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ABSTRACT: Kelps are important foundation species in many cold-temperate coastal systems, and the loss of these organisms is a threat to ecosystem structure and function. The abundance of sugar kelp *Saccharina latissima* has recently declined in Northern Europe, which has been explained by increasing water temperature. We tested heat tolerance of sugar kelp exposed to simulated heatwave scenarios of 15, 18, 21 and 24°C for 3 wk, followed by a 2 wk recovery period at 15°C. Growth rate and photosynthetic performance decreased significantly with increasing temperature, while mortality remained low among treatments except at 24°C, where >90% of the algae died within a few days. Although exposure to 18 and 21°C had limited effect on mortality, kelps exposed to these temperatures had negative growth and continued to show impaired photosynthesis during the subsequent recovery period. Reductions in growth were strongly correlated to reduced carbon acquisition and, hence, photosynthetic performance, which was strongly correlated to heat-related changes in pigmentation. We suggest that reduced performance after exposure to elevated but non-lethal temperatures was caused by oxidative stress resulting from a discrepancy between light absorption and photosynthesis. Our results show that exposure to high but sub-lethal temperatures can have significant long-term effects, which may cause loss of biomass and leave sugar kelp susceptible to other stressors.

KEY WORDS: Climate change · Sugar kelp · Heat stress · Physiology

1. INTRODUCTION

Kelps constitute important foundation species in cold-temperate rocky coastal ecosystems (Wernberg et al. 2019). High productivity and the 3-dimensional structure of kelp forests make them ecologically important as a source of organic matter and a habitat-structuring agent that provides shelter and nursery grounds for various marine species (Christie et al. 2009). Sugar kelp *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl and G. V. Saunders is an arctic and cold-temperate kelp species with a wide range distribution across the Northern Hemisphere (Lüning 1990). Sugar kelp constitutes one of the dominant foundation species in Northern Europe, where it is essential to marine biodiversity and ecosystem productivity (Moy & Christie 2012). The abundance of sugar kelp has decreased in Northern Europe during the last 2 decades (Karlsson 2007, Moy & Christie 2012), and evidence suggests that rising water temperature in summer is the dominant driver of the observed decline (Moy et al. 2008, Pehlke & Bartsch 2008, Müller et al. 2009, Moy & Christie 2012). The decline of sugar kelp in Northern Europe may be part of a large shift where kelps are retreating towards the poles and are

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being replaced by more warm-adapted species (Kirihara et al. 2006, Müller et al. 2009, Wernberg et al. 2010, 2011, Merzouk & Johnson 2011, Díez et al. 2012, Gao et al. 2015).

The average annual water temperature in the North Sea has increased by ca. 1°C during the last 5 to 6 decades (Hawkins et al. 2003, Wiltshire et al. 2008) and is expected to continue to increase during the 21st century (IPCC 2013). However, the effect of ocean warming on the distribution of kelps is not controlled by the average annual water temperature, but rather by periods of extreme heat during summer (i.e. marine heatwaves; Wethey et al. 2011, Wernberg et al. 2013, Hobday et al. 2016). The average sea surface temperature in the Baltic Sea and the North Sea has increased 2 to 5 times faster in summer than in other seasons since the 1980s (Mackenzie & Schiedek 2007). As a result, marine heatwaves (MHWs) have increased in both frequency and intensity (Meehl & Tebaldi 2004, Perkins et al. 2012, Oliver et al. 2018). As the southern distribution limit of sugar kelp is controlled by maximum summer temperature (Lüning 1990, Müller et al. 2009), detailed information on the physiological response of the kelp to extreme heat events is particularly important in order to predict how climate change will affect this foundation species.

