TEMPERATURE ACCLIMATION AND HEAT TOLERANCE OF PHOTOSYNTHESIS IN NORWEGIAN *SACCHARINA LATISSIMA* (LAMINARIALES, PHAEOPHYCEAE)

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Key words: global warming, heat tolerance, kelp, kelp deforestation, PAM, photosynthesis, *Saccharina latissima*, temperature.

Abbreviations: α: 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}: non-photosynthetic 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 respration rate.

Abstract

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. 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.

Saccharina 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 had suffered substantial tissue deterioration and showed reduced net photosynthetic capacity. The reduced photosynthetic capacity was 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 extraordinary warm summers, 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.

Introduction

Ongoing climate changes leads to increasing sea temperatures. The average global sea surface temperature has increased by 0.6 ±0.2 °C over the last century (IPCC 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 & Lüning 1988). Ocean warming is therefore expected to cause changes in the range distribution of many marine algae (van den Hoek et al. 1990, Adey & Steneck 2001, Müller et al. 2009). Such changes have been documented for inter-tidal seaweeds (e.g. 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 & Johnson 2011, Wernberg et al. 2011).

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 used to form extensive, sub-tidal meadows extending from the Swedish border in the east, along the south coast of Norway and up 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 1990'ies 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 also, that they used to recovered within few years. The production of spores in *S. lattisima* 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 sea water as the most important driver, while others state that coastal eutrophication might be more important.

The optimal growth temperature for *Saccharina latissima* is variable, but seems to range from 10 to 15°C while poor growth is typically observed above 20°C (e.g. Fortes and Lüning 1980, Bolton and Lüning 1982). Increasing temperature will facilitate algal metabolism. If respiration is stimulated more than photosynthesis, then more light and/or a more efficient photosynthesis becomes necessary to maintain a positive carbon budget. In addition to affecting the carbon balance, high temperature may be harmful by disturbing enzyme driven processes and affecting 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 (see e.g. Campbell et al. 2007). If compensating mechanisms are present in *S. latissima,* it may have the ability 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). This range distribution means that 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 ÷1.5 to 0°C in NE Greenland; Borum et al. 2002). The wide distribution of *S. latissima*

99 may indicate the existence of ecotypes (as a result of adaptations) and/or a high capacity for
100 thermal acclimation of photosynthesis and other metabolic processes. Davison and Davison
101 (1987) studied the thermal acclimation in *S. latissima* collected at Helgoland (Germany) and
102 found similar rates of light saturated photosynthesis and respiration in plants grown at
103 temperatures ranging from 0 to 20°C for longer periods. These results were later confirmed
104 for rates of photosynthesis obtained at low light and for light requirements (I_C) in plants
105 grown at 5 and 15°C, respectively (Davison et al. 1991). It was hypothesized that iso-enzymes
106 (in the Calvin Cycle) with different temperature optima made it possible for *S. latissima* to
107 acclimate to a wide range of temperatures (Davison and Davison 1987, Machalek et al. 1996).
108 Gerard and Du Bois (1988), on the other hand, found that *Saccharina latissima*
109 growing near its southern boundary in the NW Atlantic (New York, USA) were more tolerant
110 to high temperatures than plants from colder regions (Maine, USA). The heat tolerance in
111 plants from the southern population was explained by adaptation through improved ability to
112 maintain high N reserves and, thus, enzyme systems that could aid the production of heat
113 shock proteins (Gerard 1997). Similar results have been reported for *Saccharina japonica*
114 (Liu and Pang 2009). It seems therefore that the ability of *S. latissima* to cope with a wide
115 range of water temperatures depends on a combination of local adaptations and a high
116 capacity for thermal acclimation.
117 The present study aimed to examine the capacity of Norwegian *Saccharina latissima*
118 to acclimate to and cope with high temperatures (here 20°C). Plants were collected from three
119 different locations along the south coast of Norway (Dröbak in the east, Grimstad in the south
120 and Bergen in the west) and subsequently exposed to three growing temperatures (10, 15 and
121 20°C) for an extended period (4-6 weeks) after which we measured the photosynthetic
122 performance at five different assay temperatures (5, 10, 15, 20 and 25°C). The photosynthetic
123 capacity, chlorophyll a fluorescence and pigment concentrations and composition in the plants

were finally compared across the respective growth temperatures and sampling sites.

Materials and Methods

Sampling sites: Young sporophytes (5-25 cm long, FW 3.93±4.04 g) of *Saccharina latissima* were harvested at 5-10 m depth in the vicinity of Bergen, Grimstad and Dröbak in March 2010 (Figure 1). These sites vary with respect to water temperature (Figure 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 ±1.6°C) than near Grimstad (17.4 ±1.6°C) and Dröbak (18.4 ±1.5°C) (repeated measures ANOVA; $F = 135.2$, $p < 0.001$, all sites different from each other). Average August temperatures in the surface water occasionally exceeded 20°C near Grimstad and Dröbak, but never so near Bergen. Water temperatures did also decrease with depth; water at 20 m of depth was significantly colder than at the surface, i.e. 13.0 ±2.1 versus 15.6 ±1.6°C near Bergen (paired t-test; $t = 8.52$, $df = 25$, $p < 0.001$) and 15.6 ±0.8 versus 17.4 ±1.6°C near Grimstad (paired t-test; $t = 8.49$, $df = 26$, $p < 0.001$). Water temperatures have increased substantially all along the south coast of Norway from 1980 to 2006 with an annual increase in the surface water temperatures ranging from 0.07°C (near Bergen) to 0.12°C (near Grimstad and Dröbak), corresponding to an average increase of ca. 2.01 – 3.15°C in 27 years.

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 four weeks, before being used in the experiments. Plants were held in aquaria (volume 20 L) where they were tied to small PVC-plates with non-toxic silicon strings. Eighteen aquaria (the main experimental units), each holding 5 replicate kelp plants from each sampling site (15 individuals per aquarium), were placed in six temperature regulated water baths (3 aquaria per bath and 2 baths per temperature, making 6 aquaria per temperature in

151 total). The water temperature in the water baths was controlled by the combined use of
152 thermostat regulated heaters (Julabo ED, Julabo Labortechnik GmbH, Germany) and coolers
153 (P Selecta, J.P. Selecta, Spain) that kept the water temperature constant within ±0.2°C. The
154 aquaria were filled with GFC-filtered sea-water with salinity 30-32 PSU and the water was
155 replenished weekly. The initial temperature in the cultures equaled the *in situ* water
156 temperature at the time of collection (8-9°C). Temperatures were subsequently changed by
157 1°C per day until the warranted growth temperatures were reached (i.e. 10, 15 and 20°C). Our
158 first attempt to establish cultures at 20°C failed as most of the involved plants died. The
159 acclimating process was therefore repeated with a slower increase in temperature (ca. 0.5°C
160 per day). We had too few plants from Dröbak to replace the lost ones, but plants from
161 Grimstad and Bergen were fully represented at 20°C. Each water bath was illuminated by
162 eight Halogen spots (OSRAM Decostar 51; 12V, 35W) which provided 56 µmol photons m^{-2}
163 s^{-1} (PAR) in a 12 h light 12 h dark cycle. The plants were kept at their final growth
164 temperature for 3-4 weeks before being used for any measurements.
165 Measurements of photosynthetic performance, chlorophyll a fluorescence, pigment
166 concentrations and total N-content were carried out on four replicate plants from each
167 combination of growth temperature and collection site. The measurements were executed
168 after the acclimating period of 3-4 weeks. Replicate plants within the same growth
169 temperature were collected from separate aquaria.
170
171 **Photosynthetic performance:** Measurements of (dark) respiration and photosynthesis
172 were performed in 800 mL gas tight, transparent chambers. Each chamber was equipped with
173 a circulation pump (AquaBee, 300 L h^{-1}) that ensured circulation of water within the chamber.
174 One thallus was fixed within the chamber, which was filled with artificial seawater (salinity
175 30 PSU). Pencilin G-sodium salt and Streptomycin sulfate salt were added to the water

(concentration = 50 mg L^{-1} for each) to reduce bacterial growth in the chamber. The water was bubbled with N_2 to reduce the initial O_2 concentration to ca. 60% of air saturation in order to prevent high O_2 concentrations to build-up during incubations. The chamber was finally submerged into a water bath keeping a constant temperature (5, 10, 15, 20 or 25° C, respectively). The water bath held two replicate chambers at a time.

