima* forest demise. The temperatures have now, however, been  
50 normal for several years, and regrowth is probably no longer hindered  
51 by high sea water temperature [see e.g. 44].

52 Recent surveys have shown that a benthic community shift oc-  
53 curred when the kelp disappeared, resulting in complete dominance of  
54 filamentous red and brown algae (turf algae) [29]. Moy and Christie  
55 [29] reported that healthy *S. latissima* populations still remained in  
56 wave exposed areas, and these should have been able to disperse and  
57 recolonize adjacent, deforested areas. Rapid forest recovery has oc-  
58 curred after large scale disturbances in the past, indicating that recol-  
59 onization by this species used to be effective [29]. At present however,  
60 the turf algae communities seem persistent along the south coast, and  
61 there are currently no signs of kelp forest recovery in the deforested  
62 areas [29]. The importance of competitive interactions has been doc-  
63 umented in kelp forests [16, 10], but in relation to *S. latissima* forests  
64 in Norway such interactions are poorly studied.

65 The strait running between the south east coast of Norway, the  
66 south west coast of Sweden, and the Jutland peninsula of Denmark  
67 is called Skagerrak. As in many coastal areas around the world, the  
68 water in Skagerrak has become increasingly turbid during the past  
69 decades (i.e. darkening of the water) [7, 12], and the depth to which  
70 sunlight penetrate has therefore been reduced. A substantial change  
71 in the vertical distribution of photosynthesizing species (including *S.*  
72 *latissima*) has also occurred in Skagerrak over time [36, 32, 9, 29], and  
73 these changes have been coupled to the reductions in light availability.

74 In addition to the water darkening, extensive epiphytism seems to  
75 be an increasing problem, and epibionts may deprive their host algae  
76 of much light. The effects these organisms have on kelp are relatively  
77 poorly known [see however 24, 25, 18, 19, 39, 42], but Sogn Andersen  
78 et al [45] suggested that their impact may be important in relation to  
79 the kelp loss in Skagerrak.

80 *S. latissima* forests also deteriorated along the west coast of Nor-

81 way in the late 1990's [29]. However, the Norwegian monitoring  
82 programs have since documented a gradient of ecosystem recovery, from  
83 mainly disintegrated and lacking forests on the south east coast and  
84 in part on the south west coast, to healthy forests in many areas on  
85 the mid west coast of Norway. This means that the west coast kelp  
86 have been able to disperse and recolonize while kelp in Skagerrak have  
87 not. The explanation for this may lie in environmental differences  
88 between the areas, or in physiological differences between the local  
89 populations. Physiological traits in *S. latissima* populations may vary  
90 geographically (e.g. different environments may lead to adaptations  
91 resulting in ecotypic differentiation) [14, 13, 15], and kelp individuals  
92 from different parts of the Norwegian gradient may therefore respond  
93 to stressors like low light and extreme temperatures in different ways.  
94 Temperature responses were tested in a recent study, and individuals  
95 from the intermediate and both extremities of the Norwegian gradient  
96 (south east to west) showed very similar photophysiological responses  
97 to temperature stresses [44]. The question of whether the tolerances  
98 differ when the kelp plants are subjected to the whole range of stres-  
99 sors encountered *in situ* is however not yet answered.

100 The present study investigated the relationship between depth and  
101 patterns of growth and survival in kelp from the south and west coast  
102 of Norway. These relationships were investigated in two areas; one  
103 site on the west coast representing the westernmost part of the recov-  
104 ery gradient, where kelp forests are able to recover; and another site  
105 representing the south-eastern (Skagerrak) part of the gradient, where  
106 recovery is poor and kelp forests are scarce. In a crossed experiment,  
107 kelp plants from both areas were translocated on rigs and monitored  
108 at six depths for seven months. The main objective was to find out if  
109 kelp from the two populations responded in different ways.

110 Secondly, we investigated the extent of shading caused by different  
111 forms of epibionts that are commonly found on *S. latissima* in Sk-  
112 agerrak. The amount of shading was compared to the light reductions

113 measured with increasing water depth in Grimstad. This comparison  
114 was used in combination with the investigations of growth and sur-  
115 vival, to indicate the impact epibiont shading may have had on the  
116 kelp. Particular attention was therefore given to the lower depth limit  
117 of *S. latissima*. We hypothesize that epibionts deprive their host of  
118 light, and that the deprivation may become lethal.

## 119 2 Materials and Methods

### 120 2.1 Growth and survival

#### 121 2.1.1 Experimental sites

122 Two areas (hereafter called experimental sites) were chosen to repre-  
123 sent the Skagerrak (south eastern) and the west coast (western) parts  
124 of what might be a *S. latissima* recovery gradient in Norway. The ex-  
125 perimental site in Skagerrak was located at 58°19'N, 8°35'E (WGS84  
126 datum) nearby Grimstad, while the experimental site on the west coast  
127 was located at 60°15'N, 5°12'E (WGS84 datum) nearby Bergen. The  
128 two areas had water depths of approximately 30 m and were classified  
129 as sheltered according to the wave exposure model developed by Isæus  
130 [21].

131 At both experimental sites, four stations were picked for the de-  
132 ployment of the experimental rigs (eight locations in total). Each sta-  
133 tion had a water depth of 30 m and was separated from other stations  
134 by more than 500 m.

#### 135 2.1.2 Sampling of kelp plants

136 Adult *S. latissima* sporophytes were collected at 6 m depth within a  
137 radius of 2 km from each of the experimental sites in February 2009.  
138 Sheltered sites within Skagerrak (i.e exposed to the same level of wave  
139 action as the experimental sites) are still largely devoid of kelp, and  
140 kelp plants had to be sampled in more exposed areas. On the west

141 coast, the sampling site was sheltered (same level of exposure as the  
142 experimental site). For simplicity, kelp sampled on the Skagerrak coast  
143 will hereafter be referred to as SCK, while kelp sampled on the west  
144 coast will be referred to as WCK.

145 Half of the kelp plants sampled were transported to the opposite  
146 coast, so that each experimental site had both native and transported  
147 samples. The samples were transported in sea water, and contained  
148 in dark transport coolers that kept the temperature low (0-5 °C).

### 149 **2.1.3 Rig deployment**

150 Kelp from different sampling sites (WCK or SCK) were mounted on  
151 separate rigs. On each rig, four kelp individuals were attached at each  
152 of the six depths: 1, 3, 6, 9, 15 and 24 m. At each station two rigs,  
153 one with WCK and one SCK were deployed, 20-30 m apart from each  
154 other. Thus, at each experimental site, eight rigs were monitored (384  
155 kelp individuals in total).

156 In experiments where organisms are handled, method control should  
157 be applied in order to separate the effect of the handling from the ef-  
158 fect of the surrounding environment. For method control in relation  
159 to rig treatment, it would have been necessary to measure responses in  
160 individuals in natural populations at both sites and compare these re-  
161 sults to the responses measured in individuals mounted on rigs within  
162 each site and at the same depths. Since the sites were largely void of  
163 natural populations at the time, this was impossible. As a suboptimal  
164 approach, treatment control could have been conducted within the  
165 sampling sites. This would have expanded the amount of work done  
166 by SCUBA diving in wave exposed areas, and the logistics required  
167 to execute this safely (especially in winter) was not feasible within  
168 our budget. However, our main objective was to test the relative ef-  
169 fect of depth on growth and survival. The monitoring was continued  
170 for seven months, and the effects observed late in the study were less  
171 likely to be caused by the translocation *per se*. Our results apply,

172 in the strictest sense, only to translocated kelp on rigs. However, we  
173 would argue that the relative differences among kelp plants mounted  
174 at the different depths are relevant in relation to effects in natural  
175 populations.

#### 176 **2.1.4 Measurements**

177 The frond length and frond width of each kelp plant was measured  
178 at the initiation of the experiment. A small hole was punched 10  
179 cm above the transition zone, between stipe and frond, to be able  
180 to measure elongation according to the method described in Fortes  
181 and Lüning [11]. Growth ( $G_{rate}$ ) as rate of daily areal increase was  
182 calculated according to formula 1;

$$G_{rate} \sim \frac{E W_1}{L W_2 d}, \quad (1)$$

183 where  $L$  and  $E$  are the total length and elongation of the lamina  
184 respectively,  $W_1$  and  $W_2$  are the lamina widths 10 cm and 50 cm  
185 above the basis respectively, and  $d$  is the number of days since the  
186 last measuring.

187 The extent of epibiont covers on the kelp frond (in percentages)  
188 was recorded throughout the experiment. Survival was recorded as a  
189 binary response, and kelp individuals that had been torn off and indi-  
190 viduals with severely perforated and bleached meristems were recorded  
191 as dead.

192 The rigs were monitored for seven months (February 2009 - Septem-  
193 ber 2009), and measurements were executed in spring, summer and  
194 fall according to Table 1.

#### 195 **2.1.5 Statistical analyses**

196 The relationships between growth in the sample populations (WCK  
197 and SCK), depth and season at both experimental sites were investi-  
198 gated. Because growth was measured as percentage change in size, the

Table 1: **Dates** (2009) in which measurements were executed.

<b>Location</b>	Initiation	Spring	Summer	Fall
Skagerrak	February 22 <sup>nd</sup>	March 26 <sup>th</sup>	May 19 <sup>th</sup>	September 25 <sup>th</sup>
West coast	February 20 <sup>th</sup>	April 14 <sup>th</sup>	June 10 <sup>th</sup>	Rigs lost

199 response was log transformed and analyzed assuming Normal errors  
 200 [see chapter 28 in 8, page 514]. The statistical analyses were per-  
 201 formed using the protocol of Zuur et al [see chapter 5 in 51, page 127]  
 202 for mixed linear modelling, first including all explanatory variables  
 203 (Site, Population, Depth and Season) and their interactions. Because  
 204 spatial autocorrelation was likely to occur, we could not assume that  
 205 the response (growth) was independent within a station. Station was  
 206 therefore included as a random factor in the model selection process.  
 207 The Akaike's Information Criterion (AIC) was used in order to select  
 208 between model alternatives (model selection step 1), and backwards  
 209 model selection was used in excluding parameters (selection step 2)  
 210 [51]. Finally, the variance structures (i.e. normality and homogeneity  
 211 of errors) were evaluated by visual inspection of residual plots [51].

212 Upon inspecting the model residuals, both depth and season de-  
 213 pendent error structures were revealed, and these dependencies vio-  
 214 lated the underlying assumption of linear models meaning that we  
 215 could not trust the p-values. A second degree polynomial term was  
 216 therefore added to improve model fit with depth. An additional  
 217 weights term (varIdent in the *nlme* package of R) was also added, to  
 218 allow for seasonal differences in variance [see chapter 4 in 51]. These  
 219 measures dealt with the issues of heterocedasticity (selection step 3).

220 We also investigated the patterns of epibiont covers and survival  
 221 by the end of the experiment. The relationships between epibiont  
 222 covers (response in percentages), survival (success or not) and depth  
 223 in both WCK and SCK were tested by the use of generalized linear  
 224 mixed modelling with binomial distributions. Station was included

225 as a random factor in both models for the same reason as mentioned  
226 above.

227 We used the R computer software [35] for all computations. In the  
228 statistical analyses we used the *nlme*[33] and *lme4* [3] R packages.

## 229 **2.2 Shading by epibionts**

### 230 **2.2.1 Epibiont covers and densities**

231 Fifty kelp plants were harvested (at approx. 6 m depth) from one  
232 of the few remaining forest patches in Skagerrak in October 2009.  
233 The harvest site was located close to the sample site used in the rig  
234 experiment.

235 Dominating epibionts found in Skagerrak are vase tunicates (*Ciona*  
236 *intestinalis*), encrusting bryozoans (*Electra pilosa*) and filamentous  
237 algae (mostly red algae) [45, 29]. All of these were present on the  
238 individuals we harvested. Vase tunicates and bryozoans form colonies  
239 that are almost uniform in density, while the densities of the epiphytic  
240 algal layers vary considerably. Vase tunicate and bryozoan covers were  
241 therefore regarded as either present or absent, while the densities of the  
242 algal covers were measured in dry weight per substrate (kelp sample)  
243 area (DW cm<sup>-2</sup>).

### 244 **2.2.2 The measuring equipment**

245 To estimate the amount of shading caused by epibionts, a series of light  
246 measurements were performed. We used a TriOS RAMSES (TriOS  
247 Optical Sensors, Germany) with 198 channels to measure light in the  
248 range of wavelengths from 310 to 950 nm. The sensor was connected  
249 to a field-PC with MSDA software to record the readings. A cylinder  
250 of plexi-glas served as a measuring chamber, and a lamp (FieldCal)  
251 that was fitted on top of the measuring chamber served as the light  
252 source. Each measurement was repeated three times, and the values  
253 were averaged. This was done in order to reduce the influence of noise

254 that could occur in the readings (judged by previous experience with  
255 the equipment).

### 256 **2.2.3 Light measurements**

257 The measuring chamber was filled with sea water ( $\sim 10$  °C). A kelp  
258 sample was then cut from the a harvested individual with a cork borer  
259 and fitted into the measuring chamber. The extent of shading caused  
260 by each type of epibiont cover (either vase tunicate, bryozoan or algal)  
261 was estimated from nine replicate samples. In addition, we performed  
262 a series of measurements in which varying amounts of epiphytic algae  
263 were transplanted on top of a kelp sample. In this case, epibionts  
264 from the harvest was used to ensure a realistic composition of algal  
265 species. This additional step was done in order to further investigate  
266 the relationship between the density of algal epiphytes and shading.

267 To estimate the light deprivation caused by epibionts, we had to  
268 compare light measurements from samples *with* epibiont covers to  
269 measurements *without* them. The latter will hereafter be called the  
270 reference readings. In the measuring process, the reference readings  
271 had to be obtained in different ways. In the case of vase tunicates,  
272 the animals were gently removed from each sample before the refer-  
273 ence readings were performed. Bryozoan crusts and filamentous algae  
274 on the other hand, were impossible to remove without scarring the  
275 kelp lamina, and scars would have affected the light measurements.  
276 In these cases, clean tissue samples (from each kelp) were therefore  
277 taken to serve as references.

278 The amount of light that penetrated each sample was estimated by  
279 calculating the integral of the light intensities measured in all wave-  
280 lengths. The integrals were calculated by the trapezoidal rule approx-  
281 imation method and the amount of shading was estimated according  
282 to formula 2.

$$\int_a^b f(x) dx - \int_a^b g(x) dx, \quad (2)$$

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in which  $x$  is wavelength,  $a = 310$  nm,  $b = 950$  nm, and  $f$  and  $g$  are the curves describing light penetrating profile in the reference and samples covered by epibionts respectively.

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#### 2.2.4 Statistical analyses

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The effect of either vase tunicate or bryozoan presence (i.e. presence or absence) on shading was analysed using a generalised linear model with a binomial distribution. In a separate analysis, the effect of different algal densities on shading was described by an asymptotic function fitted by non-linear least square regression. For both analyses we used the *stats* [35] R package.

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### 2.3 Ambient temperature and light

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Temperature data were retrieved from the operational ocean forecast database at the Norwegian Meteorological Institute.

The ambient light condition in the water column was recorded throughout the experiment. Small, light logging HOBO-pendants (UA-002-08, Onset Computer Corporation, USA) were mounted at five depths (3, 6, 9, 15 and 24 m) on two rigs within both experimental sites (20 loggers in total). During each measuring event, the pendants were wiped clean of fouling organisms that could affect the sensors. At the termination of the experiment the pendants were brought back to the lab, and data from seven days following each cleaning was extracted and pooled.

The rigs with light loggers were unfortunately lost from the west coast site.

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## 3 Results

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### 3.1 Growth

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In analysing growth rates, several statistical models were tested against each other (see Table 2), but in the end a linear model, without random terms, was considered the best fit because it obtained the lowest AIC value [51]. The residuals did not show any trend with station (the random factor) which also indicated that mixed effect modeling was not required [see 51].

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*S. latissima* from the Skagerrak population (SCK) grew slightly slower than kelp from the west coast population (WCK) (Table 3 and Figure 1). This difference was consistent at all depths and throughout the experiment, which means that the "reaction" to the depth treatment and season had been the same in both sample populations. The difference in growth between SCK and WCK was also consistent among experimental sites, which indicated that transport and relocation had not affected these results.

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The depth related pattern of growth changed from spring to fall (shown by the significant interaction between depth and season in Table 3). In spring and summer, growth generally decreased with depth (Figure 1 and Table 3). In fall however, the pattern was reversed in Skagerrak, and growth was faster at 15 m than at 3 m depth. All rigs in the west coast area were unfortunately lost by the end of the summer, and data from this area in fall could therefore not be retrieved.

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### 3.2 Survival

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Survival was analyzed in relation to depth. It was also important to find out if the depth related pattern of survival differed between the two sample populations (WCK and SCK). By the end of the summer, most kelp plants had survived at all depths (Table 4 and Figure 2).

