

Response to experimental warming in northern eelgrass populations: comparison across a range of temperature adaptations

P. Beca-Carretero^{1,2,*}, B. Olesen³, N. Marbà¹, D. Krause-Jensen^{4,5}

¹Department of Global Change Research, IMEDEA (CSIC-UIB), Institut Mediterrani d'Estudis Avancats, Miquel Marqués 21, 07190 Esporles (Illes Balears), Spain

²Botany and Plant Science, School of Natural Sciences, and Ryan Institute, National University of Ireland Galway, University Road, Galway H91 TK33, Ireland

³Department of Bioscience, Aarhus University, Ole Worms Allé 1, Building 1135, 8000 Aarhus C, Denmark

⁴Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

⁵Arctic Research Centre, Aarhus University, Ny Munkegade 114, Building 1540, 8000 Aarhus C, Denmark

ABSTRACT: Global warming may exert diverging effects on eelgrass (*Zostera marina* L.) populations originating from the northern versus the central part of the distribution range and on populations growing at saturating versus limiting light. We experimentally examined growth and physiological temperature responses of 3 eelgrass populations adapted to different temperature regimes in subarctic Greenland (2 populations) and in Denmark (1 population). Shoots were incubated at 5 different temperatures (10, 15, 20, 25 and 28°C) for 15 to 16 d at a saturating irradiance (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and one of the populations was also incubated at a limited irradiance of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. All populations exhibited optimum temperatures of 20 to 25°C for photosynthesis and growth under saturating light, while light limitation reduced the optimum by 5 to 10°C. When compared at their respective *in situ* summer temperature (i.e. 10, 15 and 20°C), all populations exhibited similar relative growth rates, indicating a capacity for local adaptation. The 2 subarctic populations exhibited higher activation energy for growth and, hence, greater responsiveness to warming than the centrally located population. However, subarctic populations were also more sensitive to extreme high temperatures, showing faster increases in respiration rates and declines in photosynthesis. Sensitivity to warming varied across light conditions with light-limited plants being most vulnerable to extreme temperatures, causing a negative carbon budget. In conclusion, projected warming would stimulate the performance of subarctic eelgrass populations but could eventually compromise populations in the center of the distribution range, which currently grow close to their temperature optimum.

KEY WORDS: Experimental warming · Latitude comparison · Production · Energy activation · Photosynthetic response · Greenland · Denmark · Seagrass · *Zostera marina*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The globe is warming at unprecedented rates and models project warming to continue through the 21st century in response to scenarios of greenhouse gas

emissions (Intergovernmental Panel on Climate Change (IPCC) 2014). Warming is affecting ecosystems worldwide (IPCC 2014) and signs of species migration poleward have been reported over recent decades (Walther et al. 2002, Poloczanska et al. 2013)

in response to isotherm migration (Burrows et al. 2011). To facilitate predictions of future distribution ranges of species there is a demand for studies on their temperature response. Most predictions have assumed a similar temperature response over the species distribution range (Müller et al. 2009, Jueterbock et al. 2013), but recent studies have highlighted the need for taking into account possible changes in temperature response over this range due to local species adaptation (Bennett et al. 2015).

Eelgrass *Zostera marina* L. is the most widely distributed seagrass in the northern hemisphere (Den Hartog 1979), where it plays a key role in the coastal zone in terms of high productivity, stimulation of biodiversity, protection of the coastline, carbon storage and nutrient retention (Serrano et al. 2011, Duarte et al. 2013, Cole & Moksnes 2016). *Z. marina* occurs from subtropical regions of southern Portugal and Spain (Setchell 1935, Den Hartog 1979, Diekmann & Serrao 2012) with summer seawater temperatures close to 27°C (Newton & Mudge 2003) to subarctic regions with summer temperatures around 10 to 15°C (Olesen et al. 2015), and to even colder Arctic regions. The wide geographical distribution range of this species demonstrates a broad temperature tolerance, but warming emerges as a potential stress on eelgrass populations from the southern and central part of the distribution range, where population declines after periods of high temperature have been observed (Phillips et al. 1983, Durako & Moffler 1987, Reusch et al. 2005). At the northern distribution limit of the species, by contrast, warming is likely to stimulate growth and success of sexual reproduction and, hence, a northward expansion, as has also been predicted for fucoid seaweed species (Jueterbock et al. 2013, Olesen et al. 2015).

Responses to warming depend on the species' thermal tolerance range and its capacity to adapt to local temperature conditions (Short & Neckles 1999). A wide range of metabolic rates increase exponentially with warming and this uniformity in response facilitates comparisons of the sensitivity of metabolic processes to warming expressed as the thermal energy causing a given percent change in process rate, i.e. the activation energy (Brown et al. 2004, Dell et al. 2011). While warming stimulates both photosynthesis and respiration, rates of respiration tend to increase faster than photosynthesis rates per 1°C of warming (Marsh et al. 1986, López-Urrutia et al. 2006), and warming may thereby increase the risk of rendering the carbon budget of the plants negative. Acclimation (phenotypic plasticity) and/or local adaptation (genetic adaptation) may to some extent

compensate such negative effects of warming (Davison et al. 1991, Bergmann et al. 2010, Bennett et al. 2015).

In addition to the different temperature regimes across the geographical distribution range, northern and central/southern eelgrass populations are also exposed to marked differences in the light climate. While subtropical and temperate populations receive light year round, eelgrass growing near the northern limit has to cope with severe light limitation for major parts of the year (e.g. McRoy 1969) due to the long Arctic night in combination with sea ice cover. Warming of the Arctic, occurring at twice the global average rate (IPCC 2014), has already caused major reductions in Arctic sea ice extent and projections indicate that the Arctic summers will be ice free within a few decades (Wang & Overland 2009). As a result, more light may reach the seafloor annually and stimulate growth of eelgrass in shallow Arctic coastal areas (Krause-Jensen & Duarte 2014, Olesen et al. 2015). Light limitation of eelgrass growth is most pronounced near the depth limit where light energy on an annual basis only just enables a positive carbon budget (Dennison 1987). The critical light requirement of seagrass to maintain growth is expected to increase with temperature (Lee et al. 2007). Hence, in a warming scenario, the deep-growing, light-limited seagrass ecosystems are more likely than light-saturated populations to shift from autotrophic to heterotrophic metabolism.

The aim of this study was to compare the temperature response of 3 eelgrass populations adapted to growth across a broad thermal gradient from near the northern distribution limit to centrally in the distribution range. We also aimed to investigate how temperature affects the performance of eelgrass growing at saturating light versus at light limitation reflecting the conditions near the lower depth limit of the meadow, and in particular, how warming affects the minimum light requirement for photosynthesis. We hypothesized (1) that eelgrass adapted to low-temperature environments have lower optimum temperature for growth, photosynthesis and respiration, and are more sensitive to thermal stress as compared to populations adapted to higher temperature, and (2) that shaded plants are more sensitive to thermal stress than light-saturated plants, resulting in a higher critical light level to maintain a positive carbon balance with increasing temperature. The study was performed by comparing the response of 3 distinct eelgrass populations—2 near the northern distribution limit growing at summer temperatures of 10 and 15°C, respectively, and 1 from the center of the

biogeographical distribution growing at summer temperatures of around 20°C—to the same range of water temperatures (10–28°C). Also, the temperature effect on shoots acclimated to saturating versus limiting light was examined in 1 population. Growth rates were measured as leaf elongation as well as shoot biomass increase, to evaluate the potential effect of higher respiratory carbon losses (of leaves and rhizome/roots) relative to carbon assimilation with increasing temperature. The novelty of this study resides in the aim to evaluate the role of local adaptation in thermal performance in *Z. marina*, the seagrass species with the widest geographical distribution range.