Each chamber was equipped with a Clark-type O_2 microelectrode (model OX-500, Unisense, Denmark) that was connected to a pico-amperemeter (model Picoammeter PA2000, Unisense, Denmark) and a Pico Technology ADC-16 high-resolution data logger. The O_2 concentration was recorded every minute throughout incubations. A lamp with 6 halogen spots (OSRAM Decostar 51; 12V, 35W) 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: 0-375 µmol photons m^{-2} s^{-1} PAR). Rates of O_2 consumption or release were calculated from incubation periods with constant changes in O_2 concentration over a minimum of 10 min. Incubations (providing a full PI-curve) lasted for 3-4 hours and four replicate PI-curves were run at each assay temperature. Photosynthetic rates were expressed in units of µmol O_2 g^{-1} FW h^{-1}.

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

Chlorophyll a fluorescence: Chlorophyll a fluorescence was measured using pulse amplitude modulated (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 non-photochemical quenching; NPQ_{Max}) of PSII (Maxwell and Johnson 2000). The fluorescence parameters (i.e. F_v/F_m and NPQ_{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 minutes, 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 0-460 μmol photons m^{-2} s^{-1} (PAR). 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, Effentrich, Germany). 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 (chlorophyll a, chlorophyll c and fucoxanthin) and, the two xanthophyll cycle pigments violaxanthin and zeaxanthin. Whole

plants were sampled, freeze-dried and ground to a fine powder. Ten mg sample was suspended in 2 mL MeOH followed by 30 min sonication. Pigment separation was carried out on a 4.6 x 150 mm Water Spherisorb ODS 2 column (C18, 3 μm 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 μg pigment g^{-1} 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, Italy), in order 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}, α 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 *Saccharina 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 requires a fully balanced design. We divided therefore 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

The photosynthetic performance was similar in plants grown and assayed at 10 and 15°C, respectively, but changed markedly in plants grown and assayed at 20°C (Table 1). Maximum photosynthetic rate (P_{Max}), the photosynthetic efficiency (α) and respiration (R_D) remained almost the same at 10 and 15°C, but P_{Max} and α dropped and R_D increased substantially in plants from Bergen and Grimstad when these were held and assayed at 20°C (Figure 3). The low α-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 (Figure 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 at 10 and 15°C while the performance in plants from Bergen and Grimstad was similar at all growth temperatures. 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 and not by the interaction between growth temperature and site (Table 2). The composite response of Fv/Fm and NPQ_{max} in plants grown at 20°C differed significantly from that in plants grown at 10 and 15°C. Average Fv/Fm (across sites) decreased from 0.62 in plants grown at 10°C to 0.55 in plants grown at 20°C (Figure 5). The average heat dissipation (across sites) efficiency of PSII (NPQ_{max}) decreased, in contrast, almost 50% with increasing temperature, being 0.047 in plants grown at 10°C and 0.024 in plants grown at 20°C (Figure 5).

Pigment content. The concentrations of all pigments (chlorophyll a and c, fucoxanthin, violaxanthin + zeaxanthin) were significantly affected by growth temperature

294 and by site, but not by the interaction between growth temperature and site (Table 3). The
295 average concentration of chlorophyll a (across sites) decreased with 60% from ca. 183 μg Chl
296 a g^{-1} DW in plants grown at 10°C to ca. 73 μg Chl a g^{-1} DW in plants grown at 20°C (Figure
297 6). Concentrations of chlorophyll c and fucoxanthin were even more affected by increasing
298 growth temperature, as shown by the marked change in pigment ratios with increasing growth
299 temperature (Figure 5); plants grown at 20°C had less fucoxanthin, chlorophyll c and
300 violaxanthin + zeaxanthin relative to chlorophyll a than plants grown at 10 and 15°C,
301 respectively. Plants from Dröbak had higher pigment concentrations than those from
302 Grimstad and Bergen when compared at 10 and 15°C.
303
304 **N content.** Tissue N content varied from 1.20 to 1.82% of DW in plants by the end of
305 the experiment (Table 4). The N-content was affected by growth temperature
306 (PERMANOVA; $p = 0.001$), but not by site, nor by the interaction between growth
307 temperature and site ($p > 0.859$ and $p > 0.094$, respectively). The lowest average N-content
308 was found in plants grown at 15°C independent of site, indicating that these plants had grown
309 faster than plants held at 10 and 20°C, respectively.
310
311 **Survival.** Most plants survived through the experimental period. However, more
312 individuals (ca. 15%) died at 20°C than in the 10 (ca. 1%) and 15°C (ca. 2%) treatments.
313 Plants grown at 20°C were more feeble than those grown at lower temperatures; the distal part
314 of the fronds had lost their pigmentation, they were perforated and fragile, but the lower part
315 of the blade and the zone between the stipe and the blade (the meristem) seemed intact.
316
317 **Photosynthetic response to abrupt, short-term changes in temperature.** Growth
318 temperature, assay temperature, site and the interactions between these factors all had a

significant effect on the photosynthetic response of *Saccharina latissima* (Table 5). Assay temperature affected photosynthesis across all growth temperatures and sites. Photosynthetic rates at high light (P_{Max}) and photosynthetic efficiency (α) showed an almost unimodal response to assay temperature (Figure 7); high rates were obtained at assay temperatures equal or close to the growth temperature in plants grown at 10 and 15°C, respectively. Lower or higher assay temperatures generally caused a reduction in P_{Max} 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 Figure 7).

Respiration rates (R_D) in plants grown at 10 and 15°C were relatively similar across sites and assay temperature. R_D in these plants was lowest at assay temperatures close to the growth temperature and increasing with higher assay temperatures (Figure 7). The increase in R_D with increasing assay temperature was, however, rather small and could not explain the decrease in net P_{Max} with increasing assay temperature. Plants grown at 20°C had much higher (5 to 10-fold) R_D than plants grown at 10 and 15°C independent of assay temperature.

The variation in photosynthetic performance with changing assay temperature caused variations in the light compensation point (I_C) and the saturating light intensity (I_{Sat}) as well(Figure 8). I_C 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_C was much higher for each level of assay temperature in plants grown at 20°C than in those grown at 10 and 15°C. I_{Sat} increased with increasing assay temperature regardless of site and growth temperature (Figure 8). However, the 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

344 grown at 15°C; plants from Bergen needed higher light intensities than plants from the other
345 sites to saturate photosynthesis.
346

Discussion

Sachharina 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 correlates to a rise in sea surface temperature in northern Skagerak, where temperatures now exceed 20°C for weeks in most summers. The question 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, which 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, cause irreversible damages including denaturation of proteins, malfunctioning of enzyme systems and injury of membranes, which will affect the survival of the plant (Wahid et al. 2007, Davison 1991).

Optimum temperatures for photosynthesis and growth in different organisms vary. Most plants can acclimate to changes in temperature within their limits of tolerance. Thermal acclimation in plants relies on regulation of pigment levels, the amount of photosynthetic units and the amount and activity of enzymes (Wahid et al. 2007, Davison 1991, Salvucci & Crafts-Brandner 2004). Furthermore, tolerance limits and optimum temperatures may vary within the same species as a function of geographic and/or regional origin. Such variations are caused by genotypic adaptation, resulting in the presence of distinct ‘eco-types’ with different tolerance limits and optimum temperatures (Davison 1991).

