



Effects of increasing temperature on the photosynthetic activity and oxygen balance of sheath-covered seagrass *Zostera marina* seeds

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ABSTRACT: The seed sheaths of eelgrass *Zostera marina* L. have recently been shown to possess photosynthetic capacity that can alleviate intra-seed hypoxic conditions and thereby enhance biosynthetic activity. However, nothing is known about how increasing seawater temperatures affect physiological responses in developing *Z. marina* seeds. We used an optical multi-analyte sensor system in combination with O₂-sensitive sensor spots to measure rates of photosynthesis and dark respiration within custom-made gas exchange chambers. Exponential saturation models were then applied to determine key photosynthetic parameters, such as maximum photosynthesis rate, light use efficiency, saturating photon irradiance (E_K), compensation photon irradiance, and net diel O₂ budget. Our results showed that both photosynthetic activity and dark respiration rates in sheath-covered seeds increased with increasing seawater temperature (from 10 to 25°C) but with a 2-fold stronger response in dark respiration than in gross photosynthesis over the measured temperature range. These temperature responses resulted in increasing light requirements (from 47 to 183 μmol photons m⁻² s⁻¹ in E_K) and decreasing net diel O₂ budgets (from -5.4 to -126 nmol O₂ mg wet weight [WW]⁻¹ h⁻¹) of the eelgrass seeds with increasing temperature. Eelgrass seed exposure to high temperature led thus to highly reduced net diel O₂ balances, which is expected to have detrimental effects on seed development and germination success owing to negative effects on synthesis rates of storage products in the endosperm.

KEY WORDS: Global warming · Marine heat waves · Oxygen budget · Photosynthesis · Respiration · Seagrass · Seed development

1. INTRODUCTION

Seagrass meadows are important coastal ecosystems that provide several key ecosystem services in coastal waters, such as enhancing marine biodiversity by functioning as nursery habitats (Bertelli & Unsworth 2014, Olesen et al. 2024), reducing eutrophication via nutrient uptake (e.g. N and P; McRoy et al. 1972, Pernice et al. 2016), and mitigating climate change through efficient carbon sequestration rates (Duarte et al. 2005, Fourqurean et al. 2012, Rohr et al. 2016, Leiva-Dueñas et al. 2023). Seagrasses mainly grow in shallow water as they have relatively high

light requirements (~20% of incident irradiance at the sea surface for *Zostera marina* in Danish waters; Duarte 1991) and can reproduce both asexually (via vegetative propagation) and sexually by producing seeds (Harwell & Orth 2002, Marion & Orth 2012, Hosokawa et al. 2015). In recent years, seagrass habitat restoration has attracted increasing attention, given that seagrasses are in decline worldwide (Orth et al. 2006, Waycott et al. 2009) and have considerable potential for contributing to reversing climate change and the biodiversity crisis (Easterling et al. 2000, Cullen-Unsworth & Unsworth 2018). Here, seed-based restoration seems to be a promising technique

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for sowing new seagrass meadows with high genetic diversity and rapid recovery of vital ecosystem services (Orth et al. 2020). However, limited knowledge exists on how changing environmental conditions affect the physiological response of developing seagrass seeds.

Photosynthetic activity in eelgrass *Z. marina* L. seed sheaths during light exposure has recently been shown to increase the O₂ availability in the central parts of the seed containing the developing plant embryo, which enables enhanced respiratory energy generation for biosynthetic activity (Brodersen & Kühl 2023). In the sheath-covered *Z. marina* seeds, the photosynthetic activity only occurs in the sheath itself, which generates steep internal gradients of photosynthesis and O₂ concentration from high O₂ availability in the photosynthetically active seed sheath to low O₂ availability in the endosperm and embryo region (Brodersen & Kühl 2023). Such O₂ production in sheath-covered eelgrass seeds is thus important for alleviating hypoxic stress conditions inside the seed and may likely increase endosperm storage activity, thereby improving the physiological conditions for successful seed development and maturation (Brodersen & Kühl 2023). Indeed, compared to terrestrial seeds, seed sheath photosynthesis may be even more important for the development of eelgrass seeds, as gas diffusion is much slower in water than in air (~10 000 times; Madsen & Sand-Jensen 1991), which may limit the passive O₂ supply from the surrounding water to the inner parts of the developing seed. Very low intra-seed O₂ conditions, and of course anoxic conditions, may limit respiration rates and thus the endosperm storage activity (Rolletschek et al. 2005, Borisjuk & Rolletschek 2009). Endosperm storage products, like proteins and carbohydrates, are essential during seed germination, and low storage activity or reserves can therefore have negative impacts on seed development and, subsequently, seedling establishment (e.g. Celdran & Marin 2013). Oxygen produced by eelgrass seed sheaths, increasing the internal O₂ availability and likely the supply of respiratory energy, is thus expected to positively affect successful sexual reproduction in eelgrass (Brodersen & Kühl 2023).