MATERIALS AND METHODS

Experimental plants

We collected the experimental plants of *Zostera marina* L. (eelgrass) from 3 meadows, 2 located at the reported northernmost distribution limit in Greenland (64° N) in the extensive Godthåbsfjord system on the southwest coast near Nuuk (Kobbe Fjord and Kapisillit), and 1 in the center of the distribution range (56° N) in Denmark (Århus Bay Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m589p059_supp.pdf). In spite of their relatively short geographic distance apart (~80 km), the 2 populations from Greenland were adapted to different environmental conditions (Olesen et al. 2015). The Kobbe Fjord (64° 09' N, 51° 33' W) population was growing at *in situ* summer temperatures of 8.5 to 10°C and at a salinity of 34, while the Kapisillit (64° 28' N, 50° 13' W) population was adapted to *in situ* summer temperatures of 11.6 to 15°C and a salinity of 20 at the time of collection in late August. For more information on the Greenland eelgrass meadows see Olesen et al. (2015). Summer temperatures in Århus Bay (56° 23' N, 16° 33' E) ranged between 18 and 22°C and the salinity was 25.

Shoots from both Greenland populations were sampled at approximately 2 to 3 m water depth at mean water level (tidal range 14.5 m, Richter et al. 2011). To avoid pseudo-replication by resampling the same genotypes, the shoots were randomly collected across the meadows from a boat at intervals of 5 to 10 m using a small rake attached to a rope that was thrown and manually dragged for about 1 m on the sea floor. The Greenland shoots were transported to the laboratory in Århus, Denmark, wrapped in salt-water-wetted tissue in open plastic bags, loosely

packed in cold bags and transferred to aquaria upon arrival. Eelgrass shoots from the Århus population were collected by hand at approximately 1 m depth in early September using a similar sampling strategy as for the Greenland populations to reduce the risk of resampling the same genotype. The shoots were transferred to cooling bags with seawater and directly transported to the laboratory.

Experimental design

We kept the harvested shoots under laboratory conditions at 5°C for 15 d (Greenland populations) and at 15°C for 9 d (Århus population), a few degrees below their *in situ* temperatures to avoid them exceeding their local summer temperature conditions. The irradiance during this initial phase was 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in a 16 h light:8 h dark cycle. We used artificial seawater (Marinemix professional, HW Wiegand) with a salinity of 25, as an intermediate level of the salinity at the 3 sampling locations. The incubation medium was enriched with nutrient solution (Plant Nutrition+, Tropica Aquarium Plants) to yield a concentration of 96 μM nitrogen (N) and 3.2 μM phosphorous (P) to ensure sufficient levels of nutrients for plant growth.

Prior to experimental start, shoots from each population were progressively acclimated to the target incubation temperatures of 10, 15, 20, 25 and 28°C. The shoots were kept for 3 d at a given temperature before they were directly transferred to a 5°C colder or warmer aquarium until reaching the target temperature. At the onset of the experiment, shoots had acclimated to a specific experimental temperature for at least 6 d, except for those transferred from 25 to 28°C, which had an acclimation period of 3 d. At the onset of the experiment, we measured the initial shoot fresh weight (FW) and length, counted the number of leaves per shoot, adjusted the number of rhizome segments to 3 and pierced a hole through the leaves at the top of the leaf sheath with a hypodermic needle for measurement of growth rate (Sand-Jensen 1975, Short & Duarte 2001). Initially, the shoots from Kobbe Fjord, Kapisillit and Århus Bay had 3.98 ± 0.1 , 4.3 ± 0.1 and 3.2 ± 0.7 leaves per shoot (mean \pm SE) and weighed 0.67 ± 0.05 , 1.32 ± 0.06 and 1.24 ± 0.05 g FW, respectively. A randomly selected subsample of shoots from each population was kept for assessment of the initial fresh weight: dry weight ratio (FW:DW).

For each treatment, 3 apical shoots (attached to 3 rhizome internodes with roots) were then placed in

each of three 3 l cylindrical Perspex chambers (internal diameter 10 cm, height 30 cm), resulting in a total of 9 shoots per temperature for each population. The shoots were attached to plastic nets at the base of the chambers to keep them in an upright position. The chambers with seawater medium, as described above, were continuously aerated with atmospheric air. Water level and salinity were measured every second day and the chambers were refilled with fresh water to compensate for evaporation. Half of the water in each chamber was renewed once a week to ensure sufficient levels of nutrients. Saturating irradiance of 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Olesen & Sand-Jensen 1993) below the water surface was provided by fluorescent tubes (Philips TL5HO, 39 W, 830/840) in a 16 h light:8 h dark cycle. Another set of shoots from Kapisillit was incubated over the same temperature range at an irradiance of 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to imitate summer irradiance conditions near the deep edge of the meadow (Staeher & Borum 2011). The low-irradiance treatment was obtained by covering the chambers with a shading screen. Hence, for each temperature, the set-up included 3 replicate chambers per population exposed to saturating light and 3 additional replicate chambers for the low irradiance treatment of Kapisillit shoots (see Fig. S2 in the Supplement). The 12 Perspex chambers from each temperature treatment were placed in a temperature-controlled water tank (length: 60 cm, width: 40 cm, height: 30 cm) equipped with a heater, a thermostat (IKS Aquastar Basis-System) and a water circulation pump. Experimental temperature was continuously logged by HOBO loggers (UA-002-64, Onset) placed in 1 of the 12 cylinders.

The growth experiment was run for 15 to 16 d to ensure adequate formation of new leaf and rhizome tissue for the photosynthesis measurements. We then quantified a set of response parameters (see details in the sections below) related to growth (leaf formation rate, leaf and rhizome elongation rates and relative growth rate) as well as a set of physiological response parameters (net rate of leaf photosynthesis, P_{max} , and respiration, R , leaf $P_{\text{max}}:R$ ratio, and rhizome and root respiration rates). The optimum temperatures for growth and photosynthesis were estimated as the temperature interval with the highest rates measured and for respiration the interval with the lowest rates. Activation energy was calculated for all the above process rates. In addition, we measured photosynthesis versus light response for entire shoots from Kapisillit grown at saturating and limiting irradiance levels. Chlorophyll *a* and *b* content was also measured for these plants.

Growth responses

Leaf formation rate (new leaves d^{-1}) was quantified as the number of new leaves, identified as those without a punched hole, divided by the incubation period (in d). Leaf elongation rate per shoot ($\text{cm shoot}^{-1} \text{ d}^{-1}$) was quantified as cm of new leaf material produced, measured as the length of new leaves (i.e. leaves without holes) plus, for each growing leaf, the distance between the punched hole and the reference hole in the oldest leaf and divided by the incubation period (Short & Duarte 2001). Rhizome elongation rate ($\text{cm rhizome apex}^{-1} \text{ d}^{-1}$) was calculated as the increase in rhizome length divided by the incubation period. The relative growth rate (RGR, d^{-1}) was calculated as:

$$\text{RGR} = -\ln(B_f/B_i)/t \quad (1)$$

where B_i is the initial and B_f the final DW of the shoot (including rhizomes, new shoots formed during the incubation and leaves shed during incubation) after freeze drying for 24 h to constant weight, and t is the incubation period in d.