372 **Thermal acclimation.** Previous studies have shown that *Saccharina latissima* has a
373 high capacity for thermal acclimation. Davison & Davison (1987) showed that plants
374 collected at Helgoland (Germany) obtained similar rates of net photosynthesis and growth
375 when cultured and assayed at temperatures ranging from 5 to 20°C. This acclimation was
376 correlated to changes in the amount and activity of Rubisco and other Calvin cycle enzymes,
377 and on changes in pigment concentrations (Davison & Davison 1987, Davison et al. 1991,
378 Machalek et al. 1996).
379 The optimal temperature for photosynthesis in *Sachharina latissima* from southern
380 Norway ranged between 10 and 15°C whereas plants exposed to 20°C for weeks showed
381 poorer performance and suffered relatively high mortality, which corresponds well to the
382 temperature ranges reported for *S. latissima* in Müller et al. (2009). Net photosynthetic rates
383 (P_N) of plants grown and assayed at 10 and 15°C were almost identical. Short-term exposure
384 to a broad range of temperatures showed that P_N increased with increasing temperature until
385 the optimum temperature was reached, above which it declined. The same was evident for
386 respiration (R_D), where low rates were observed at temperatures close to the growth
387 temperature. The changes in P_{Max}, α and R_D caused I_C to be low near to the growth
388 temperature, but increasing at higher assay temperatures, which indicates thermal acclimation.
389 The poor performance of plants grown at 20°C, however, shows that *S. latissima* from
390 southern Norway were unable to acclimate to the highest temperature. Results obtained for
391 other purposes showed further that plants from Bergen and Grimstad grown at 5°C for months
392 had significantly lower P_{Max} (3-6 μmol O_2 g FW^{-1} h^{-1}) and α (0.13-0.21 μmol O_2 g FW^{-1} h^{-1}
393 [μmol m^{-2} s^{-1}]$^{-1}$), but higher R_D (ca. 2.6 μmol O_2 g FW^{-1} h^{-1}) than plants held at 10 and 15°C,
394 respectively (M.F. Pedersen, unpublished data). Together, these results show that *S. latissima*
395 from southern Norway can optimize net photosynthesis to temperatures ranging between 10
396 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 and Davison 1987, Davison 1991, Machalek et al. 1997). 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 chlorophyll a decreased slightly as the growth temperature was raised from 10 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 & 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 *Sacharina latissima* grown at 10 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 *Sachharina latissima*. Respiration (R_D) increased substantially when the growth temperature was raised from 15 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 (ca. 15 μmole O_2 g^{-1} FW h^{-1}) could not be explained by the increase in R_D (ca. 4 μmole O_2 g^{-1} FW 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 & Crafts-Brandner 2004) and lower gross photosynthesis at 20°C may thus have been caused partly by reduced enzyme activity. Also, PSII is the most thermo-labile part of the photosynthetic apparatus (Wahid et al. 2004) 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

422 was accompanied by a significant decrease in maximum quantum yield (F_v/F_m) and non-
423 photosynthetic quenching (NPQ_{max}) indicating reduced functionality of PSII (Maxwell and
424 Johnson 2000). Although the decrease in F_v/F_m was relatively small (ca. 10%) as the growth
425 temperature was raised from 10 to 20°C, it corresponded very well to changes observed in
426 *Saccharina latissima* from North America and *Laminaria japonica* from China when these
427 were exposed to high temperatures (Gerard 1997, Liu & Pang 2009). The marked decrease
428 (ca. 50%) in NPQ_{max} in plants grown at high temperature supported the hypothesis that PSII
429 did not function properly at 20°C. Low photosynthetic rates in plants held at 20°C may thus
430 have been caused by a combination of reduced activation of Rubisco and impaired
431 functionality of PSII. The photosynthetic efficiency (α) was also reduced substantially in
432 plants grown at high temperature. The level of α is mainly, but not entirely, determined by
433 pigment concentrations and the observed drop in α correlated well with the observed decline
434 in chlorophyll a and other light harvesting pigments at high growth temperature. Loss of
435 pigments is a common response during severe heat stress in plants (Wahid et al. 2004) and
436 may partly be due to membrane injuries. Low rates of net photosynthesis in plants grown at
437 high temperature seem therefore to have been caused by a combination of increasing
438 respiration rates, lower pigment concentrations, injured PSII and most likely also impaired
439 Rubisco activity.

440 Our results showed that 3-4 weeks of exposure to 20°C was harmful to *Saccharina*
441 *latissima* from Southern Norway. We do not, however, know for how long plants can tolerate
442 20°C before they become injured and we do not know if such plants would be able to recover
443 if subsequently exposed to lower temperatures.

444

445 **Adaptation.** We found only a few marginal differences when we compared the
446 photosynthetic response of plants across sampling site. Plants from the warmest site, Dröbak,

had slightly higher P_{Max} and α and contained more pigments than those from the other sites when grown at 10 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_{Sat}) were also consistently lower than in plants from the other sites when grown at 10°C (across all assay temperatures). This pattern reversed in plants grown at 15°C; plants from Bergen had higher I_{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, which is also supported by the fact that plants from Bergen performed significantly better (higher P_{Max} and α, 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 difference in temperature regimes may simply have been too small to cause different adaptations among sampling sites. In contrast, Gerard & Du Bois (1988) found considerably variation in the optimum temperature and in the upper tolerance limit among North American populations of *S. latissima* (Maine *versus* Long Island), and concluded that this was due to adaptation rather than acclimation. Borum et al. (2002) provided another strong evidence for adaptation to local temperature regimes in *S. latissima* collected in Young Sund (NE Greenland). These plants live in water with constantly low temperatures (from -1.4 to 0.0°C), but their photosynthetic performance was very similar to that of plants from southern Norway when grown at 10 and 15°C. These findings provide strong evidence 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 and rise above 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 *Saccharina 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 (Figure 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, potentially stressful, factors (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 2-3 decades (Moy and Christie 2012). The blade of *S. latissima* is heavily fouled by epiphytes in summer and these deprive the host plants of light (Sogn Andersen et al. 2011). 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 density of drift macroalgae and/or epiphytes may therefore impair the 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 *Saccharina latissima* in southern

Norway is partly a result of extraordinary warm summers. It is, however, important to keep in mind that temperature interacts with other potentially stressful factors, all of which vary on both temporal and geographical scales. This complicates any attempt to make general and large-scale predictions. The changes in kelp distributions potentially following changes in sea temperature may not only be mediated by acclimation and local adaptations, but also affected by potentially confounding factors such as coastal eutrophication and by biological interactions. Attempts to make predictions for the future distribution of *S. latissima* and other kelp species are likely to fail unless all these variables are accounted for.

Acknowledgements

The authors would like to thank Hartvig Christie, Stein Fredriksen and two anonymous referees for their helpful and constructive comments on this manuscript. We also like to thank people in the laboratory at Roskilde University for all their help and encouraging pats on the back. The project was funded by grant no 178681 from The Norwegian Research Council.

513 **Reference**

514 Adey, W. H. & Steneck, R. S. 2001. Thermogeography over time creates biogeographic
515 regions: a temperature/space/time-integrated model and an abundance-weighted test
516 for benthic marine algae. *J. Phycol.* 37:677–698.
517 Anderson, M. J., Gorley, R. N. & Clarke, K. R. 2008. PERMANOVA+ for PRIMER. Guide to
518 software and statistical methods. PRIMER-E Ltd. Plymouth, U.K.
519 Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C. M., Buck, B. H., Eggert, A., Feuerpfeil, P.,
520 Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M. Y., Schubert,
521 H., Schumann, R., Valentin, K., Weinberger, F. & Wiese, J. 2008. The genus Laminaria
522 sensu lato: recent insights and developments. *Eur. J. Phycol.* 43:1–86.
523 Bolton, J. J. & Lüning, K. 1982. Optimal growth and maximal survival temperatures of
524 Atlantic Laminaria species (Phaeophyta) in culture. *Mar. Biol.* 66:89–94.
525 Borum, J., Pedersen, M. F., Krause-Jensen, D., Christensen, P. B. & Nielsen, K. 2002.
526 Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord,
527 NE Greenland. *Mar. Biol.* 141:11–19.
528 Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O. & Hurry, V. 2007.
529 Acclimation of photosynthesis and respiration is asynchronous in response to changes
530 in temperature regardless of plant functional group. *New. Phytol.* 176: 375–89.
531 Cie, D. K. & Edwards, M. S. 2011. Vertical distribution of kelp zoospores. *Phycologia*.
532 50:340–50.
533 Davison, I. R. 1987. Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to
534 changes in growth temperature. *J. Phycol.* 23:273–83.
535 Davison, I. R., Greene, R. M. & Podolak, E. J. 1991. Temperature acclimation of respiration
536 and photosynthesis in the brown alga *Laminaria saccharina*. *Mar. Biol.* 110:449–54.
537 Davison, I. R. 1991. Environmental effects on algal photosynthesis: temperature. *J. Phycol.*