Increasing seawater temperatures, as a result of global warming and/or marine heat waves, represent an increasing challenge for seagrasses owing to e.g. negative effects of high temperatures on internal plant aeration (Pedersen et al. 2016, Hansen et al. 2022). Reduced internal aeration at high temperatures is due to adverse effects on photosynthesis owing to protein denaturation and enzyme (e.g. RuBisCO)

capacity limitations (for *Z. marina*, the temperature optimum for photosynthesis ranges between 20° and 25°C; Nejrup & Pedersen 2008, Staehr & Borum 2011, Beca-Carretero et al. 2018), while respiration continues to increase with increasing temperature (Pedersen et al. 2016), which can cause insufficient internal aeration (Raun & Borum 2013, Hansen et al. 2022). Adequate internal aeration is essential for seagrasses, as they grow in reduced, anoxic sediment and therefore need to transport O₂ down to below-ground tissues to maintain aerobic metabolism and provide protection against H₂S intrusion (Colmer 2003, Brodersen et al. 2015, Koren et al. 2015, Brodersen et al. 2018). In developing seagrass seeds, increasing temperatures could thus promote hypoxic and/or anoxic stress conditions, limiting endosperm storage products owing to reduced photosynthetic activity in combination with increased respiratory needs. However, nothing is known about the potential negative or beneficial effects of increasing seawater temperatures on the photosynthesis and respiration rates of sheath-covered seagrass seeds.

In this study, we used gas exchange measurements to determine the effects of increasing seawater temperatures on photosynthesis, dark respiration (*R*), and diel O₂ budgets (DOBs) of sheath-covered *Z. marina* seeds. We hypothesized that environmentally relevant increases in temperature would lead to higher rates of photosynthesis and *R* in the immature eelgrass seeds. We further expected a stronger response in *R* than in photosynthesis with increasing temperature, which should result in enhanced light requirements and negative DOBs at higher temperatures.

2. MATERIALS AND METHODS

2.1. Sampling and storage of seagrass seeds

Zostera marina L. flowering shoots with seeds were sampled from shallow, brackish coastal waters (<2 m depth; salinity of ~20) near Helsingør, Denmark (56° 03' 29.2" N, 12° 34' 40.7" E) in July 2023. Here, the seawater temperature generally ranges between 12° and 22°C during the summer months (June–August; <https://www.da.seatemperature.net>). After sampling, immature sheath-covered seeds were collected from the spathes of the flowering *Z. marina* shoots and stored in small, illuminated seawater reservoirs (photosynthetically active radiation [PAR] photon irradiance [400–700 nm] of 50 μmol photons m⁻² s⁻¹; 14 h light: 10 h dark cycle, temperature of 15°C, salinity of 20) containing aerated and filter-sterilized seawater (0.2 μm),

thus mimicking the physicochemical conditions within the eelgrass meadow at the sampling site.

2.2. Gas exchange measurements

O₂ production and consumption rates of immature *Z. marina* seeds with sheaths (n = 3) were measured in custom-made gas exchange chambers (1.8 ml glass vials) containing deoxygenized and pre-sterilized seawater (0.2 μm, salinity of 20, air saturation of ~50%), employing calibrated O₂-sensitive sensor spots (OXSP5, PyroScience) connected via optic-fibers to an optical multi-analyte sensor system (FireSting PRO, PyroScience) that was interfaced to a PC running dedicated software for data acquisition (PyroWorkbench, PyroScience) (Hansen et al. 2022, Brodersen & Kühl 2023, Molin et al. 2023). Deoxygenated seawater was used to ensure that the O₂ concentrations within the closed measuring chambers did not vastly exceed 100% air saturation during light–dark measurements to minimize undesired photorespiration. The O₂-sensitive sensor spots were 2-point calibrated in anoxic (0% air saturation; obtained by using the O₂ scavenger sodium sulfite) and air-saturated (100% air saturation) seawater at experimental temperature and salinity. Water circulation within the measuring chambers was maintained with small magnets (one in each chamber) that were controlled by a magnet stirrer (VMS-C7 Advanced; VWR International). For gas exchange measurements, closed measuring chambers containing sheath-covered *Z. marina* seeds (protected from the stirring magnets via a small plankton mesh; 0.2 mm mask width) were submerged into a glass aquarium containing deionized water, wherein the temperature could be manipulated and controlled via a heating and cooling system (Titan 1500; Aqua Medic). A total of 4 measuring chambers with sensor spots were used; one control chamber to ensure that there was no undesired O₂ consumption or production in the filter-sterilized seawater and 3 chambers with one sheath-covered seagrass seed within each measuring chamber. Experimental temperatures for photosynthesis and respiration measurements were 10°, 15°, 20°, and 25°C, respectively. The average seawater temperature at the sampling site during seed development is 10°C in May, 15°C in June, 18°C in July, 18°C in August, and 17°C in September, with maximum seawater temperatures of 22°C during July and August (<https://www.da.seatemperature.net>). Hence, the 25°C treatment represents a future ocean scenario; for example,

during a marine heatwave. Photon irradiances of 0, 25, 50, 100, 300, and 600 μmol photons m⁻² s⁻¹ (PAR) were provided by a fiber-optic tungsten halogen lamp equipped with a trifurcated fiber bundle; each optical fiber equipped with a collimating lens (KL-2500 LED, Schott). Lamp settings for defined photon irradiance levels were determined using a calibrated irradiance meter (ULM-500, Walz) with a photon scalar irradiance sensor (3 mm diameter). The highest measured photon irradiance represents average mid-day light intensities at the depth of the seagrass meadow within the region (Staehr et al. 2018). Prior to dark measurements, the sheath-covered eelgrass seeds were acclimated to total darkness for more than 10 min to ensure that the first measurements represented *R* (Hansen et al. 2022, Molin et al. 2023). Respiration, net photosynthesis (NP), and gross photosynthesis (GP) rates (in nmol O₂ mg wet weight [WW]⁻¹ h⁻¹) were calculated from the measured O₂ fluxes (i.e. consumption or production rates) as $GP(E) = NP(E) + |R|$, with blanks serving as controls (i.e. measuring chambers only containing pre-sterilized seawater). Briefly, the rates of NP and *R* were calculated from the steady-state linear O₂ concentration slopes as a function of time at the given incident irradiances (*E*), by multiplying the O₂ concentration slope (nmol l⁻¹ h⁻¹) by the volume of seawater in the measuring chamber (*l*) divided by the WW of the respective samples. Rates of GP were then calculated by adding the NP rates to the absolute value of *R* (Hansen et al. 2022, Brodersen & Kühl 2023, Molin et al. 2023). All oxygen production and consumption data were normalized to mg WW⁻¹, where the WW of the seeds was determined after the gas exchange measurements on a high-precision analytical balance after the seeds were gently blotted of water.