The physiological response to super-optimal temperatures in algae depends on their ability to sustain distinct periods of extreme stress and whether they can recover when the temperature declines again. We investigated the performance of sugar kelp when exposed to different heatwave (HW) scenarios followed by a subsequent recovery period. The temperatures of the 3 HW scenarios were chosen to represent a typical Danish summer (18°C), a rare Danish and/or near-future summer situation (21°C) and an extreme future scenario (24°C). The performance of sugar kelp at the targeted HW temperatures was compared to the performance at 15°C, which is considered within the optimal temperature range for growth and photosynthesis in sugar kelp from the NE Atlantic (Fortes & Lüning 1980, Bolton & Lüning 1982, Davison & Davison 1987). We expected that exposure to 18°C would have little or no effect on sugar kelp, that exposure to 21°C would have a significant negative effect and that exposure to 24°C would have a strong negative effect including high mortality. The aim was to provide a deeper physiological understanding of how prolonged heat stress affects survival, growth and photosynthetic performance of sugar kelp.

2. MATERIALS AND METHODS

2.1. Estimation of heatwave metrics

A marine heatwave (MWH) is defined as a discrete prolonged anomalously warm water event (Hobday et al. 2016). Data on sea surface temperature (SST) from the upper 1 m of central Limfjorden (56° 53′ N, 8° 37′ E) from 1975 to 2014 were obtained from the Danish Nature Agency, and the frequency, duration and intensity of MWHs were estimated according to the methods presented by Hobday et al. (2016). MHWs are defined as periods (minimum 5 d) where SST exceeds the climatological 90th percentile threshold based on a long baseline period (here 40 yr). MHWs can therefore also occur during periods of relatively low water temperature (e.g. in winter) where an extraordinary rise in temperature will have little effect on the biota. We therefore only report MHW metrics for the summer period (i.e. from 1 June to 30 September) each year from 1975 to 2014.

2.2. Experimental setup

Approximately 200 sugar kelp sporophytes (1 yr old) with a frond length of 12–20 cm were obtained from Hjarnø Havbrug (Southern Kattegat, Denmark, 55° 49′ N, 10° 5′ E) on 5 January 2015. The algae were immediately transported to Roskilde University in insulated polystyrene boxes containing 7°C natural seawater with a salinity of 25 PSU (*in situ* conditions at time of collection).

The algae were first preconditioned for 16 d. All algae were distributed equally among 20 l aquaria (n = 6) containing seawater from the North Sea (salinity 25 PSU and approximately 97 µM PO_4^{3-} and 80 µM dissolved inorganic nitrogen [DIN: NO_3^- plus NH^{4+}]; Jepsen et al. 2019). Temperature was regulated using heating tanks filled with fresh water, into which the aquaria were placed. A combination of thermostat-regulated heaters (Julabo ED, Julabo Labortechnik) and coolers (P Selecta, JP Selecta) maintained the temperature in the tanks within ±0.4°C of the target temperatures. Water temperatures were continuously logged using temperature loggers placed in each aquarium (HOBO Pendant Temperature/Light Data logger, Onset Computer). The initial water temperature was 7°C and was raised by 1°C every second day until it reached 15°C. The aquaria were illuminated with Halogen spots (OSRAM Decostar 51) providing an initial light intensity of ca. 50 µmol photons m^{-2} s^{-1} (measured with an underwater 2π censor [(LiCor Li-

192SA, connected to a LiCor Li-1000 light meter]) in a 16:8 h light:dark cycle. The light intensity was increased 3 times during the preconditioning period by reducing the distance between lamps and the aquaria to reach near-saturating illumination (141 ± 17 µmol photons m^{-2} s^{-1}; Lüning 1979) by the end of the preconditioning period. The water in all aquaria was exchanged weekly, and water circulation was ensured by bubbling the water with atmospheric air through aquarium air-stones.