538 27:2–8.
539 Diez, I., Muguerza, N., Santolaria, A., Ganzedo, U. & Gorostiaga, J. M. 2012. Seaweed
540 assemblage changes in the eastern Cantabrian Sea and their potential relationship to
541 climate change. *Est. Coast. Shelf Sci.* 99:108-120.
542 Druehl, L. D. 1970. The pattern of Laminariales distribution in the northeast Pacific.
543 *Phycologia*. 9:237–47.
544 Fork, D. C., Murata, N. & Sato, N. 1979. Effects of growth temperature on the lipid and fatty
545 acid composition, and the dependence on temperature of light-induced redox reactions
546 of cytochrome f and light energy distribution in the thermophillic blue-green algae
547 *Synechococcus lividus*. *Plant Phys.* 63:524-530.
548 Fortes, M. D. & Lüning, K. 1980. Growth rates of North Sea macroalgae in relation to
549 temperature, irradiance and photoperiod. *Helgoländer Meeresun*. 34:15-29.
550 Gaylord, B., Reed, D. C., Raimondi, P. T. & Washburn, L. 2006. Macroalgal spore dispersal in
551 coastal environments: mechanistic insights revealed by theory and experiment. *Ecol.*
552 *Monogr.* 76:481-502.
553 Gerard, V. A. 1997. The role of nitrogen nutrition in high-temperature tolerance of the kelp
554 *Laminaria saccharina* (Chromophyta). *J. Phycol.* 33:800–10.
555 Gerard, V. A. & Du Bois, K. R. 1988. Temperature ecotypes near the southern boundary of the
556 kelp *Laminaria saccharina*. *Mar Biol*. 97:575–80.
557 van den Hoek, C. & Donze, M. 1967. Algal phytogeography of the European Atlantic coasts.
558 *Blumea*. 15:63–89.van den Hoek, C., Luning, K., 1988. Biogeography of marine
559 benthic algae — preface. *Helgoländer Meeresun*. 42, 131–132.
560 van den Hoek, C., Breeman, A.M., Stam, W.T., 1990. The geographic distribution of seaweed
561 species in relation to temperature — present and past. In: Beukema, J.J., Wolff, W.J.,
562 Brouns, J.J.W. (Eds.), Expected Effects of Climatic Change on Marine Coastal

563 Ecosystems, pp. 55–67. Kluwer Academic. Dordrecht, The Netherlands.
564 Johnson, C. R., Banks, S. C., Barrett, N. S., Cazassus, F., Dunstan, P. K., Edgar, G. J., Frusher,
565 S. D., Gardner, C., Haddon, M., Helidonotis, F., Hill, K. L., Holbrook, N. J., Hosie, G.
566 W., Last, P. R., Ling, S. D., Melbourne-Thomas, J., Miller, K., Pecl, G. T., Richardson,
567 A. J., Ridgway, K. R., Rintoul, S. R., Ritz, D. A., Ross, D. J., Sanderson, J. C.,
568 Shepherd, S. A., Slotwinski, A. Swadling, K. M. & Taw, N. 2011. Climate changes
569 cascades: Shifts in oceanography, species' ranges and subtidal community dynamics in
570 eastern Tasmania. *J. Exp. Mar. Biol. Ecol.* 400:17-32.
571 Liu, F. & Pang, S. J. 2009. Performances of growth, photochemical efficiency, and stress
572 tolerance of young sporophytes from seven populations of *Saccharina japonica*
573 (Phaeophyta) under short-term heat stress. *J. Appl. Phycol.* 22:221–9.Lüning, K. 1984.
574 Temperature tolerance and biogeography of seaweeds: The marine algal flora of
575 Helgoland (North Sea) as an example. *Helgoländer Meeresun*. 38:305–317.
576 Machalek, K. M., Davison, I. R. & Falkowski, P. G. 1996. Thermal acclimation and
577 photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant,*
578 *Cell Environ.* 19:1005–16.
579 Maxwell, K. & Johnson, G. N. 2000. Chlorophyll fluorescence - a practical guide. *J. Exp. Bot.*
580 51:659–68.
581 Merzouk, A. & Johnson, L. E. 2011. Kelp distribution in the northwest Atlantic Ocean under a
582 changing climate. *J. Exp. Mar. Biol. Ecol.* 400:90–8.
583 Moy, F. E. & Chrisite, H. 2012. Large-scale shift from sugar kelp (*Saccharina latissima*) to
584 ephemeral algae along the south and west coast of Norway. *Mar. Biol. Res.* 8:309-321.
585 Moy, F. E., Chrisite, H., Steen, H., Stålnacke, P., Aksnes, D., Alve, E., Aure, J., Bekkby, T.,
586 Fredriksen, S., Gitmark, J., Hackett, B., Magnusson, J., Pengerud, A., Sjøtun, K.,
587 Sørensen, K., Tveiten, L., Øygarden, L. & Åsen, P. 2008. *Sukkertareprosjektets*

588 *sluttrapport*. NIVA.
589 Müller, R., Laepple, T., Bartsch, I. & Wiencke, C. 2009. Impact of oceanic warming on the
590 distribution of seaweeds in polar and cold-temperate waters. *Bot. Mar.* 52:617–38.
591 Papageorgiou, G. C. & Govindjee, 2004. Chlorophyll a fluorescence: a signature of
592 photosynthesis. Springer.
593 Schiel, D. R. & Foster, M. S. 2006. The population biology of large brown seaweeds:
594 Ecological consequences of multiphase life histories in dynamic coastal environments.
595 *Ann. Rev. Ecol. Evol. Syst.* 37:343–72.
596 Quinn, G. P. & Keough, M. J. 2002. Experimental design and data analysis for biologists.
597 Cambrigde University Press. Cambridge, U.K.
598 Salvucci, E. S. & Crafts-Brandner, S. J. 2004. Relationship between the heat tolerance of
599 photosynthesis and the thermal stability of Rubisco activase in plants from contrasting
600 thermal environments. *Plant. Phys.* 134:1460-1470.
601 Sogn Andersen, G., Steen, H., Christie, H., Fredriksen, S. & Moy, F. E. 2011. Seasonal
602 patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima*
603 in Skagerrak, Norway: Implications for forest recovery. *J. Mar. Biol.* 2011:1–8.
604 Staehr, P. A. & Wernberg, T. 2009. Physiological responses of *Ecklonia radiata*
605 (Laminariales) to a latitudinal gradient in ocean temperature. *J. Phycol.* 45:91-99.
606 Underwood, A. J. 1997. Experiments in ecology. Their logical designs and interpretation
607 using analysis of variance. Cambridge University Press. Cambridge, U.K.
608 Wahid, A., Gelani, S., Ashraf, M. & Foolad, M. R. 2007. Heat tolerance in plants: an
609 overview. *Env. Exp. Bot.* 61:199-223.
610 Wernberg, T., Thomsen, M. S., Tuya, F., Kendrick, G. A., Staehr, P. A. & Toohey, B. D. 2010.
611 Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential
612 implications for a warmer future. *Ecol. Let.* 13:685-694.

613 Wernberg, T., Russel, B. D., Moore, P. J., Ling, S. D., Smale, D. A., Cambell, A., Coleman,
614 M. A., Steinberg, P. D., Kendrick, G. & Connel, S. D. 2011. Impacts of climate change
615 in a global hotspot for temperate marine biodiversity and ocean warming. *J. Exp. Mar.*
616 *Biol. Ecol.* 400:7-17.
617

Table 1. Results of partly nested PERMANOVAs testing the effect of growth temperature (GT) and sampling site (Si) on the photosynthetic response variables (P_{Max}, α 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.