2.3. Data calculations and analysis

Parameter estimates of maximum rates of GP and NP (GP_{max} and NP_{max}, respectively) and *R* were obtained from plots of photosynthetic rates versus *E* using non-linear regression in the software program OriginPro v.2017 (OriginLab Corporation). Parameter estimates of GP_{max}, NP_{max}, and *R* are presented with 95% *t*-based confidence intervals (i.e. 2.5 and 97.5 percentiles from the *t*-distribution with n – 2 or n – 3 df). R² is not a valid measure for goodness of fit in non-linear regression (Kvålseth 1985), so we used reduced (Red) Chi² as a measure for goodness of fit for each regression, calculated as:

$$\text{Chi}_{\text{Red}}^2 = \frac{\sum_i \frac{(O_i - C_i)^2}{\sigma^2}}{\nu} = \frac{\text{Chi}^2}{\nu} \quad (1)$$

where O_i is the observed data, C_i is the calculated data, σ^2 is the variance, and ν is the df (the number of points in the regression minus the number of parameter estimates). Chi^2 values of ≤ 1 indicate a good fit between observed and calculated data.

For the GP rates as a function of E , an exponential saturation model (Webb et al. 1974) was fitted:

$$\text{GP}(E) = \text{GP}_{\text{max}} \left[1 - \exp \left(-\alpha \frac{E}{\text{GP}_{\text{max}}} \right) \right] \quad (2)$$

For fitting NP rates as a function of E , a similar exponential saturation model with an additional term to account for respiration (Spilling et al. 2010) was used:

$$\text{NP}(E) = \text{NP}_{\text{max}} \left[1 - \exp \left(-\alpha \frac{E}{\text{NP}_{\text{max}}} \right) \right] + R(E) \quad (3)$$

where GP_{max} and NP_{max} are the calculated maximum GP and NP rates at saturating photon irradiance, respectively, α is the light use efficiency, and R is the dark respiration rate.

The saturating photon irradiance (E_K); that is, the photon irradiance at the onset of photosynthesis saturation, was determined as:

$$E_K = \text{NP}_{\text{max}} / \alpha \quad (4)$$

The compensation photon irradiance (E_C); that is, the level of E at which the photosynthetic O_2 production just meets the respiratory needs (i.e. $\text{NP} = 0$), was calculated from the photosynthetic parameters achieved from the fitted exponential saturation models as:

$$E_C = \text{NP}_{\text{max}} \log_{10} \left(\frac{1 + \frac{R}{\text{NP}_{\text{max}}}}{-\alpha} \right) \quad (5)$$

Calculations of Q_{10} values for GP_{max} and R (that is, to evaluate the photosynthetic parameters' sensitivity to temperature increases), were determined as:

$$Q_{10} = (\text{Parameter}_{T_2} / \text{Parameter}_{T_1})^{[10/(T_2 - T_1)]} \quad (6)$$

where Parameter_{T_2} and Parameter_{T_1} are the photosynthetic parameters GP_{max} or R , calculated at the given temperatures T_2 and T_1 , respectively; here, calculated for the temperature intervals of 10–20° and 15–25°C.

Finally, we estimated the total DOB of the sheath-covered eelgrass seeds at the investigated temperatures from the fitted exponential saturation models for NP and R , using *in situ* data on diel E originating from Staehr et al. (2018):

$$\text{DOB} = \left[\sum_{t=1}^{t=7} \text{NP}(E) \right] - (R \times 10) \quad (7)$$

where $\text{NP}(E)$ is the net O_2 production during light exposure (here, 14 h of light exposure divided into seven 2 h light intervals; i.e. $t_1 - t_7$, with differing averaged photon irradiances for each time interval following the *in situ* measured bell-shaped light curve), R is the dark respiration (during 10 h of darkness), and t represent the respective time intervals (further details provided in Hansen et al. 2022, Molin et al. 2023).

3. RESULTS

3.1. Temperature effects on measured rates of photosynthesis

GP rates of sheath-covered eelgrass seeds increased both with increasing photon irradiance until saturation and with increasing temperature (Fig. 1). The highest GP rate (mean \pm SE: 5.98 ± 1.08 nmol O_2 mg $\text{WW}^{-1} \text{h}^{-1}$) was obtained at a photon irradiance of $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and temperature of 25°C, while the highest GP rates at 10°, 15°, and 20°C were 1.29 ± 1.12 , 1.85 ± 0.24 , and 3.06 ± 0.37 nmol O_2 mg $\text{WW}^{-1} \text{h}^{-1}$, respectively ($n = 3$, Fig. 1).