The preconditioning period was followed by a 16 d acclimation period during which all algae were kept under the same conditions (i.e. 15°C, 25 PSU, 141 ± 17 µmol photons m^{-2} s^{-1} in a 16:8 h light:dark cycle). At the beginning of the acclimation period, 180 healthy looking individuals were selected for use in the experiment. The frond of each alga was pruned to a length of 10 cm by cutting off the distal part, and the algae were subsequently attached to acrylic plates with non-toxic silicon strings and distributed among 20 l aquaria (n = 12, each with 15 individual plants). The 12 aquaria were placed into 4 heating tanks (i.e. 3 replicate aquaria in each tank). The water in all aquaria was exchanged weekly with water from the North Sea (salinity 25 PSU and approximately 97 µM PO_4^{3-} and 80 µM DIN) to reduce the risk of severe nutrient limitation.

2.3. Heatwave simulation

The HW treatment was initiated on Day 32 after collection of the algae. The target temperatures of the 4 HW treatments were: 15°C (control), 18, 21 and 24°C (Fig. 1). The HWs were initiated by increasing the temperature in each tank by 1°C d^{-1} until the target temperatures were reached to avoid acute heat shock. The gradual increase in temperature meant that it took longer to reach the target temperature in HW treatments with the highest temperatures, and the exposure time at the target temperatures was therefore set to last for 20 d in the 18°C treatment, 19 d in the 21°C treatment and 18 d in the 24°C treatment. The 24°C treatment was terminated after 8 d of exposure to 24°C because 91% of the algae had died and the fronds of the few survivors had started to disintegrate. At the end of the remaining HW treatments (i.e. 18 and 21°C), temperature was lowered by 1.5°C every day until 15°C was reached, and all surviving individuals were subsequently kept at this temperature for a 15 d recovery period (Fig. 1).

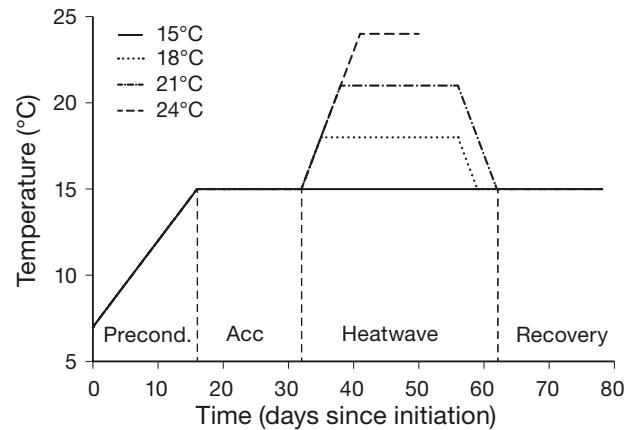Mortality and growth were measured during the acclimation, HW and recovery periods. All other end

Fig. 1. Temperature development of the 4 temperature treatments during preconditioning (Precond.), acclimation (Acc), heatwave (HW) simulation and recovery. The pre-conditioning period lasted for 16 d and was followed by an acclimation period of 16 d where all algae were kept at the same temperature (15°C). The HW period lasted 23–27 d (including time where the temperature was changing) depending on treatment level and was followed by a 5 d recovery period where all algae were kept at 15°C

points (i.e. photosynthetic performance, chlorophyll *a* [chl *a*] fluorescence, pigmentation, tissue nutrients and mannitol) were measured 4 times during the experiment: (1) at the end of the acclimation period; (2) midway through the HW; (3) at the end of the HW; and (4) at the end of the recovery period.

2.4. Mortality and growth

Kelp individuals with lost fronds were considered deceased. Frond elongation was measured using the punch hole method (Parke 1948); 1 hole (5 mm in diameter) was punched in the blade 5 cm above the transition between the stipe and the blade at the onset of the experiment, and elongation rate was expressed as linear growth in units of cm d^{-1}. Specific growth rate was estimated from changes in fresh weight biomass assuming exponential growth:

$$\text{Specific growth rate} = (\ln FW_2 - \ln FW_1)\,(t_2 - t_1)^{-1} \quad (1)$$

where FW_1 and FW_2 are the fresh weight biomass at times t_1 and t_2, respectively.