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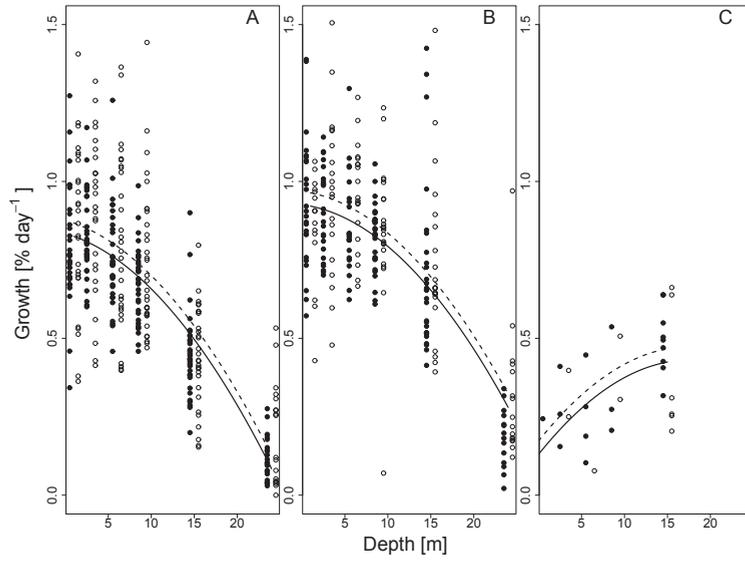


Figure 1: **Growth.** Seasonal growth rates in relation to depth. Pane A is spring growth, pane B is summer growth and pane C is growth in fall. Site did not explain a significant part of the variation, and the results from the experimental sites were pooled in these presentations. Growth rates in SCK and WCK are represented by dots and open circles respectively. The lines are model predictions from the growth model (Table 3); solid line for SCK, and dashed line for WCK.

337 By fall, survival was low both close to the surface and at 24 m  
338 depth in Skagerrak (< 20 %), but much higher at 15 m depth (70-80  
339 %) (Figure 2). The model predicted an unimodal pattern of survival  
340 with the highest probabilities between 10 and 15 m depth in this area  
341 (Table 4 and Figure 3).

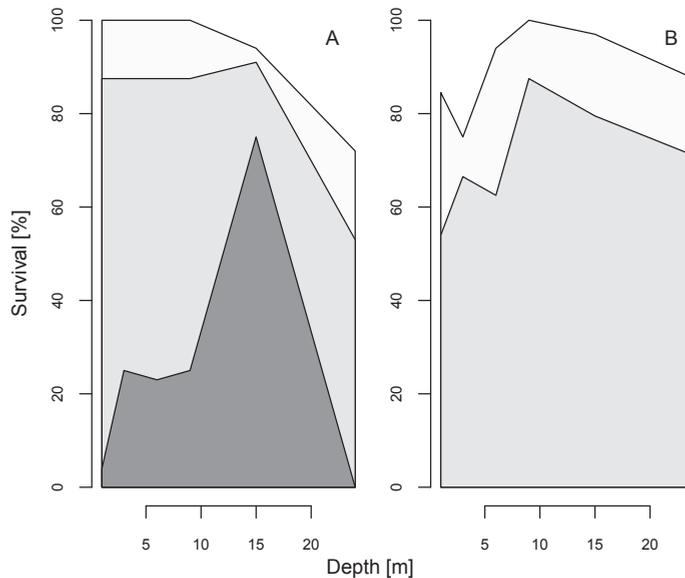


Figure 2: **Survival.** Kelp survival was recorded at the different sampling times in Skagerrak (pane A) and on the west coast (pane B). Light gray area shows survival as recorded in spring, intermediate gray area shows survival as recorded until summer, and dark gray area shows survival as recorded from the initiation of the experiment and until fall.

342 The kelp fronds were fouled by heavy loads of epibionts, and the  
343 general condition of the remaining kelp plants in fall was poor.

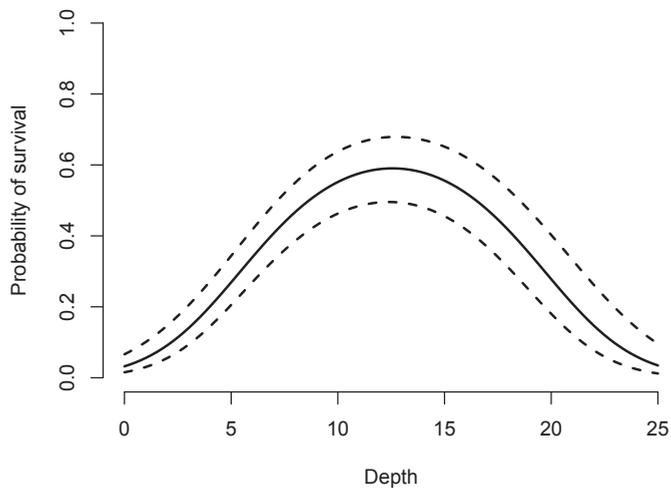


Figure 3: **Probability of survival in fall.** Predicted survival of *S. latissima* on rigs in Skagerrak in fall (solid line), according to the model presented in Table 4. The standard errors of the model predictions are represented by the upper and lower curves (dashed).

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### 3.3 Epibionts

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In spring, kelp plants from both sample populations appeared clean and healthy in both areas. Scattered turfs of epiphytic algae and colonies of bryozoans were observed in both areas in summer, while vase tunicates were only observed in Skagerrak. The amounts were in every case too low to be estimated as covers.

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In fall (Skagerrak only) the situation had changed dramatically, and all remaining SCK and WCK at all depths were covered with bryozoans (Figure 4). On kelp that had been kept at depths ranging from 1 to 9 m, one side (probably the side that had been facing upwards) was covered by vase tunicates infiltrated by turfs of filamentous algae. At 15 m depth, the densities of epibionts were significantly scarcer (Figure 4, Table 5). The statistical analysis also showed that there was a positive relationship between bryozoan cover and the cover of vase tunicates (see Table 5). The bryozoans form crusts on which vase tunicates may grow, while bryozoans never covered the vase tunicates.

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### 3.4 Shading by epibionts

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The shading caused by epicovers was substantial. Bryozoan cover appeared to be the least light depriving epibiont form, and the light reduction caused by a single layer of encrusting bryozoans (*Electra pilosa*) was 11 % (95 % confidence interval [1 % – 59 %], binomial distribution). Far more light, averagely 91 %, was deprived by a single layer of vase tunicates (*Ciona intestinalis*) (95 % confidence interval [35 % – 99 %], binomial distribution). Increasing the densities of epiphytic algae (DW cm<sup>-2</sup>) caused rapid increases in shading (Figure 5), and the light reduction was well described by an exponential decay function ( $t = -8.884$ ,  $P < 0.001$ ,  $RSS = 0.479$ ). The naturally occurring epiphyte density in October (not sampled on rigs, but in the field) reduced the light availability by averagely 85 % according to this model (Figure 5).

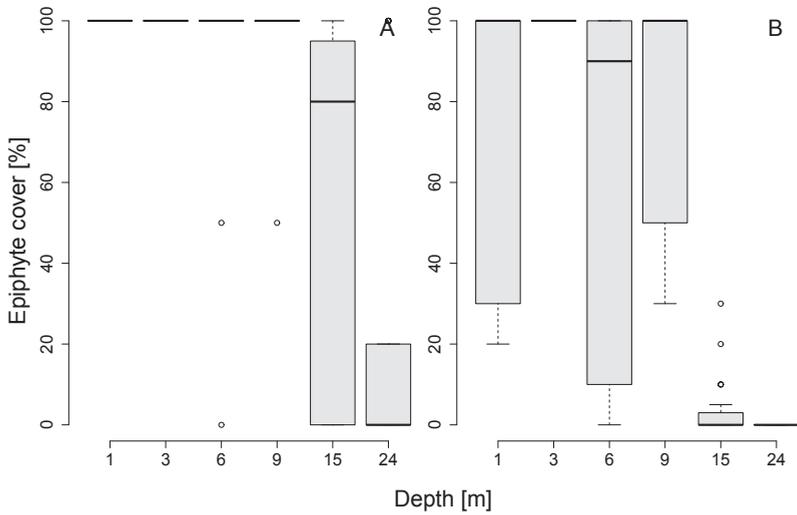


Figure 4: **Epibiont cover.** Covers of *Electra pilosa* (pane A) and *Ciona intestinalis* (pane B) observed on kelp at six depths in Skagerrak in fall. Medians are represented by the horizontal line in each box, and the boxes comprise the first and third quartiles of each data group. Whiskers extend to the extreme data point which is no more than 1.5 times the interquartile range from the box. Recorded values that fall outside this range is represented by circles.

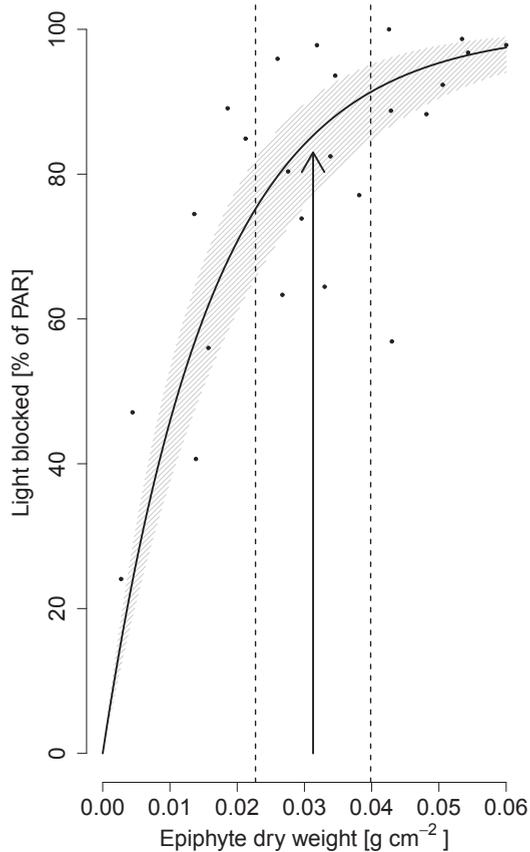


Figure 5: **Shading by algal epiphytes.** The vertical arrow represent the mean dry weight of algal epiphytes ( $\text{g cm}^{-2}$ ) found naturally occurring on *S. latissima* in Skagerrak in October. The vertical dashed lines represent the 95 % confidence interval of this mean. The relationship between epiphyte density (X) and light blocking (Y) is described by the model;  $Y \sim 100(1 - e^{-61.4X})$ , and represented by the solid line. The shaded area depicts the 95% confidence interval of the model predictions.

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### 3.5 Temperature and light

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The daily means of ocean temperatures (at 0, 5, 10, 20 and 30 m depth) in Skagerrak from March to August 2009 are presented in Figure 6. The period with the highest sea water temperatures spanned from June (summer) to August (fall). The average sea water temperature at the surface was ( $17.6^{\circ}\text{C} \pm 0.2 \text{ SE}$ ) from July to August, and the difference down to 20 m depth was relatively small ( $-2.8^{\circ}\text{C} \pm 0.2 \text{ SE}$ ).

The 24-hour light dynamic schemes varied with depth and season (Figure 7). The shape of the curves were similar all seasons, while the intensity and the rate of reduction with depth varied due to seasonal changes in the irradiance and the solar elevation angle. In fall, light decreased rapidly with depth, and the intensity was 90 % lower at 15 m depth as compared to at 3 m depth (Figure 8).

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## 4 Discussion

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The present study showed that kelp from the west coast (WCK) and the Skagerrak populations (SCK) responded similarly to the depth treatment and seasonal changes in the environment while mounted on rigs. Secondly, it showed that that the light deprivation caused by epibionts may become lethal. Beyond that, further studies are needed in order to determine how epibionts impact natural populations, and how the impact varies with depth on a larger scale. That said, the following discussion supports the hypothesis that the recovery of *Saccharina latissima* forests in Skagarrak is hindered by high levels of environmental stress, and that fouling is likely to be an important stressor.

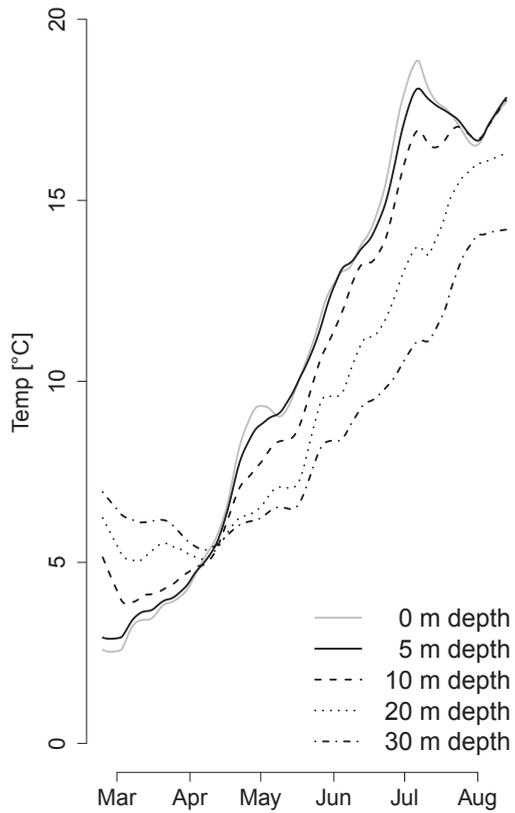


Figure 6: **Temperatures.** Temperatures from March to August 2009 at five depths as provided by the Norwegian Meteorological Institute.

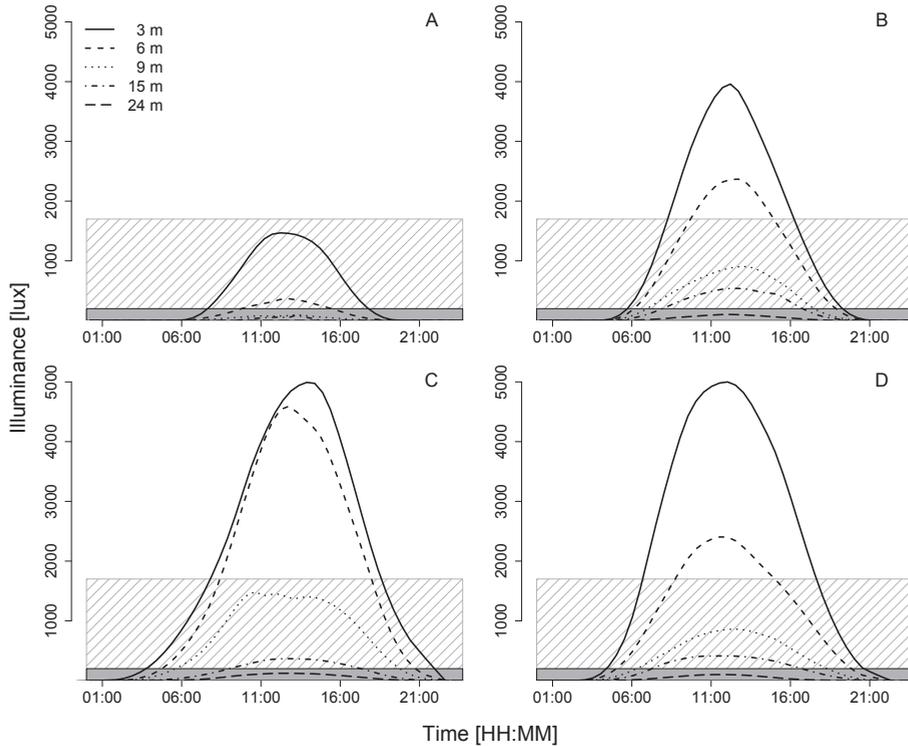


Figure 7: **Light.** Daily light dynamics in the water column (at 3, 6, 9, 15 and 24 m depth) in Skagerrak in winter (pane A), spring (pane B), summer (pane C) and fall (pane D) of 2009. Light intensities were measured by HOBO-pendants and the lines were drawn using locally weighted polynomial regressions (the `lowess` function in R). The shaded areas represent light intensities lower than the compensation points of *Saccharina lattissima* grown at 10°C (solid gray) and 20°C (diagonal lines) respectively (according to Sogn Andersen et al [44]).