3.2. Photosynthetic parameters analyzed via exponential saturation models

GP_{max} of the eelgrass seeds increased 5.9-fold when the temperature was raised from 10° to 25°C (Fig. 2), corresponding to Q_{10} values of 2.99 and 3.22 in the temperature range of 10–20° and 15–25°C, respectively (Table 1). In comparison, R increased 10.7-fold when the temperature was raised from 10° to 25°C (Fig. 2), which corresponded to Q_{10} values of 6.57 and 3.11 in the temperature range of 10–20° and 15–25°C, respectively (Table 1). Hence, there was an ~2-fold higher increase in R compared to photosynthesis in the eelgrass seeds that mostly occurred in the lower temperature range (Fig. 2, Table 1).

Eelgrass seeds exhibited the highest light use efficiency of 0.04 ± 0.01 nmol O_2 mg $\text{WW}^{-1} \text{h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) $^{-1}$ at a temperature of 15°C ($n = 3$, Fig. 3), corresponding to the acclimation temperature during cultivation. Likewise, the lowest E_K of the seeds ($39 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was observed at 15°C (Fig. 3). Light use efficiency did not seem to be affected by the increasing temperature (Fig. 3), which was in strong contrast to E_K , which increased ~4-fold with increasing temperature from 47 μmol

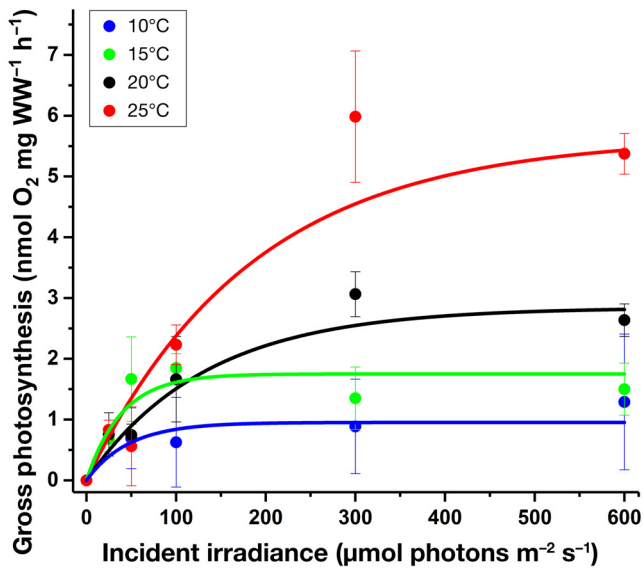


Fig. 1. Gross photosynthesis rates of sheath-covered seagrass *Zostera marina* seeds as a function of photon scalar irradiance under increasing seawater temperatures. The rates of gross photosynthesis were measured for photon irradiances (photosynthetically active radiation, 400–700 nm) of 0, 25, 50, 100, 300, and 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and temperatures of 10°, 15°, 20°, and 25°C. Symbols with error bars show mean values \pm SE ($n = 3$ biological replicates). All exponential saturation models (represented by the colored lines) had reduced Chi^2 values of <1 (Table A1, see Appendix; $n = 5\text{--}6$)

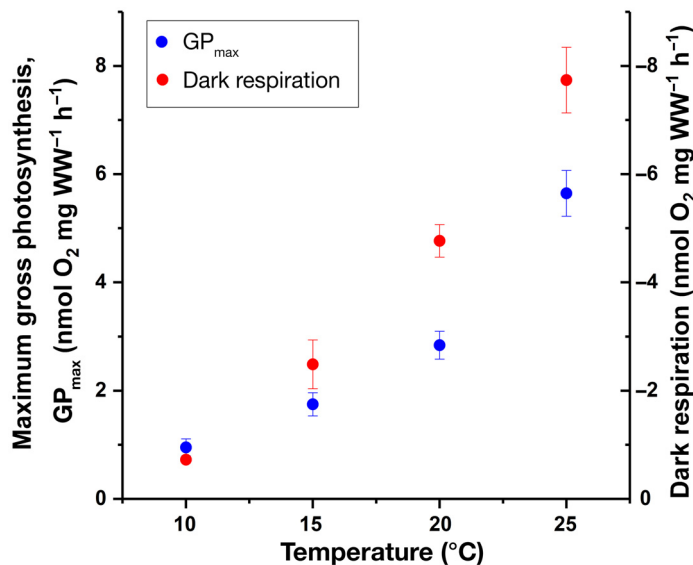


Fig. 2. Rates of maximum gross photosynthesis (GP_{max}) and dark respiration of sheath-covered seagrass *Zostera marina* seeds as a function of increasing seawater temperatures (10°, 15°, 20°, and 25°C, respectively). All rates are parameter estimates (mean values \pm SE) extracted from the exponential saturation models shown in Eqs. (2) and (3) (reduced Chi^2 values of <1 ; $n = 5\text{--}6$). The 95% confidence intervals of the photosynthetic parameters are provided in Fig. A2 (see Appendix; $n = 5\text{--}6$), as further statistical analysis of the derived parameters

Table 1. Q_{10} values for maximum gross photosynthesis (GP_{max}) and dark respiration (R) rates of sheath-covered seagrass *Zostera marina* L. seeds calculated for temperature ranges of 10–20°C and 15–25°C. Values for GP_{max} and R are parameter estimates from the fitted exponential saturation models (Eqs. 2 and 3; all with reduced Chi^2 values of <1 , $n = 3$ biological replicates) at the respective temperatures

Temperature range (°C)	GP_{max}	R
10–20 (normal) ^a	2.99	6.57
15–25 (extreme)	3.22	3.11

^aAverage summer seawater temperature at the sampling site (Helsingør, Denmark) is $\sim 17^\circ\text{C}$, with an average temperature of $\sim 15^\circ\text{C}$ in June during seed development (<https://www.da.seatemperature.net>)

photons $\text{m}^{-2} \text{s}^{-1}$ at a seawater temperature of 10°C to a maximum E_K of 183 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 25°C (Fig. 3). The observed E_C of the sheath-covered eelgrass seeds was 29 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 10°C, while it was impossible to calculate E_C values at 15°, 20°, and 25°C because of the negative rates of NP even in the carboxylation-limited phase of the photosynthesis–irradiance curve (Fig. A1 in the Appendix).