Physiological responses: photosynthesis and respiration

Light-saturated photosynthesis (P_{max}) and respiration were measured on segments (10–15 cm) of the second youngest leaf as oxygen production or consumption at the end of the experiment. In addition, respiration rates were measured on the 2–3 youngest rhizome internodes including attached roots. The leaf and rhizome segments of 3 replicate shoots from each treatment were gently cleaned of algae and dead plant material and placed in 30 ml glass-stoppered bottles with artificial seawater (salinity 25) at the same temperature as during the growth experiment. Bottles were placed on a rotating wheel in a thermostat incubator. Each bottle was provided with 2–3 glass beads to ensure stirring and thereby minimize diffusion limitation. Dark respiration of leaves and rhizomes was measured during incubation in total darkness for 90–120 min prior to photosynthesis measurements of leaves, which were conducted at a saturating irradiance of 240 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ provided by fluorescent tubes. The reason for measuring the plants grown under low light at saturating irradiance was to evaluate the effect of temperature on photosynthetic light acclimation by comparing the photosynthetic capacity of shoots from the 2 light treatments. The

incubation medium in the bottles was completely renewed with temperature-adjusted and aerated saltwater medium between measurements. To reduce the risk of photorespiration, the water for photosynthetic measurements was purged with N₂ gas to lower initial O₂ concentration to approximately 80% saturation (Pedersen et al. 2013). Incubation time for photosynthetic measurements (45–60 min) was adjusted in order to maintain final oxygen concentration below 120% saturation and thereby prevent bubble formation. At the end of the incubation, the dissolved oxygen concentration was measured with oxygen microelectrodes (Type OX-500, Unisense A/S). Oxygen consumption and production were subsequently calculated by subtracting the oxygen change in control bottles incubated without plant material. For each temperature, the electrode was calibrated to 0 and 100% of air saturation.

Photosynthetic light response was measured on whole shoots from the Kapisillit population grown at high and low irradiance. Three shoots from each temperature and light treatment were incubated at their experimental temperature in a 200 ml closed Perspex chamber and oxygen production or consumption was measured with Fiber-Optic Oxygen Microsensors connected to an OXY-4 4 channel fiber-optic oxygen meter (PreSens Precision Sensing). We used 3 separate chambers allowing 3 parallel measures. The chambers were placed in a temperature-controlled water bath, and irradiance was provided from fluorescent tubes (Philips TL5HO, 39 W, 830/840) in the range of 0 to 380 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Neutral density filters were used to obtain 7 different photon flux densities. The chambers were stirred with a magnetic stirrer bar and a plastic net (mesh size 10 mm) was used to separate the stirrer from the eelgrass shoot. Following a 30 min acclimation period to darkness prior to incubation, the dark respiration was measured over a 30–40 min period, after which photosynthetic measurements proceeded at gradually increasing irradiances. Oxygen concentration in the chambers were logged every 15 s and metabolic rates were calculated by fitting a linear regression line to the data omitting the first period (5–10 min) at the initiation of each irradiance treatment before rates stabilized. The oxygen concentration in the chambers was in the range of 80 to 150% saturation. Measurements were aimed at identifying light use efficiency, respiration rates and light compensation points of whole shoots while maximum photosynthetic rates (P_{max}) were disregarded, as saturation of whole shoots was not always obtained at the highest irradiances of 380 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

applied. The photosynthetic efficiency (α) was calculated from the initial slope of the P-I curve between 0 and 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 10 and 15°C, and between 0 and 105 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 20, 25 and 28°C. Dark respiration rate (R) was estimated from the intercept of the regression line on the ordinate and the light compensation point (I_c) from the intercept on the abscissa. At the end of the measurements, the shoots were freeze-dried for 24 h and weighed. The freeze-dried material was ground and the concentrations of chlorophyll *a* and *b* were determined spectrophotometrically after extraction for 24 h in darkness in 96% ethanol according to Lichtenthaler (1987).

Activation energy

The activation energy was calculated to quantify the thermal sensitivity of the eelgrass and to examine if it differed among populations. The metabolic theory of ecology (MTE) suggests that the Boltzmann–Arrhenius model from chemical reaction kinetics can be used to predict the rise of many biological rates with warming (Brown et al. 2004). According to the MTE, the scaling of a biological rate (BR) with temperature (T , in Kelvin), is:

$$\text{BR} = \text{BR}_i \cdot e^{(E/kT)} \quad (2)$$

where E is the activation energy, k is Boltzmann's constant, and BR_i is the initial biological rate. We used a similar approach to also examine the sensitivity of the fall of biological rates to warming when optimum temperature is exceeded, following Dell et al. (2011).

Statistical analysis

All values are reported as means \pm SE. Differences in eelgrass performance between temperature treatments, populations or light levels were tested using 2-way analysis of variance (ANOVA), and a 1-way ANOVA was used to test differences among populations in their performance at ambient temperature. Tukey's test was applied to identify the treatments that differed significantly. Data was checked for homogeneity of variance using Bartlett's test. We used regression analysis on log-transformed parameters to estimate the activation energy of the various growth/physiological rates in each population. All data treatment and statistical analysis was performed using the IBM SPSS, v.11.

RESULTS

Growth response at saturating light

The 3 eelgrass populations grew at relatively similar rates when experimentally exposed to their *in situ* temperature (i.e. $\sim 10^{\circ}\text{C}$ for Kobbe Fjord, $\sim 15^{\circ}\text{C}$ for Kapisillit and $\sim 20^{\circ}\text{C}$ for Århus Bay) despite the 10°C range in *in situ* temperatures (Fig. 1, left panels; Table S1 in the Supplement). Hence, for all growth variables (leaf formation, leaf and rhizome elongation, relative growth), rates at the respective *in situ* temperature did not differ significantly between populations even though the population from the coldest subarctic site (Kobbe Fjord) exhibited slightly lower mean levels (Fig. 1, left panels, Table S1).

However, subarctic eelgrass grew significantly faster than cold-temperate eelgrass when compared

across the full experimental temperature range, which represented an overall warming for the subarctic eelgrass (Fig. 1, left panels, Table S2). Hence, subarctic Greenland populations produced new leaves faster (on average by 60% for Kobbe Fjord and 80% for Kapisillit) than the cold-temperate Danish population (Århus Bay, Fig. 1, left panels). Similarly, rhizomes from Greenland populations elongated, on average, 2.5-fold faster than those of the Danish populations when exposed to the experimental temperature range (Fig. S3, left panel). The amplitude of leaf, rhizome and overall plant growth fluctuations (coefficient of variation, Table S3) across the experimental temperature range also tended to be wider in subarctic than cold-temperate populations.

There was no consistent difference in optimum growth temperature across subarctic and cold-temperate populations (Table S3). Warming enhanced