2.5. Photosynthesis

Photosynthesis and dark respiration were measured in 800 ml gas-tight transparent acrylic chambers filled with filtered seawater (using Whatman GF/C glass microfiber filters). The chambers were equipped with

a water circulation pump (AquaBee UP300, AquaBee Aquarientechnik) and submerged in a water bath to keep the temperature similar to the growth temperature. Change in O_2 concentration inside the chambers was measured using a Clark-type O_2 microelectrode (OX-500; Unisense) connected to a pico-amperemeter (Picoammeter PA2000; Unisense) and a Pico Technology ADC-16 high-resolution data logger.

For each measurement, 2 randomly selected individuals (from the same aquarium) were fixed inside the chamber. The water in the chambers was initially bubbled once with N_2 to reduce the O_2 concentration to ~60% of air saturation to prevent O_2 super-saturation during the incubations. Concentrations of O_2 were recorded every minute, and rates of O_2 evolution or consumption were calculated using linear regression from incubation periods with a linear O_2 development over at least 10 min. Dark respiration rate (R_D) was measured in darkness while net photosynthetic rate (P_N) was measured at 14, 20, 38, 90, 149 and 225 µmol photons m^{-2} s^{-1} using shade screens with different densities. Light utilization efficiency (α) and the light compensation point (I_C) were calculated from linear regression on the data obtained at the 5 lowest light intensities (i.e. 0, 14, 20, 38 and 90 µmol photons m^{-2} s^{-1}).

2.6. Daily carbon acquisition

Rates of carbon (C) uptake and losses of C through respiration were derived from O_2 evolution or consumption using a photosynthetic quotient (PQ) of 1.48 for brown algae (Table 1 in Pedersen et al. 2010). Daily net C acquisition was calculated using the 16:8 h light:dark cycle applied in the experiment:

$$\text{C acquisition} = (P_{N149} \times 16) - (R_D \times 8) \qquad (2)$$

where P_{N149} is the hourly net photosynthetic rate measured at 149 µmol photons m^{-2} s^{-1} (i.e. equivalent to the light intensity in the aquaria) and R_D is the hourly respiration rate in darkness.

2.7. Chl *a* fluorescence

Chl *a* fluorescence was measured using pulse-amplitude modulation (PAM) fluorometry (Walz Imaging-PAM, Walz; Maxwell & Johnson 2000). Four randomly selected individuals from each aquarium were dark adapted for >20 min. Each alga was then placed in a petri dish with enough seawater to keep it moist, and fluorescence was measured 1–3 cm above the meristematic region at 14 levels of illumination (0, 4,

24, 59, 104, 156, 204, 334, 414, 504, 604, 714, 838, 964 µmol photons m^{-2} s^{-1}). Fluorescence parameters, i.e. maximum quantum yield (F_v/F_m), relative electron transport rate at 156 µmol m^{-2} s^{-1} ($rETR_{156}$; Delebecq et al. 2011) and non-photosynthetic quenching at 156 µmol m^{-2} s^{-1} (NPQ_{156}), were obtained from fluorescence images of each alga using the Walz ImagingWin software.

2.8. Pigment concentrations

Concentrations of chl *a*, chl *c*, fucoxanthin, β-carotene, violaxanthin and zeaxanthin were determined using a high-performance liquid chromatograph (HPLC). Pigments were extracted with acetone (90%) from 18–30 mg of freeze-dried and ground kelp samples. Samples were centrifuged at 9000 × g (5 min), after which the supernatant was analyzed on an HPLC (Thermo/Dionex, UltiMate 3000) with UV and fluorescence detectors. Absorbance was measured at 430, 440, 450 and 470 nm with a bandwidth of 22 nm, while fluorescence was measured at 450 nm excitation and 630 nm emission from 0–5 min, 450 nm excitation and 650 nm emission from 5–18.8 min and 430 nm excitation and 670 nm emission from 18.8–35 min.

