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Implications of rising temperatures for gametophyte performance of two kelp species from Arctic waters

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Abstract: The aim of this study was to determine the temperature effects on photosynthesis, growth and reproduction in gametophytes of *Alaria esculenta* (Linnaeus) Greville and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl *et* G.W. Saunders from the Artic waters. After 24 days of culture, no gametophytes of either species survived at 20°C. Most growth parameters were greater at 10–15°C than at 5°C. Length and width were similar for both species, but area was greater for *A. esculenta* and cell number was greater for *S. latissima*. Female gametophytes were larger than male gametophytes in width and area, but the opposite was observed in cell number. In *A. esculenta*, but not for *S. latissima*, the percentage of female gametophytes decreased with increasing temperature. *Alaria esculenta* female gametophytes produced more sporophytes at 5°C than at 10°C, with no sporophytes at 15°C. In *S. latissima*, all female gametophytes produced sporophytes at both 5°C and 10°C, with a small percentage of sporophytes at 15°C. *Saccharina latissima* still had a measurable F_v/F_m at 20°C, while the F_v/F_m of *A. esculenta* was zero at this temperature. Maximum relative electron transport rate ($rETR_{max}$) and non-photochemical quenching (NPQ) were constant in the male and female gametophytes of *A. esculenta* and the male gametophytes of *S. latissima* at temperatures between 5°C and 15°C. Photosynthesis was saturated at a higher irradiance in *A. esculenta* than in *S. latissima*.

Keywords: *Alaria esculenta*; Arctic; climate warming; *Saccharina latissima*; temperature.

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Introduction

Climate change is one of the major issues of environmental concern because of its likely widespread and severe ecological impacts. The change in global climate will reportedly progress at an unprecedented rate, with a projected increase of 4.8°C over the course of this century (IPCC 2014). The implications of this rise for marine life are of particular concern, as the amplitude of daily and seasonal temperature fluctuations in aquatic environments is considerably less than in terrestrial environments. Typically, as aquatic organisms are adapted to smaller temperature ranges than terrestrial organisms, they are likely to be more susceptible to the expected extent of temperature change (Wernberg et al. 2012).

Polar regions have been most strongly affected by global warming (Wiencke and Clayton 2009). There is evidence that the Arctic environment is being profoundly affected, with average temperatures rising almost twice as fast as in other parts of the world (Serreze and Francis 2006). Temperature is one of the most important environmental factors affecting the photosynthetic activity, growth, reproduction, recruitment, and survival of seaweeds (e.g. Lüning 1990, Wiencke et al. 1994). Species-specific differences in response to temperature are known to be important determinants of geographical distribution (van den Hoek 1982). Due to their long history of exposure to cold water, polar seaweeds are particularly well adapted to low temperatures (Wiencke et al. 2007, Wulff et al. 2009), but, with predictions of an ultimate increase of 3–7°C in average annual temperature (IPCC 2014) and a 4.8°C elevation for waters off Spitsbergen, the geographical distribution of seaweeds will undoubtedly be affected (Müller et al. 2009).

The coastal Arctic ecosystem of Kongsfjord is a model system for monitoring effects of climate change (Svendsen et al. 2002, Wiencke et al. 2004b), and the kelps *Alaria esculenta* (Linnaeus) Greville and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl *et* G.W. Saunders have been identified as key subtidal species (Wiencke et al. 2007). *Alaria esculenta* is found in the North Atlantic and Arctic oceans, and extends southward to the 16°C summer isotherm (Lüning 1990). *Saccharina latissima* occurs throughout much of the Arctic and North Atlantic oceans, and its southern boundary is determined by summer temperatures of 19–20°C and winter temperatures of 13–14°C (Breeman 1988, Müller et al. 2009).

The maintenance of kelp populations depends on the survival, growth, and reproduction of both the gametophyte and sporophyte phases of the life cycle. Studies of the effects of temperature on the early stages of various kelp species from the Arctic have been reported (Müller et al. 2008, Fredersdorf et al. 2009, Roleda 2009). From a review of the published information on temperature demands of Arctic endemic or cold-temperate kelp species, Müller et al. (2009) concluded that zoospores of *Laminaria solidungula* and *S. latissima* germinate optimally at temperatures between 2°C and 12°C, and that gametophytes of *L. solidungula*, *Saccorhiza dermatodea*, and *S. latissima* survive at 19–20°C, 21°C, and 23–25°C, respectively. The fertility of gametophytes of *L. solidungula* and *S. dermatodea* in terms of oogonium or embryonic sporophyte formation occurs at 0–5°C and 0–10°C, respectively. The temperature ranges for the growth of sporophytes are 0–15°C for *L. solidungula* and 3–10°C for *S. dermatodea* (Müller et al. 2009).

Recruitment of kelp forests is directly related to the successful survival and growth of early stages. During unfavorable conditions, gametophytes are able to persist with reduced metabolic activity, and then re-establish activity and produce sporophytes when conditions improve. For example, the giant kelp, *Macrocystis pyrifera*, releases zoospores during the winter months when nitrate concentration is low, resulting in a significant increase of gametophytes, so that sporophytes can be recruited once nutrients increase (Reed et al. 1996, Carney and Edwards 2010). As the gametophyte phase thus plays a key role as a form of "seed bank" by postponing the formation of sexual cells (Müller et al. 2012), it is extremely important to understand how gametophytes respond to unfavorable environmental conditions (i.e. high temperature) in terms of growth and photosynthesis. There are some reports on the maturation of gametophytes of Arctic seaweeds (Gómez et al. 2009, Müller et al. 2009), but the upper temperature limits for gametophyte growth and photosynthesis are as yet unknown.

The objectives of this study were to compare the effects of temperature on photosynthesis, growth, and reproduction in gametophytes of *A. esculenta* and *S. latissima* from the Arctic, to determine the thresholds of thermal tolerance, and to evaluate the potential consequences of climate change on their geographical distribution.