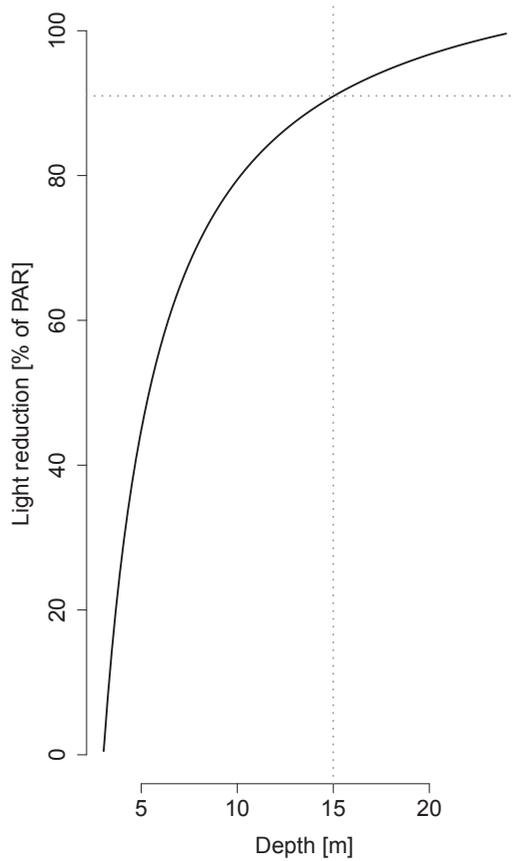


Figure 8: **Light reduction with depth.** Light reductions calculated from 3 m and down to 24 m depth in fall. The horizontal dotted line indicates the light reduction experienced by a kelp at 3 m depth overgrown by a vase tunicate colony (*Ciona intestinalis*). The vertical dotted line indicates the depth equivalent in regards to light intensity.

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## 4.1 Growth and survival in the sample populations

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The growth rates were quite high and did not differ among the two study sites in spring and summer, while poor growth and high mortality was recorded in Skagerrak in fall. Information about differences between the study sites in fall would have been valuable, but the rigs on the west coast were unfortunately lost.

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Growth was consistently slower in SCK as compared to in WCK, but care must be taken in the interpretation of this result. Growth was measured as relative areal increase, and there are morphological differences between the west coast and the Skagerrak populations which leads to differences in biomass per thallus area. *S. latissima* individuals from Skagerrak have thicker laminas and appear sturdier than the individuals from the west coast (personal observations). Direct comparisons of growth rates as a measure of differences in production of new tissue may therefore be inappropriate.

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We were, however, more interested in the effect depth had on the growth rates. The statistical analyses showed that the interaction between sample population and depth was not significant (Table 2), which indicate that the depth related growth response in the two populations were approximately the same. The consistency in responses (both growth and survival) between WCK and SCK in Skagerrak, suggests that both sample populations were equally poorly adapted to handling the environment at the Skagerrak site.

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## 4.2 Depth related patterns of growth and survival

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The depth limit of *S. latissima* is controlled by light availability, and a considerable upwards change in Skagerrak has been imputed to reduced water clarity [36, 47]. Recent surveys have documented that the distribution of *S. latissima* patches stops at depths of 15 m in

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429 the north-eastern part of Skagerrak [29, and references therein]. Even  
430 though survival was good at 15 m depth in our study, the low light  
431 conditions may have posed a challenge had we continued the exper-  
432 iment through the winter. Although *S. latissima* in arctic areas are  
433 able to endure very low light conditions [4], we do not know how the  
434 populations along the Norwegian mainland respond to similar condi-  
435 tions.

436 In order to estimate the success of a species in a given habitat,  
437 growth is ecologically significant because it integrates many physio-  
438 logical processes, of which photosynthetic activity is very important  
439 [2]. The organism has to maintain a positive carbon budget in order  
440 to grow, and when the expenditure (i.e. respiration) increases,  
441 more light and/or more efficient photosynthesis is needed. The growth  
442 rates in individuals from both sample populations slowed down with  
443 increasing depth in spring and summer. This pattern was expected  
444 as a consequence of reduced light availability. Contrastingly, faster  
445 growth was observed at 15 m as compared to at shallower depths  
446 in fall. High temperatures reduce the kelps net photosynthesis [44],  
447 and higher temperatures in shallow waters could have explained the  
448 slower growth. However, the light intensities recorded at 3 and 6 m  
449 depth should have been sufficient to support photosynthetic gain in  
450 *S. latissima* (Figure 7 C-D), and the sea water temperatures were not  
451 particularly high in 2009 (well below 20°C, Figure 6). Low growth and  
452 high mortality at shallow depths may therefore also have been caused  
453 by other factors. The densities of epibionts in fall were very high close  
454 to the surface, and epibiont covers are likely to have negative impacts  
455 on kelp growth and survival.

### 456 **4.3 Effects of epibiont fouling**

457 Epibionts have been shown detrimental to canopy forming marine macro-  
458 phytes in other areas of the world [40, 42, 41]. Poor conditions of kelp  
459 in forest patches and high kelp mortality have coincided with fouling

460 in Skagerrak [45, 29], suggesting that fouling organisms may have neg-  
461 ative impacts in this area as well. Epibionts may deprive their host  
462 of light through shading, and reduced light availability may cause en-  
463 ergy deficiency in photosynthesizing organisms like kelp. Our study  
464 showed that the extent of shading caused by ascidian and algal covers  
465 was considerable, while the effect of a bryozoan cover was relatively  
466 modest. However, a positive relationship between bryozoan and vase  
467 tunicate covers, suggested that bryozoan crust may modify the kelp  
468 surface and facilitate settlement of other, more light absorbant species.

469 The kelp individuals monitored from 1 to 9 m depth in the rig study  
470 were densely covered by vase tunicates in fall, and the laboratory study  
471 showed that these covers may have deprived the individuals of as much  
472 as 90 % of the available light. The difference in light intensity between  
473 3 and 15 m depth in fall was also 90 %. Thus, the heavily fouled kelp  
474 plants located at 3 m depth may have received light amounts equal  
475 to those expected at approximately 15 m depth. Growth was slower  
476 and mortality much higher at 3 m as compared to 15 m depth in  
477 fall, which may indicate either that the covers deprived the kelp of  
478 more light than estimated, or that other factors than light influenced  
479 our results. Higher temperature at 3 m depth may have reduced the  
480 photosynthetic gain in the kelp, causing slower growth, and/or the  
481 epibionts may have had additional negative impacts on *S. latissima*.

482 Epibionts are likely to increase the diffusion boundary layers of or-  
483 ganisms [23, 38], and therefore prevent nutrient and carbon inflow from  
484 the surrounding sea water. Nutrient and carbon limitations will affect  
485 the growth of photosynthesizing hosts negatively, which could explain  
486 the slow growth on heavily fouled kelp. Because nitrogen nutrition has  
487 been coupled to heat tolerance in *S. latissima* [14], a reduction of the  
488 kelps resilience against heat stress may be a particularly relevant con-  
489 sequence. If epibionts reduce heat tolerance of their host, and covers  
490 are most extensive in shallow waters, one might expect a downward  
491 push in the upper growth limit of *S. latissima* farther than predicted

492 from temperature studies performed on clean kelp (i.e. most studies).  
493 The accumulation of epibionts may also increase the brittleness of the  
494 lamina, which results in defoliation during mechanical disturbance like  
495 storms [24, 25, 39, 42]. Dense covers of bryozoans may finally reduce  
496 the reproductive output of kelp [37] impairing recruitment and, by  
497 extension, forest recovery.

498 The present study does not fully answer the question of how epibionts  
499 affect *S. latissima* growing on the sea floor, but it does suggest that  
500 the effect of epibionts should be incorporated into future research deal-  
501 ing with the distribution of kelp forests. Though it could be argued  
502 that the rig treatment may have affected the development of epibiont  
503 communities, procedural controls in another study showed that dis-  
504 lodgement of kelp and moving them to an unfamiliar location did not  
505 affect epibiont covers [27]. As epibiont settlement in the present study  
506 occurred several months after the translocation (in concurrence with  
507 [45]) and is commonly found on *S. latissima* in situ [29], we consider  
508 the covers observed on translocated individuals to be the effect of lo-  
509 cation rather than the methodology applied. Further, the densities of  
510 algal epiphytes reported from the laboratory study were measured on  
511 kelp individuals sampled in the field in October, and these samples  
512 had not been subjected to rig treatment at all.

#### 513 **4.4 Final remarks**

514 The WCK and the SCK sample populations did not exert different  
515 responses in relation to neither the depth treatment nor seasonal  
516 changes, they were equally fouled by epibionts and showed similar  
517 patterns of survival when exposed to the same environment. The re-  
518 gional difference in kelp forest recovery seems therefore most likely  
519 caused by environmental differences between the areas.

520 Growth and survival of *S. latissima* in Skagerrak are likely to be  
521 reduced by heavy loads of epibionts. While epibionts reduce the light  
522 availability to levels that may become lethal in shallow areas, espe-

523 cially during warm periods in summer and fall, depths where epibionts  
524 are sparse (i.e. around 15 m) may be close to the lower limit of the  
525 kelps depth distribution. This suggest that a vertical squeeze, or nar-  
526 rowing of the distribution range of *S. latissima* may be occurring in  
527 Skagerrak. Epibionts are sparser and the distribution of *S. latissima*  
528 spans into deeper waters on the west coast [46], which may explain  
529 why kelp forests in this area are more successful than in Skagerrak.  
530 Large-scale and long-term studies of natural populations are however  
531 needed, in order to test these hypotheses.

532 Although the kelp growth model was deemed appropriate for the  
533 purpose of the present discussion, it should not be applied for further  
534 predictions. Depth was used as a proxy for an intricate web of interac-  
535 tions of which epibiont densities, light and temperature are important  
536 contributing factors, all of which vary on geographical scales.

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Table 2: **Model selection in relation to growth.** Both generalised least square models (*gls*) and linear mixed effect models (*lme*) were tested. Parameters marked with an x were included in the model. Site and Pop annotates experimental site and sample population (WCK or SCK) respectively. In step 1, saturated models were fitted by maximizing the restricted log-likelihood (REML). The model with the lowest AIC was chosen for further parameter selection. Selection of parameters was performed in step 2, where models were fitted by maximizing log-likelihood (ML). In step 3, heterocedasticity was dealt with by including a second degree polynomial (Depth<sup>2</sup>) and an additional weights term, to allow for different variances between seasons.

Selection	<i>Step 1</i>			<i>Step 2</i>			<i>Step 3</i>
	1a	1b	1c	2a	2b	2c	Final
R function	<i>gls</i>	<i>lme</i>	<i>lme</i>	<i>gls</i>	<i>gls</i>	<i>gls</i>	<i>gls</i>
Site	x	x	x	x	x		
Pop	x	x	x	x	x	x	x
Depth	x	x	x	x	x	x	x
Depth <sup>2</sup>							x
Ssn (Season)	x	x	x	x	x	x	x
<i>Site</i> × <i>Pop</i>	x	x	x	x			
<i>Site</i> × <i>Depth</i>	x	x	x				
<i>Pop</i> × <i>Depth</i>	x	x	x				
<i>Pop</i> × <i>Ssn</i>	x	x	x				
<i>Ssn</i> × <i>Depth</i>	x	x	x	x	x	x	x
Random		Station	Station				
Random int		x	x				
Random slope			x				
Weights							Season
Method	REML	REML	REML	ML	ML	ML	ML
AIC	-5737	-5735	-5731	-5958	-5959	-5961	<b>-5996</b>

Table 3: **Final growth model.** Estimates from A) the growth rate model with the best AIC fit (see Table 2) and B) a separate model excluding data from fall. The baselines (Intercept) in the gaussian models were A) growth rates in the west coast sample population (WCK) in Fall and B) growth rates in WCK in spring. Experimental site was not significant, and excluded in the model selection process.

	Parameter	Estimate	S.E.	t	p
A	Intercept (WCK in Fall)	0.0017	0.0006	2.70	0.0071
	Population (SCK vs. WCK)	-0.0004	0.0002	-2.64	0.0085
	Depth	0.0003	0.0001	5.48	< 0.0001
	Depth <sup>2</sup>	-0.0001	0.0000	-5.99	< 0.0001
	Season (Spring vs. Fall)	0.0070	0.0006	10.89	< 0.0001
	Season (Summer vs. Fall)	0.0080	0.0007	12.17	< 0.0001
	<i>Depth × Season (Spring vs. Fall)</i>	-0.0004	0.0001	-7.35	< 0.0001
	<i>Depth × Season (Summer vs. Fall)</i>	-0.0004	0.0001	-6.52	< 0.0001
<i>Without data from fall:</i>					
B	Intercept (WCK in Spring)	0.0087	0.0002	36.48	< 0.0001
	Population (SCK vs. WCK)	-0.0005	0.0002	-2.98	0.0030
	Depth	-0.0001	0.0000	-1.80	0.0721
	Depth <sup>2</sup>	-0.0000	0.0000	-6.06	< 0.0001
	Season (Summer vs. Spring)	0.0009	0.0002	3.41	0.0007
	<i>Depth × Season (Summer vs. Spring)</i>	0.0000	0.0000	1.84	0.0658

Table 4: **Analysis of survival.** Estimates from the analysis of survival on the west coast (in summer) and in Skagerrak (in fall). The baselines (Intercept) in the binomial models were in both cases survival in the west coast sample. Station was included as a random factor.

Parameter	Estimate	S.E.	z	p
<i>Summer (on the west coast)</i>				
Intercept (WCK)	15.86	93.84	0.169	0.866
Pop (SCK vs WCK)	36.12	>100	0.000	1.000
Depth	$\sim 0$	8.662	0.000	1.000
Depth <sup>2</sup>	$\sim 0$	33.42	0.000	1.000
<i>Fall (in Skagerrak)</i>				
Intercept (WCK)	-3.722	0.811	-4.586	< 0.001
Pop (SCK vs WCK)	0.320	0.481	0.664	0.507
Depth	0.600	0.141	4.258	< 0.001
Depth <sup>2</sup>	-0.024	0.006	-4.131	< 0.001

Table 5: **Analysis of epibiont covers.** Estimates from the analysis of covers on *S. latissima* survivors in Skagerrak in fall (percentages). The baseline (Intercept) in both binomial models was cover on individuals from the west coast sample population. Station was included as a random factor influencing both the intercept and the slope of the models.

Parameter	Estimate	S.E.	z	p
<i>Cover of vase tunicates</i>				
Intercept	-0.937	2.404	-0.390	0.697
Pop (SCK vs WCK)	-1.330	0.736	-1.808	0.070
Depth	-0.303	0.081	-3.739	≪ 0.001
Bryozoan cover	4.638	2.380	1.949	0.051
<i>Cover of bryozoans</i>				
Intercept	0.550	1.843	0.298	0.765
Pop (SCK vs WCK)	-1.320	0.782	-1.688	0.092
Depth	-0.068	0.069	-0.983	0.326
Tunicate cover	8.345	4.215	1.980	0.048





## Patterns of *Saccharina latissima* recruitment

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### Abstract

The lack of recovery in Norwegian populations of the kelp *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders after a large-scale disturbance that occurred sometime between the late 1990s and early 2000s has raised considerable concerns. Kelp forests are areas of high production that serve as habitats for numerous species, and their continued absence may represent the loss of an entire ecosystem.

Some *S. latissima* populations remain as scattered patches within the affected areas, but today, most of the areas are completely devoid of kelp. The question is if natural recolonization by kelp and the reestablishment of the associated ecosystem is possible.

Previous studies indicate that a high degree of reproductive synchrony in macrophytes has a positive effect on their potential for dispersal and on the connectivity between populations, but little is known about the patterns of recruitment in Norwegian *S. latissima*. More is, however, known about the development of fertile tissue (sori) on adult individuals, which is easily observed.

The present study investigated the degree of coupling between the appearance of sori and the recruitment on clean artificial substrate beneath adult specimens. The pattern of recruitment was linked to the retreat of visible sori (i.e. spore release) and a seasonal component unrelated to the fertility of the adults.

The formation and the retreat of visible sori are processes that seem synchronized along the south coast of Norway, and the link between sori development and recruitment may therefore suggest that the potential for *S. latissima* dispersal is relatively large. These results support the notion that the production and dispersal of viable spores is unlikely to be the bottleneck preventing recolonization in the south of Norway, but studies over larger temporal and spatial scales are still needed to confirm this hypothesis.

## Introduction

Kelps, seaweeds, and seagrasses provide important ecosystem services in coastal areas, and the loss of these macrophytes is a global concern [1–5]. In Norway, the disappearance of the perennial kelp *Saccharina latissima* has raised considerable concerns both within and outside the scientific community [6]. The Norwegian authorities have called on researchers for plans of action, and some of the preliminary suggestions involve measures aimed at facilitating natural recolonization. Successful kelp recruitment is a prerequisite for recolonization, yet there are few studies of *S. latissima* recruitment *in situ*.

Kelps exhibit life histories in which large diploid sporophytes (benthic spore producing stages) alternate with microscopic haploid gametophytes (benthic sexual stages) via flagellated spores (planktonic dispersal stages). The production of spores in *S. latissima* is significant, and kelp spores may disperse over great distances [7,8]. Although most settle near the mother plants [9,10], large-scale oceanographic processes may serve as key drivers of connectivity (exchange of genetic material) between kelp populations [11]. Kelp recolonization of barren grounds [12] and colonization patterns on artificial reefs [pers.com. Hartvig Christie] in Norway are also consistent with long-distance dispersal of *S. latissima*, and rapid forest recovery after *S. latissima* deforestation in the past indicate that natural recolonization was once possible [6]. Today, turf algae communities loaded with sediments seem persistent in most of the deforested areas, while *S. latissima* remains absent.