2.9. Tissue carbon, nitrogen and mannitol

Tissue concentrations of total C and N were measured using an EA 1110 CHNS elemental analyzer (CE Instruments) on 3–4 mg freeze-dried, ground kelp samples. Mannitol content was measured according to Vaskovsky & Isay (1969) on 3–5 mg freeze-dried, ground kelp samples that were extracted for 15 min in isopropanol (50%) and centrifuged at 9000 × g (5 min). Rhamnose solution, containing periodic acid, was added to the supernatant to react for 5 min, after which the reaction was stopped by adding Nash reagent. The reaction between mannitol and periodic acid was measured at 412 nm on a spectrophotometer (Shimadzu UV-1601).

2.10. Statistics

Data were analyzed using group-by-trials repeated measures ANOVA (Quinn & Keough 2002) testing for the effect of temperature (fixed, between-subjects factor), time (fixed, within-subject factor) and their interaction. Temperature included 3 levels because

data from the 24°C treatment were left out from the analysis due to high mortality early in the experiment, while time included 3 levels for mortality and growth and 4 levels for all other end points. Assumptions of variance homogeneity were tested using Levene's test, while normality was tested using the Kolmogorov-Smirnoff test. Assumptions of sphericity were tested using Mauchly's test, and Greenhouse-Geiser-corrected p-values were used when these assumptions were not met. Tukey's test was used to identify pairwise differences between levels of temperature or time when ANOVA yielded significant results. Relationships between growth rate and daily C acquisition, P_{G225} and chl *a* content, α and chl *a* content, and tissue N content and chl *a* content were analyzed using Pearson correlation analysis. Data were ln-transformed when necessary to obtain linear relationships or bi-variate, normally distributed data. All tests were conducted with SYSTAT v. 13 using a significance level of $\alpha = 0.05$.
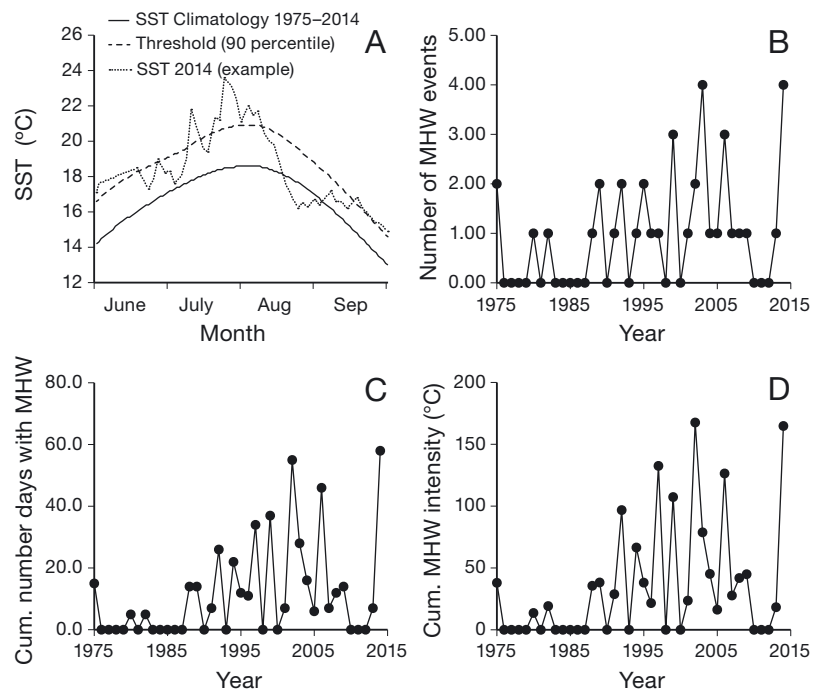