Materials and methods

Cultures

Fertile sporophytes of *Alaria esculenta* and *Saccharina latissima* were collected near Blomstrandhalvøya, close to Ny Ålesund (Spitsbergen, 78° 55′ N, 11° 56′ E), where the typical summer surface temperature is 5–6°C (Hanelt et al. 2001). Release of spores was induced and spore solutions were prepared as previously described by Han and Kain (1993). The plants were stored overnight in a polythene bag in a controlled temperature room maintained at 5°C before they were wiped with soft paper towels to remove diatoms and mucus. Suitable fertile blades were rinsed in two or three separate baths of artificial seawater and immersed with fresh artificial seawater, with magnetic stirring to stimulate the release of zoospores. Meiospores were incubated in artificial seawater (Coralife, Energy Savers, Franklin, TN, USA) and transported in 50-ml centrifuge tubes to Korea. On arrival, the artificial seawater was decanted and the tubes filled with enriched artificial seawater (Coralife plus 1 mM KNO_3 and 0.1 mM K_2HPO_4). The tubes were maintained at 5°C in the dark, and the medium was changed monthly.

The developing gametophytes were removed from the walls of the centrifuge tubes, transferred to 10 ml of enriched artificial seawater, and distributed into Petri dishes containing coverslips (19×19 mm). After 24 h in the dark, each coverslip with gametophytes attached was transferred to single Petri dishes, containing 10 ml of medium. The medium was replaced at 3-day intervals.

Three replicate Petri dishes were incubated at each of four temperatures (5, 10, 15, and 20°C). All cultures were maintained at 30 µmol photons $m^{-2} s^{-1}$ of fluorescent light (TLD 18W/865, Philips, Aachen, Germany) with a 12:12 h light : dark photoperiod. It should be noted that there is the possibility that the upper survival temperature (UST) obtained for this experimental protocol may be slightly lower than under long-day (16:8 h light:dark) conditions as the light period can alter temperature tolerance (tom Dieck 1993).

Growth measurements

After 24 days of culture, 10 haphazardly selected female and male gametophytes were examined on each of three coverslips from each temperature treatment. For each gametophyte, length, width, and projected area were measured and the number of cells was counted using an image analysis system (Ista-Video Test Ltd., St. Petersburg, Russia). The areas of eggs and developing sporophytes were excluded from the measurements of female gametophytes.

Sex ratios and sporophyte development

Sex ratios at each temperature were determined by counting the number of female and male gametophytes on one haphazardly selected 1-mm^2 area on each of the three coverslips from each temperature treatment. The number of sporophytes produced was counted by examining randomly selected 100 female gametophytes on each of the three coverslips from each temperature treatment.

Photosynthesis measurements

The quantum efficiency of photosystem II (PS II) of gametophytes cultured for 24 days was measured as the ratio of variable ($F_v = F_m - F_o$) to maximum fluorescence (F_m), using microscopic-pulse-amplitude-modulated (PAM) fluorometry (Walz, Effeltrich, Germany). A coverslip with attached gametophytes was placed in the reservoir of a microslide with 100 µl of medium. The algae were dark-adapted for 15 min to measure F_o, then exposed to a pulse of saturating irradiance (I_k) to measure F_m. Rapid light curves were generated to determine relative electron transport rate (rETR) from PS II vs. photosynthetically active radiation (PAR). A coverslip with gametophytes was irradiated with actinic light for 10 s at each of eight irradiances from 16 to 244 µmol photons m^{-2} s^{-1}. No temperature change in the reservoir was detected during the actinic light exposures. Maximum relative electron transport rate ($rETR_{max}$) and efficiency of electron transport (α^{ETR}) were used to calculate minimum I_k according to the study by Platt et al. (1980). Non-photochemical quenching (NPQ) was assessed by measuring Stern-Volmer quenching.

Statistical analyses

Two-way analyses of variance (ANOVA) were conducted to compare parameters for different temperature treatments of each species and differences between the two species cultured under the same conditions. When the results indicated a significant difference ($p < 0.05$), the least significant difference (LSD) was calculated as a *post hoc* test to compare the mean values of various treatment groups (Sokal and Rohlf 1969).

Results

Growth

No gametophytes of either species survived at 20°C. Female and male gametophytes of *Saccharina latissima* and female gametophytes of *Alaria esculenta* grew significantly longer at 10–15°C than at 5°C after 24 days (Figures 1 and 2). The longest male gametophytes of *A. esculenta* were encountered at 15°C. Temperature had no significant effect on the width of female or male gametophytes of either *A. esculenta* or *S. latissima*. In *A. esculenta*, the area of female gametophytes was greater at 10–15°C than at 5°C, but that of male gametophytes was greatest at 15°C (Figure 1). In *S. latissima*, the area of female gametophytes was greatest at 10°C, while the area of male gametophytes did not differ among different temperatures (Figure 2). The greatest mean number of cells observed in *A. esculenta* was 8 for female gametophytes at 10–15°C, and 20 for male gametophytes at 15°C (Figure 1). Female gametophytes of *S. latissima* averaged 10–15 cells, and male gametophytes averaged 20–25 cells at all temperature treatments tested (Figure 2). In general, *S. latissima* gametophytes had greater length, area, and number of cells than *A. esculenta* gametophytes.

Percent of female and male gametophytes and sporophyte development

In *Alaria esculenta*, the percentage of female gametophytes decreased from 61% to 50% with increasing temperature, while in *Saccharina latissima*, the percentage (40%–41%) was not affected by temperature (Figure 3). Reproduction of gametophytes was also influenced by temperature. At 5°C, the females of both species were composed of relatively few cells, most of which developed into oogonia, whereas at higher temperatures, they developed into multi-cellular branched filaments. In *A. esculenta*, 100% of female gametophytes had produced a single sporophyte at 5°C after 24 days, but only 40% produced a sporophyte at 10°C (Figure 4). In *S. latissima*, all female gametophytes had produced sporophytes at both