*Saccharina latissima* growing along the Skagerrak coast of Norway initiate the formation of sori (fertile tissue) in October, and sori are usually observed until spring (March-April) [13]. The distinct pattern of sori development in Norwegian *S. latissima* (similar to that of *Pterygophora californica* in the study by Reed et al. [14]) may indicate reproductive synchrony among adjacent populations. Supporting this notion, previous observations indicate that the amount of *S. latissima* recruits *in situ* varies considerably through the kelps' reproductive period [Henning Steen, unpublished]. This result may be important to validate, because a high degree of reproductive synchrony has a positive effect on the dispersal potential of kelp populations [10,14].

The present study recorded *S. latissima* recruitment on clean artificial substrate directly beneath adult sporophytes (parent kelps) throughout a reproductive period. The aim of the study was to determine the extent of coupling between the development of fertile tissue (sori) on the parent kelp and recruitment, and to investigate whether the recruitment showed seasonal variation that was unrelated to the fertility of

the parent sporophytes. The influence of such a seasonal component would mean that the probability of transition from spore to sporophyte varied throughout the reproductive period. To aid the interpretations, the recruitment on substrate that had been exposed to *in situ* spore settlement for one, two and three months was recorded. In addition, a series of laboratory experiments was performed to investigate recruitment both early and late in the reproductive period of the kelp. The aim was to investigate the seasonal differences in the time-related pattern of recruitment following spore release, because such differences would affect the proportion of recruits settling directly beneath the kelp *in situ*.

## Materials and Methods

### Ethics statement

The deployment of rigs, the sampling and the laboratory work was executed in accordance with Norwegian regulations. The study did not involve endangered or protected species and the research complied with all applicable Norwegian laws.

### Field study

The pattern of recruitment and the extent of coupling to sori development on the parent kelp was investigated in a field experiment.

Fifteen adult ( $> 1$  yr) *Saccharina latissima* individuals were sampled from a persisting population just outside Grimstad on the south coast of Norway ( $58^{\circ}19'N$ ,  $8^{\circ}35'E$ , WGS84 datum). The kelp plants were mounted on separate rigs and deployed at three sites inside a deforested area ( $> 30$  m between rigs and  $> 300$  m between sites). The specimens were kept at a 3 m depth and monitored for approx. one year to detect seasonal patterns of sori formation. Sori formation was estimated as the percentage cover of the kelps' blade area. A narrowing of the blade somewhere close to the distal part was visible on all blades. This narrowing represents the transition between two growth periods. The blade area from the base of the blade to this narrowing was considered new tissue ( $< 1$  year old), while the remaining distal part of the blade was considered old tissue (probably  $> 1$  year old).

Trays with a series of wrenched holes were mounted on the rigs at a 5 m depth (2 m below the kelp), one tray on each rig. Removable plexiglass quadrats (3 x 3 cm, termed tiles hereafter) were attached to the trays with polypropylene screws. The surface of the tiles had been ground with sandpaper to

make them suitable for *S. latissima* spore attachment. After one, two, and three months' deployment in the field, the tiles were collected. The comparison of recruitment on tiles that had been in the field for different durations allowed for an evaluation of density effects. The collection of tiles, and the subsequent deployment of new ones, was executed every month from December 2007 until May 2008. After collection, the tiles were stacked onto wrenched bolts (approx. length of 10 cm) with slender spacers in-between each tile (1-2 mm spacing). Clean tiles were placed at each end of the stacks to protect the spores/recruits on the first and last recruitment tile in each stack. Each stack was kept tight on the bolt with a nut to ensure minimal disturbance of the tile surfaces during transport to the laboratory. The samples were protected from direct sunlight at all times and transported in dark coolers containing seawater. In the laboratory, the tiles were separated and put in individual petri dishes containing IMR/2 medium. The petri dishes were kept at a temperature of 12 °C (close to the optimal temperature for gametophyte and sporophyte growth [2]) in a 16 : 8 hour light-dark cycle under 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for approximately one week. *S. latissima* recruits (juvenile sporophytes) on each tile were finally counted using a light microscope.

The parent kelps were trimmed down to 1 m length at each collection event. The trimming served two purposes: 1) the blade area of each kelp was kept similar in size throughout the experiment, making comparisons of sori covers easier and 2) the disturbance caused by long blades sweeping the spore collecting devices was avoided. Trimming was, however, seldom needed in the reproductive period as this is also a period of minuscule growth in *S. latissima*.

## Laboratory study

In order to interpret the seasonal aspect of the field experiment, a laboratory experiment was conducted. The aim was to investigate the time-related pattern of recruitment in the minutes and hours following spore release, and to detect whether the pattern changed during the reproductive period of the kelp. Such a change would affect the recruitment observed directly beneath the parent kelp, and thus be vital for the interpretation of the field results.

Mature sporophytes (6 individuals) with visible sori were collected from the persistent population just outside Grimstad on the south coast of Norway in January 2009, April 2009, February 2011, and March 2011. A spore solution (mixture of kelp spores and seawater) was prepared from the fertile tissue, following the same procedure at every sampling event. From each kelp individual, one sample of fertile tissue (6  $\text{cm}^2$ ) was taken. The samples were rinsed in fresh water for five seconds and blotted dry with

paper towels for two minutes before they were submersed in 1 L of filtered seawater. The tissue samples were left in the water for 45 minutes (to ensure spore release), and spores from the different tissue samples were mixed. To create the spore solution, 110 mL of the spore mixture was added to 990 mL of filtered seawater. This dilution was performed because induced spore release tends to cause very high concentrations of spores, and very high concentrations are more difficult to work with (based on own experience with kelp cultivation).

The number of spores was counted manually in six subsamples of the solution using a hemocytometer, and the spore concentration was estimated. A 25 mL sample of the spore solution was added to four petri dishes containing one standard cover slip for microscopy each (18 mm x 18 mm). Each petri dish represented the onset of one series. At given time intervals, the spore solution in each petri dish was carefully poured into a new petri dish with a new cover slip. The transfer was executed at 10 min intervals for one hour, at 20 min intervals for the second hour, at 30 min intervals for the next four hours, every hour for four subsequent hours, every second hour for six hours, and, finally, every third hour until the series was discontinued after 25 hours. The time schedule was based on experiences with a pilot study in January 2009. After every spore solution transfer, the preceding petri dish was filled with growth medium (IMR/2) to ensure growth of the settled spores. All cover slips were kept at 6 °C in a 16 : 8 light-dark cycle under 50  $\mu\text{mol photons } m^{-2}s^{-1}$  (PAR). The average sea water temperature at a 10 m depth was 5.04 ( $\pm 1.2$  SD) from January to April, so the temperature in the culture room was similar to natural conditions.

After one month in culture, the number of recruits (juvenile sporophytes) was counted using a light microscope (10 x 10 magnification), scrolling one lane across each cover slip. No particular edge effect was observed. If present, but undetected, such an effect would be likely to affect all samples and be of little consequence for the overall results. All recruits within a lane were therefore counted. The area on each cover slip in which the recruits were counted (1.25 mm x 18 mm) will hereafter be called the counting field. The rate of recruitment was determined by dividing the number of recruits observed in a counting field with the amount of time spore settlement had been allowed to occur on that particular slip (the interval duration).

## Method development for the laboratory study

Two different treatments were tested in the pilot project in January 2009. Spore solutions were first poured into petri dishes containing two cover slips. One of the cover slips in each dish was then gently removed and placed in a new petri dish containing growth medium. The remaining cover slip in the old dish was treated as described in the previous paragraph, and the spore solution was transferred to a new petri dish containing two additional cover slips. The differences in recruitment between the two treatments were analyzed using a generalized linear model (GLM) of the Poisson family. The analysis proved the effect of treatment to be nonsignificant ( $P > 0.1$ ).

## Statistical analyses

All statistical analyses were conducted with the R statistical software [15]. The recruitment in the field was analyzed with hurdle models based on the *pscl* package [16]. The recruitment patterns observed in the laboratory were analyzed with generalized linear mixed models (GLMM) via PQL, based on the *MASS* package [17], while the spore concentrations were compared using a GLM of the Poisson family. Datasets and R-scripts are publicly available in a GitHub-repository (<http://bit.ly/SAndersen2013>).

The numbers of recruits observed on the field tiles were analyzed in relation to the time of tile deployment (November - May), the duration of deployment (1, 2, or 3 months) and sori cover on the parent kelp. The traditional methods for analyzing count data (such as the GLM of the Poisson or Quasipoisson family) was considered, but the data contained many zeros, and the model predictions were over-dispersed. The use of a hurdle model was a much better option for several reasons. The idea underlying the hurdle model is that the observed response (in this case, the number of kelp recruits) is the result of two ecological processes [18]. In the context of the present study, one process was assumed to cause the presence or absence of recruits (i.e., the presence or lack of spores in the seawater), and where recruits were present, a second process was expected to influence the actual number of them (e.g., spore viability, grazing, or density controlling mechanisms). Model selection was performed using the Akaike Information Criterion as a measure of relative goodness of fit [19]. The model residuals were plotted and inspected, especially in relation to station and rig, to detect any patterns suggesting that mixed modeling was required. No residual pattern was shown in relation to either factor, and mixed effect modeling was therefore deemed unnecessary. Hence, the data from the three different stations were pooled.

The rate of recruitment observed in the laboratory experiment was analyzed in relation to time after spore release (Time), the number of recruits left in the spore solution above the counting field (Density), and the timing within the reproductive period of *S. latissima* (early or late). The main goal was to look at the seasonal differences, and year (2009 or 2011) was therefore included as a random factor. The density of spores could not be measured in the solutions during the experiment. Instead, a proxy was calculated by adding the number of recruits observed in a counting field to the number of recruits observed in the subsequent counting fields within the series. Hence, "Density" in the models reflected the number of potential recruits left in the solution at the beginning of the given interval. How interval duration affected the rates was not known, but stochastic processes changing with duration could potentially influence the results. To look at the effects of season, time, and the density proxy, the data were grouped according to interval duration and one analysis was performed per data group. The rates appeared to be gamma distributed, and GLMMs of the Gamma family was therefore used. Finally, the residuals were inspected to detect any autocorrelation within the series, but no patterns were found, and the analyses were deemed appropriate.

## Results

### Field study

Scattered spots of visible sori were observed on all kelp sporophytes in November, when the field study was initiated. By December, the spots had grown to larger areas, and the development of visible spore forming tissue continued until February. New parts of the blade formed sori early, while older (more distal) parts of the blade formed sori late in the reproductive period (Figure 1 B and C). In April, all of the dark areas were gone, indicating that most of the sporangia (the compartments containing spores) were empty.

Recruitment was observed throughout the period when the kelps had visible sori (Figure 1 A), suggesting that spores were released from the adult plant continuously. The hurdle model, however, revealed that the recruitment on the tiles varied (Table 1).

The hurdle model is based on the assumption that two processes determine the response. In this study, one process was assumed to determine the probability of finding recruits, and *if* recruits were found, another process was assumed to have determined the actual number of recruits. The duration of

tile deployment (1, 2, or 3 months in the field) did not seem to significantly affect either the probability of observing kelp recruits on the tiles nor the count (Table 1). In contrast, the time of deployment greatly affected both the probability of recruitment and the number of juvenile kelp plants that was observed on a tile. The amount of new blade sori affected the probability but not the number, while the amount of old blade sori affected both. The model accounted for approximately 68% of the observed variation.

To visualize the effects of each continuous explanatory variable in the model, a set of predictions were made based on a constructed dataset in which only one continuous variable was allowed to vary at a time. The outcome of these predictions are shown in (Figure 2).

Both the presence and density of recruits were related to time of deployment and worked in favor of higher recruitment densities early in the reproductive period (in the Northern Hemisphere winter) (Figure 2 A, D, and G). The fertility of the parent kelp also seemed important, but the relationship depended on which parts of the blade were covered by sori. The predicted effect of increased cover on the new part of the blade (<1 yr) was negative in relation to both the probability of observing recruits and the number of recruits (Figure 2 B, E, and H), though not statistically significant in the latter case (Table 1). In contrast, the predicted effect of increased sori cover on the old part of the blade (> 1 yr) was strongly positive (Figure 2 C, F and I). The extent of this effect did, however, depend on the amount of new blade sori (see interaction term in Table 1).

In summary, the recruitment of *Saccharina latissima* was highest early in the reproductive period. Recruitment was low beneath kelp plants that still contained spores in the newest part of the blade (i.e., had visible new blade sori), while recruitment was high beneath kelp plants that had begun to develop spores in the older part of the blade.

## Laboratory study

The concentration of spores in the spore solutions was  $1\,349\,000\text{ mL}^{-1}$  early in the kelps' reproductive period and  $17\,240\text{ mL}^{-1}$  late in the reproductive period in 2011. The volume of spore solution directly above each counting field at the initiation of the experiment was  $450\text{ mm}^3$ , which translates to 0.45 mL and brings the estimated amount of spores potentially settling in one whole series up to numbers of 607 050 and 7 523 in the respective periods. It was assumed that half of the spores were female and that all females produced sporophytes (although both sex-ratios and success rates may vary somewhat [20, 21]), and the number of potential recruits was estimated to be  $303\,525 (\pm 20\% \text{ SD})$  and  $3\,761 (\pm 14\% \text{ SD})$ , respectively.

The number of realized recruits observed in the series was far less than the estimated potential both months, approx 0.6% of the expected number early in the reproductive period and 64% later on. Despite the lower spore concentration in the spore solution, the recruitment of juvenile sporophytes was therefore much higher late in the reproductive period ( $2\,399 \pm 8\%$  SD as compared to  $1\,739 \pm 12\%$  SD earlier on). More realized recruits were observed in 2009, but the concentrations of spores in these solutions were unfortunately not measured.

The rates of recruitment were similar in both seasons, except for after 6 hours, when significantly higher rates were observed late compared to early in the reproductive period (in the Northern Hemisphere winter). Seasonal differences were also observed in the relationships between recruitment, time, and density in some of the duration intervals (Table 2, see also Figure 4 in Appendix).

The rate of recruitment on the cover slips was generally high shortly after the spores had been released from the parent kelp and subsided very rapidly. The rate then stabilized and remained almost constant within the 20 minute and 30 minute interval groups, i.e., from 1-6 hours into the experiment. This pattern was similar both seasons. After 6 hours, the rate subsided with time late in the reproductive period, while it remained almost constant early in the reproductive period of the kelp (except for at a very high density of potential recruits) (Table 2, see also Figure 4 in Appendix). These results indicate that seasonal differences in the time related recruitment patterns did occur and that the effect appeared quite a long time after the spores had been released. After 10 hours, the recruitment became sporadic, and no significant differences were found between the cover slips. Because the amounts of unsuccessful spores were quite large in both seasons, this indicates that the probability of transition from spore to recruit may have been reduced after 10 hours in the laboratory. The same pattern could have been the consequence of spore depletion and density limits on recruits early on. However, the frequency of recruit counts throughout the study was highest at the low end of the scale (Figure 3), so in most cases, the cover slides were probably large enough for the accumulated spores to develop.

The density of potential recruits in the spore solution affected recruitment rates, but had a significant effect on the time-related pattern (Time x Density in Table 2) only at the initiation of the experiment. As time progressed within the first hour, the predicted effect of Density became increasingly positive (Figure 5 in Appendix). This result may indicate that density-regulating mechanisms (either in mortality or settlement behavior) occurred shortly after spore release during both seasons, but caution is recommended in the interpretation of these results. There are probably better and more ecologically

relevant ways to assess density-controlling mechanisms in the recruitment of *S. latissima*. In the time span from 1 to 6 hours after the initiated spore release, high densities of potential recruits had a positive effect on recruitment, but this effect was significant only early in the reproductive period. The rates of recruitment thus seemed rather stable within this time frame (that is, within the 20 and 30 minute intervals).

In summary, lower spore densities but more recruits were found late compared to early in the reproductive period of *S. latissima*. The time-related patterns of recruitment were however similar in both periods (except for after 6 hours).

## Discussion

*Saccharina latissima* sporophytes translocated into a deforested area in Skagerrak were able to produce fertile tissue and release viable spores. Dense *in situ* recruitment of juvenile *S. latissima* was observed on clean substrate, which further indicated that environmental conditions in the water column did not prevent kelp recruitment. The recruitment of juvenile sporophytes *in situ* can be viewed as the result of two processes: one process that determines the presence of recruits and another process that determines the number of recruits. The present study shows that both processes depend, to different degrees, on the development of spore-producing tissue (sori). Furthermore, the study indicates that a seasonal component independent of spore production in the parental plants also affects recruitment.

The recruitment of juvenile *S. latissima* sporophytes in the field was highest in December and January. Seemingly contradictory, the laboratory study showed much lower kelp recruitment during the peak season than later in the reproductive period of the kelp. This result was not only contradictory but also counter-intuitive as a much higher density of spores was found in the spore solution prepared at that time. Spores must, however, be contained in the sori for a certain amount of time to fully develop [22]. A spore contained in any sporangium was therefore more likely to be fully developed at the end of the reproductive period (March/April) than earlier on (January/February). Because forced release by freshwater rinsing and drying is an effective way of inducing spore release [23], it was assumed that most of the sporangia within the sori were emptied in the process. And the greater number of fully developed spores contained in the sori collected, may thus have accounted for the greater recruitment success in the laboratory late in the reproductive period of the kelp.