	Omitting 20 °C				Omitting Dröbak			
Source of variation	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			

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Table 2. Results of partly nested PERMANOVAs testing the effect of growth temperature (GT) and sampling site (Si) on Chlorophyll a fluorescence data (Fv/Fm 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.

	Omitting 20 °C				Omitting Dröbak			
Source of variation	df	MS	Pseudo-F	P	df	MS	Pseudo-F	P
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		

620 Pairwise test GT: 20 ≠ 10 & 15.

621

622

Table 3. Results of partly 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.

	Omitting 20 °C				Omitting Dröbak			
Source of variation	df	MS	Pseudo-F	P	df	MS	Pseudo-F	P
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 Site: D ≠ B&G				Pairwise test GT: 20 ≠ 10 & 15			

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624

Table 4. Tissue N content in *Saccharina latissima* collected near Bergen, Grimstad and Dröbak, and grown in cultures at 10, 15 and 20 °C. Mean values ±sd (n = 4).

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

625

626

Table 5. Results of partly nested PERMANOVAs testing the effect of growth temperature (GT), assay temperature (AT) and sampling site (Si) on the photosynthetic response variables (P_{Max}, α 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.

	Omitting 20 °C				Omitting Dröbak			
Source of variation	df	MS	Pseudo-F	P	df	MS	Pseudo-F	P
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 × AT	4	7.491	4.979	<0.001	8	7.535	8.353	<0.001
GT × Si	2	6.738	4.479	<0.001	2	3.369	3.735	0.004
AT × Si	8	3.605	2.396	0.002	4	2.509	2.781	0.004
GT × AT × Si	8	4.516	3.002	<0.001	8	2.611	2.895	<0.001
Res	84	1.504			81	0.902		

627

Figure captions

Figure 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.

Figure 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 ±sd (n=27). Small horizontal bars represent observed minimum and maximum mean temperatures in August.

Figure 3. Photosynthetic parameters (A: P_{Max}; B: α; C: R_D) in plants from Bergen, Grimstad and Dröbak measured at the respective growth temperatures (10, 15 and 20 °C). Mean values ±sd (n=4).

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

Figure 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, respectively. Mean values ±sd (n = 4).

Figure 6. Pigments. Chlorophyll a content (A) and the ratios between Chlorophyll c (B), Fucoxanthin (C), Viola+Zeaxanthin (D) and Chlorophyll a in plants from Bergen, Grimstad and Dröbak, grown at 10, 15 and 20 °C, respectively. Mean values ±sd (n = 4).

Figure 7. Photosynthetic parameters (A: P_{Max}; B: α; 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, respectively). Mean values ±sd (n=4). Solid lines describe the mean situations across sites.

Figure 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, respectively). Mean values ±sd (n=4). Solid lines describe the mean situations across sites.

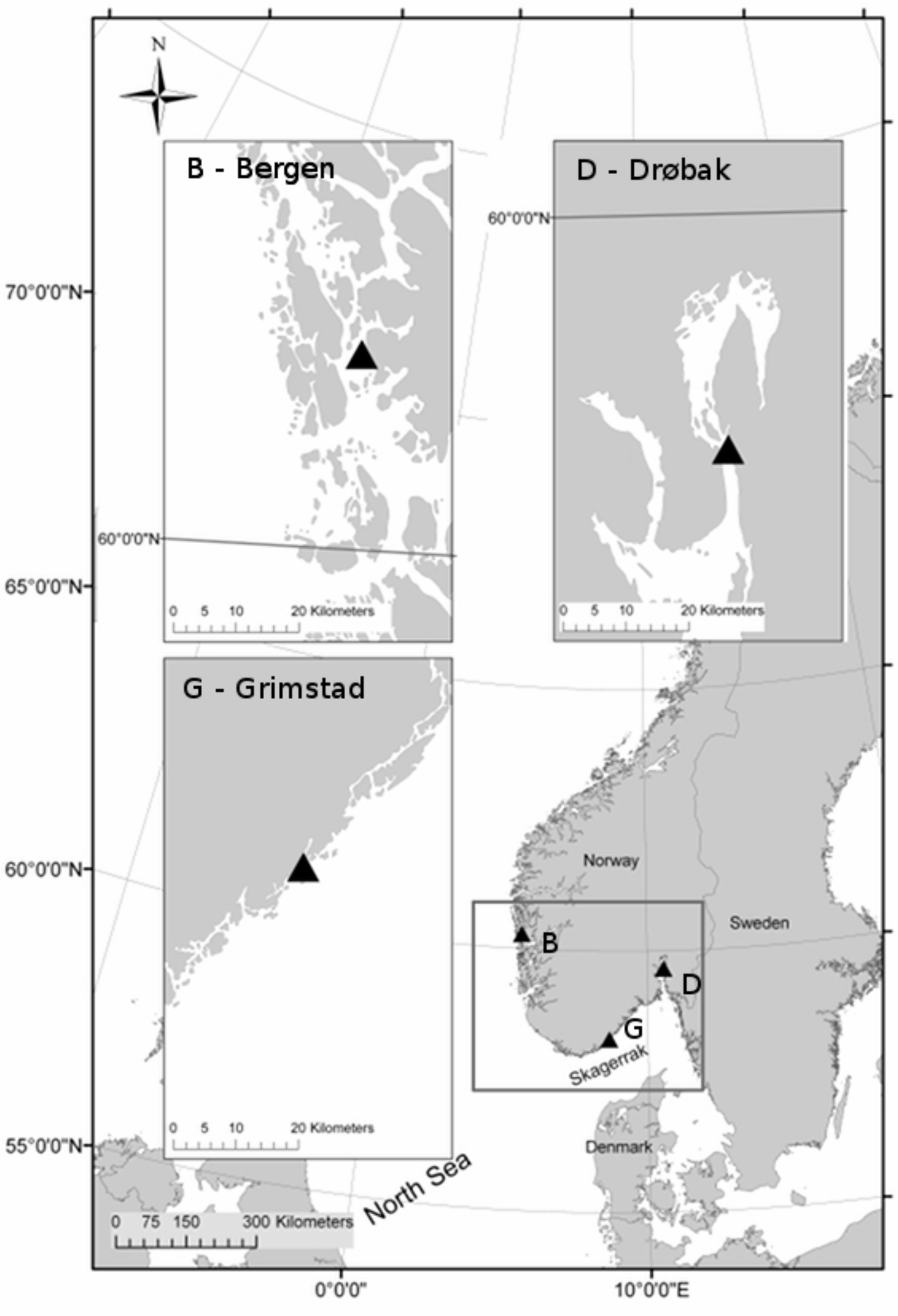

N
B - Bergen
D - Drøbak
G - Grimstad
60°0'0"N
70°0'0"N
65°0'0"N
60°0'0"N
55°0'0"N
0 5 10 20 Kilometers
0 5 10 20 Kilometers
0 5 10 20 Kilometers
0 75 150 300 Kilometers
Norway
Sweden
Denmark
North Sea
Skagerrak
B
D
G
0°0'0"
10°0'0"E

Figure 2.