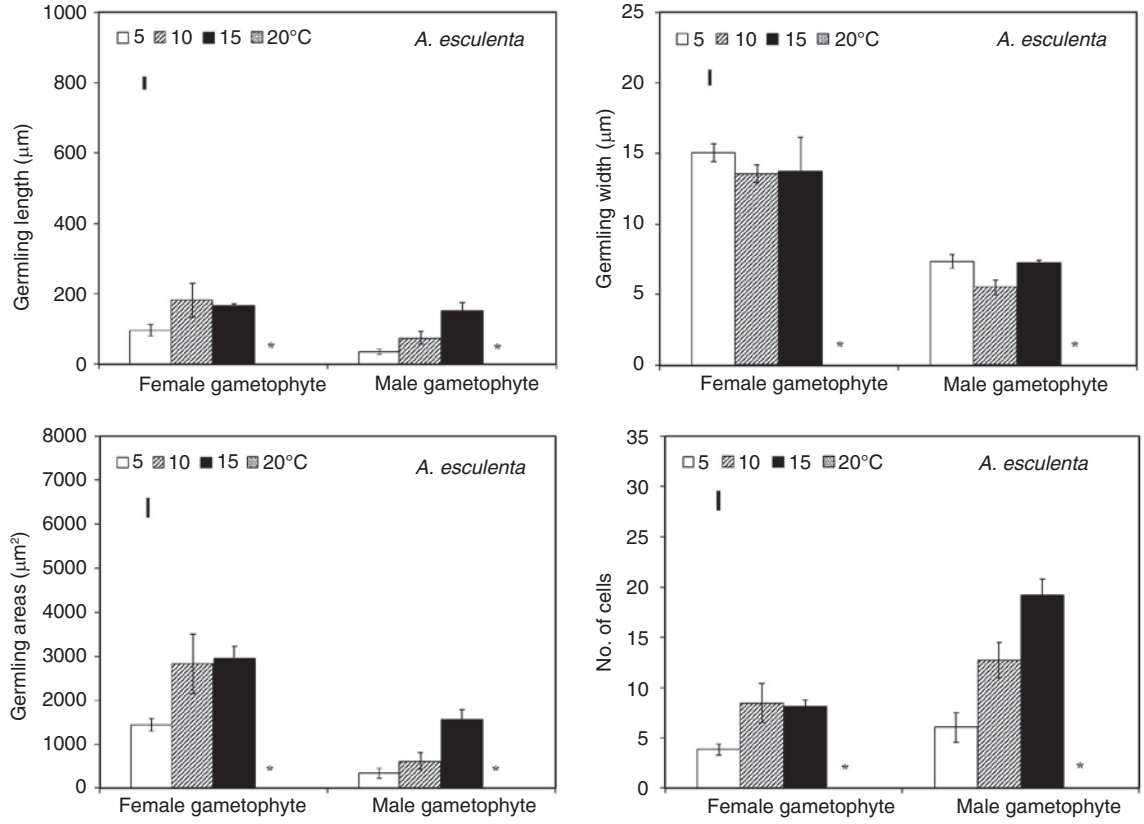

Figure 1: Effect of temperature on length, width, area, and cell number of Arctic *Alaria esculenta* gametophytes after 24 days in culture. Mean and 95% confidence intervals are shown (n = 30 for each treatment). *Indicates no germlings found. Vertical bar on the top left of each panel represents the least significant difference at p = 0.05.

5°C and 10°C after 18 days. At 15°C, no fertile gametophytes or sporophytes were observed in *A. esculenta*, but a small percentage of *S. latissima* females produced sporophytes, although none survived beyond 12 days.

Photosynthetic parameters

F_v/F_m ranged from 0.42 to 0.50 for gametophytes of *Alaria esculenta* and *Saccharina latissima* grown at 5–15°C (Figures 5 and 6), and there were no significant differences between different temperature treatments or species. No F_v/F_m signal was found in *A. esculenta* gametophytes grown at 20°C, whereas *S. latissima* had F_v/F_m values in the range of 0.20–0.25.

$rETR_{max}$ ranged from 4.99 to 6.77 for gametophytes of *A. esculenta* and showed no significant difference among temperature treatments from 5°C to 15°C. At 20°C, $rETR_{max}$ was zero. In *S. latissima*, $rETR_{max}$ values were highest at 5–10°C, lower at 15°C, and lowest at 20°C. In *A. esculenta* gametophytes, α was significantly lower at 5°C than at 10°C or 15°C. In *S. latissima* gametophytes, α^{ETR} was relatively constant at 5–15°C, and declined by 50% at 20°C. I_k values were higher in *A. esculenta* than in S. *latissima* grown at 5–15°C, and were not strongly affected by temperature, except for significantly higher values in female gametophytes of *S. latissima* grown at 5°C. NPQ tended to be highest in gametophytes of both species grown at 5°C, and there was a consistent decline with increasing temperature in *S. latissima* but not *A. esculenta*.

Discussion

In this study, gametophytic growth of *Alaria esculenta* and *Saccharina latissima* at a range of temperatures was compared from measurements of length, width, area, and cell number. Differences between treatments and species were not consistent among the four measured parameters, but length and cell number agreed most closely in *A. esculenta*, and area and cell number in *S. latissima*. Therefore, cell number appears to be the most consistent growth parameter of those used in the present study. Cell number has also been used to measure gametophyte growth in several previous studies (Bolton and Levitt 1985,