Fig. 2. Marine heatwave (MHW) metrics for the summer period (June to October) in central Limfjorden from 1975 to 2014. (A) Sea surface temperature (SST) climatology (i.e. average daily mean SST based on data from a 40 yr period), the 90th percentile MHW threshold (from the same period) and SST time series for 2014 as an example. (B) Number of MHW events yr^{-1} between June and October. (C) Cumulative duration of MHWs per year between June and October. (D) Cumulative intensity of MHWs between June and October

3. RESULTS

3.1. Heatwave metrics

Average (across 40 yr) summer SST in Limfjorden ranged from 12.8 to 18.6°C while the 90th percentile threshold ranged from 14.8 to 21.6°C (Fig. 2A). The frequency of MHW events between June and October ranged from 0 to 4 yr^{-1} and tended to increase over time (Fig. 2B); there were 11 summer MHW events between 1975 and 1994 with a cumulative duration of 108 d (Fig. 2C) and a cumulative intensity of 337.4°C (Fig. 2D) while in the following 20 yr (1995–2014) there were 27 MHW events with a total duration of 338 d and a cumulative intensity of 1056.2°C.

3.2. Mortality and growth

All kelp individuals survived the acclimation period (Fig. 3A). Mortality rate remained low and did not differ significantly among algae from the 15, 18 and 21°C treatments during the HW simulation (0.11,

0.13 and 3.64 3% d^{-1} at 15, 18 and 21°C; Table 1); however, mortality was high among those exposed to 24°C (5.38% d^{-1}), where 91% were dead after 8 d at this temperature. The blades of the few survivors were bleached and disintegrating, which is why we terminated the 24°C treatment at this point. All algae surviving the HW treatments at 15 and 18°C also survived the subsequent recovery period, while a single individual from the 21°C treatment died during the recovery period.

Frond elongation rate and specific growth rate averaged 0.160 ± 0.028 cm d^{-1} and 0.024 ± 0.004 d^{-1}, respectively, during the acclimation period, and both decreased significantly with time and increasing temperature (Fig. 3B,C, Table 1). Frond elongation rate in algae exposed to 18 and 21°C was, respectively, 51 and 89% lower than in those continuously kept at 15°C and was still affected during the recovery period, where rates in algae from the 18 and 21°C treatments were 25 and 72% lower than in those kept at 15°C. Specific growth rate was also affected by the HW treatment, being respectively 22 and 48% lower in algae exposed to 18 and 21°C than in those
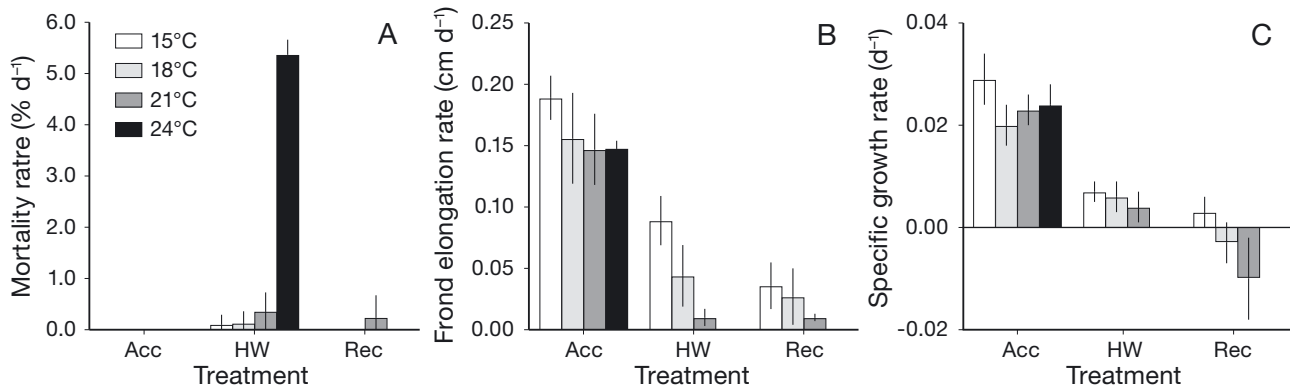
Fig. 3. Mortality rate and growth in sugar kelp exposed to a control treatment (15°C) and 3 simulated heatwave (HW) scenarios (18, 21 and 24°C): (A) mortality rate, (B) frond elongation rate and (C) specific growth rate. Acc: acclimation period (15°C); HW: HW period; Rec: recovery period (15°C). Mean values ± SD (n = 3)