The first appearance of sori on the parental *S. latissima* tended to be located in the middle of the frond, which was considered new tissue. The probability of observing recruitment in the field increased as the sori cover on the new parts of the parental plant disappeared. When sori disappear, it is because spores are released, so increased recruitment was not a surprising effect. Older tissue developed sori later than new tissue (Figure 1 C vs B), and increased sori cover on the old parts of the parental plant seemed to increase both the probability of recruitment and the number of recruits beneath the plant. However, these effects may not be linked to old blade sori cover *per se*. As sori develop in old tissue, increasing amounts of spores contained in the younger parts of the blade would have matured and therefore been released at increasing rates. The significant interaction (SO x SN in the hurdle model) also indicated that the correlation between recruitment and visible sori in the old parts of the kelp plant depended on the visibility of sori in the newer tissue. The high recruitment coinciding with sori development in the parents' old tissue was therefore largely a consequence of spore release from the younger tissue.

The probability of finding recruits in the field dropped markedly between March and April, independently of sori cover (see Figure 2 A). This result indicated that some seasonal mechanism, unrelated to the fertility of the parental sporophyte, affected the recruitment on the tiles. The explanation could have been seasonal differences in the physiology of the released spores, but the results from the laboratory study indicate that the differences were small. The time-related recruitment patterns were almost identical in both seasons during the first 6 hours of the laboratory experiment. Because the recruitment tiles were located directly beneath the parent kelp, this was also the time frame in which differences would have been most likely to affect the results in the field. The seasonal component may be explained by variation in the interactions between physical and biological factors and the kelps microscopic stages in the field [24], but this discussion lies well beyond the scope of the present study.

The duration of tile deployment in the field was expected to increase the number of kelp recruits because the tiles would have had more time to accumulate spores. The effect was, however, relatively small and statistically insignificant throughout the study, which indicates that some sort of density-controlling mechanism was constantly at play. This mechanism could be related to the experimental design: Because the tiles were always clean when deployed, but probably accumulated sediment, bacteria, and other organisms in the field, the quality of the tile as substrate may have been progressively reduced during deployment. Other possible explanations are intra- and inter-specific competition, but this discussion also goes beyond the scope of the present study.

The extent of connectivity along the south coast of Norway is not known, but previous records [6] indicate that dispersal from remnant populations and subsequent recovery was once possible. Connectivity between kelp populations is reinforced by reproductive synchrony because higher densities of spores in the currents increase the probability of long-distance dispersal [14]. The seasonal development and demise of visible sori in *S. latissima* are processes that largely overlap along the south coast of Norway [13,25, pers. com. Stein Fredriksen]. The present study showed that *S. latissima* recruitment was tightly linked to this pattern and may therefore also support the notion that the potential for connectivity between *S. latissima* populations in Norway is high. If this is true, forest regeneration through facilitation of natural recolonization may be feasible because remnant populations still exist and the environmental conditions in the water seem to permit it.

Whether the present-day bottom conditions permit kelp recruitment in the deforested areas is, however, a different matter. Sediment covers and turf algae communities have been shown to impair kelp recruitment in other areas of the world [26–28]. The persistence of sediment-loaded carpets of turf algae and the lack of forest recovery in most deforested areas in Skagerrak [6] suggest that this may be the case in Norway as well. Other mechanisms may also obstruct the transition from juvenile to mature kelp: The juvenile sporophytes may, for instance, experience high temperature stress and overgrowth by epiphytic organisms from June to September (the Northern Hemisphere summer). These stressors may reduce the kelps' chance of survival [13,29] and thereby hinder the formation of populations able to sustain themselves. To evaluate management strategies involving facilitation of kelp recruitment and the probability of their success, further studies on the impacts of multiple stressors are needed.

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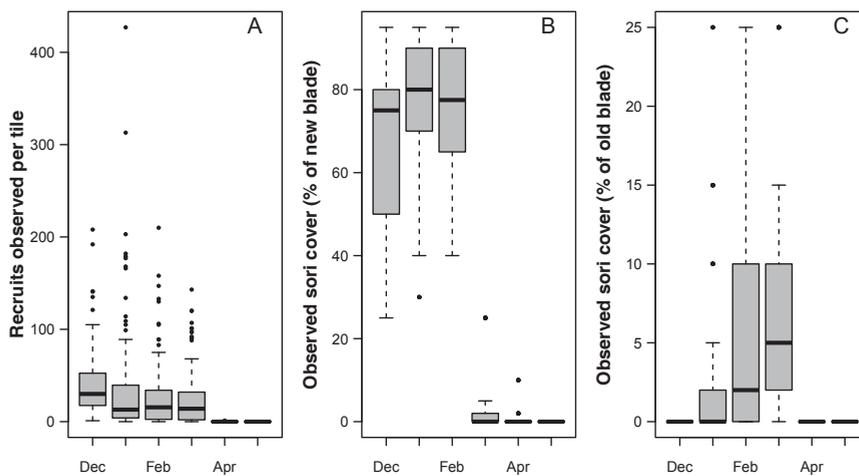
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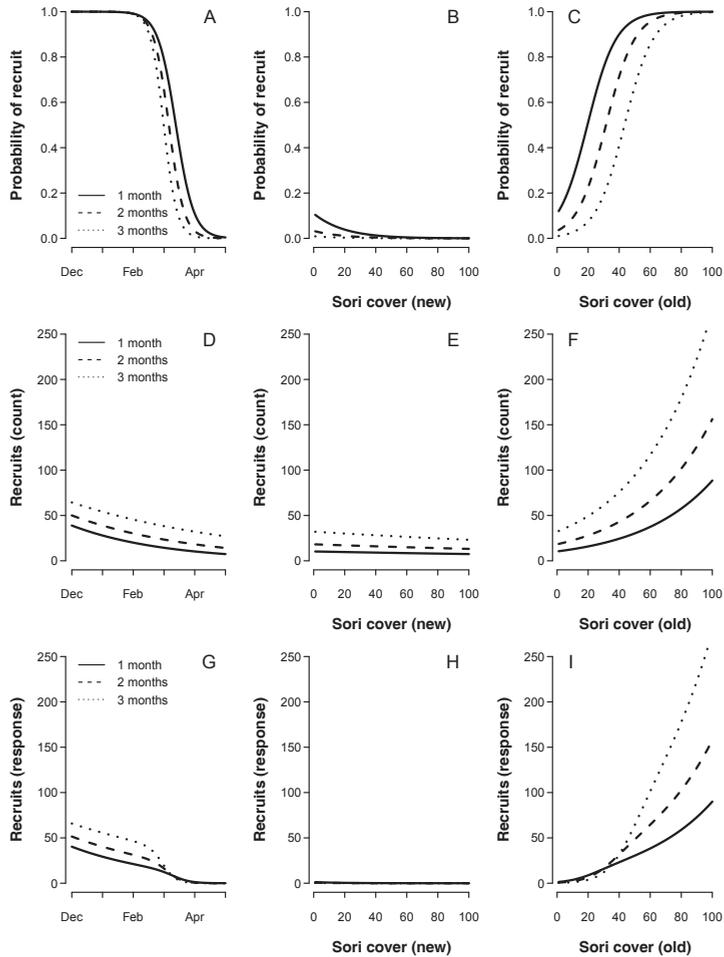
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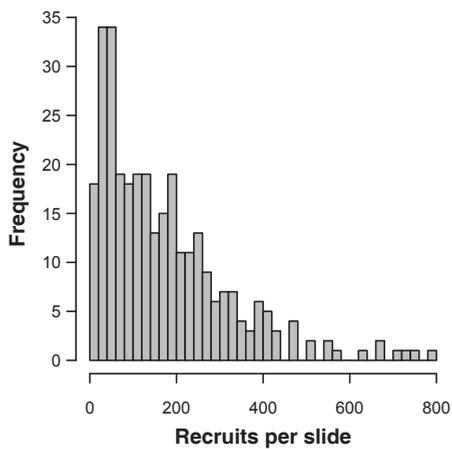
## Figures



**Figure 1. Summary of results from the field experiment** (December 2007 - May 2008). Boxplot A shows the number of recruits observed on the tiles. Boxplot B shows sori coverage observed on the new parts of each blade, while C shows sori coverage observed on the older parts of each blade.



**Figure 2.** Graphical presentation of the model predictions in relation to varying time of tile deployment, sori cover on new tissue, and sori cover on old tissue. Different line types represent different durations of tile deployment. A, B, and C show the predicted probability of observing recruitment. D, E, and F show the predicted number of recruits where recruits are present, while G, H, and I show overall count predictions.



**Figure 3.** Histogram showing the frequency of kelp recruit counts on the cover slides.

## Tables

**Table 1. Hurdle model selection.**

<i>Model</i>	<i>HP1</i>		<i>HNb1</i>		<i>HNb3a</i>	
	Count	Zero	Count	Zero	Count	Zero
Which						
Family	Pois	Bin	Negbin	Bin	Negbin	Bin
Duration (D)	<b>0.002</b>	0.154	0.251	0.154	0.233	0.154
Month (M)	≪ <b>0.001</b>	≪ <b>0.001</b>	< <b>0.001</b>	≪ <b>0.001</b>	< <b>0.001</b>	≪ <b>0.001</b>
Sori new (SN)	0.0705	≪ <b>0.001</b>	0.218	≪ <b>0.001</b>	0.109	≪ <b>0.001</b>
Sori old (SO)	≪ <b>0.001</b>	<b>0.012</b>	0.065	<b>0.012</b>	<b>0.010</b>	<b>0.012</b>
D x M	≪ <b>0.001</b>	0.058	0.119	0.058	0.132	0.058
SN x SO	≪ <b>0.001</b>	<b>0.016</b>	0.613	<b>0.016</b>		<b>0.016</b>
AIC	20833		5372		5370	

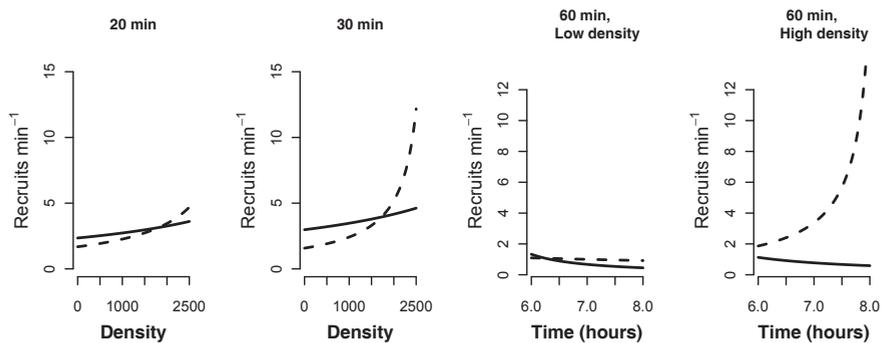
Models with different error distributions were tested (Pois = Poisson, Bin = Binomial, Negbin = Negative Binomial). The Zero part of each model predicts the probability of recruitment, while the Count part predicts the number of recruits given  $\text{Zero} \neq 0$ . Significant P-values are indicated by bold formatting. The interactions between Month and Sori (both new and old) were not significant in any of the models. Because it had the lowest AIC value, the best model was *HNb3a*.

**Table 2. GLMM models of recruitment in the lab.**

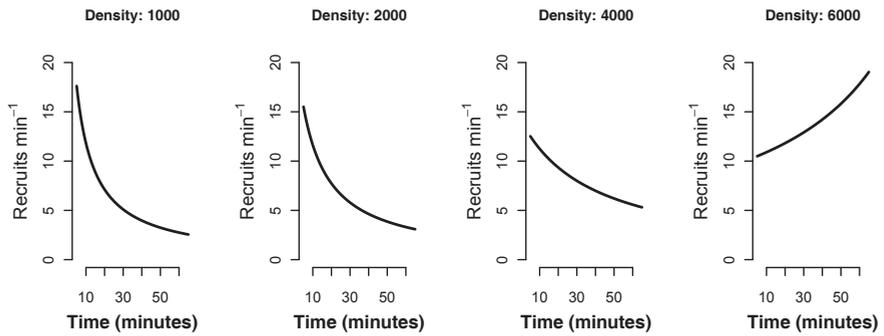
Hours after initiation	<1 hrs		1 – 6 hrs		> 6 hrs	
	10 min	20 min	30 min	60 min	120 min	
Interval duration						
Intercept (late)	0.536	0.728	<b>0.041</b>	< <b>0.001</b>	0.379	
Season (early vs late)	0.154	0.539	0.053	< <b>0.001</b>	0.319	
Time (late)	< <b>0.001</b>	0.333	0.437	<b>0.001</b>	0.970	
Density (late)	<b>0.008</b>	0.816	0.465	<b>0.002</b>	0.791	
Time x Season (early vs late)	0.0506	0.886	0.411	<b>0.001</b>	0.792	
Density x Season (early vs late)	0.8051	<b>0.009</b>	< <b>0.001</b>	0.065	0.407	
Time x Density	< <b>0.001</b>	0.208	0.871	<b>0.027</b>	0.053	

One model was made per interval duration. The interval durations were correlated with Time because the intervals were shorter early in the experiment and subsequently longer as time progressed. After the 120 min intervals were completed (16 hours into the experiment), too little settlement was observed to perform any analysis. Season refers to early (Northern Hemisphere winter) and late (Northern Hemisphere spring) in the reproductive period. Year was included as a random factor. Significant P-values are indicated by bold formatting. Plots showing the effects of the significant parameters on the predictions are presented in the Appendix.

## Appendix



**Figure 4.** The significant effects on the GLMM model predictions in the 20, 30 and 60 minute interval groups. Solid lines represent effects late, while dashed lines represent effects in early in the reproductive period of *S. latissima*. Predicted recruitment increased with increasing density of potential recruits, and the effect was significantly different early compared to late in the reproductive period in all three interval groups. Predicted recruitment decreased with time in the 60 minute interval group, except for during the reproductive peak season at high densities, when predictions increased with time.



**Figure 5.** The effect of time on the GLMM model predictions varied with the density of potential recruits in the 10 minutes interval group. The positive effect of time at the highest densities may suggest the possibility that recruitment was limited by substrate availability at the initiation of the experiment.







## TEMPERATURE ACCLIMATION AND HEAT TOLERANCE OF PHOTOSYNTHESIS IN NORWEGIAN *SACCHARINA LATISSIMA* (LAMINARIALES, PHAEOPHYCEAE)<sup>1</sup>

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Kelps, seaweeds and seagrasses provide important ecosystem services in coastal areas, and loss of these macrophytes is a global concern. Recent surveys have documented severe declines in populations of the dominant kelp species, *Saccharina latissima*, along the south coast of Norway. *S. latissima* is a cold-temperate species, and increasing seawater temperature has been suggested as one of the major causes of the decline. Several studies have shown that *S. latissima* can acclimate to a wide range of temperatures. However, local adaptations may render the extrapolation of existing results inappropriate. We investigated the potential for thermal acclimation and heat tolerance in *S. latissima* collected from three locations along the south coast of Norway. Plants were kept in laboratory cultures at three different growth temperatures (10, 15, and 20°C) for 4–6 weeks, after which their photosynthetic performance, fluorescence parameters, and pigment concentrations were measured. *S. latissima* obtained almost identical photosynthetic characteristics when grown at 10 and 15°C, indicating thermal acclimation at these temperatures. In contrast, plants grown at 20°C suffered substantial tissue deterioration, and showed reduced net photosynthetic capacity caused by a combination of elevated respiration and reduced gross photosynthesis due to lowered pigment concentrations, altered pigment composition, and reduced functionality of Photosystem II. Our results support the hypothesis that extraordinarily high temperatures, as observed in 1997, 2002, and 2006, may have initiated the declines in *S. latissima* populations along the south coast of Norway. However, observations of high mortality in years with low summer temperatures suggest that reduced population resilience or other factors may have contributed to the losses.