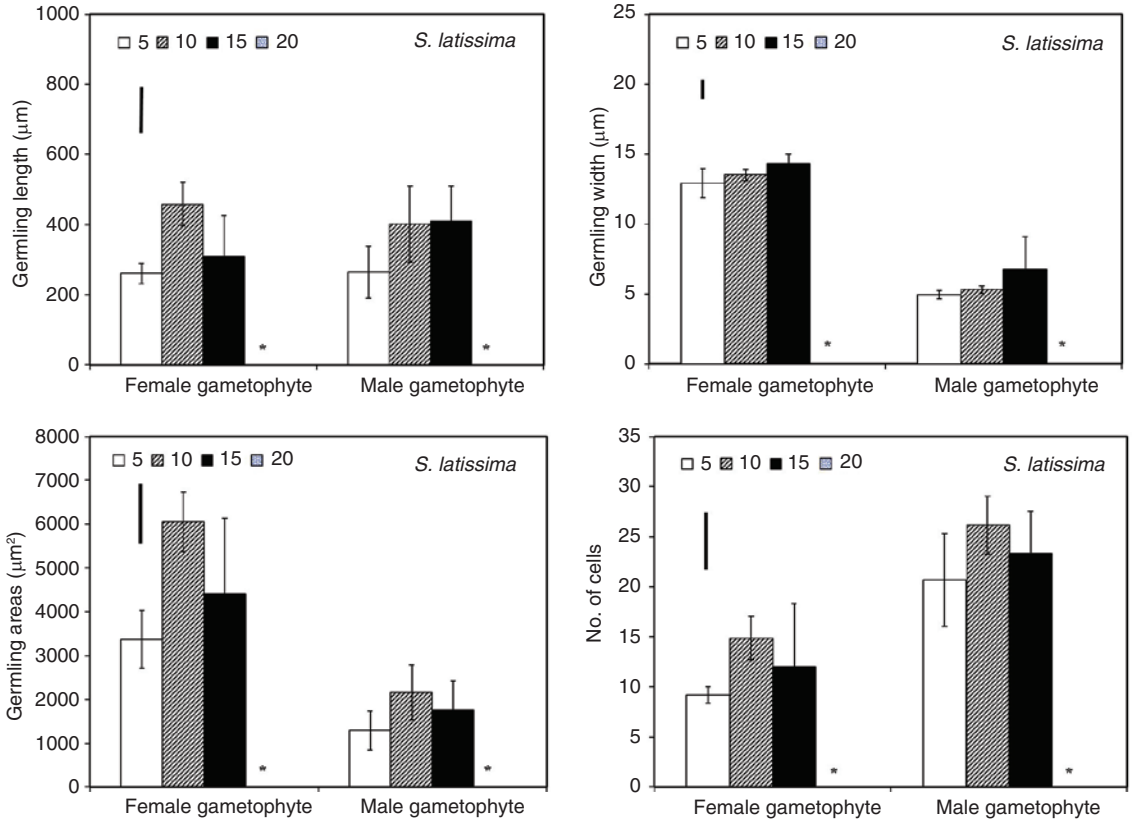

Figure 2: Effect of temperature on length, width, area, and cell number of Arctic *Saccharina latissima* gametophytes after 24 days in culture. Mean and 95% confidence intervals are shown (n = 30 for each treatment). *Indicates no surviving germlings found. Vertical bar on the top left of each panel represents the least significant difference at p = 0.05.

Kain 1979). Based on cell number, similarities and differences in the effects of temperature on the two species are apparent.

Gametophytes of *A. esculenta* exhibited reduced growth at 5°C and grew fastest at 15°C, while *S. latissima* gametophytes showed no significant temperature effect at 5–15°C. Considering the ambient temperature of 7°C in the Arctic (Roleda 2009), gametophytes of both species appear to carry out active growth at temperatures up to double the ambient. *Saccharina latissima* is well known to have a plastic physiological capacity toward temperature, and is thus able to have a broad geographical distribution pattern from the high Arctic to temperate regions (40°N) in the North Atlantic (Müller et al. 2009). Gametophytes of Arctic *A. esculenta* were reported to have a UST of 19–21°C, showing no correspondence with their ambient environmental conditions (tom Dieck 1993). Members of the Laminariales are thought to have evolved in the North Pacific and to have adapted to Arctic conditions only after the beginning of the glaciation period about 2 million years ago (tom Dieck 1993). Thus, their optimum growth at higher-than-ambient temperatures may reflect their relatively recent arrival in Arctic, and increasing ambient temperatures, due to climate change, would actually not affect gametophyte growth in either species.

While tom Dieck (1993) found that gametophytes of Arctic *A. esculenta* survived for 14 days at 20°C, in the present study, no gametophytes of this species survived after 24 days' exposure to 20°C. This apparent difference is most likely related to the length of exposure, with a 2-week exposure period not sufficient to "saturate" the UST (Wiencke et al. 1994).

As found in a previous study (Lee and Brinkhuis 1988), the sex ratio of *S. latissima* gametophytes was not temperature-dependent at 5–15°C. In *A. esculenta*, however, the percentage of female gametophytes decreased with increasing temperature over this range. With differential thermal sensitivity expressed at higher temperatures, males appear to survive better under adverse conditions than females (Kain 1979). The switch between the vegetative and reproductive material can be explained as follows. When environmental conditions are optimal for reproduction, gametophytes stop vegetative growth (Cosson 1975,

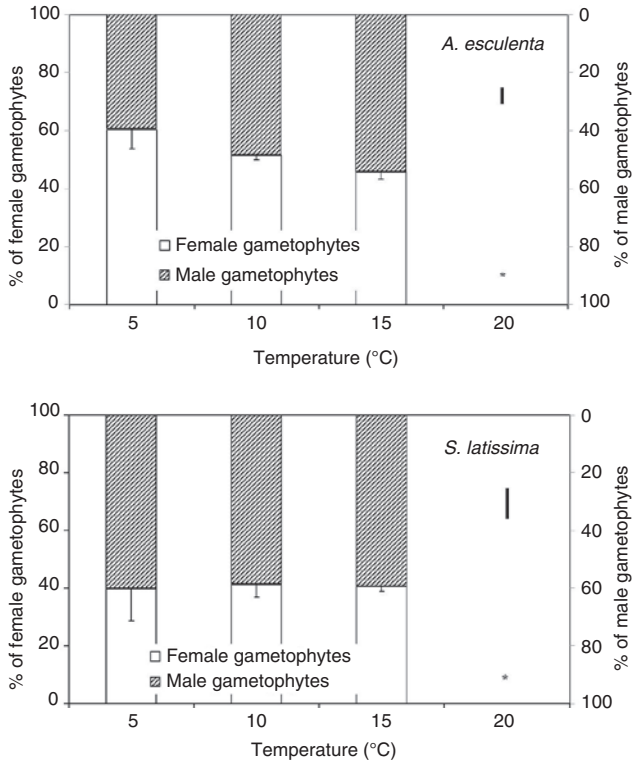

Figure 3: Effects of temperature on % of female and male gametophytes of Arctic *Alaria esculenta* and *Saccharina latissima*.
Mean and 95% confidence intervals are shown (n = 100 for each treatment). Vertical bar on the right of each panel represents the least significant difference at p = 0.05.