3.3. Effect of temperature on the oxygen budget of sheath-covered seagrass seeds

The DOB of the seeds showed an increasing O_2 deficit (i.e. negative oxygen budget) with increasing temperature (Fig. 4). Hence, DOB decreased >23 -fold from $-5.4 \text{ nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$ at 10°C to $-126 \text{ nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$ at 25°C. Separating the DOB into O_2 balances in light and darkness, respectively (Fig. 4), elucidated that seeds only exhibited an O_2 surplus ($1.8 \text{ nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$) in light at 10°C while showing an O_2 deficit at all higher temperatures during light exposure. The O_2 production by seeds in light at 10°C accounted for $\sim 25\%$ of the O_2 consumption during darkness (Fig. 4). The O_2 balance of seeds in darkness (Fig. 4) exhibited a >10 -fold increase in O_2 consumption with increasing temperature, from $-7.3 \text{ nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$ at 10°C to $-77.4 \text{ nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$ at 25°C. The positive differences in O_2 balance from light to dark conditions were 14.1, 13.1, and 28.8 $\text{nmol O}_2 \text{ mg WW}^{-1} \text{ h}^{-1}$ at 15°, 10°, and 25°C, respectively (Fig. 4), thus demonstrating the importance of eelgrass seed sheaths photosynthetic capacity for the O_2 balance of the growing immature seeds.

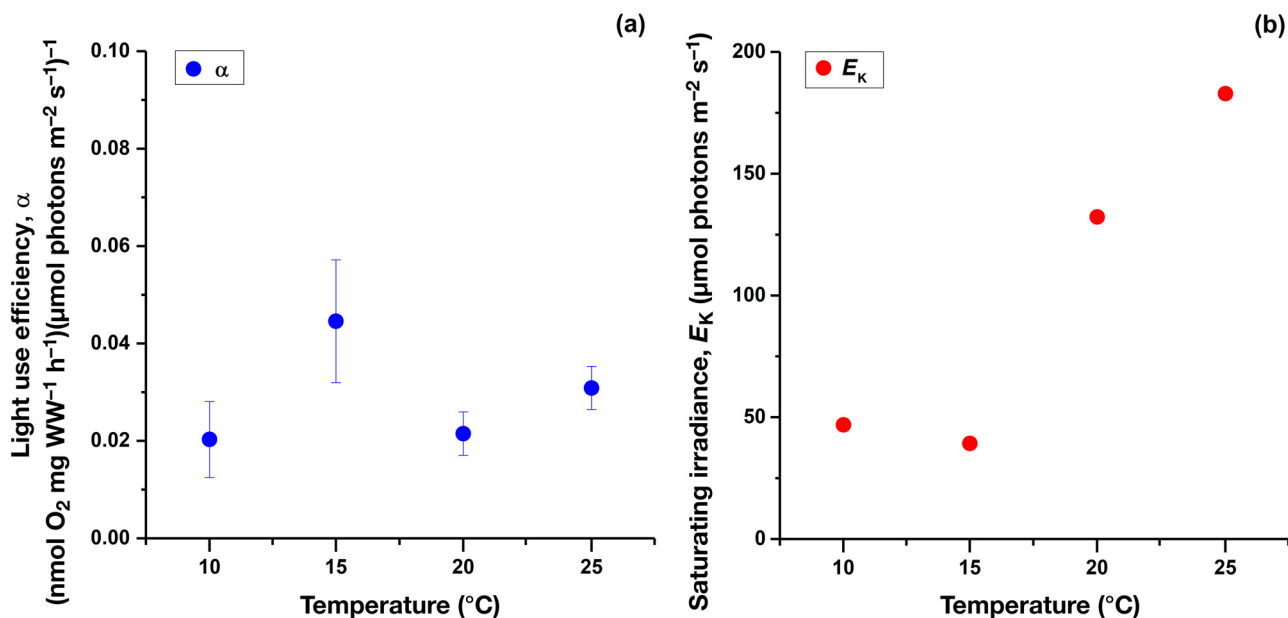


Fig. 3. Light use efficiency (α) and saturating photon irradiance (E_K ; i.e. photon irradiance at the onset of photosynthesis saturation) of sheath-covered seagrass *Zostera marina* seeds as a function of increasing seawater temperatures (i.e. 10°, 15°, 20°, and 25°C). Values for α are parameter estimates (mean values \pm SE) extracted from the exponential saturation models shown in Eq. (2) (with reduced Chi² values of <1; n = 5–6), while values for E_K were calculated as NP_{\max}/α (n = 3 biological replicates). The 95% confidence intervals of the photosynthetic parameters are provided in Fig. A2 (n = 5–6), as further statistical analysis of the derived photosynthetic parameters