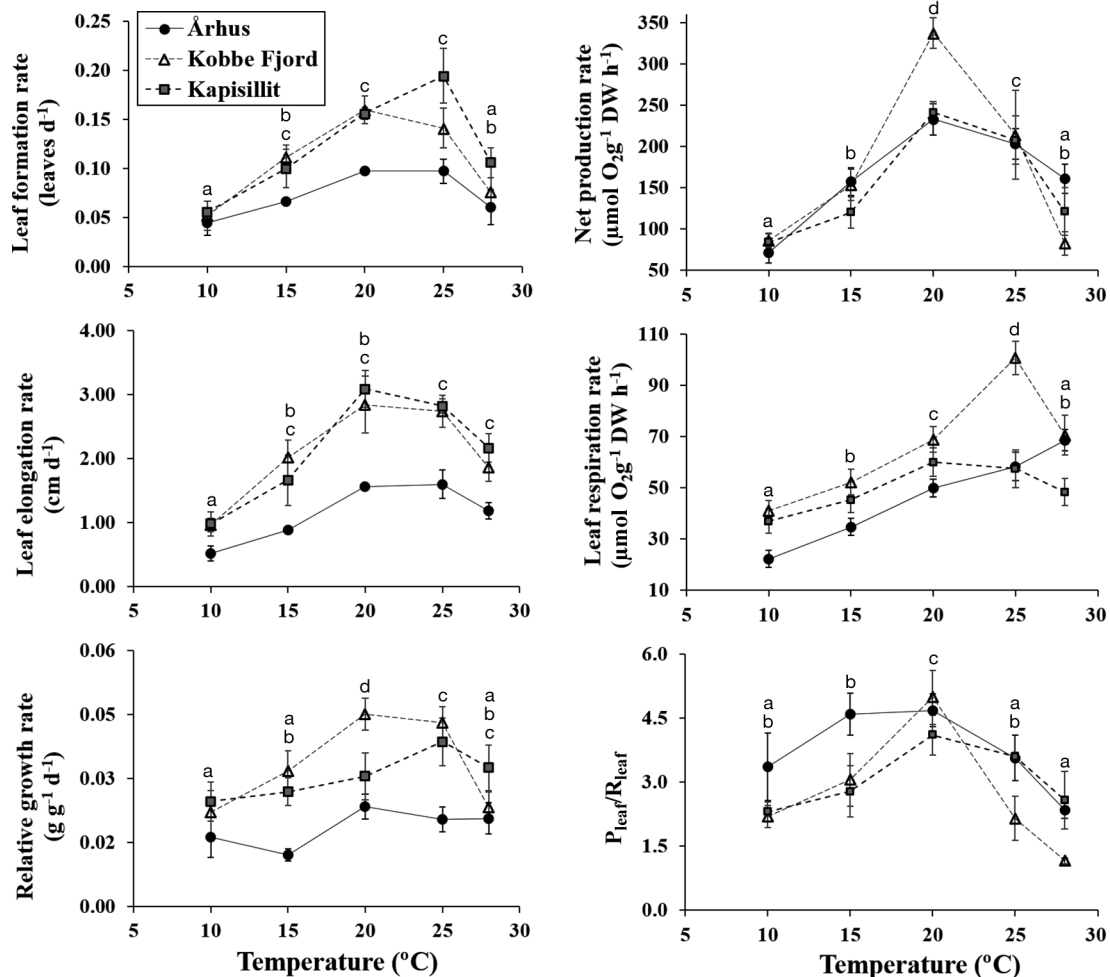


Fig. 1. Growth responses (left columns) and physiological responses (right columns) of *Zostera marina* populations from Greenland (Kobbe Fjord, Kapisillit) and Denmark (Århus) to a range of experimental temperatures including the *in situ* temperature at the time of collection of each of populations, i.e. 10°C for Kobbe Fjord, 15°C for Kapisillit, and 20°C for Århus. Letters indicate significant differences between temperatures according to the 2-way ANOVA (see Table S2 in the Supplement)

eelgrass growth rates up to optimum temperatures ranging between 20 and 25°C, whereas minimum growth rates were observed at the lowest (10°C) and the highest (28°C) temperature treatments (Fig. 1). Only shoots exposed to 28°C showed stages of leaf necrosis in all the populations. However, subarctic eelgrass populations were more sensitive than cold-temperate ones to warming up to optimum temperature, as reflected by their generally higher growth activation energies (Table S3). The sensitivity of eelgrass growth to increased temperature also differed depending on whether warming occurred below or above optimum temperature. The decline of growth rates when warming exceeded optimum temperature tended to be more abrupt (activation energy: 0.49–1.41 eV) than growth stimulation as temperatures increased to the optimum (activation energy: 0.22–0.80 eV, Table S3). These ‘de-activation energies’ did not show consistent differences between subarctic and cold-temperate populations.

Photosynthesis and respiration response at saturating light

Eelgrass from the cold-temperate Århus Bay population exhibited significantly higher net leaf photosynthesis and leaf $P_{\max}:R$ ratio at its *in situ* temperature (i.e. 20°C) than did subarctic Greenland populations at their *in situ* temperature (10°C for the Kobbe Fjord population, 15°C for the Kapisillit population), whereas leaf and rhizome respiration rates were similar among populations (Table S1).

The physiological response to the experimental temperature range differed between populations (Fig. 1, right panels, Table S2). Respiration rates of leaves and rhizomes were significantly higher for the coldest-adapted subarctic population (Kobbe Fjord) while P_{\max} was similar among populations (Table S2). This resulted in generally lower leaf $P_{\max}:R$ ratio along the experimental temperature range for the coldest-adapted subarctic population (Fig. 1, Table S2), although all 3 populations maintained autotrophic leaf metabolism during day hours for the entire temperature range tested ($P_{\max}:R > 1$, Fig. 1).

P_{\max} and the leaf $P_{\max}:R$ ratio showed bell-shaped responses to temperature with similar optimum temperature (20°C) for subarctic and cold-temperate eelgrass populations, but with the highest amplitude of fluctuations in the coldest subarctic population (Kobbe Fjord, Fig. 1, Table S3). Eelgrass rhizome respiration increased with increasing temperature along the entire experimental temperature range in all

populations (Fig. S4), and leaf respiration responded similarly in the cold-temperate population (Århus Bay, Fig. 1), while that of the subarctic populations exhibited a bell-shaped temperature response peaking at 20°C (Kapisillit) and 25°C (Kobbe Fjord, Fig. 1).

At saturating light, the activation energy for P_{\max} ranged between 0.77 eV and 0.99 eV while warming up to optimum temperature, and did not vary consistently with the *in situ* summer temperature of the populations (Table S3). However, when warming exceeded the optimum temperature, P_{\max} of subarctic populations was more sensitive to temperature than that of the cold-temperate population, with de-activation energies being 2- to 4-fold higher (see Table S3). The activation energy of eelgrass rhizome respiration (0.53–0.67 eV) and leaf respiration (0.27–0.46 eV) was lower than that of P_{\max} , and kept increasing across all treatment temperatures (Table S3). The sensitivity of the $P_{\max}:R$ ratio to warming beyond 20°C was highest for the coldest subarctic population (Kobbe Fjord), which had highest activation energy (Table S3), and thus was most prone to shift to heterotrophy in extreme warming conditions, which, however, are unlikely at those latitudes.

Effect of light limitation on growth, photosynthesis and respiration

Light limitation significantly reduced average eelgrass growth rates, except leaf elongation rate, across the experimental temperature range (Fig. 2, Table S4). Shading also caused a significant reduction, of 5 to 10°C, in optimum growth temperature of light-limited compared to light-saturated eelgrass (Fig. 2, Table S3). The effect of low light was similar across the temperature gradient, showing no significant interaction between temperature and light treatments (Table S4). There was no consistent difference between the activation energies of growth rates under saturating and limiting light (see Table S3).

Limiting light also altered the physiological response of eelgrass to warming (Fig. 2, right panel). Hence, P_{\max} and the $P_{\max}:R$ ratio of eelgrass leaves grown under low light were significantly enhanced when incubated under saturating light at all temperatures except at 10°C (Fig. 2, Table S4). By contrast, limiting light did not significantly affect eelgrass leaf and rhizome respiration across the tested temperature range (Table S4). The amplitude of the fluctuations in P_{\max} and the $P_{\max}:R$ ratio in response to temperature was markedly wider in eelgrass grown