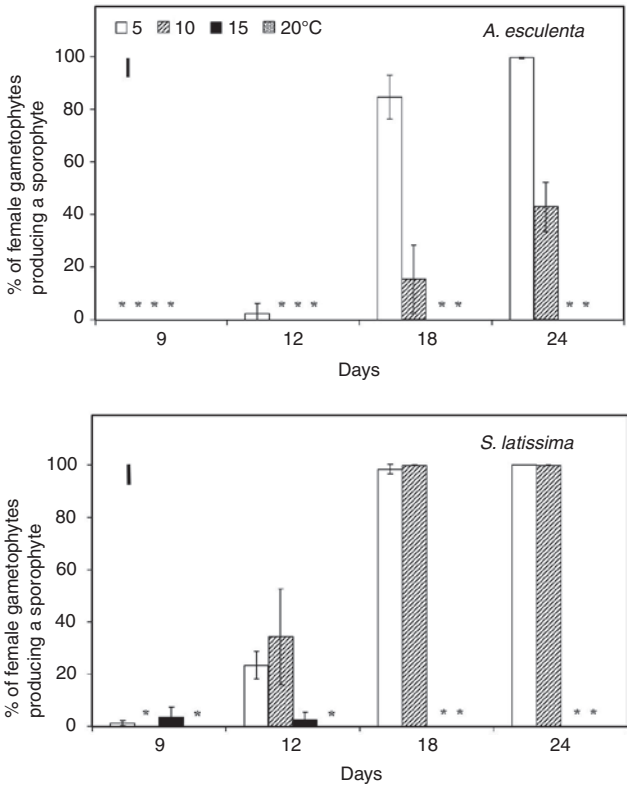

Figure 4: Effects of temperature on % of female gametophytes producing a sporophyte in Arctic *Alaria esculenta* and *Saccharina latissima*.
Mean and 95% confidence intervals are shown (n = 100 for each treatment). *Indicates no sporophyte production observed. Vertical bar on the top left of each panel represents the least significant difference at p = 0.05.

Izquierdo et al. 2002), but vegetative growth may be a means by which laminarian gametophytes survive relatively warm summers in cold-temperate zones. Increasing ambient temperature will result in more vegetative growth, thus forming a seed bank of microscopic forms as a means of maintaining Arctic kelp populations under unstable environmental conditions (tom Dieck 1993). Our experiment was less than 1 month in duration and we did not determine if the gametophytes grown at high temperature remained fertile. Although the kelp gametophytes are very persistent and may not lose their expected ability to form sporophytes (Carney and Edwards 2006), a long-term study simulating the current and future temperature conditions in the Arctic will be needed to confirm the seed bank idea suggested in this study.

Temperature effects on sporophyte production differed between *A. esculenta* and *S. latissima*. Sporophyte production was higher at 5°C than at 10°C in *A. esculenta*, and no sporophytes were produced at 15°C where gametophyte growth was fastest. These results suggest that resource allocation in the gametophytes was switched from reproduction to vegetative growth with increasing temperature. In contrast, *S. latissima* consistently produced sporophytes at both temperatures and even showed some production at 15°C, i.e. resources were allocated to both reproduction and vegetative growth at higher temperatures. While instantaneous switching from vegetative growth to reproduction, as in *A. esculenta*, may maximize reproductive output, the gradual switching observed in *S. latissima* suggests that every vegetative cell could produce a sporophyte by the end of the reproductive season. This would be the optimum strategy in an environment where the reproductive period is limited and more or less constant from year to year (Suzuki and Ohnishi 2006).

Fertile sporophylls of *A. esculenta* are present throughout the summer at Kongsfjorden. The released spores germinate with high rates between May and August but, in September, germination rates decrease strongly (Müller et al. 2008, Roleda et al. 2006, Wiencke et al. 2007). Fertile sporophytes of *S. latissima* occur predominantly in winter but are also found between May and August (Wiencke et al. 2004b, Müller et al. 2008, Roleda et al. 2006). As