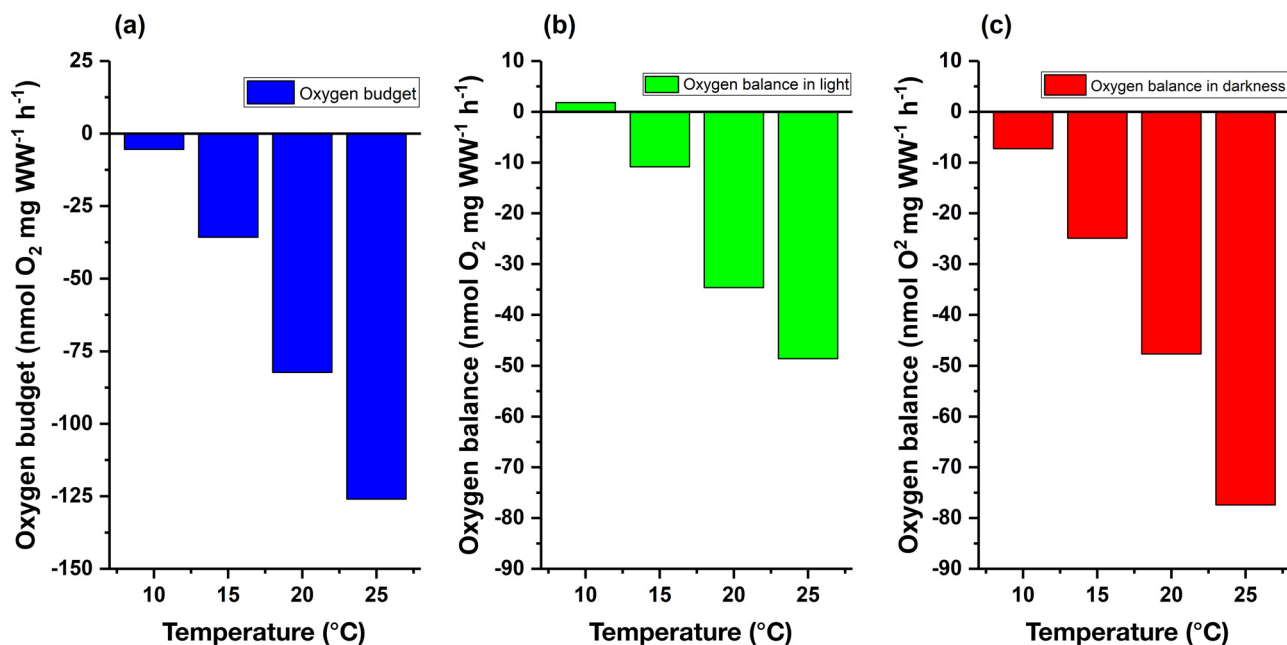


Fig. 4. Diel oxygen budgets and balances in light and darkness of sheath-covered seagrass *Zostera marina* seeds as a function of increasing temperatures (10°, 15°, 20°, and 25°C, respectively). The daily net O₂ production and consumption was calculated from fitted exponential saturation models at the 4 experimental temperatures (Eq. 3; all with reduced Chi² values of <1, n = 5–6) using a 14 h light:10 h dark cycle measured *in situ* during summer (Staehr et al. 2018). The black line at y = 0 demonstrates a neutral O₂ balance. Note the slightly different scaling on the y-axis between the O₂ budget and the O₂ balances

4. DISCUSSION

Our results provide the first experimental evidence that rates of photosynthesis and R in sheath-covered eelgrass seeds increase with increasing temperature. However, the temperature response in R was stronger than for photosynthetic rates, which led to a shift from a net O_2 surplus to a deficit at temperatures above 10°C in the light. In general, the O_2 demand and O_2 deficit of the seeds increased with increasing temperature. Furthermore, increasing temperatures above 15°C resulted in markedly increased values of E_K in the sheath-covered eelgrass seeds.

4.1. Temperature effects on photosynthesis and respiration in sheath-covered seagrass seeds

The GP_{\max} of *Zostera marina* seed sheaths increased about 6-fold within the investigated temperature range of $10\text{--}25^\circ\text{C}$. The observed increase in GP of the seeds with increasing temperature is likely caused by an optimization of the biochemical processes underlying the seeds' photosynthetic efficiency (e.g. Way 2019, Nguyen et al. 2021), which includes temperature-enhanced enzyme activity and increased membrane fluidity that enhances the mobility of photosynthetic proteins embedded in the thylakoid membrane (Nguyen et al. 2021). Such an increase in seed photosynthesis with increasing temperature is similar to what has been observed in adult seagrass plants until reaching the optimum temperature for photosynthesis (Staeher & Borum 2011, Pedersen et al. 2016, Hansen et al. 2022), above which rates of photosynthesis decrease owing to limitations in enzyme capacity, constrained RuBisCO activase activity, and finally, denaturation of proteins (Staeher & Borum 2011). Hence, the temperature optimum for photosynthesis in the summer-acclimated *Z. marina* seeds used in this study seemed to lie above 25°C (as no photosynthetic temperature optimum with increasing temperature was reached), which is very unlikely to occur for long durations within Danish waters. Seagrass can protect themselves from excess energy in the photosynthetic apparatus and from heat stress by activating the xanthophyll cycle (i.e. photo-protective mechanism), whereby an increased proportion of violaxanthin is converted to antheraxanthin and zeaxanthin (i.e. photo-protective pigments), which leads to increased energy dissipation and thus protection of the photosynthetic apparatus (Nguyen et al. 2021).