**Key index words:** global warming; heat tolerance; kelp; kelp deforestation; PAM; photosynthesis; *Saccharina latissima*; temperature

**Abbreviations:**  $\alpha$ , light affinity; DW, Dry weight;  $F_v/F_m$ , maximum quantum yield of photosynthesis; FW, Fresh weight;  $I_C$ , Compensation irradiance;  $I_{SAT}$ , Saturation irradiance;  $NPQ_{max}$ , nonphotosynthetic quenching; PAM, pulse amplitude modulated; PAR, Photosynthetic active radiation; P-I, Photosynthesis-irradiance;  $P_{max}$ , Maximum photosynthetic rate;  $P_N$ , Net photosynthetic rate; PSII, Photosystem II;  $R_D$ , dark respiration rate,

Ongoing climate change has led to average global sea surface temperature increasing by  $0.6 \pm 0.2^\circ\text{C}$  over the last century (Parry et al. 2007), and larger increases have been reported from polar and cold-temperate areas and from shallow coastal waters and estuaries. The global distribution of marine algae is largely determined by water temperature (Lüning 1984, van den Hoek and Luning 1988), and ocean warming is therefore expected to cause changes in the distribution and abundance of many marine algae (van den Hoek et al. 1990, Adey and Steneck 2001, Müller et al. 2009, Harley et al. 2012). Such changes have been documented for intertidal seaweeds (e.g., Wernberg et al. 2011a, Diez et al. 2012), but similar data for kelps are rare. Most kelp species are cold-temperate species and ocean warming is expected to drive a pole-ward change in their distribution (Müller et al. 2009). No studies have yet documented changes in the range distribution of kelp caused by elevated sea temperatures (but see Johnson et al. 2011), most likely because proper base-line data and extensive time-series are lacking (Merzouk and Johnson 2011, Wernberg et al. 2011b).

*Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl, and G.W. Saunders was previously the most common (>60% cover in the sub-littoral zone) kelp species along the south-coast of Norway where it formed extensive, subtidal meadows extending from the Swedish border in the east to Bergen on the southern part of the Norwegian west-coast (Moy and Christie 2012). These populations have declined dramatically since the end of the

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1990s and especially so in 2002 and 2006 when substantial losses were recorded (Moy and Christie 2012). *S. latissima* is still present in the area, but mostly as single individuals or small scattered populations. Local reports indicate that these kelp forests have disappeared occasionally in the past, but that they used to recover within few years. The production of spores in *S. latissima* is large and kelp spores may disperse over great distances (Schiel and Foster 2006, Cie and Edwards 2011), although most settle near the mother plants (Gaylord et al. 2006). The few scattered populations that still remain along the south coast of Norway should therefore be able to support recovery of the deforested areas. However, after a decade with barren grounds seasonally covered by mud, silt and filamentous algae there is presently no sign of recovery. The continued loss of the kelp populations in southern Norway has stimulated strong debate on the potential causes; some emphasize warmer seawater as the most important driver, while others state that coastal eutrophication might be more important.

The optimal growth temperature for *S. latissima* is variable, but seems to range from 10°C to 15°C while poor growth is typically observed above 20°C (e.g., Fortes and Lüning 1980, Bolton and Lüning 1982). If increasing temperature stimulates respiration more than photosynthesis, then more light and/or a more efficient photosynthesis become necessary to maintain a positive carbon budget. In addition to affecting the carbon balance, high temperature may disturb enzyme driven processes and affect the stability of the lipid membranes that contain the photosynthetic apparatus. However, most plants can to some degree compensate for the negative effects of increasing temperatures (e.g., Campbell et al. 2007). If compensating mechanisms are present in *S. latissima*, it may be able to acclimate and thereby maintain a positive carbon budget and tolerate relatively high temperatures.

*Saccharina latissima* can tolerate a broad range of temperatures. In the N Atlantic, it is distributed from New York State (USA) and Portugal in the south to NE Greenland in the north (van den Hoek and Donze 1967, Druehl 1970, Borum et al. 2002, Bartsch et al. 2008). Some populations experience large annual variations in water temperature (e.g., from a few degrees to more than 20°C in temperate populations; Gerard and Du Bois 1988), whereas others are exposed to constantly low temperatures (e.g., from -1.4°C to 0°C in NE Greenland; Borum et al. 2002). The wide distribution of *S. latissima* may indicate the existence of ecotypes (as a result of adaptations) and/or a high capacity for thermal acclimation of photosynthesis and other metabolic processes. Davison (1987) studied the thermal acclimation in *S. latissima* collected at Helgoland (Germany) and found similar rates of light saturated photosynthesis and respiration in plants grown at temperatures ranging from 0°C to 20°C for

longer periods. These results were later confirmed for rates of photosynthesis obtained at low light and for light requirements ( $I_C$ ) in plants grown at 5°C and 15°C, respectively (Davison et al. 1991). It was hypothesized that iso-enzymes (in the Calvin Cycle) with different temperature optima made it possible for *S. latissima* to acclimate to a wide range of temperatures (Davison 1987, Machalek et al. 1996).

Gerard and Du Bois (1988), on the other hand, found that *S. latissima* growing near its southern boundary in the NW Atlantic (New York, USA) were more tolerant to high temperatures than plants from colder regions (Maine, USA). The heat tolerance in plants from the southern population was explained by adaptation through improved ability to maintain high N reserves and, thus, enzyme systems that could aid the production of heat shock proteins (Gerard 1997). Similar results have been reported for *S. japonica* (Liu and Pang 2009). It seems therefore that the ability of *S. latissima* to cope with a wide range of water temperatures depends on a combination of local adaptations and a high capacity for thermal acclimation.

This study aimed to examine the capacity of Norwegian *S. latissima* to acclimate to and cope with high temperatures (here 20°C). Plants were collected from three different locations along the south coast of Norway (Dröbak in the east, Grimstad in the south and Bergen in the west) and subsequently exposed to three growing temperatures (10, 15 and 20°C) for an extended period (4–6 weeks) after which we measured the photosynthetic performance at five different assay temperatures (5, 10, 15, 20, and 25°C). The photosynthetic capacity, chl *a* fluorescence and pigment concentrations and composition in the plants were compared across the respective growth temperatures and sampling sites.

#### MATERIALS AND METHODS

**Sampling sites.** Young sporophytes (5–25 cm long, FW  $3.93 \pm 4.04$  g) of *S. latissima* were harvested at 5–10 m depth in the vicinity of Bergen, Grimstad and Dröbak in March 2010 (Fig. 1). These sites vary with respect to water temperature (Fig. 2); the average (over the period 1980–2006) mean temperature of the surface water (1 m depth) in the warmest month (August) was significantly lower near Bergen ( $15.6 \pm 1.6^\circ\text{C}$ ) than near Grimstad ( $17.4 \pm 1.6^\circ\text{C}$ ) and Dröbak ( $18.4 \pm 1.5^\circ\text{C}$ ; repeated measures ANOVA;  $F_{(2, 52)} = 135.2$ ,  $P < 0.001$ , all sites different from each other). Average August temperatures in the surface water occasionally exceeded 20°C near Grimstad and Dröbak, but never near Bergen. Water temperatures also decreased with depth; water at 20 m of depth was significantly colder than at the surface, e.g.,  $13.0 \pm 2.1^\circ\text{C}$  versus  $15.6 \pm 1.6^\circ\text{C}$  near Bergen (paired *t*-test;  $t_{25} = 8.52$ ,  $P < 0.001$ ) and  $15.6 \pm 0.8^\circ\text{C}$  versus  $17.4 \pm 1.6^\circ\text{C}$  near Grimstad (paired *t*-test;  $t_{26} = 8.49$ ,  $P < 0.001$ ). Surface water temperatures have increased substantially all along the south coast of Norway from 1980 to 2006 with annual increases ranging from 0.07°C (near Bergen) to 0.12°C (near Grimstad and Dröbak), corresponding to an increase of ~2.01°C–3.15°C in 27 years.

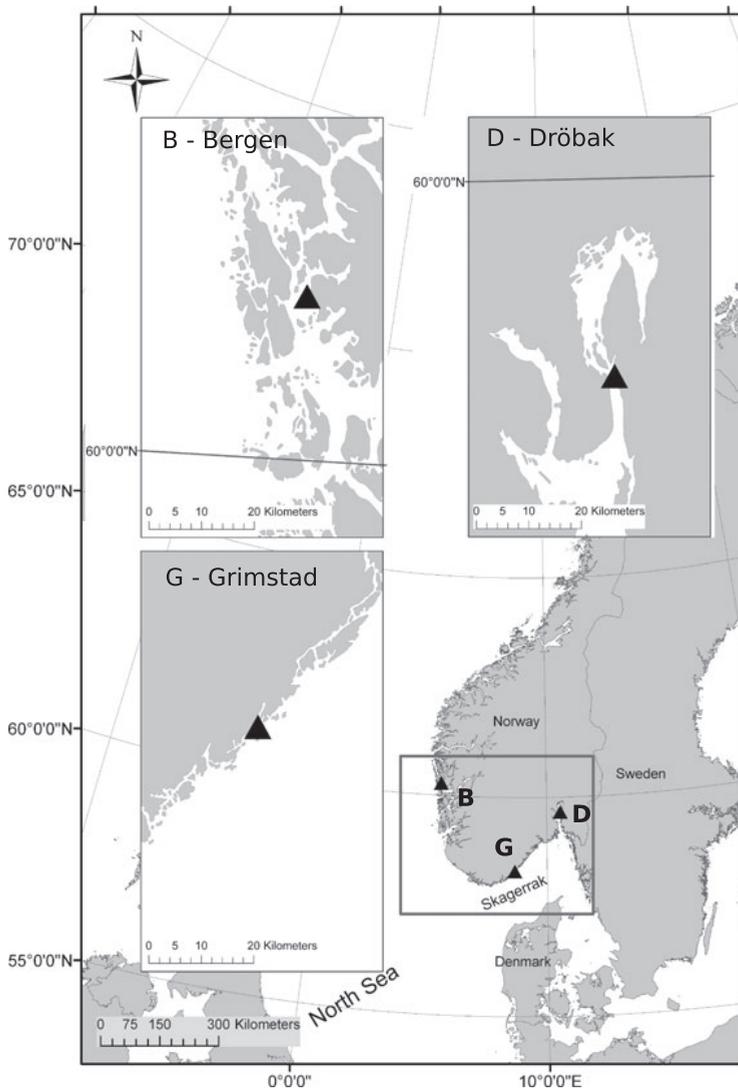


FIG. 1. Map of sample sites. B pinpoints the sample site in vicinity of Bergen at the south-west side of Norway, G pinpoints the southern-most sample site in vicinity of Grimstad and D the south-east sample site close to Dröbak.

*Overall experimental design.* The collected plants were kept in transport boxes with water from the collection sites and immediately transported to the culture facility in Roskilde (Denmark) where they were kept at constant temperature (see below) and light conditions for at least 4 weeks prior to being used in the experiments. Plants were held in 20 L aquaria where they were tied to small PVC-plates with non-toxic silicon strings. Eighteen aquaria (the main experimental units), each holding five replicate kelp plants from each sampling site (15 individuals per aquarium), were placed in six temperature regulated water baths (three aquaria per bath and two baths per temperature, making six aquaria per tem-

perature in total). The water bath temperature was controlled by the combined use of thermostat regulated heaters (Julabo ED, Julabo Labortechnik GmbH, Seelbach, Germany) and coolers (P Selecta, J.P. Selecta, Abrera, Spain) that kept the water temperature constant within  $\pm 0.2^{\circ}\text{C}$ . The aquaria were filled with GFC-filtered sea-water with salinity 30–32 and the water was replenished weekly. The initial temperature in the cultures equaled the in situ water temperature at the time of collection ( $8^{\circ}\text{C}$ – $9^{\circ}\text{C}$ ). Temperatures were subsequently changed by  $1^{\circ}\text{C}$  per day until the intended growth temperatures were reached (i.e., 10, 15 and  $20^{\circ}\text{C}$ ). Our first attempt to establish cultures at  $20^{\circ}\text{C}$  failed as most of the involved plants

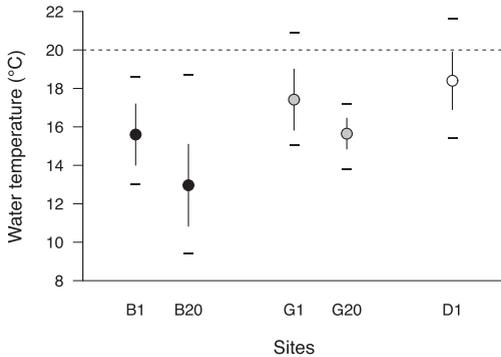


Fig. 2. Average water temperature in August at 1 and 20 m depth, respectively, near the three sampling sites (B: Bergen, G: Grimstad, D: Dröbak). Data cover the period from 1980 to 2006. Mean values  $\pm$  SD ( $n = 27$ ). Small horizontal bars represent observed minimum and maximum mean temperatures in August.

died. The acclimating process was therefore repeated with a slower increase in temperature ( $-0.5^{\circ}\text{C}$  per day). We had too few plants from Dröbak to replace the lost ones, but plants from Grimstad and Bergen were fully represented at  $20^{\circ}\text{C}$ . Each water bath was illuminated by eight Halogen spots (OSRAM Decostar 51; 12 V, 35 W) which provided  $56 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (PAR) in a 12:12 h light:dark cycle. The plants were kept at their final growth temperature for 3–4 weeks before taking any measurements.

Measurements of photosynthetic performance, chl *a* fluorescence, pigment concentrations and total N-content were carried out on four replicate plants from each combination of growth temperature and collection site. Replicate plants within the same growth temperature were collected from separate aquaria.

**Photosynthetic performance.** Measurements of (dark) respiration and photosynthesis were performed in 800 mL gas tight, transparent chambers. Each chamber was equipped with a circulation pump (AquaBee model UP300, AquaBee Aquariertechnik, Zerbst, Germany,  $300 \text{ L} \cdot \text{h}^{-1}$ ) that ensured circulation of water within the chamber. One thallus was fixed within the chamber, which was filled with artificial seawater (salinity 30). Penicillin G-sodium salt and Streptomycin sulfate salt were added to the water (concentration =  $50 \text{ mg} \cdot \text{L}^{-1}$  for each) to reduce bacterial growth in the chamber. The water was bubbled with  $\text{N}_2$  to reduce the initial  $\text{O}_2$  concentration to  $\sim 60\%$  of air saturation to prevent high  $\text{O}_2$  concentrations from building up during incubations. The chamber was finally submerged into a water bath keeping a constant temperature (5, 10, 15, 20 or  $25^{\circ}\text{C}$ , respectively). The water bath held two replicate chambers at a time.

Each chamber was equipped with a Clark-type  $\text{O}_2$  microelectrode (model OX-500; Unisense, Aarhus, Denmark) that was connected to a picoammeter (model Picoammeter PA2000; Unisense) and a Pico Technology ADC-16 high-resolution data logger. The  $\text{O}_2$  concentration was recorded every minute throughout incubations. A lamp with six halogen spots (OSRAM Decostar 51; 12 V, 35 W) illuminated the set-up, and variable levels of irradiance were obtained by using shade screens with different densities. Incubations were initiated by measuring respiration in darkness. Photosynthesis was subsequently measured at increasing levels of irradiance (range:  $0\text{--}375 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  PAR). Rates of  $\text{O}_2$  consumption or release were calculated from incubation periods with constant changes in  $\text{O}_2$  concentration over a

minimum of 10 min. Incubations (providing a full PI-curve) lasted for 3–4 h and four replicate PI-curves were run at each assay temperature. Photosynthetic rates were expressed in units of  $\mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ .

Respiration ( $R_D$ ) was measured in darkness while  $P_{\text{max}}$  was measured at the highest light intensity ( $375 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) which is above the saturating light intensity ( $100\text{--}150 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) reported for *S. latissima* (Fortes and Lüning 1980, Borum et al. 2002). The light utilization efficiency ( $\alpha$ ) and the light compensation ( $I_C$ ) point were estimated from linear regression on six data points obtained at low light (range:  $0\text{--}55 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) while the light saturation point ( $I_{\text{SAT}}$ ) was estimated as the intercept between  $\alpha$  and  $P_{\text{max}}$ .

**Chl *a* fluorescence.** Chl *a* fluorescence was measured using PAM fluorometry (Maxwell and Johnson 2000, Papageorgiou and Govindjee 2004). The level of stress and the photo-protective response in the plants was evaluated from changes in the maximum quantum yield ( $F_v/F_m$ ) and the heat dissipation efficiency (i.e., maximum  $\text{NPQ}_{\text{max}}$ ) of PSII (Maxwell and Johnson 2000). The fluorescence parameters (i.e.,  $F_v/F_m$  and  $\text{NPQ}_{\text{max}}$ ) were measured on four replicate plants from each combination of sampling site and growth temperature by the end of the experiment. Plants were initially placed in darkness for 15 min, keeping the water temperature stable at the growth temperature. A disc (3 cm in diameter) was cut from the middle of each thallus immediately before measuring the fluorescence parameters  $F_0$ ,  $F_m$  and  $F'_m$  (Maxwell and Johnson 2000) using a Walz Imaging-PAM (Walz, Effentrich, Germany). The discs were placed at the bottom of a petri dish filled with seawater at a fixed distance from the camera of the Imaging-PAM during the measurements. Each PAM-run consisted of measurements at 13 levels of illumination spanning from  $0\text{--}460 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Each illumination lasted 10 s and each PAM-run was completed within 2 min. Three circular areas on the resulting fluorescence image of each thallus disc were subsequently selected and numerical values of the fluorescence parameters were extracted using the ImagingWin software (Walz). These were used to calculate mean values of  $F_0$ ,  $F_m$  and  $F'_m$  for each disc.