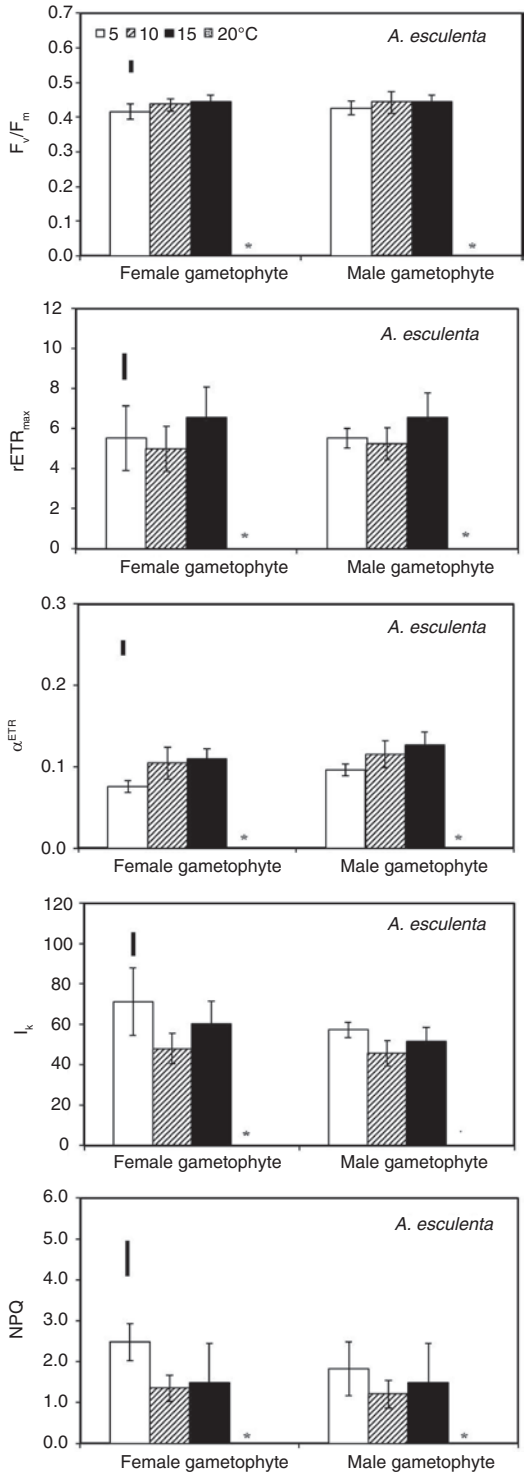

Figure 5: Effects of temperature on photosynthetic parameters of female and male gametophytes of Arctic *Alaria esculenta*: ratio of variable to maximum fluorescence (F_v/F_m), maximum relative electron transport rate ($rETR_{max}$), efficiency of electron transport (α^{ETR}), saturating irradiance (I_k), and non-photochemical quenching (NPQ). Mean and 95% confidence intervals are shown (n = 5 for each treatment). *Indicates no measurable activity recorded. Vertical bar on the top left of each panel represents the least significant difference at p = 0.05.

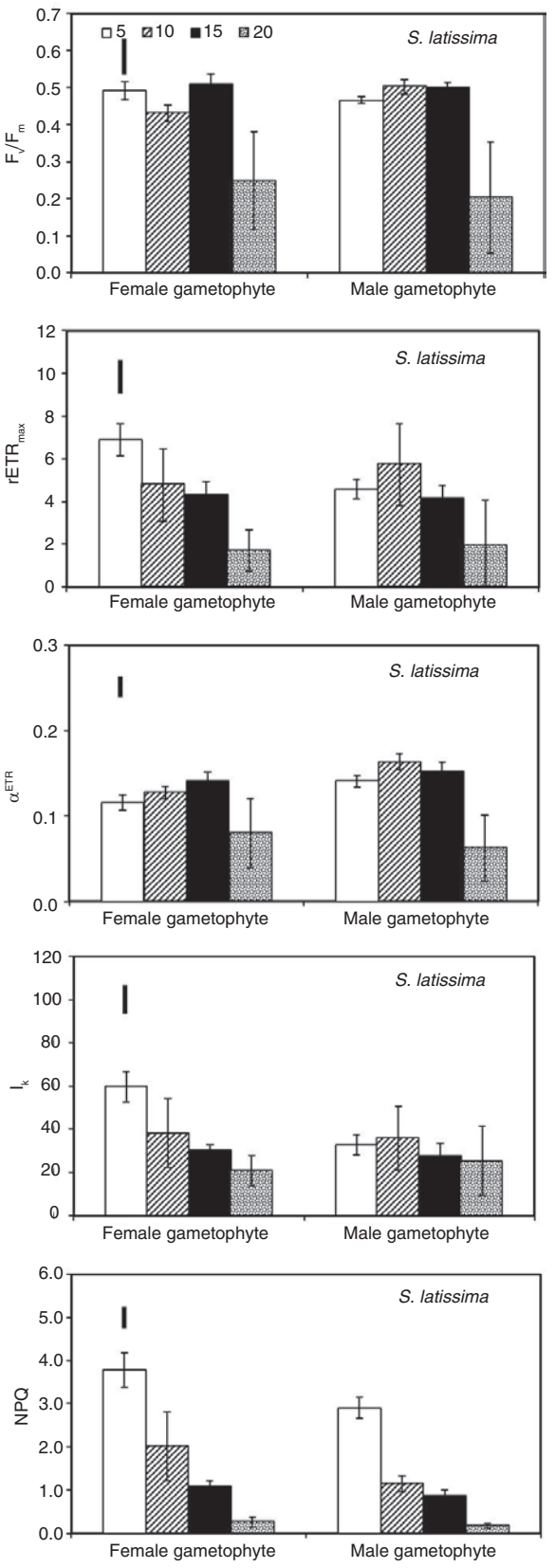

Figure 6: Effects of temperature on photosynthetic parameters of female and male gametophytes of Arctic *Saccharina latissima*. All details as in Figure 5.

gametophyte fertility is not controlled by photoperiod in either species, juvenile sporophytes can be formed under the ambient temperature regime of Kongsfjorden (between −1.8°C and 6°C) throughout the year (Hanelt et al. 2001). Thus, under conditions of oceanic warming, sexual reproduction in *A. esculenta* will be reduced in summer, whereas it will not be affected in *S. latissima*.

Photosynthetic parameters

The traditional technique of measuring O_2 evolution was previously used to estimate the average photosynthetic performance of cultures of kelp gametophytes (Kain 1969, Lüning and Dring 1975, Fain and Murray 1982, Campbell et al. 1999), but not of individual plants. Using microscopic-PAM chlorophyll fluorescence, the present study was able to determine photosynthetic characteristics of individual gametophytes in contrast to Roleda (2009) who, using a WATER-PAM, examined chlorophyll fluorescence of kelp zoospores.

The maximum quantum yield of PS II (F_v/F_m) for gametophytes of *Alaria esculenta* and *Saccharina latissima* grown at 5–15°C ranged from 0.4 to 0.5, values slightly lower than those reported by previous studies of kelp gametophytes (Dring et al. 1996, Fredersdorf et al. 2009, Roleda 2009), perhaps due to the use of different instruments (microscopic-PAM for this study vs. mini-PAM and imaging PAM for the other studies). However, *S. latissima* had reduced, but measurable, F_v/F_m at 20°C, while F_v/F_m of *A. esculenta* was zero at this temperature. Thus, deleterious effects on photosynthesis may explain the inability of *A. esculenta* gametophytes to survive at 20°C, but do not necessarily account for the same inability in *S. latissima*. Interestingly, the opposite trend in high temperature tolerance was found in zoospores of the same species. Based on chlorophyll fluorescence characteristics, *A. esculenta* exhibited greater tolerance of 19°C than *S. latissima* (Roleda 2009). A systematic survey of the thermal optima for different life stages of Arctic kelp species appears to be required.