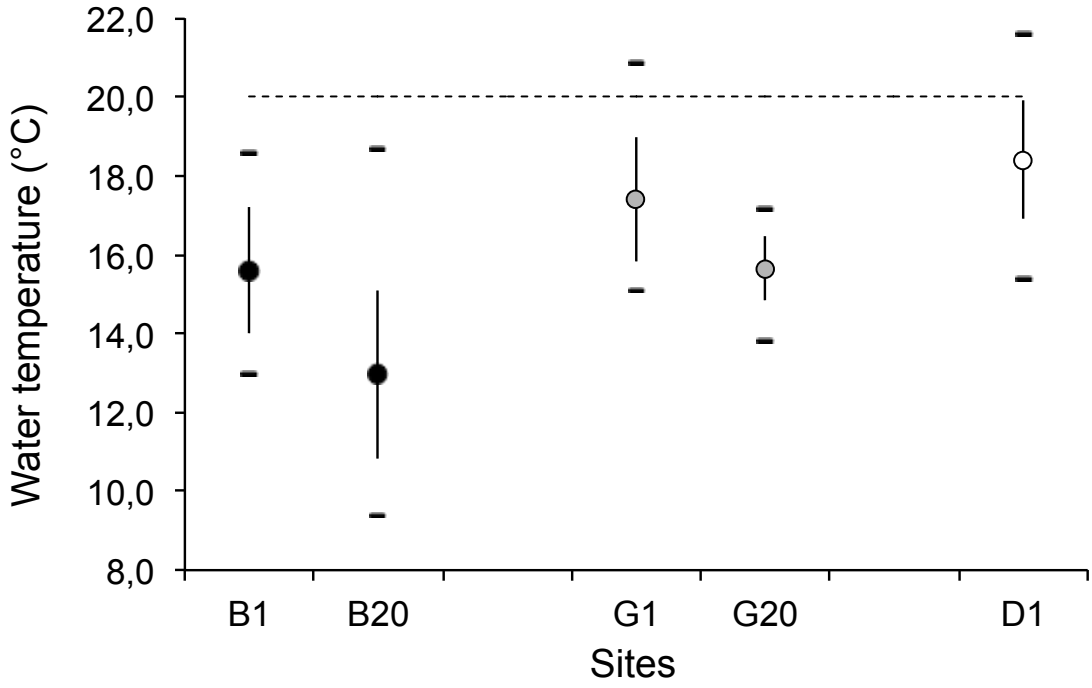

22,0
20,0
18,0
16,0
14,0
12,0
10,0
8,0
Water temperature (°C)
B1
B20
G1
G20
D1
Sites

Figure 3.

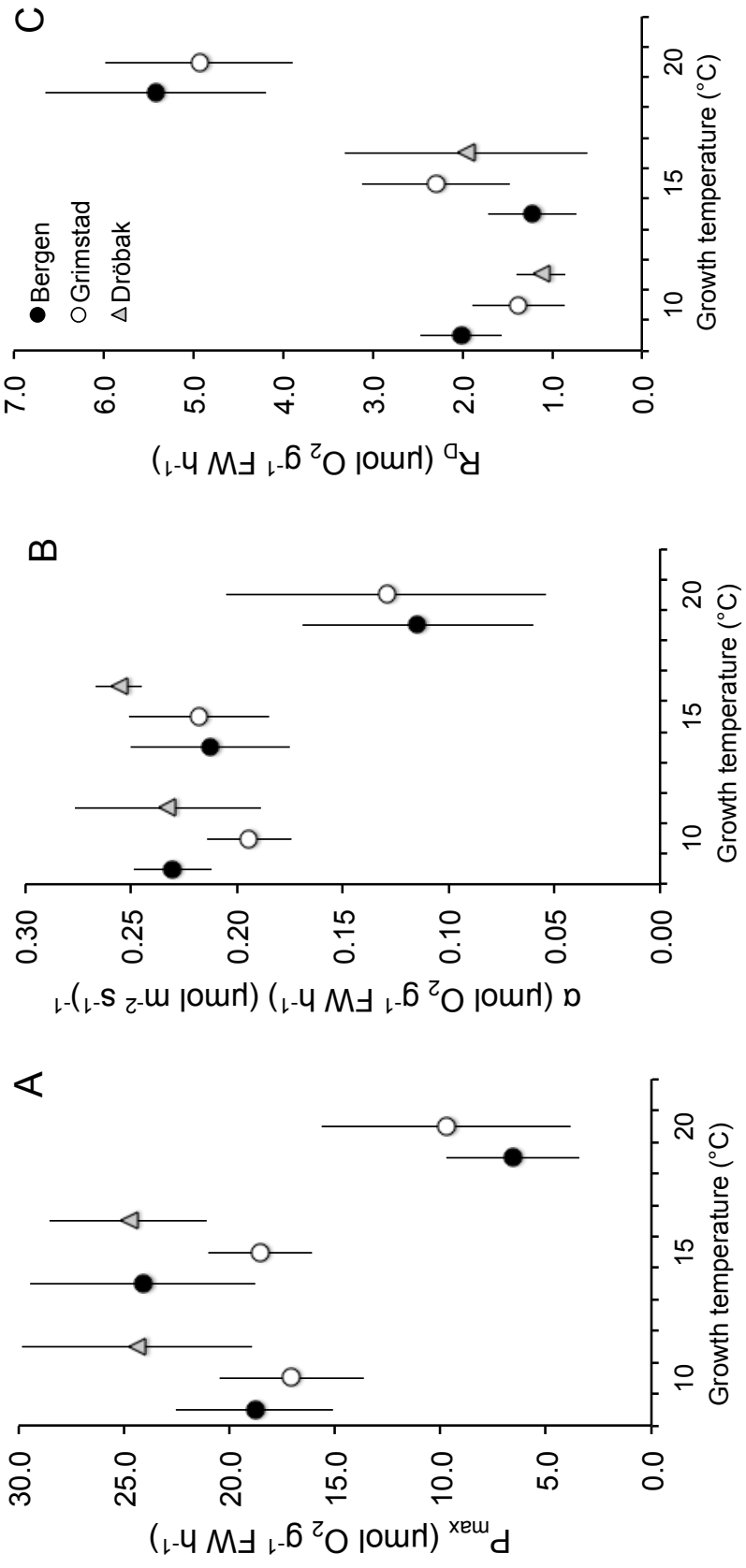

A
B
C
P_{max} (µmol O_2 g^{-1} FW h^{-1})
α (µmol O_2 g^{-1} FW h^{-1}) (µmol m^{-2} s^{-1})$^{-1}$
R_D (µmol O_2 g^{-1} FW h^{-1})
Growth temperature (°C)
Bergen
Grimstad
Dröbak

Figure 4.

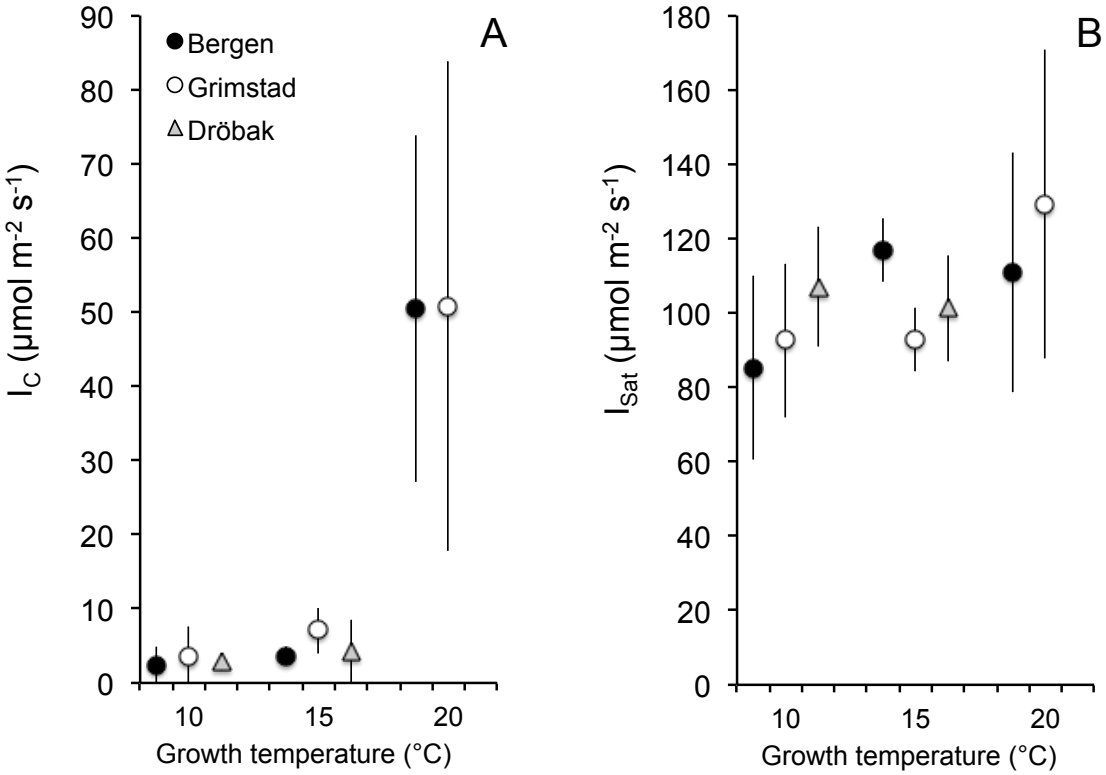

A
Bergen
Grimstad
Dröbak
I_C (µmol m^{-2} s^{-1})
Growth temperature (°C)
B
I_{Sat} (µmol m^{-2} s^{-1})
Growth temperature (°C)

Figure 5.