**Pigment concentrations.** Pigment concentrations were measured on four replicate plants from each combination of sampling site and growth temperature by the end of the experiment. High performance liquid chromatography (HPLC) was used to separate and quantify the content of light harvesting pigments (chl *a*, chl *c* and fucoxanthin) and the xanthophyll cycle pigments violaxanthin and zeaxanthin. Whole plants were freeze-dried and ground to a fine powder. A 10 mg sample was suspended in 2 mL MeOH followed by 30 min sonication. Pigment separation was carried out on a  $4.6 \times 150 \text{ mm}$  Water Spherisorb ODS 2 column (C18,  $3 \mu\text{m}$  particle size) fitted on a Dionex Summit HPLC system (Dionex, Hvidovre, Denmark). Elution was performed by applying a gradient method using two eluents: (A) 80:20 (v/v) methanol and 1 M ammonium acetate and (B) 90:10 (v/v) methanol and acetone. A gradient elution was run for 10 min changing from 50:50 composition of eluent A and B to 100% eluent B followed by 11 min elution on 100% eluent B. The column was recalibrated for 9 min on the initial composition of eluent A and B. Pigment concentrations were expressed in units of  $\mu\text{g pigment} \cdot \text{g}^{-1} \text{DW}$ .

**Nitrogen content.** Tissue N-content was measured on freeze-dried and ground samples (same as those used for pigment analyses) using an EA 1110 CHNS elemental analyzer (CE Instruments, Milano, Italy) to check for the possibility of N-limitation in the cultures.

**Survival.** Plant condition and survival was evaluated from pigmentation and consistency of the tissue. Plants with severely perforated or bleached meristems were considered deceased.

**Statistical analyses.** The effect of growth temperature, sampling site, and assay temperature on the photosynthetic performance ( $P_{\max}$ ,  $\alpha$  and  $R_D$ ), fluorescence parameters ( $F_v/F_m$  and  $NPQ_{\max}$ ), and pigment concentrations were analyzed by use of permutational multivariate analyses of variance (PERMANOVA). This approach was chosen because several response variables were obtained from each analysis of photosynthetic performance, fluorescence, and pigment concentrations, respectively, and because parameters were likely inter-correlated. Data were normalized to minimize scale differences among response variables before analysis. PERMANOVAs were executed using Type I (sequential) sum of squares on geometric (Euclidean) distances using unrestricted permutation of raw data (Anderson et al. 2008).

The experimental design represents a partly nested design (Quinn and Keough 2002). *Aquaria* (random factor) were nested in the "between subject" factor growth temperature (fixed). The "within subject" factors, i.e., sampling site (fixed) in the case of photosynthetic performance measured at growth temperatures, fluorescence and pigment concentrations or, sampling site and assay temperature (both fixed) in the case of photosynthetic performance measured at various assay temperatures, were un-replicated in each aquarium. Site was considered a fixed factor because sites were chosen to represent the entire distributional range of *S. latissima* in southern Norway and, therefore, did not represent a random sample of all potential sites in the area.

The loss of all 20°C plants from Dröbak prevented us from running the full statistical analyses described above, because PERMANOVA inserts cell/block averages where data are missing. In our case, where an entire cell of data was missing, the approach would have inflated the significance of site (and temperature) in the analyses. We therefore divided each statistical analysis into two; one including data from all sites but omitting the 20°C treatment and, one including data from all growth temperatures but omitting plants from Dröbak (Underwood 1997, Quinn and Keough 2002).

## RESULTS

Photosynthetic performance was similar in *S. latissima* grown and assayed at 10°C and 15°C, respectively, but changed markedly in plants grown and assayed at 20°C (Table 1).  $P_{\max}$ , the photosynthetic efficiency ( $\alpha$ ) and respiration ( $R_D$ ) were similar at 10°C and 15°C, but  $P_{\max}$  and  $\alpha$  dropped and  $R_D$  increased substantially in plants from Bergen and Grimstad when these were held and assayed at 20°C (Fig. 3). The low  $\alpha$ -values and high respiration rates obtained at 20°C lead to a higher light compensation point ( $I_C$ ) whereas the saturating light intensity

( $I_{SAT}$ ) remained little affected by growth temperature (Fig. 4). Sampling site had a marginal effect on the photosynthetic performance; plants from Dröbak performed slightly better (i.e., higher  $P_{\max}$  and lower  $R_D$ ) than plants from Grimstad and Bergen. Plants from the latter sites performed similarly. We found no interaction effect between growth temperature and site (Table 1).

**Light efficiency and protection of PSII.** Fluorescence parameters were significantly affected by growth temperature, but not by sampling site or the interaction between growth temperature and site (Table 2). The composite response of  $F_v/F_m$  and  $NPQ_{\max}$  in plants grown at 20°C differed significantly from that in plants grown at 10°C and 15°C. Average  $F_v/F_m$  (across sites) decreased from 0.62 at 10°C to 0.55 at 20°C (Fig. 5). Across sites, the average heat dissipation efficiency of PSII ( $NPQ_{\max}$ ) decreased almost 50% with increasing temperature, being 0.047 in plants grown at 10°C and 0.024 in plants grown at 20°C (Fig. 5).

**Pigment content.** The concentrations of all pigments (chl *a* and *c*, fucoxanthin, violaxanthin + zeaxanthin) were significantly affected by growth temperature and by site, but not by the interaction between growth temperature and site (Table 3). The average concentration of chl *a* (across sites) decreased 60% from ~183  $\mu\text{g chl } a \cdot \text{g}^{-1} \text{ DW}$  in plants grown at 10°C to ~73  $\mu\text{g chl } a \cdot \text{g}^{-1} \text{ DW}$  in plants grown at 20°C (Fig. 6). Concentrations of chl *c* and fucoxanthin were even more affected by increasing growth temperature, as shown by the marked change in pigment ratios with increasing growth temperature (Fig. 5); plants grown at 20°C had less fucoxanthin, chl *c* and violaxanthin + zeaxanthin relative to chl *a* than plants grown at 10°C and 15°C, respectively. Plants from Dröbak had higher pigment concentrations than those from Grimstad and Bergen when compared at 10°C and 15°C.

**N content.** Tissue N content varied from 1.20% to 1.82% of DW in plants by the end of the experiment (Table 4). The N-content was affected by growth temperature (PERMANOVA;  $P = 0.001$ ), but not by site, nor by the interaction between growth temperature and site ( $P > 0.859$  and  $P > 0.094$ , respectively). The lowest average N-content was found in plants grown at 15°C, indicating that these

TABLE 1. Results of partially nested PERMANOVAs testing the effect of growth temperature (GT) and sampling site (Si) on photosynthetic response variables ( $P_{\max}$ ,  $\alpha$  and  $R_D$ ) in *Saccharina latissima*. Due to a missing cell (Dröbak 20°C), two tests were conducted: one including all sites (Bergen, Grimstad and Dröbak) but omitting 20°C and, one including all temperatures (10, 15 and 20°C) but omitting Dröbak.

Source of variation	Omitting 20°C				Omitting Dröbak			
	df	MS	Pseudo-F	P	df	MS	Pseudo-F	P
GT	1	3.381	1.448	0.266	2	23.411	45.845	<0.001
AQ (GT)	6	2.336	0.969	0.498	9	0.511	0.333	0.977
Si	2	6.338	2.631	0.043	1	0.294	0.192	0.832
GT × Si	2	5.009	2.079	0.097	2	1.739	1.134	0.360
Residuals	12	2.409			9	1.535		
		Pairwise test Site: D ≠ B&G				Pairwise test GT: 20 ≠ 10&15		

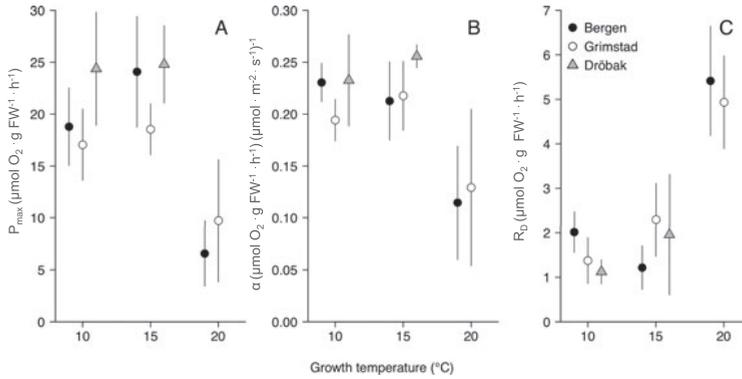


FIG. 3. Photosynthetic parameters (A: P<sub>Max</sub>; B: α; C: R<sub>D</sub>) in plants from Bergen, Grimstad and Dröbak measured at three growth temperatures (10, 15 and 20°C). Mean values ± SD (n = 4).

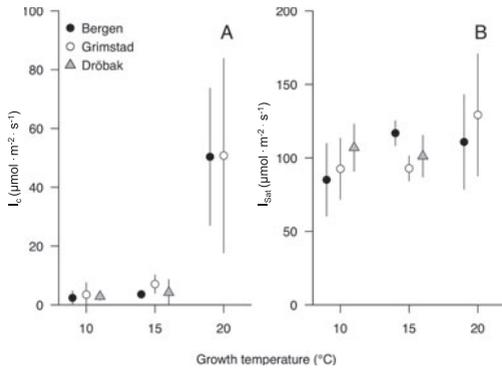


FIG. 4. Compensation (A) and saturating (B) irradiance in plants from Bergen, Grimstad and Dröbak measured at three growth temperatures (10, 15 and 20°C). Mean values ± SD (n = 4).

plants had grown faster than plants held at 10°C and 20°C, respectively.

**Survival.** Most plants survived through the experimental period. However, more individuals (~15%) died at 20°C than in the 10°C (~1%) and 15°C (~2%) treatments. Plants grown at 20°C were more feeble than those grown at lower temperatures; the distal part of the fronds had lost their pigmentation, they were perforated and fragile, but the lower part of the blade and the zone between the stipe and the blade (the meristem) seemed intact.

**Photosynthetic response to abrupt, short-term changes in temperature.** Growth temperature, assay temperature, site and the interactions between these factors all had a significant effect on the photosynthetic response of *S. latissima* (Table 5). Assay temperature affected photosynthesis across all growth temperatures and sites. Photosynthetic rates at high light (P<sub>max</sub>) and photosynthetic efficiency (α) showed an almost unimodal response to assay temperature

(Fig. 7); high rates were obtained at assay temperatures equal or close to the growth temperature in plants grown at 10°C and 15°C, respectively. Lower or higher assay temperatures generally caused a reduction in P<sub>max</sub> and α. This pattern differed for plants grown at 20°C, which performed best at low assay temperatures and poorer with increasing temperatures. The interaction between assay temperature and site revealed that plants from Bergen tended to have higher photosynthetic rates than those from Grimstad and Dröbak at the lowest assay temperature (see Fig. 7).

Respiration rates (R<sub>D</sub>) in plants grown at 10°C and 15°C were relatively similar across sites and assay temperature. R<sub>D</sub> in these plants was lowest at assay temperatures close to the growth temperature and increasing with higher assay temperatures (Fig. 7). The increase in R<sub>D</sub> with increasing assay temperature was, however, rather small and could not explain the decrease in net P<sub>max</sub> with increasing assay temperature. Plants grown at 20°C had much higher (5- to 10-fold) R<sub>D</sub> than plants grown at 10°C and 15°C independent of assay temperature.

The variation in photosynthetic performance across assay temperatures led to variation in the light compensation point (I<sub>c</sub>) and the saturating light intensity (I<sub>SAT</sub>) as well (Fig. 8). I<sub>c</sub> was low at low assay temperatures (i.e., 5, 10 and 15°C) and especially so at the growth temperature, but increased substantially with increasing temperatures. This pattern was consistent across sites and growth temperatures, except that I<sub>c</sub> was much higher for all assay temperatures in plants grown at 20°C. I<sub>SAT</sub> increased with increasing assay temperature regardless of site and growth temperature (Fig. 8). However, photosynthesis in plants from Bergen saturated at lower light intensities than in those from Grimstad and Dröbak when grown at 10°C. The opposite was true for plants grown at 15°C; plants from Bergen needed higher light intensities

TABLE 2. Results of partially nested PERMANOVAs testing the effect of growth temperature (GT) and sampling site (Si) on chl *a* fluorescence data ( $F_v/F_m$  and  $NPQ_{max}$ ) in *Saccharina latissima*. Due to a missing cell (Dröbak 20°C), two tests were conducted: one including all sites (Bergen, Grimstad and Dröbak) but omitting 20°C and, one including all temperatures (10, 15 and 20°C) but omitting Dröbak.

Source of variation	Omitting 20°C				Omitting Dröbak			
	df	MS	Pseudo-F	<i>P</i>	df	MS	Pseudo-F	<i>P</i>
GT	1	4.454	2.880	0.108	2	6.043	4.066	0.021
AQ (GT)	6	1.578	0.745	0.706	9	1.486	0.802	0.679
Si	2	1.893	0.984	0.483	1	1.829	0.987	0.422
GT × Si	2	1.391	0.657	0.641	2	1.016	0.548	0.687
Residuals	12	2.118			9	1.853		
					Pairwise test GT: 20 ≠ 10 & 15			

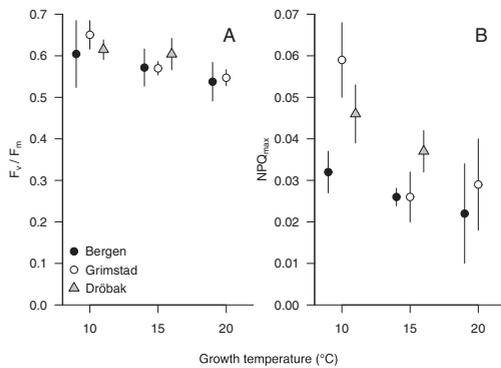


FIG. 5. Chlorophyll fluorescence variables.  $F_v/F_m$  (A) and  $NPQ_{max}$  (B) in plants from Bergen, Grimstad and Dröbak grown at 10, 15 and 20°C. Mean values  $\pm$  SD ( $n = 4$ ).

than plants from the other sites to saturate photosynthesis.

#### DISCUSSION

*Saccharina latissima* used to be the dominant kelp species along the south coast of Norway, but most populations have disappeared over the last decade. This loss followed a rise in sea surface temperature in northern Skagerak, where temperatures now exceed 20°C for weeks in most summers. The ques-

tion is whether the observed increase in temperature can explain the extensive loss of these kelp forests.

Most plants can tolerate (i.e., survive) a broad range of temperatures. Metabolic rates (including net photosynthesis and growth) increase with increasing temperature until the optimal temperature is reached, above which the rates decline. Temperatures slightly above the optimum may cause reversible physiological responses in the plant, for example, a larger increase in respiration than in gross photosynthesis. Such temperatures are not lethal per se, but reduce net photosynthesis, growth, and fitness, and may leave the plants more susceptible to other stressors (Wernberg et al. 2010). Temperature increases beyond the upper limit of tolerance may, on the other hand, affect the survival of the plant by causing irreversible damage to proteins, enzyme systems, and membranes (Davison 1991, Wahid et al. 2007).

Optimum temperatures for photosynthesis and growth vary among organisms. Most plants can acclimate to changes in temperature within their limits of tolerance. Thermal acclimation in plants relies on regulation of pigment levels, the quantity of photosynthetic units and the amount and activity of enzymes (Davison 1991, Salvucci and Crafts-Brandner 2004, Wahid et al. 2007). Tolerance limits and optimum temperatures may also vary within the geographic range of a species as a function of genotypic adaptation, resulting in the presence of distinct “eco-types” with different tolerance limits and optimum temperatures (Davison 1991).

TABLE 3. Results of partially nested PERMANOVAs testing the effect of growth temperature (GT) and sampling site (Si) on pigment data (chl *a*, chl *c*, fucoxanthin, violaxanthin and zeaxanthin) in *Saccharina latissima*. Due to a missing cell (Dröbak 20°C), two tests were conducted: one including all sites (Bergen, Grimstad and Dröbak) but omitting 20°C and, one including all temperatures (10, 15 and 20°C) but omitting Dröbak.

Source of variation	Omitting 20°C				Omitting Dröbak			
	df	MS	Pseudo-F	<i>P</i>	df	MS	Pseudo-F	<i>P</i>
GT	1	23.004	8.056	0.026	2	27.442	13.812	<0.001
AQ (GT)	6	2.856	1.366	0.301	9	1.987	1.170	0.398
Si	2	10.718	5.126	0.031	1	0.475	0.280	0.730
GT × Si	2	2.669	1.276	0.304	2	1.735	1.021	0.386
Residuals	12	2.091			9	1.699		
Pairwise test GT: 10 ≠ 15					Pairwise test GT: 20 ≠ 10 & 15			
Pairwise test Site: D ≠ B&G								

**Thermal acclimation.** Previous studies have shown that *S. latissima* has a high capacity for thermal acclimation. Davison (1987) showed that plants collected at Helgoland (Germany) obtained similar rates of

net photosynthesis and growth when cultured and assayed at temperatures ranging from 5°C to 20°C. This acclimation was correlated to changes in the amount and activity of Rubisco and other Calvin cycle enzymes, and on changes in pigment concentrations (Davison 1987, Davison et al. 1991, Machalek et al. 1996).