Relatively constant values of $rETR_{max}$ in all *A. esculenta* gametophytes and in the male gametophytes of *S. latissima* grown at 5–15°C were consistent with the lack of temperature effects on F_v/F_m. It was notable, however, that female gametophytes of *S. latissima* exhibited significantly higher $rETR_{max}$ at 5°C than at 10–15°C, which was similar to results reported by Machalek et al. (1996) for *S. latissima* sporophytes. The temperature effect on sporophytes was attributed to higher ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) concentrations in algae grown at lower temperatures, and the same may be true for gametophytes. In fact, a recent study has shown that Rubisco activity and content in *Laminaria japonica* were higher in haploid gametophytes than in diploid sporophyte stages (Wang et al. 2011). However, it would be interesting to find out why the increase appears to occur only in females. Overall, the decreasing $rETR_{max}$ in *S. latissima* gametophytes with increasing temperature and the full inhibition of photosynthesis at 20°C in *A. esculenta* gametophytes reflect the observed growth pattern in both species.

Alaria esculenta gametophytes had significantly lower values of electron quantum yield at 5°C than at 10–15°C. Based on the corresponding increase in NPQ at 5°C, this may be due, in part, to dissipation of absorbed energy through the xanthophyll cycle prior to PS II excitation. In *S. latissima*, electron quantum yield was not affected by temperatures between 5°C and 15°C, in contrast to previous observations on *S. latissima* sporophytes, although there was a significant decline in electron quantum yield at 20°C (Machalek et al. 1996).

Photosynthesis was saturated at slightly higher irradiances in *A. esculenta* than in *S. latissima*, indicating some adaptation to low light in the latter species. This result reflects the ecological differences between the two species: *A. esculenta* is restricted to depths between 1.5 m and 13.5 m where it grows as a canopy species. In contrast, *S. latissima* is a subdominant undergrowth species in shallow waters becoming dominant only in deeper waters, where *A. esculenta* does not occur (Wiencke et al. 2004a, 2007).

NPQ of chlorophyll fluorescence is an indicator of non-radiative dissipation in the light-harvesting antenna of PS II, and physiological stress can increase NPQ. Gametophytes of *A. esculenta* grown at 10–15°C showed constant levels of NPQ, indicating that temperature was not a stress factor. In contrast, NPQ values for *S. latissima* gametophytes were significantly higher at 5°C than at 10–15°C, despite similar F_v/F_m values. Although F_v/F_m is the most frequently used fluorescence parameter to measure maximal PS II quantum yield, it provides little information on the PS II reaction centers, characterized as heterogenous populations (Lu and Zhang 1998). The increased NPQ in *S. latissima* gametophytes grown at 5°C may be related to a change in the grana membranes, with light-harvesting-complex (LHC) proteins becoming disconnected from the PS II reaction center (Betterle et al. 2009). Considering the average summer temperature and high light conditions in Spitsbergen, a capacity for inducing high NPQ at 5°C may

have ecological significance in *S. latissima* with regard to photoprotection.

Conclusions

The typical summer temperature for surface waters around Spitsbergen is 5–6°C (Hanelt et al. 2001) and so the predicted warming of about 4.8°C will raise summer temperatures to about 10°C. Based on the results of the present study, gametophytes of *Alaria esculenta* and *Saccharina latissima* will continue to survive, grow, and reproduce in the Arctic, even under the maximum predicted warming. However, populations at lower latitudes in the North Atlantic Ocean, which are already limited by high summer temperatures, may be negatively impacted by further temperature increases.

Sporophyte production by gametophytes of *A. esculenta* and *S. latissima* was the most temperature-sensitive process of those studied, and the projected 4.8°C rise in temperature may affect sexual reproduction of Arctic *A. esculenta*. Our findings suggest that the gametophytes of *A. esculenta* are most likely constricted by temperature. Gametophytes of *S. latissima*, however, are less likely to be affected by the temperature increase in the Arctic.

Our results indicate a switch from reproduction to vegetative growth of gametophytes as temperature increases. It is important to note that other factors, such as egg production, spermatazoid production, and fertilization, are also likely to be affected by the temperature changes. Although these parameters were indirectly estimated in the present study by measuring the sporophyte production, additional studies will be required to determine the details.

Although temperature rise accompanying climate change may be a predominant and influential factor affecting these two Arctic kelp species, simultaneous influences of other environmental stressors, including UV radiation, salinity, and pollutants, must also be considered when making predictions about changes in the population dynamics (Roleda 2009). Therefore, more studies on the interactive effects of stressors are required to assess how climate change will alter the ecology of kelp species.

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References

Betterle, N., M. Ballottari, S. Zorzan, S. Bianchi, S. Cazzaniga, L. Dall'Osto, T. Morosinotto and R. Bassi. 2009. Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. *J. Biol. Chem. 284*: 15255–15266.

Bolton, J.J. and G.J. Levitt. 1985. Light and temperature requirements for growth and reproduction in gametophytes of *Ecklonia maxima* (Alariaceae: Laminariales). *Mar. Biol. 87*: 131–135.

Breeman, A.M. 1988. Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phenological evidence. *Helgol. Meersunters 42*: 199–241.

Campbell, S.J., J.S. Bite and T.R. Burridge. 1999. Seasonal patterns in the photosynthetic capacity, tissue pigment and nutrient content of different developmental stages of *Undaria pinnatifida* (Phaeophyta: Laminariales) in Port Phillip Bay south-eastern Australia. *Bot. Mar. 42*: 231–241.

Carney, L.T. and M.S. Edwards. 2006. Cryptic processes in the sea: A review of delayed development in the microscopic life stages of marine macroalgae. *Algae 21*: 161–168.

Carney, L.T. and M.S. Edwards. 2010. Role of nutrient fluctuations and delayed development in gametophyte reproduction by *Macrocystis pyrifera* (Phaeophyceae) in southern California. *J. Phycol. 46*: 987–996.

Cosson, J. 1975. Action des conditions d'éclairement sur la croissance des gamétophytes de *Laminaria digitata* (L.) Lamouroux (Phéophycée, Laminariale). *Soc. Phycol. de France Bull. 20*: 50–54.

Dring, M.J., V. Makarov, E. Schoschina, M. Lorenz, and K. Lüning. 1996. Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Mar. Biol. 126*: 183–191.