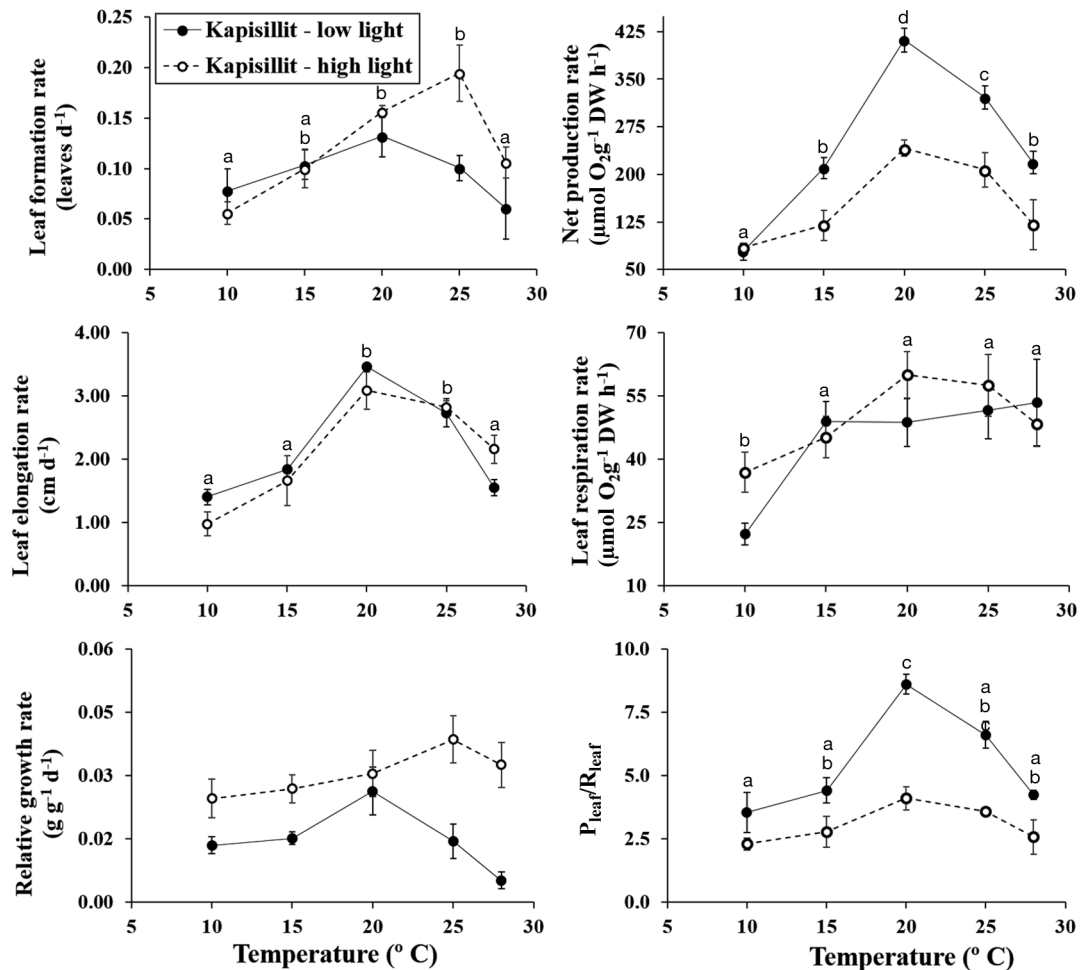


Fig. 2. Growth responses (left columns) and physiological responses (right columns) of *Zostera marina* from subarctic Greenland (Kapisillit) populations grown under low (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to different temperatures. Letters indicate significant differences between temperatures according to the 2-way ANOVA (see Table S4 in the Supplement)

under low than high light, whereas that of the respiration rates was similar between light treatments (Table S3). Eelgrass grown under low light exhibited a similar optimum temperature (20°C) for P_{max} and the $P_{\text{max}}:R$ ratio as those grown under high light (Table S3). The activation energies of P_{max} and the $P_{\text{max}}:R$ ratio below the optimum temperature were about 50% higher for plants grown under low than high light, while those of rhizome and leaf respiration were relatively similar (Table S3).

The parallel measurements of the photosynthetic response of whole plants from Kapisillit grown under low and high irradiance provided more detail on photosynthesis under low irradiance. The size of the plants did not vary significantly between light- and temperature treatments prior to the photosynthetic measurements, and the overall biomass averaged $0.230 \pm 0.014 \text{ g DW shoot}^{-1}$ and the leaf to rhizome +

root biomass ratio averaged 2.06 ± 0.13 . The photosynthetic efficiency (α) showed no significant difference between temperatures or between light- and shade-acclimated plants across the experimental temperature range in spite of the slightly higher α of shaded plants at intermediate temperatures relative to lower and higher temperatures (Fig. 3, Table S5). Respiration rates (R) were also similar for light- and shade-grown plants, and were unaffected by temperatures up to 25°C, whereas they increased markedly upon further increase in temperature. Combining α and R resulted in the minimum light requirement for photosynthesis (I_c) being slightly but significantly lower (average $31.1 \pm 3.2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 10–25°C) for shade-acclimated plants compared to light-acclimated plants (average $42.3 \pm 3.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 10–25°C). Above 25°C, the light compensation point increased for

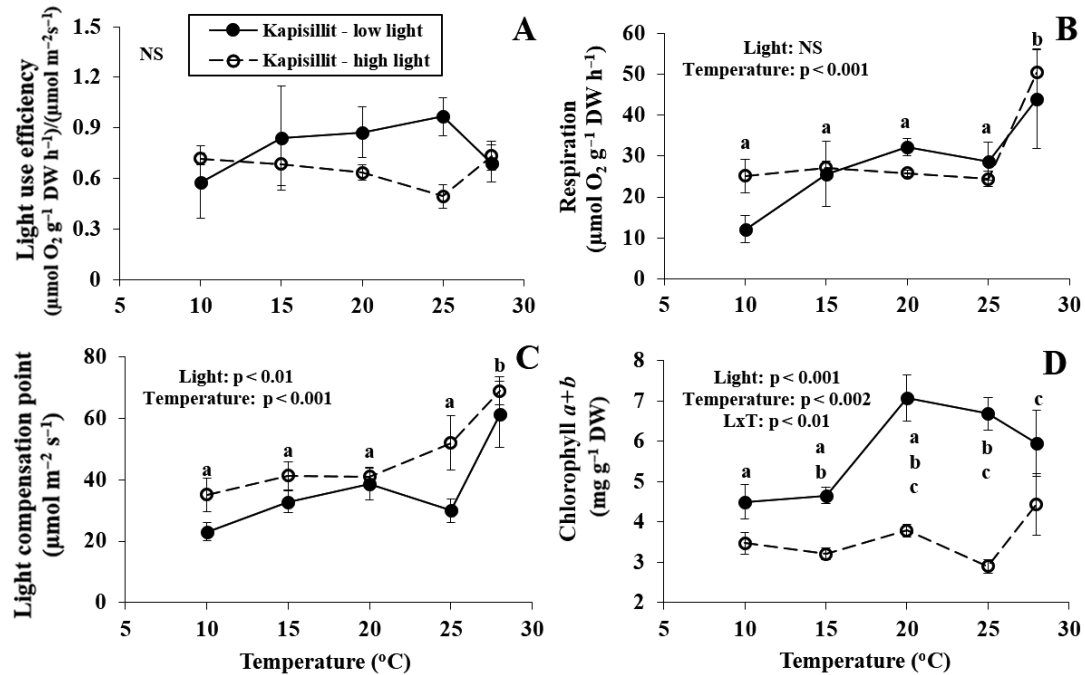


Fig. 3. Photosynthetic response of whole *Zostera marina* plants from subarctic Greenland (Kapisillit) grown under low and high light. (A) Photosynthetic efficiency, α , (B) respiration rate, R , (C) minimum light requirements for photosynthesis, I_c , and (D) chlorophyll content. Letters indicate significant differences between temperatures according to the 2-way ANOVA (see Table S5 in the Supplement). NS indicates no significance

both plant types (Fig. 3). Chlorophyll content was significantly affected by light and temperature conditions during growth with higher concentrations in shade-acclimated compared to light-acclimated plants. For shade-acclimated plants, the chlorophyll content was higher at 20 to 28°C than at 5 and 10°C, whereas the chlorophyll content in light-acclimated plants only varied slightly with temperature. This difference in temperature effect was reflected in a significant light–temperature interaction (Fig. 3, Table S5).

DISCUSSION

Temperature optimum and tolerance ranges across populations

The majority of the tested growth and physiological variables of the 3 eelgrass populations showed a characteristic bell-shaped temperature-response pattern (e.g. Dell et al. 2011) and were significantly affected by temperature in spite of the low number of replicates ($n = 3$). Remarkably, the Greenland populations growing near the northern distribution limit and the Danish population growing centrally in the distribution range exhibited relatively similar optimum temperatures ranging from 20 to 25°C for

growth and photosynthesis variables when grown under high irradiance. Had we included smaller temperature intervals than the 5°C intervals used in our experiment, it is likely that slight differences in optimum temperature among the 3 populations would have been found. The observed temperature optimum is within the range observed in most other similar experimental studies on eelgrass from various locations across the geographical distribution range (Table 1, Lee et al. 2007).