The optimal temperature for photosynthesis in *S. latissima* from southern Norway ranged between 10°C and 15°C whereas plants exposed to 20°C for weeks showed poorer performance and suffered relatively high mortality. These results correspond well to the temperature ranges reported for *S. latissima* in Müller et al. (2009). Net photosynthetic rates ( $P_N$ ) of plants grown and assayed at 10°C and 15°C were almost identical. Short-term exposure to a broad range of temperatures showed that  $P_N$  increased with increasing temperature until the optimum temperature was reached, above which it declined. The same was evident for respiration ( $R_D$ ), where low rates were observed at temperatures close to the growth temperature. The changes in  $P_{max}$ ,  $\alpha$  and  $R_D$  caused  $I_C$  to be low near to the growth temperature, particularly relative to higher assay temperatures, which may indicate thermal acclimation. The poor performance of plants grown at 20°C, however, shows that *S. latissima* from southern Norway were unable to acclimate to the highest temperature. Results obtained for other purposes showed further that plants from Bergen and Grimstad grown at 5°C for months had significantly lower  $P_{max}$  ( $3\text{--}6 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ ) and  $\alpha$  ( $0.13\text{--}0.21 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1} [\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}]^{-1}$ ), but higher  $R_D$  ( $\sim 2.6 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ ) than plants held at 10°C and 15°C, respectively (M.F. Pedersen, unpublished data). Together, these results show that *S. latissima* from southern Norway can optimize net photosynthesis to temperatures ranging between 10°C and 15°C, but probably not beyond these limits. The range of optimal temperatures in these plants seems thus to be narrower than in plants from Helgoland.

Increasing temperatures should lead to higher pigment levels and lower amounts or lower activity of Calvin cycle enzymes (Davison 1987, 1991,

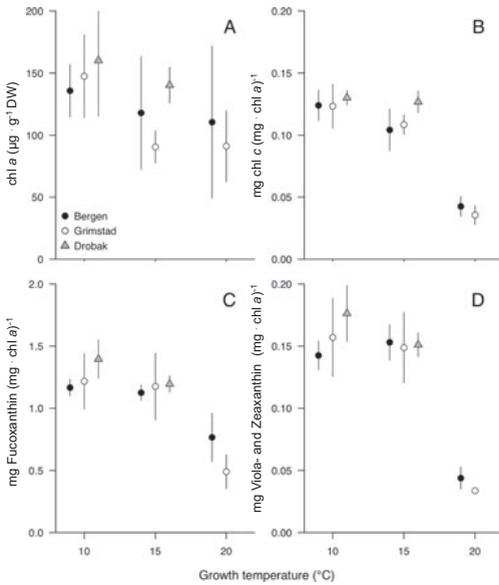


FIG. 6. Pigments. Chl *a* content (A) and the ratios between chl *c* (B), Fucoxanthin (C), Viola+Zeaxanthin (D) and chl *a* in plants from Bergen, Grimstad and Drøbak grown at 10, 15 and 20°C. Mean values  $\pm$  SD ( $n = 4$ ).

TABLE 4. Tissue N content in *Saccharina latissima* collected near Bergen, Grimstad and Drøbak, and grown in cultures at 10, 15 and 20°C. Mean values  $\pm$  SD ( $n = 4$ ).

Nutrient		10°C	15°C	20°C
(% DW)	Site			
Nitrogen	Bergen	1.69 $\pm$ 0.23	1.20 $\pm$ 0.25	1.72 $\pm$ 0.30
	Grimstad	1.54 $\pm$ 0.11	1.26 $\pm$ 0.13	1.82 $\pm$ 0.18
	Drøbak	1.39 $\pm$ 0.26	1.39 $\pm$ 0.25	na

TABLE 5. Results of partly nested PERMANOVAs testing the effect of growth temperature (GT), assay temperature (AT) and sampling site (Si) on photosynthetic response variables ( $P_{max}$ ,  $\alpha$  and  $R_D$ ) in *Saccharina latissima*. Due to a missing cell (Drøbak 20°C), two tests were conducted: one including all sites (Bergen, Grimstad and Drøbak) but omitting 20°C and, one including all temperatures (10, 15 and 20°C) but omitting Drøbak.

Source of variation	Omitting 20°C				Omitting Drøbak			
	df	MS	Pseudo-F	<i>P</i>	df	MS	Pseudo-F	<i>P</i>
GT	1	0.987	0.577	0.609	2	60.250	186.980	<0.001
AQ (GT)	6	1.712	1.138	0.329	9	0.322	0.357	0.996
AT	4	24.460	16.260	<0.001	4	14.705	16.302	<0.001
Si	2	6.567	4.366	0.002	1	3.769	4.178	0.015
GT $\times$ AT	4	7.491	4.979	<0.001	8	7.535	8.353	<0.001
GT $\times$ Si	2	6.738	4.479	<0.001	2	3.369	3.735	0.004
AT $\times$ Si	8	3.605	2.396	0.002	4	2.509	2.781	0.004
GT $\times$ AT $\times$ Si	8	4.516	3.002	<0.001	8	2.611	2.895	<0.001
Res	84	1.504			81	0.902		

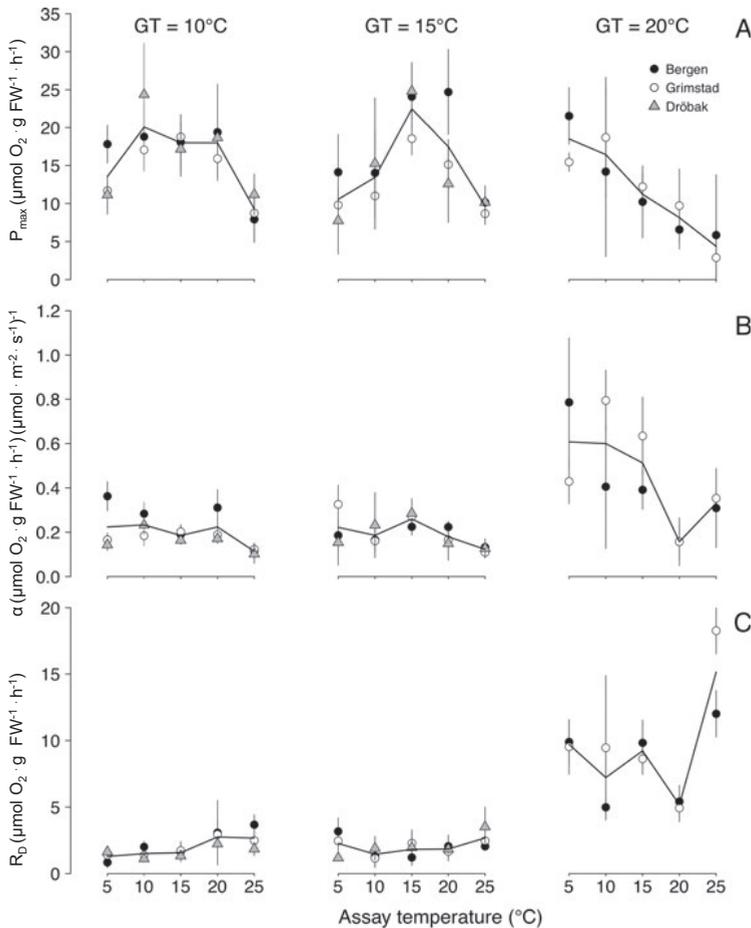


FIG. 7. Photosynthetic parameters (A:  $P_{max}$ ; B:  $\alpha$ ; C:  $R_D$ ) in plants from Bergen, Grimstad and Dröbak grown at 10, 15 or 20°C and assayed over a broad range of temperatures (5, 10, 15, 20 and 25°C). Mean values  $\pm$  SD ( $n = 4$ ). Solid lines indicate the means across sites.

Machalek et al. 1996). We did not measure the amount and/or the activity of Rubisco or other enzymes, but pigment concentrations and the ratio between antenna pigments and chl *a* decreased slightly as the growth temperature was raised from 10°C to 15°C. Although several studies have documented positive correlations between pigment concentrations in algae and temperature, other studies have shown the opposite trend. Staehr and Wernberg (2009) found, for example, a negative correlation between pigment concentration and in situ temperature in the Australian kelp *Ecklonia radiata*. The observed thermal acclimation in *S. latissima* grown at 10°C and 15°C may therefore occur mainly through changes in the amount and activity of Rubisco and other enzymes.

*The negative effects of high temperature.* The low performance and high mortality observed among plants grown at 20°C indicate that this temperature is close to the upper tolerance limit of Norwegian *S. latissima*. Respiration ( $R_D$ ) increased substantially when the growth temperature was raised from 15°C to 20°C, indicating severe thermal stress. High  $R_D$  caused a decrease in net photosynthesis ( $P_N$ ), but the observed drop in net  $P_{max}$  at 20°C ( $\sim 15 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ ) could not be explained by the increase in  $R_D$  (ca.  $4 \mu\text{mol O}_2 \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ ) alone. Gross  $P_{max}$  is mainly determined by the amount and activity of Rubisco or, rather, by Rubisco activase that is sensitive to high temperatures (Salvucci and Crafts-Brandner 2004). Lower gross photosynthesis at 20°C may thus have been caused

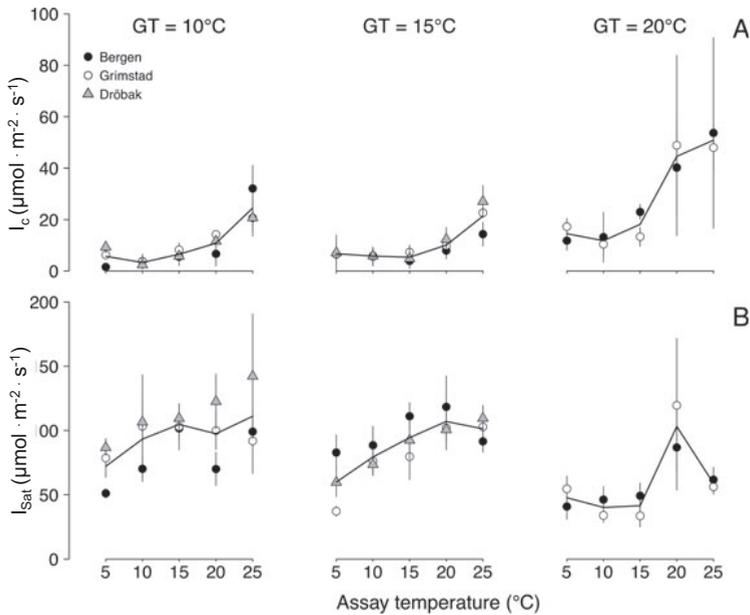


FIG. 8. Compensation (A) and saturating (B) irradiance in plants from Bergen, Grimstad and Dröbak grown at 10, 15 or 20°C and assayed over a broad range of temperatures (5, 10, 15, 20 and 25°C). Mean values  $\pm$  SD ( $n = 4$ ). Solid lines indicate the means across sites.

partly by reduced enzyme activity. Also, PSII is the most thermo-labile part of the photosynthetic apparatus (Wahid et al. 2007) and reduced photosynthesis at high temperatures is often related to the malfunctioning of PSII (Fork et al. 1979). The observed drop in photosynthesis at 20°C was accompanied by a significant decrease in maximum quantum yield ( $F_v/F_m$ ) and  $\text{NPQ}_{\text{max}}$  indicating reduced functionality of PSII (Maxwell and Johnson 2000). Although the decrease in  $F_v/F_m$  was relatively small (~10%) as the growth temperature was raised from 10°C to 20°C, it corresponded very well to changes observed in *S. latissima* from North America and *Laminaria japonica* from China that were exposed to high temperatures (Gerard 1997, Liu and Pang 2009). The marked decrease (~50%) in  $\text{NPQ}_{\text{max}}$  in plants grown at high temperature supported the hypothesis that PSII did not function properly at 20°C. The photosynthetic efficiency ( $\alpha$ ) was also reduced substantially in plants grown at high temperature. The level of  $\alpha$  is mainly, but not entirely, determined by pigment concentrations and the observed drop in  $\alpha$  correlated well with the observed decline in chl *a* and other light harvesting pigments at high growth temperature. Pigment loss is a common response during severe heat stress in plants (Wahid et al. 2007) and may partly be due to membrane injuries. Low rates of net photosynthesis in plants grown at high temperature may therefore

have been caused by a combination of increased respiration, lower pigment concentrations, PSII malfunction, and, most likely, impaired Rubisco activity as well.

Our results showed that 3–4 weeks of exposure to 20°C was harmful to *S. latissima* from Southern Norway. We do not, however, know for how long plants can tolerate 20°C before they become injured and we do not know if such plants would be able to recover if subsequently exposed to lower temperatures.

**Adaptation.** We found only a few marginal differences in photosynthetic responses among sampling sites. Plants from the warmest site, Dröbak, had slightly higher  $P_{\text{max}}$  and  $\alpha$  and contained more pigments than those from the other sites when grown at 10°C and 15°C. This suggests that plants from Dröbak may be able to handle high temperatures better. Conversely, plants from Bergen seemed more able to handle low temperatures. These plants had higher photosynthetic rates than plants from the other sites when exposed to low assay temperature (5°C) and their light requirements ( $I_{\text{SAT}}$ ) were also consistently lower than in plants from the other sites when grown at 10°C. This pattern reversed in plants grown at 15°C; plants from Bergen had higher  $I_{\text{SAT}}$  than those from the other sites. These results indicate that plants from Bergen performed somewhat better than those from other sites when grown at

low temperature; plants from Bergen also performed significantly better (higher  $P_{\max}$  and  $\alpha$ , lower  $R_D$  and  $I_C$ ) than plants from Grimstad when grown for months at 5°C (M.F. Pedersen, unpublished data).

Overall, plants from all three sites responded almost identically to different growth and assay temperatures. Any among-site differences in temperature regime may have been too small to promote local adaptation. In contrast, Gerard and Du Bois (1988) found considerable variation in the optimum temperature and in the upper tolerance limit among North American populations of *S. latissima* (Maine vs. Long Island), and concluded that this was due to adaptation rather than acclimation. Borum et al. (2002) provided further strong evidence for adaptation to local temperature regimes in *S. latissima* collected in NE Greenland. These plants live in water with constantly low temperatures (from -1.4°C to 0.0°C), but their photosynthetic performance was very similar to that of plants from southern Norway grown at 10°C and 15°C. These findings suggest that *S. latissima* can adapt to a broad range of temperatures, which may explain its wide range distribution.

*Perspective in relation to kelp loss in Norway.* Long-term exposure to 20°C left the plants in a poor condition and with a low photosynthetic capacity. This result is ecologically relevant because sea temperatures may reach or exceed 20°C in southern Norway in summer (Moy et al. 2008, Moy and Christie 2012). Our results support the hypothesis that long periods (weeks) of high water temperature, as observed in the summers of 1997, 2002 and 2006, are harmful to *S. latissima* and may have caused substantial losses of this species along the south coast of Norway. However, there seems to be more to this story. *S. latissima* used to be abundant in the entire depth range from 1 to 20 m and water temperatures in deeper waters are significantly lower than in the surface (Fig. 2). A large proportion of the population would therefore never have experienced temperatures near 20°C in summer. High kelp mortality has also been observed in years with more tolerable summer temperatures (Sogn Andersen et al. 2011), so other factors must have contributed to the losses. Super-optimal, but sub-lethal, temperatures may lower the resilience of kelp, and high temperature may increase the impact of other potential stressors (Wernberg et al. 2010). *S. latissima* have experienced increasing competition (for light) from filamentous algae and epiphytes that have become more abundant along the south-coast of Norway over the last two to three decades (Moy and Christie 2012). The blade of *S. latissima* is heavily fouled by epiphytes in summer (Sogn Andersen et al. 2011), and preliminary results have shown that epiphytes may attenuate as much as 80%–100% of the available light (Sogn Andersen, unpublished data). High summer temperatures cause a substantial increase in  $I_C$ , which makes the plants more susceptible to light limitation, and high densities of drift macroalgae and/or epiphytes may therefore restrict

carbon acquisition and cause an imbalance in the C-budget of the plant.

Global ocean warming is expected to cause a latitudinal shift in the distribution of most kelp species and it seems likely that the recent loss of *S. latissima* in southern Norway is partly a result of high temperature events. However, temperature interacts with other potentially stressful factors, all of which vary on both temporal and geographical scales. This complicates any attempt to make general, large-scale predictions. Any changes in kelp distributions following changes in sea temperature may be mediated by acclimation and local adaptations as well as potentially confounding factors such as coastal eutrophication and biological interactions. Attempts to make predictions for the future distribution of *S. latissima* and other kelp species are likely to fail unless all of these variables are accounted for.

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