Fain, S.R. and S.N. Murray. 1982. Effects of light and temperature on net photosynthesis and dark respiration of gametophytes and embryonic sporophytes of *Macrocystis pyrifera*. *J. Phycol. 18*: 92–98.

Fredersdorf, J., R. Müller, S. Becker, C. Wiencke, and K. Bischof. 2009. Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia 160*: 483–492.

Gómez, I., A. Wulff, M.Y. Roleda, P. Huovinen, U. Karsten, M.L. Quartino, K. Dunton and C. Wiencke. 2009. Light and temperature demands of marine benthic microalgae and seaweeds in polar regions. *Bot. Mar. 52*: 593–608.

Han, T. and J.M. Kain. 1993. Blue light photoreactivation in ultraviolet-irradiated young sporophytes of *Alaria esculenta* and *Laminaria saccharina* (Phaeophyta). *J. Phycol.* 29: 79–81.

Hanelt, D., H. Tüg, K. Bischof, C. Groß, H. Lippert, T. Sawall and C. Wiencke. 2001. Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar. Biol. 138*: 649–658.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland. pp. 151.

Izquierdo, J., I.M. Pérez-Ruzafa and T. Gallardo. 2002. Effect of temperature and photon fluence rate on gametophytes and young

sporophytes of *Laminaria ochroleuca* Pylaie. *Helgol. Mar. Res. 55*: 285–292.

Kain, J.M. 1969. Aspects of the biology of *Laminaria hyperborea*. V. Comparisons with early stages of competitors. *J. Mar. Biol. Ass. 49*: 455–473.

Kain, J.M. 1979. A view of the genus *Laminaria*. *Oceanogr. Mar. Biol. Ann. Rev. 17*: 101–161.

Lee, J.A. and B.H. Brinkhuis. 1988. Seasonal light and temperature interaction effects on development of *Laminaria saccharina* (Phaeophyta) gametophytes and juvenile sporophytes. *J. Phycol. 24*: 181–191.

Lu, C. and J. Zhang. 1998. Modifications in photosystem II photochemistry in senescent leaves of maize plants. *J. Exp. Bot. 49*: 1671–1679.

Lüning, K. 1990. *Seaweeds: their environment, biogeography and ecophysiology.* John Wiley & Sons Inc., New York. pp. 55.

Lüning, K. and M.J. Dring. 1975. Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. *Mar. Biol. 29*: 195–200.

Machalek, K.M., I.R. Davidson and P.G. Falkowski. 1996. Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant Cell Environ. 19*: 1005–1016.

Müller, R., C. Wiencke and K. Bischof. 2008. Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Climate Res. 37*: 203–213.

Müller, R., T. Laepple, I. Bartsch, and C. Wiencke. 2009. Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot. Mar. 52*: 617–638.

Müller, R., C. Desel, F.S. Steinhoff, C. Wiencke and K. Bischof. 2012. UV-radiation and elevated temperatures induce formation of reactive oxygen species in gametophytes of cold-temperate/Arctic kelps (Laminariales, Phaeophyceae). *Phycol. Res. 60*: 27–36.

Platt, T., C.L. Gallegos and W.G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res. 38*: 687–701.

Reed, D.C., A.W. Ebeling, T.W. Anderson, and M. Anghera. 1996. Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. *Ecology 77*: 300–316.

Roleda, M.Y. 2009. Photosynthetic response of Arctic kelp zoospores exposed to radiation and thermal stress. *Photochem. Photobiol. Sci. 8*: 1302–1312.

Roleda, M.Y., M. Clayton and C. Wiencke. 2006. Screening capacity of UV-absorbing compounds in spores of Artic Laminariales. *J. Exp. Mar. Biol. Ecol. 338*: 123–133.

Serreze, M.C. and J.A. Francis. 2006. The Arctic amplification debate. *Clim. Change 76*: 241–264.

Sokal, R. and F.J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. pp. 859.

Suzuki, N. and Y. Ohnishi. 2006. Significance of the simultaneous growth of vegetative and reproductive organs in the prostrate annual *Chamaesyce maculate* (L.) Small (Euphorbiaceae). *Ecol. Res. 21*: 91–99.

Svendsen, H., A. Beszczynska-Moeller, J.O. Hagen, B. Lefauconnier, V. Tverberg, S. Gerland, J.B. Oerbaeck, K. Bischof, C. Papucci, M. Zajaczkowski, R. Attolini, O. Bruland, C. Wiencke, J.G. Winther and W. Dallmann. 2002. The physical environment of Kongsfjord-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res. 21*: 133–166.

tom Dieck, I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Mar. Ecol. Prog. Ser. 100*: 253–264.

van den Hoek, C. 1982. The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol. J. Linn. Soc. 18*: 81–144.

Wang, C., X. Fan, G. Wang, J. Niu, and B. Zhou. 2011. Differential expression of rubisco in sporophytes and gametophytes of some marine macroalgae. *PLoS One 6*: e16351.

Wernberg, T., D.A. Smale and M.S. Thomsen. 2012. A decade of climate change experiments on marine organism: procedures, patterns and problems. *Global Change Biol. 18*: 1491–1498.

Wiencke, C. and M.N. Clayton. 2009. Future perspectives on the investigation of polar benthic algae. *Bot. Mar. 52*: 669–671.

Wiencke, C., I. Bartsch, B. Bischoff, A.F. Peters and A.M. Breeman. 1994. Temperature requirements and biography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot. Mar. 37*: 247–259.

Wiencke, C., M.N. Clayton and M. Schoenwaelder. 2004a. Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. *Mar. Biol. 145*: 31–39.

Wiencke, C., B. Vögele, N.A. Kovaltchouk and H. Hop. 2004b. Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. *Ber. Polarforsch. Meeresforsch. 492*: 55–62.

Wiencke, C., M.N. Clayton, I.K. Iken, U.H. Lüder, C.D. Amsler, U. Karsten, D. Hanelt, K. Bischof and K. Dunton. 2007. Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Rev. Environ. Sci. Biotechnol. 6*: 95–126.

Wulff, A., K. Iken, M.L. Quartino, A. Al-Handal, C. Wiencke and M.N. Clayton. 2009. Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. *Bot. Mar. 52*: 491–507.