Maximum leaf respiration rates occurred, however, at a higher temperature for the Danish (>28°C) than the Greenland populations (20 to 25°C), matching earlier reports of maximum respiration rates occurring at 30.4 to 36.8°C for other Danish eelgrass populations (Staehr & Borum 2011). This suggests an important adaptation enabling the Danish population to better cope with the higher *in situ* temperatures. The physiological performance was measured on new leaf tissue formed at the respective experimental temperatures. However, whether a longer acclimation phase than used here (14–15 d) would increase the heat tolerance of the Greenland plants warrants further study.

Even though all the tested populations exhibited wide thermal breadths over the 14–15 d experimental period, there were signs of temperature stress at high temperatures, especially in the subarctic populations.

Table 1. Literature survey of temperature response of eelgrass *Zostera marina* to experimental temperatures. RGR: relative growth rate; P_{\max} : net rate of leaf photosynthesis; R_{leaf} : leaf respiration rate

Parameter	Latitude (°N)	Location	Length of the experiment (d)	Experimental T range (°C)	Optimum T (°C)	Source
RGR (g DW g ⁻¹ d ⁻¹)	34.6	Japan	60	0–30	20	Abe et al. (2008)
	44.4	Oregon, USA	14	4.5–23.6	18	Kaldy (2014)
	55.5	Denmark	21	18–27	21	Höffle et al. (2011)
	56	Aarhus	15–16	10–28	20	This study
	64	Kobbe Fjord	15–16	10–28	20	This study
	64	Kapisillit	15–16	10–28	25	This study
	64	Kapisillit Low	15–16	10–28	20	This study
P_{\max} ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	34.4	California, USA	0.5	4–21	21	Drew (1979)
	36.5	California, USA	21	5–30	25	Zimmerman et al. (1989)
	37.2	China	30	10–25	20	Niu et al. (2012)
	37.4	Chesapeake Bay, USA	7	8–30	19	Evans et al. (1986)
	40.5	Massachusetts, USA	4	10–28	25	Marsh et al. (1986))
	55	Izembek Lagoon, USA (subtidal)	2	0–40	30	Biebl & McRoy (1971)
	55	Izembek Lagoon, USA (intertidal)	2	0–40	35	Biebl & McRoy (1971)
	55.4	Denmark	44	10–20	10–20	Nejrup & Pedersen (2008)
	55.8	Denmark (Feb)	1–2	5–35	21.7	Staeher & Borum (2011)
	55.8	Denmark (Apr)	1–2	5–35	24	Staeher & Borum (2011)
	55.8	Denmark (Aug)	1–2	5–35	23.9	Staeher & Borum (2011)
	56	Aarhus	15–16	10–28	20	This study
	64	Kobbe Fjord	15–16	10–28	20	This study
	64	Kapisillit	15–16	10–28	20	This study
64	Kapisillit Low	15–16	10–28	20	This study	
Leaf respiration rate ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	36.5	California, USA	21	5–30	25	Zimmerman et al. (1989)
	37.2	China	30	10–25	25	Niu et al. (2012)
	40.5	Massachusetts, USA	4	0–35	15	Marsh et al. (1986))
	55.8	Denmark (Feb)	1–2	5–35	30.4	Staeher & Borum (2011)
	55.8	Denmark (Apr)	1–2	5–35	35	Staeher & Borum (2011)
	55.8	Denmark (Aug)	1–2	5–35	36.8	Staeher & Borum (2011)
	56	Aarhus	15–16	10–28	>28	This study
	64	Kobbe Fjord	15–16	10–28	25	This study
	64	Kapisillit	15–16	10–28	20	This study
	64	Kapisillit Low	15–16	10–28	15–25	This study
$P_{\max}:R_{\text{leaf}}$	36.5	California, USA	21	5–30	5–25	Zimmerman et al. (1989)
	37.2	China	30	10–25	20	Niu et al. (2012)
	40.5	Massachusetts, USA	4	0–35	5	Marsh et al. (1986)
	56	Aarhus	15–16	10–28	20	This study
	64	Kobbe Fjord	15–16	10–28	20	This study
	64	Kapisillit	15–16	10–28	20	This study
	64	Kapisillit Low	15–16	10–28	20	This study

These signs included the lower temperature optimum for leaf respiration reported above, as well as the low $P_{\max}:R$ ratio of the coldest-acclimated subarctic population (Kobbe Fjord) at temperatures above optimum. An additional sign was higher deactivation energies of subarctic populations, suggesting they were more sensitive to temperatures exceeding optimum levels, while the Danish population was better adapted to high temperatures. In line with this, previous comparisons of the temperature response along broad

thermal gradients in eelgrass identified the southern, most warm-exposed populations as being more tolerant to warm temperatures and better capable of restoring their photosynthetic apparatus after a heat shock than more northern populations (Bergmann et al. 2010, Franssen et al. 2011, 2014, Winters et al. 2011, Jueterbock et al. 2016). Overall, these results highlight the relevance of local thermal adaptation to coping with potential scenarios of global warming, with southern populations demonstrating a higher re-

silience to heat stress. As a consequence, extending the comparison of eelgrass temperature response to include warm-temperate populations in addition to subarctic and cold-temperate ones would likely show a clearer shift of temperature tolerance ranges in response to experienced local temperatures, in agreement with previous observations of variability in thermal breadth across the species distribution range in marine vegetation (Bennett et al. 2015). An increased temperature sensitivity was also observed for kelp growing along a latitude gradient in Australia with higher Q_{10} -values for gross photosynthesis (Q_{10} : 3.35 vs. 1.45) and respiration (Q_{10} : 3.82 vs. 1.65) of populations growing at colder vs. warmer *in situ* temperatures (Staehr & Wernberg 2009).

The negative effects of high temperatures on eelgrass performance probably become more distinct with increasing exposure time, which may explain some of the variability in optimum temperature observed among studies (Table 1, Lee et al. 2007). For example, Nejrup & Pedersen (2008), who applied a long experimental period (44 d), found hampered growth and markedly increased mortality at the highest test temperatures (25–30°C) and, as a consequence, relatively low optimum temperatures for growth and photosynthesis (10–20°C). Longer exposure to adverse temperature conditions may also reduce the allocation of energy reserves, such as fatty acids in leaves (P. Beca-Carretero et al. unpubl.) and carbohydrates, to the underground biomass (Fig. S1), causing a decrease in the internal carbon storage for winter survival. Consequently, the length of the period with suboptimal conditions is a critical factor for observed tolerance ranges and temperature optima.

Light limitation affects temperature response

Our study further showed that light limitation (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), simulating the conditions at the lower depth limit, lowered the optimum temperature for growth by about 5°C. More drastic declines in optimum temperature (by 10–25°C) upon irradiance reduction have been reported when seagrasses are exposed to sudden shifts in temperature (Bulthuis 1987, Masini et al. 1995), suggesting that eelgrass possesses a high potential for physiological acclimation upon long-term exposure to temperature and light conditions. Both examples emphasize, however, that plants in warmer environments ($T > 20^\circ\text{C}$) are more susceptible to shading than plants in cooler environments.