Table 1. Results of repeated measures ANOVA testing for the effect of temperature, time and their interaction. Greenhouse-Geiser-corrected p-values (indicated by GG) are reported when the assumptions of sphericity were not met. Acc: acclimation period (15°C), Mid: midway through the heatwave (HW); End: end of HW; Rec: recovery period (15°C). P_{G225} (P_{G149}): gross photosynthetic rate at 225 (149) µmol m^{-2} s^{-1}; α: light utilization efficiency; R_D: dark respiration; I_C: light compensation point; F_v/F_m: maximum quantum yield; rETR$_{156}$: relative electron transport rate measured at 156 µmol photons m^{-2} s^{-1}; NPQ$_{156}$: non-photochemical quenching measured at 156 µmol photons m^{-2} s^{-1}

Variable		df	F	p	Pairwise tests
Mortality	Temperature	2	1.6	0.274	
	Time	2	3.1	0.080	
	Temperature × Time	4	0.5	0.757	
Frond elongation	Temperature	2	4.8	0.056	
	Time	2	262.9	**<0.001**	Acc > HW > Rec
	Temperature × Time	4	3.3	**0.049**	
Specific growth	Temperature	2	19.5	**0.002**	15°C > (18°C = 21°C)
	Time	2	354.8	**<0.001**	Acc > HW > Rec
	Temperature × Time	4	6.7	**0.004**	
P_{G225}	Temperature	2	36.8	**<0.001**	(15°C = 18°C) > 21°C
	Time	3	79.8	**<0.001**	Acc > Mid > End > Rec
	Temperature × Time	6	4.5	**0.006**	
α	Temperature	2	38.9	**<0.001**	(15°C = 18°C) > 21°C
	Time	3	35.5	**<0.001**	Acc > Mid > (End=Rec)
	Temperature × Time	6	2.1	0.110	
R_D	Temperature	2	2.59	0.154	
	Time	3	7.8	**0.001**	(Acc = Mid) < (End = Rec)
	Temperature × Time	6	0.2	0.972	
I_C	Temperature	2	43.5	**<0.001**	(15°C = 18°C) < 21°C
	Time	3	11.8	**<0.001**	Acc < Mid < (End = Rec)
	Temperature × Time	6	3.9	**0.012**	
C acquisition	Temperature	2	28.3	**<0.001**	(15 = 18°C) > 21°C
	Time	3	61.3	**<0.001**	Acc > Mid > (End = Rec)
	Temperature × Time	6	4.0	**0.010**	
F_v/F_m	Temperature	2	9.7	**0.013**	(15°C = 18°C) > 21°C
	Time	3	8.8	**<0.001**	Acc > Mid > (End = Rec)
	Temperature × Time	6	1.8	0.160	
rETR$_{156}$	Temperature	2	0.6	0.565	
	Time	3	13.8	**<0.001**	Acc > (Mid = End = Rec)
	Temperature × Time	6	2.0	0.125	
NPQ$_{156}$	Temperature	2	<0.1	0.960	
	Time	3	3.3	0.109^{GG}	
	Temperature × Time	6	0.3	0.770^{GG}	
rETR$_{156}$:P_{G149}	Temperature	2	28.2	**0.001**	(15 = 18°C) < 21°C
	Time	3	20.1	**<0.001**^{GG}	Acc < Mid < (End = Rec)
	Temperature × Time	6	5.9	**0.006**^{GG}	

(Table continued on next page)

Table 1 (continued)