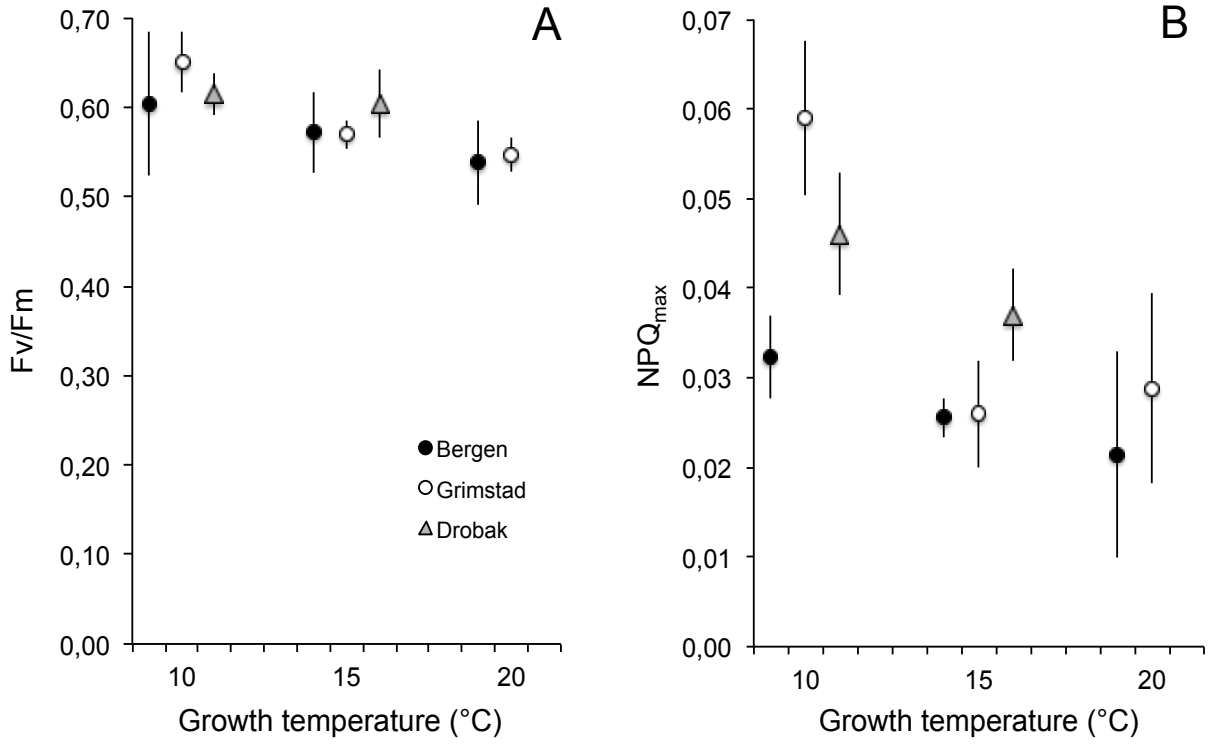

A
Fv/Fm
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
10
15
20
Growth temperature (°C)
Bergen
Grimstad
Drobak
B
NPQmax
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
10
15
20
Growth temperature (°C)

Figure 6.

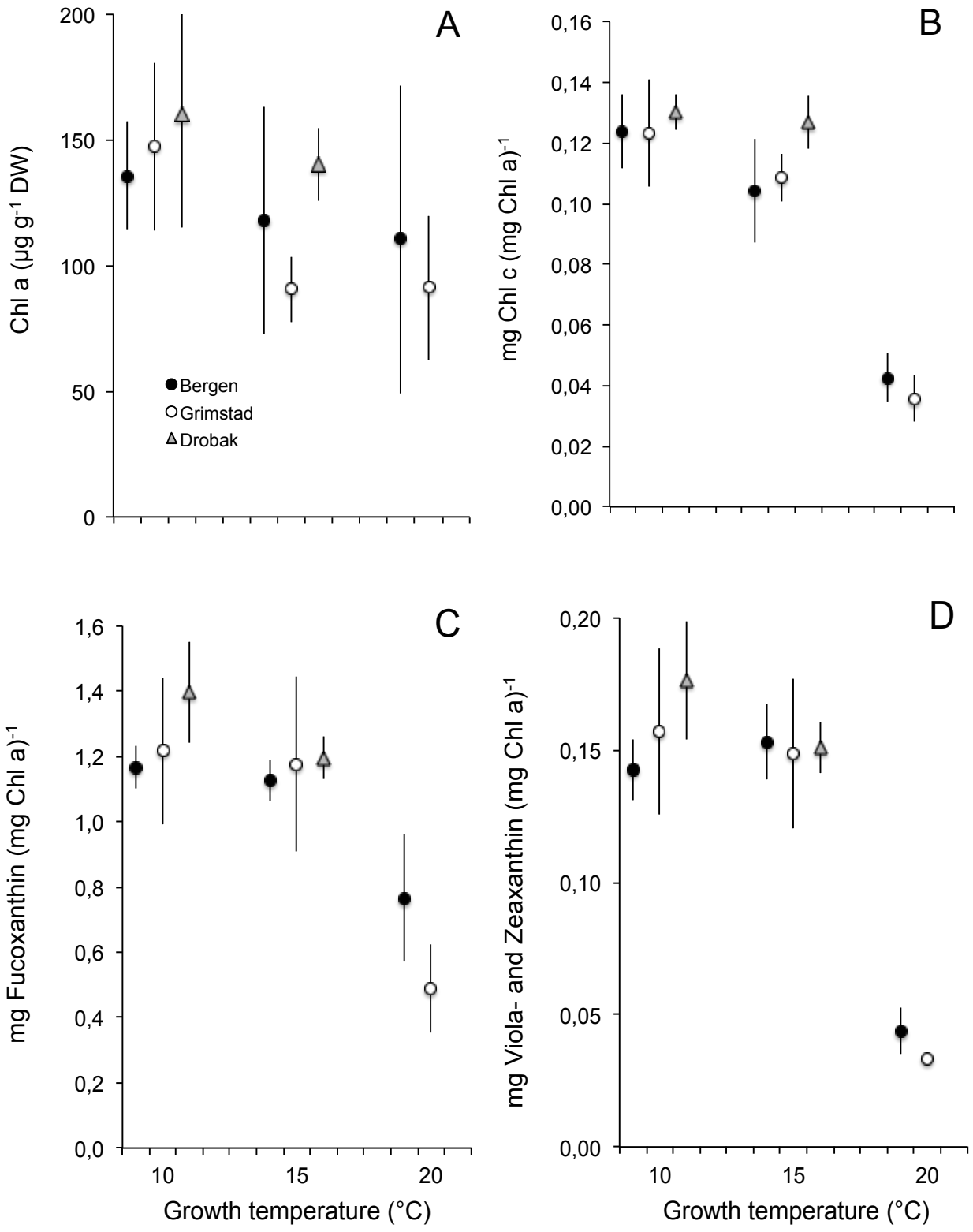

A
Chl a (µg g-1 DW)
200
150
100
50
0
Bergen
Grimstad
Drobak
B
mg Chl c (mg Chl a)-1
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
C
mg Fucoxanthin (mg Chl a)-1
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
10
15
20
Growth temperature (°C)
D
mg Viola- and Zeaxanthin (mg Chl a)-1
0,20
0,15
0,10
0,05
0,00
10
15
20
Growth temperature (°C)

Figure 7.