Rates of R in the sheath-covered seeds increased about 11-fold within the investigated temperature

range of $10\text{--}25^\circ\text{C}$. The positive correlation between temperature and seed respiration may result from increased cellular respiration due to the acceleration of enzyme-catalyzed chemical reactions (Staeher & Borum 2011, Nguyen et al. 2021). Indeed, temperature increases reduce the kinetic energy needed for engaging chemical reactions, such as cellular respiration (Staeher & Borum 2011, Nguyen et al. 2021). In addition, the O_2 solubility in seawater decreases with increasing temperature, which also enhances cellular respiration rates (Pedersen et al. 2016). In comparison, we determined an approximately 2-times stronger positive response in rates of respiration than photosynthesis in the sheath-covered *Z. marina* seeds with increasing temperature, which was mainly driven by a 2.6-fold higher Q_{10} in the lower temperature range of $10\text{--}20^\circ\text{C}$, respectively. In general, all Q_{10} values calculated in this study (i.e. between 3 and 3.2) were similar to the Q_{10} values previously recorded for adult seagrass plants (e.g. Staeher & Borum 2011, Hansen et al. 2022), except for the high Q_{10} value calculated for seed respiration in the lower temperature range (i.e. 6.6). However, this can likely be explained by the high metabolic activity of developing seeds, which is partly driven by high synthesis rates of storage products (Borisjuk & Rolletschek 2009).

The observed imbalance in the physiological response between photosynthesis and respiration resulted in substantially increased light requirements of the developing sheath-covered eelgrass seeds with increasing temperature (i.e. ~ 4 -fold increased photon irradiance at the onset of photosynthesis saturation). This implies that the photon irradiance needed for seed photosynthesis to compensate for the respiratory needs increases at high temperatures (Zimmerman et al. 1989, Brodersen & Kühl 2023), which was also indicated by the lower rates of NP with increasing temperature. Higher light requirements may lead to inadequate seed aeration and, since the O_2 conditions of the seeds are tightly coupled to the carbon balance, such negative effects of increasing temperature would reduce seed growth and development, and in the long term could be critical for seed maturation and survival (Brodersen & Kühl 2023).

4.2. Temperature effects on O_2 balances and budget in sheath-covered seagrass seeds: potential implications for seed development and germination success

We measured an extreme increase in the O_2 deficit of the eelgrass seeds with increasing temperature (i.e.

more than a 23-fold decrease in net DOB over the temperature range of 10–25°C). The only net O₂ surplus was recorded in light at the lowest temperature (10°C), where it accounted for ~25% of the respiration in darkness. However, photosynthetic O₂ production also played a beneficial role in the O₂ balance of the developing seeds at higher temperatures (i.e. 15–25°C), seen as a positive difference in the net O₂ balance of the eelgrass seeds between light and dark conditions; that is, a decrease in the negative O₂ balance of 57, 27, and 37% at temperatures of 15°, 20°, and 25°C, respectively. Our results thus indicate seriously disturbed diel O₂ balances in developing *Z. marina* seeds with increasing temperature, which can have detrimental effects on seed maturation and germination success owing to reduced biosynthetic activity (Rolletschek et al. 2002, 2005, Borisjuk & Rolletschek 2009, Brodersen & Kühl 2023). Hypoxic and/or anoxic stress in developing seeds reduces nutrient uptake and endosperm storage activity owing to the restrictions of energy-demanding transport processes and biosynthetic fluxes towards storage products (especially proteins), respectively (Borisjuk & Rolletschek 2009). Hypoxic and/or anoxic seed conditions also limit cellular energy production, which restricts mitochondrial respiration and thereby the conversion of stored energy to ATP production (Borisjuk & Rolletschek 2009). It is therefore likely that marine heat waves and future global warming of the oceans will have negative impacts on the development and germination success of heat-exposed *Z. marina* seeds, and thereby may adversely affect sexual reproduction significantly. A reduced contribution of sexual reproduction in seagrass meadows with increasing seawater temperatures and reduced water clarity may cause increased meadow vulnerability to disturbance (e.g. human activity, extreme weather and nutrient events as well as other biotic or abiotic stressors) owing to reduced genetic diversity and thus reduced capabilities to adapt to environmental changes.

4.3. Conclusions

In conclusion, we demonstrated that increasing seawater temperature leads to contrasting responses in the photosynthetic activity and rates of *R* in sheath-covered *Z. marina* seeds, as respiration is much more strongly affected. This physiological discrepancy results in increasing light requirements of the developing eelgrass seeds and decreasing net DOBs with increasing temperature. Such conditions may therefore challenge reproductive success in eelgrass and

negatively affect the recovery and resilience of affected meadows due to potentially reduced sexual reproduction and genetic diversity.