The growth of seagrasses living near the depth limit is dependent on low minimum light requirements and high photosynthetic efficiency (α) (Lee et al. 2007) to allow for a positive carbon balance. Hence, shade acclimation is often reflected in increased pigment content, increased α and a decrease in compensation irradiance, as also observed in this study (Olesen et al. 2002, Ralph et al. 2007, York et al. 2013). Warming did not affect α but increased respiration with warming resulted in markedly higher minimum light requirements above a growth temperature of 25°C. This suggests that eelgrass has the ability to acclimate its photosynthetic efficiency within the expected realized temperature range in the northern populations but little capacity to survive at low light conditions once this threshold temperature is exceeded due to high respiratory demands. A high ability for temperature acclimation of photosynthesis under low light has also been demonstrated for the kelp *Saccharina latissima*, for which specimens grown at 5 and 15°C achieved similar rates of light-limited photosynthesis and similar I_c and I_k (light intensity beyond saturation) values at their respective growth temperatures (Davison et al. 1991). Hence, within the temperature range of 10 to 25°C, the studied eelgrass populations maintained an average light compensation point for photosynthesis of 31.1 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for light-limited and 42.3 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for light-replete plants, but this increased to 61.4 and 68.9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively, at 28°C. These values are high compared to those obtained from measurements on leaves (Lee et al. 2007) but whole-plant light requirements are generally higher due to the respiratory demands of rhizomes and roots (Olesen & Sand-Jensen 1993, Staehr & Borum 2011).

Overall, the low variability we observed in the response of respiration and α in light- and shade-acclimated plants across the tested temperature range suggests a high phenotypic plasticity of eelgrass. This ability helps them survive variable light and temperature conditions across their latitudinal distribution range, across seasons (Olesen & Sand-Jensen 1993, Staehr & Borum 2011) and along depth gradients.

Reductions in underwater light due to anthropogenic and natural disturbances are the main causes of large-scale seagrass die-off (Short & Wyllie-Echeverria 1996, Waycott et al. 2009). Such negative effects of light limitation are likely to increase in a warmer future. Currently, eelgrass populations at their southern distribution limit die-back during warmer periods (Moore et al. 1996), and should be

particularly prone to experiencing additional stress from shading in a warmer future. Extreme heat waves were also found to be more detrimental for deep than shallow *Posidonia oceanica* populations (Marbà et al. 2010). At the northern distribution range of eelgrass, by contrast, warming in combination with extended open water (i.e. free of sea ice) periods, and hence more light on the seafloor, should stimulate growth and photosynthesis since, as demonstrated here, current *in situ* temperatures are far below optimum temperatures at these latitudes. Our findings thereby support recent predictions of northward expansion of eelgrass in a warmer future (Clausen et al. 2014, Olesen et al. 2015).

Acclimation and adaptation to temperature regimes

Knowledge of the potential of eelgrass for acclimation and genetic adaptation to varying temperature regimes is relevant for predicting future temperature tolerance ranges and temperature optima of the plant. Local adaptation would typically imply that thermal tolerance ranges vary in concert with local temperature ranges (Bennett et al. 2015). The broad temperature tolerance range in combination with the similarity in experimental temperature optimum among distant eelgrass populations suggest that the species' thermal breadth is largely conserved across the distribution range. However, the fact that all populations were able to grow at comparable rates at their respective *in situ* temperature, and the higher deactivation energy of photosynthesis, indicative of faster degradation processes beyond the thermal optima in the subarctic compared to the cold-temperate population, suggest some adaptation to *in situ* temperatures. Further studies are needed to identify if these differences have a genetic basis or result from phenotypic plasticity.

Overall, our results suggest a broad thermal tolerance of eelgrass along the geographical distribution range. Other broadly distributed aquatic plants, such as *Potamogeton pectinatus*, have also been characterized as thermally tolerant rather than locally adapted to prevailing temperature regimes (Pilon & Santamaría 2002). For cold-water macroalgae it has been found that temperature requirements for growth and survival relate to the climatic history of the regions and the origin of the species. Hence, cold-temperate species with a relatively short history in the polar regions show limited adaptation to the cold and, like eelgrass, have temperature

optima well above *in situ* temperatures, while the few endemic Arctic macroalgae that have been exposed to cold waters for 3 million years show a higher degree of adaptation and endemic Antarctic species, having been exposed to cold waters for at least 14 million years, show the highest level of adaptation (Wiencke et al. 1994, Wulff et al. 2009).

In conclusion, our findings suggest that warming may generate different effects on eelgrass populations from the northern versus the central part of the distribution range.

The projected increase of sea surface temperature in Greenland of 4 to 5°C by the end of this century (IPCC 2014, scenario A1B) will likely stimulate eelgrass performance with the potential for northward expansion, while projected increases of 2 to 3°C (IPCC 2014, scenario A1B) for Danish coastal waters are more critical as Danish eelgrass populations already live close to their optimal temperature. Moreover, our study pointed out that populations growing at limited light are more susceptible to further warming than those exposed to saturated irradiance. Overall, these findings have implications for the management of shallow coastal ecosystems, emphasizing the importance of ensuring good light conditions and overall good growth conditions to enhance the resilience of eelgrass meadows to elevated temperature (Lefcheck et al. 2017).

Acknowledgements. This work was supported by the European seagrass network (COST action ES0906) and the 'NOVAGRASS' project (12-132701) funded by the Danish Council for Strategic Research. It is also a contribution to the Arctic Science Partnership (www.asp-net.org).

LITERATURE CITED

- ✦ Abe M, Kurashima A, Maegawa M (2008) Temperature requirements for seed germination and seedling growth of *Zostera marina* from central Japan. *Fish Sci* 74: 589–593
- ✦ Bennett S, Wernberg T, Arackal Joy B, de Bettignies T, Campbell AH (2015) Central and rear-edge populations can be equally vulnerable to warming. *Nat Commun* 6: 10280
- ✦ Bergmann N, Winters G, Rauch G, Eizaguirre C and others (2010) Population-specificity of heat stress gene induction in northern and southern eelgrass *Zostera marina* populations under simulated global warming. *Mol Ecol* 19:2870–2883
- ✦ Biebl R, McRoy CP (1971) Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Mar Biol* 8:48–56
- ✦ Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology* 85: 1771–1789