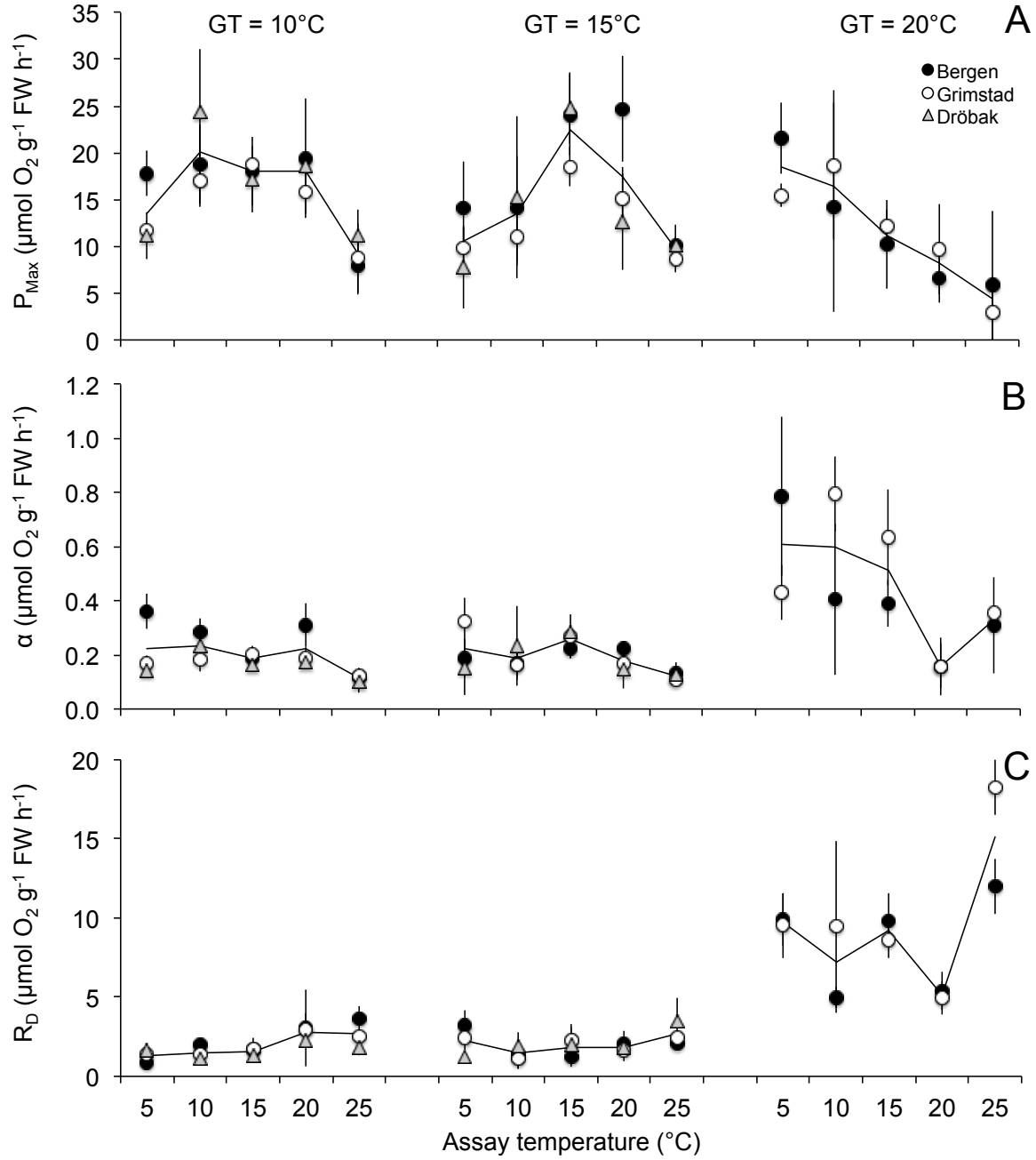
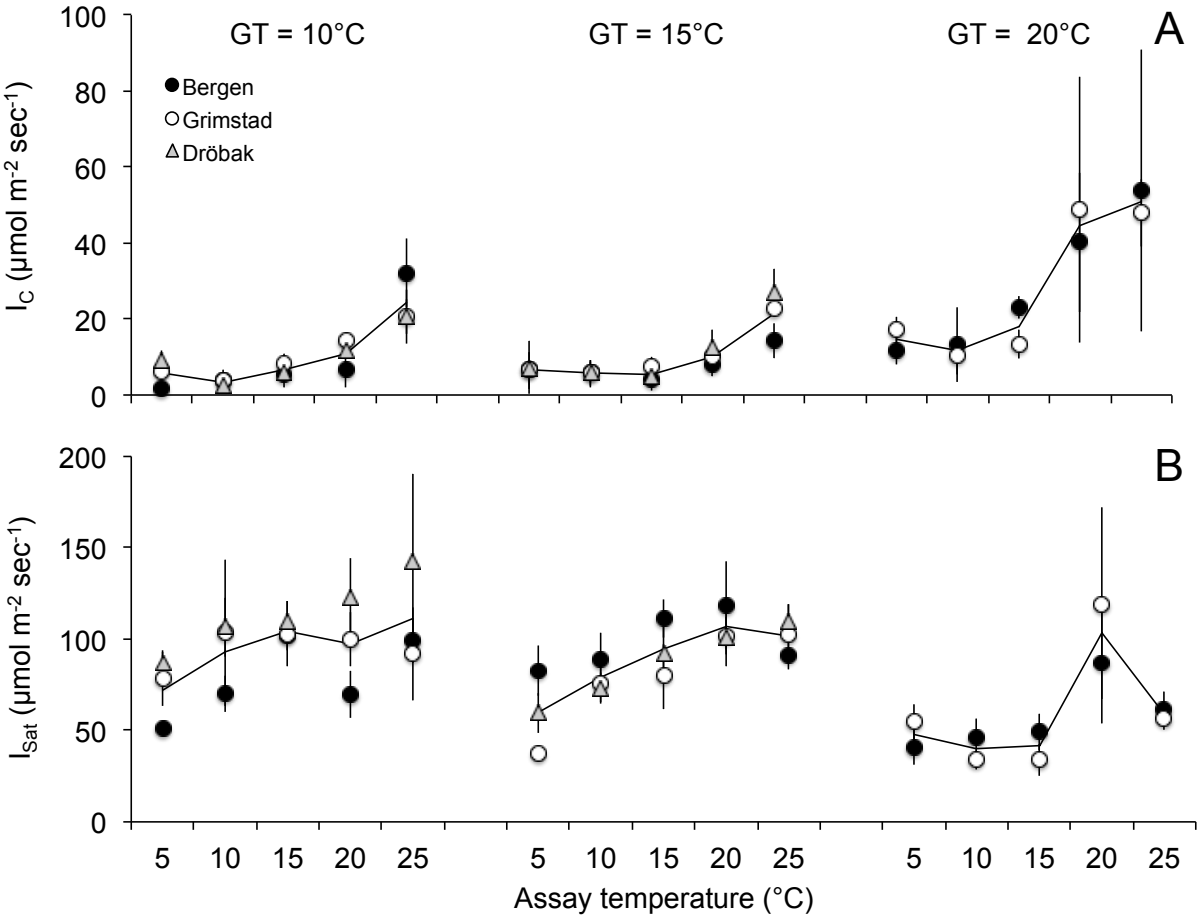

GT = 10°C
GT = 15°C
GT = 20°C
A
B
C
Bergen
Grimstad
Dröbak
P_{Max} (µmol O_2 g^{-1} FW h^{-1})
α (µmol O_2 g^{-1} FW h^{-1})
R_D (µmol O_2 g^{-1} FW h^{-1})
Assay temperature (°C)

Figure 8.


GT = 10°C
GT = 15°C
GT = 20°C
A
B
Bergen
Grimstad
Dröbak
I_C (µmol m^{-2} sec^{-1})
I_{Sat} (µmol m^{-2} sec^{-1})
0
20
40
60
80
100
50
100
150
200
5
10
15
20
25
Assay temperature (°C)