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Appendix

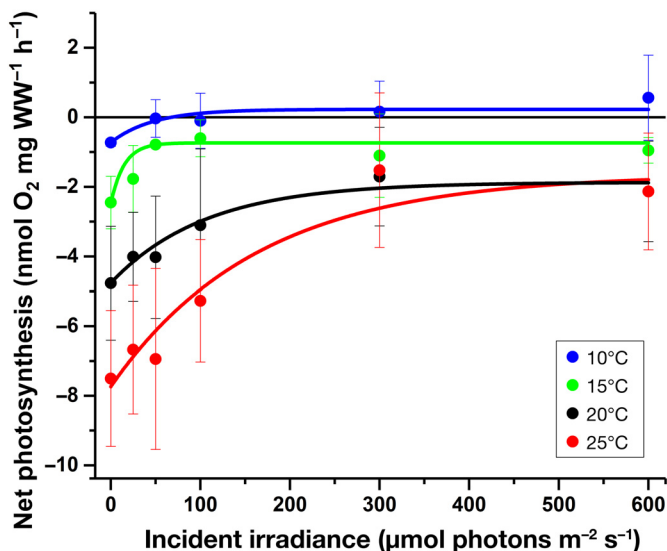


Fig. A1. Net photosynthesis rates of sheath-covered seagrass *Zostera marina* seeds as a function of photon scalar irradiance under increasing seawater temperatures. The rates of net photosynthesis were measured for photon irradiances (photosynthetically active radiation, 400–700 nm) of 0, 25, 50, 100, 300, and 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and temperatures of 10°, 15°, 20°, and 25°C. Symbols with error bars show mean values \pm SE; $n = 3$ biological replicates. All exponential saturation models (represented by the colored lines) had reduced χ^2 values of <0.36 (Table A1; $n = 5-6$)

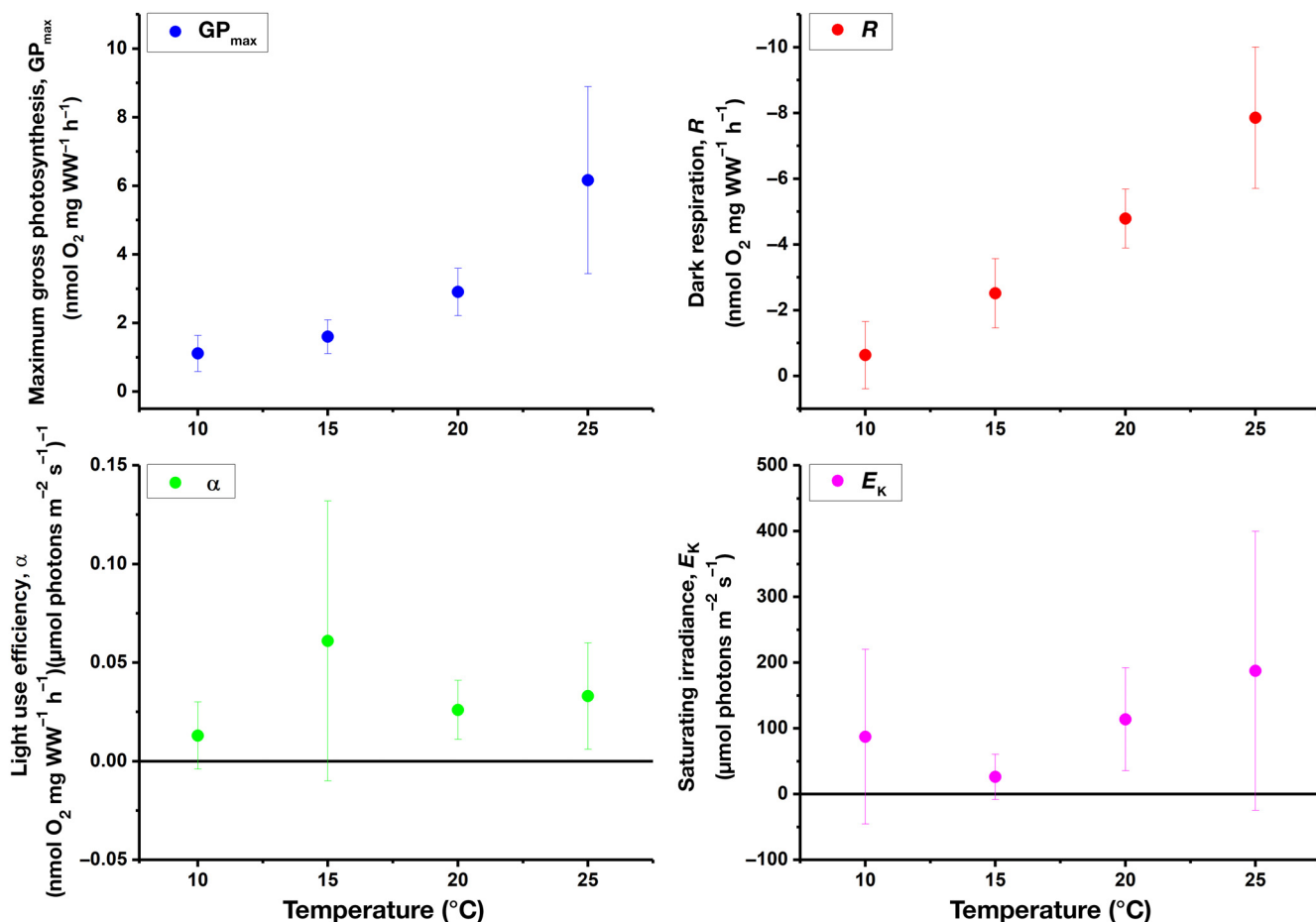


Fig. A2. Parameter estimates ($\pm 95\%$ confidence intervals) of maximum gross photosynthesis rates, dark respiration, light use efficiencies, and saturating photon irradiances of sheath-covered seagrass *Zostera marina* seeds as a function of increasing sea water temperatures (10°, 15°, 20°, and 25°C, respectively); n = 5–6

Table A1. Reduced Chi² values of all fitted exponential saturation models used for estimating photosynthetic parameters in this study. A reduced Chi² value of <1 indicates a good fit between the measured data and the fitted model

	10°C	15°C	20°C	25°C
Gross photosynthesis (Eq. 2)	0.07	0.69	0.91	0.98
Net photosynthesis (Eq. 3)	0.08	0.36	0.05	0.15

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