- ✦ Bulthuis DA (1987) Effects of temperature on photosynthesis and growth of seagrasses. *Aquat Bot* 27:27–40
- ✦ Burrows MT, Schoeman DS, Buckley LB, Moore P and others (2011) The pace of shifting climate in marine and terrestrial ecosystems. *Science* 334:652–655
- ✦ Clausen KK, Krause-Jensen D, Olesen B, Marbà N (2014) Seasonality of eelgrass biomass across gradients in temperature and latitude. *Mar Ecol Prog Ser* 506:71–85
- ✦ Cole S, Moksnes PO (2016) Valuing multiple eelgrass ecosystem services in Sweden: fish production and uptake of carbon and nitrogen. *Front Mar Sci* 2:121
- ✦ Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar Biol* 110:449–454
- ✦ Dell AI, Pawar S, Savage VM (2011) Systematic variation in the temperature dependence of physiological and ecological traits. *Proc Natl Acad Sci USA* 108:10591–10596
- ✦ Den Hartog C (1979) Seagrasses and seagrass ecosystems, an appraisal of the research approach. *Aquat Bot* 7: 105–117
- ✦ Dennison WC (1987) Effects of light on seagrass photosynthesis, growth and depth limit. *Aquat Bot* 27:15–26
- ✦ Diekmann OE, Serrao EA (2012) Range-edge genetic diversity: locally poor extant southern patches maintain a regionally diverse hotspot in the seagrass *Zostera marina*. *Mol Ecol* 21:1647–1657
- ✦ Drew EA (1979) Physiological aspects of primary production in seagrasses. *Aquat Bot* 7:139–150
- ✦ Duarte CM, Kennedy H, Marbà N, Hendriks I (2013) Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. *Ocean Coast Manage* 83:32–38
- ✦ Durako MJ, Moffler MD (1987) Factors affecting the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae). *Aquat Bot* 27:79–95
- ✦ Evans AS, Kenneth LW, Penhale PA (1986) Photosynthetic temperature acclimation in two coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. *Aquat Bot* 24: 185–197
- ✦ Franssen SU, Gu J, Bergmann N, Winters G and others (2011) Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. *Proc Natl Acad Sci USA* 108:19276–19281
- ✦ Franssen SU, Gu J, Winters G, Huylmans AK and others (2014) Genome-wide transcriptomic responses of the seagrasses *Zostera marina* and *Nanozostera noltii* under a simulated heatwave confirm functional types. *Mar Genomics* 15:65–73
- ✦ Höffle H, Thomsen MS, Holmer M (2011) High mortality of *Zostera marina* under high temperature regimes but minor effects of the invasive macroalgae *Gracilaria vermiculophylla*. *Estuar Coast Shelf Sci* 92:35–46
- IPCC (Intergovernmental Panel on 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. Geneva
- ✦ Jueterbock A, Tyberghein L, Verbruggen H, Coyer JA, Olsen JL, Hoarau G (2013) Climate change impact on seaweed meadow distribution in the North Atlantic rocky intertidal. *Ecol Evol* 3:1356–1373
- ✦ Jueterbock A, Franssen SU, Bergmann N, Gu J and others (2016) Phylogeographic differentiation versus transcriptomic adaptation to warm temperatures in *Zostera marina*, a globally important seagrass. *Mol Ecol* 25: 5396–5411
- ✦ Kaldy JE (2014) Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *J Exp Mar Biol Ecol* 453:108–115
- ✦ Krause-Jensen D, Duarte CM (2014) Expansion of vegetated coastal ecosystems in the future Arctic. *Front Mar Sci* 1:77
- ✦ Lee K, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J Exp Mar Biol Ecol* 350:144–175
- ✦ Lefcheck JS, Wilcox DJ, Murphy RR, Marion SR, Orth RJ (2017) Multiple stressors threaten the imperiled coastal foundation species eelgrass (*Zostera marina*) in Chesapeake Bay, USA. *Glob Change Biol* 23:3474–3483
- ✦ Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol* 148:350–382
- ✦ López-Urrutia A, San Martín E, Harris RP, Irigoien X (2006) Scaling the metabolic balance of the oceans. *Proc Natl Acad Sci USA* 103:8739–8744
- ✦ Marbà N, Duarte CM (2010) Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Glob Change Biol* 16:2366–2375
- ✦ Marsh JA Jr, Dennison WC, Alberte S (1986) Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *J Exp Mar Biol Ecol* 101:257–267
- ✦ Masini RJ, Cary JL, Simpson CJ, McComb AJ (1995) Effects of light and temperature on the photosynthesis of temperate meadow-forming seagrasses in Western Australia. *Aquat Bot* 49:239–254
- ✦ McRoy CP (1969) Eelgrass under Arctic winter ice. *Nature* 224:818–819
- ✦ Moore KA, Neckles HA, Orth RJ (1996) *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. *Mar Ecol Prog Ser* 142:247–259
- ✦ Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638
- ✦ Nejrup LB, Pedersen MF (2008) Effects of salinity and water temperature on the ecological performance of *Zostera marina*. *Aquat Bot* 88:239–246
- ✦ Newton A, Mudge SM (2003) Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. *Estuar Coast Shelf Sci* 57:73–85
- ✦ Niu S, Zhang P, Liu J, Guo D, Zhang X (2012) The effect of temperature on the survival, growth, photosynthesis, and respiration of young seedlings of eelgrass *Zostera marina* L. *Aquaculture* 350–353:98–108
- ✦ Olesen B, Sand-Jensen K (1993) Seasonal acclimatization of eelgrass *Zostera marina* growth to light. *Mar Ecol Prog Ser* 94:91–99
- ✦ Olesen B, Enríquez S, Duarte CM, Sand-Jensen K (2002) Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. *Mar Ecol Prog Ser* 236:89–97
- ✦ Olesen B, Krause-Jensen D, Marbà N, Christensen PB (2015) Eelgrass *Zostera marina* in subarctic Greenland: dense meadows with slow biomass turnover in cold waters. *Mar Ecol Prog Ser* 518:107–121
- ✦ Pedersen O, Colmer TD, Sand-Jensen K (2013) Underwater photosynthesis of submerged plants—recent advances and methods. *Front Plant Sci* 4:140
- ✦ Phillips RC, Grant WS, Mc Roy CP (1983) Reproductive strategies of eelgrass (*Zostera marina* L.). *Aquat Bot* 16: 1–20

- ✦ Pilon J, Santamaría L (2002) Clonal variation in the thermal response of the submerged aquatic macrophyte *Potamogeton pectinatus*. *J Ecol* 90:141–152
- ✦ Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W and others (2013) Global imprint of climate change on marine life. *Nat Clim Chang* 3:919–925
- Pulido C, Borum J (2010) Eelgrass (*Zostera marina*) tolerance to anoxia. *J Exp Mar Biol Ecol* 385:8–13
- ✦ Ralph PJ, Durako MJ, Enríquez S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. *J Exp Mar Biol Ecol* 350:176–193
- ✦ Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc Natl Acad Sci USA* 102:2826–2831
- Salo T, Pedersen MF, Boström C (2014) Population specific salinity tolerance in eelgrass (*Zostera marina*). *J Exp Mar Biol Ecol* 461:425–429
- ✦ Sand-Jensen K (1975) Biomass, net production, and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. *Ophelia* 14:185–201
- ✦ Serrano O, Mateo MA, Dueñas-Bohórquez A, Renom P, López-Sáez JA, Martínez Cortizas A (2011) The *Posidonia oceanica* marine sedimentary record: a Holocene archive of heavy metal pollution. *Sci Total Environ* 409:4831–4840
- ✦ Setchell WA (1935) Geographic elements of the marine flora of the North Pacific Ocean. *Am Nat* 69:560–577
- Short FT, Duarte CM (2001) Methods for the measurement of seagrass growth and production. In: Short FT, Coles RG (eds) *Global seagrass research methods*. Elsevier, Amsterdam, p 155–182
- ✦ Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. *Aquat Bot* 63:169–196
- ✦ Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. *Environ Conserv* 23: 17–27
- ✦ Staehr PA, Borum J (2011) Seasonal acclimation in metabolism reduces light requirements of eelgrass (*Zostera marina*). *J Exp Mar Biol Ecol* 407:139–146
- ✦ Staehr PA, Wernberg T (2009) Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature. *J Phycol* 45:91–99
- ✦ Walther GR, Post E, Convey P, Menzel A and others (2002) Ecological responses to recent climate change. *Nature* 416:389–395
- ✦ Wang M, Overland JE (2009) A sea ice-free summer Arctic within 30 years? *Geophys Res Lett* 36:L07502
- ✦ Waycott M, Duarte CM, Carruthers TJB, Orth RJ and others (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc Natl Acad Sci USA* 106:12377–12381
- ✦ Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37:247–260
- ✦ Winters G, Nelle P, Fricke B, Rauch G, Reusch TBH (2011) Effects of a simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. *Mar Ecol Prog Ser* 435:83–95
- ✦ Wulff A, Iken K, Quartino ML, Al-Handal A, Wiencke C, Clayton MN (2009) Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. *Bot Mar* 52:491–507
- ✦ York PH, Gruber RK, Hill R, Ralph PJ, Booth DJ, Macreadie PI (2013) Physiological and morphological responses of the temperate seagrass *Zostera muelleri* to multiple stressors: Investigating the interactive effects of light and temperature. *PLOS ONE* 8:e76377
- ✦ Zimmerman RC, Smith RD, Alberte RS (1989) Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). *J Exp Mar Biol Ecol* 130:93–109

Editorial responsibility: Antony Underwood,
Sydney, New South Wales, Australia

Submitted: May 19, 2017; Accepted: November 21, 2017
Proofs received from author(s): February 2, 2018