Variable		df	F	p	Pairwise tests
Chl *a*	Temperature	2	4.9	0.056	
	Time	3	53.1	**<0.001**	Acc > Mid > End > Rec
	Temperature × Time	6	1.8	0.166	
Chl *c*:chl *a* ratio	Temperature	2	3.2	0.111	
	Time	3	1.7	0.204	
	Temperature × Time	6	1.6	0.208	
Fucoxanthin:chl *a* ratio	Temperature	2	3.0	0.126	
	Time	3	11.6	**<0.001**	(Acc = Mid) < End < Rec
	Temperature × Time	6	1.8	0.156	
β-carotene:chl *a* ratio	Temperature	2	7.5	**0.023**	(15°C =18°C) < 21°C
	Time	3	6.2	**0.004**	Acc < Mid < End < Rec
	Temperature × Time	6	1.3	0.326	
Xanthophyll:chl *a* ratio	Temperature	2	7.9	**0.021**	(15°C = 18°C) < 21°C
	Time	3	17.3	**<0.001**	Acc < Mid < (End = Rec)
	Temperature × Time	6	1.6	0.216	
De-epoxidation ratio (zeaxanthin: violaxanthin ratio)	Temperature	2	13.1	**0.006**	(15°C =18°C) < 21°C
	Time	3	5.1	**0.030**[GG]	Acc < Rec, all other similar
	Temperature × Time	6	1.8	0.204[GG]	
C content	Temperature	2	4.5	0.065	
	Time	3	11.4	**<0.001**	Acc < Mid < End < Rec
	Temperature × Time	6	2.3	0.080	
N content	Temperature	2	0.8	0.485	
	Time	3	17.4	**<0.001**	Acc > Mid > (End = Rec)
	Temperature × Time	6	1.1	0.413	
Mannitol content	Temperature	2	2.7	0.147	
	Time	3	60.7	**<0.001**	Acc > Mid > (End = Rec)
	Temperature × Time	6	2.5	0.063	

kept at 15°C. Specific growth rate continued to decrease during the recovery period, where algae previously exposed to 18 and 21°C experienced a net loss of biomass.

3.3. Photosynthetic performance and C acquisition

The relationship between net photosynthetic rate (P_N) and light intensity followed a saturation curve (not shown), with saturating light intensities between 149 and 225 µmol photons m^{-2} s^{-1} depending on treatment and time. Photosynthetic performance decreased significantly over the course of the experiment and was significantly affected by elevated temperature during the HW and during the subsequent recovery period (Fig. 4, Table 1). Photosynthetic parameters (i.e. gross photosynthetic rate in high light [P_{G225}], α and R_D) were similar in algae exposed to 15 and 18°C (all p > 0.734) during the HW. P_{G225} in algae exposed to 21°C decreased by 66% relative to that in algae at 15°C, while P_{G225} in algae exposed to 24°C was reduced by 86% just before that treatment was terminated (Fig. 4A). The

negative effect of elevated temperature on gross photosynthesis persisted through the recovery period, where it was, respectively, 27 and 55% lower in algae that had been exposed to 18 and 21°C than in those exposed to 15°C. Algae exposed to 21 and 24°C had α-values that were 78 and 89% lower than in those exposed to 15°C during the HW (Fig. 4B, Table 1), and the negative effect of elevated temperature continued through the recovery period, where α was 65% lower in algae that had been exposed to 21°C than in those from the 15 and 18°C treatments. Average R_D (Fig. 4A) increased by ca. 66% over the course of the experiment but was not affected by temperature, except in algae exposed to 24°C, where R_D increased 4-fold just before that treatment was terminated. I_C increased significantly with increasing temperature and was 9-fold higher in algae exposed to 21°C than in those kept at 15°C at the end of the HW (Fig. 4C).

Daily net acquisition of C decreased, but remained positive, over the course of the experiment in the 15, 18 and 21°C treatments (Fig. 4D, Table 1). Only algae exposed to 24°C had a negative C acquisition just before this treatment was terminated. The daily net
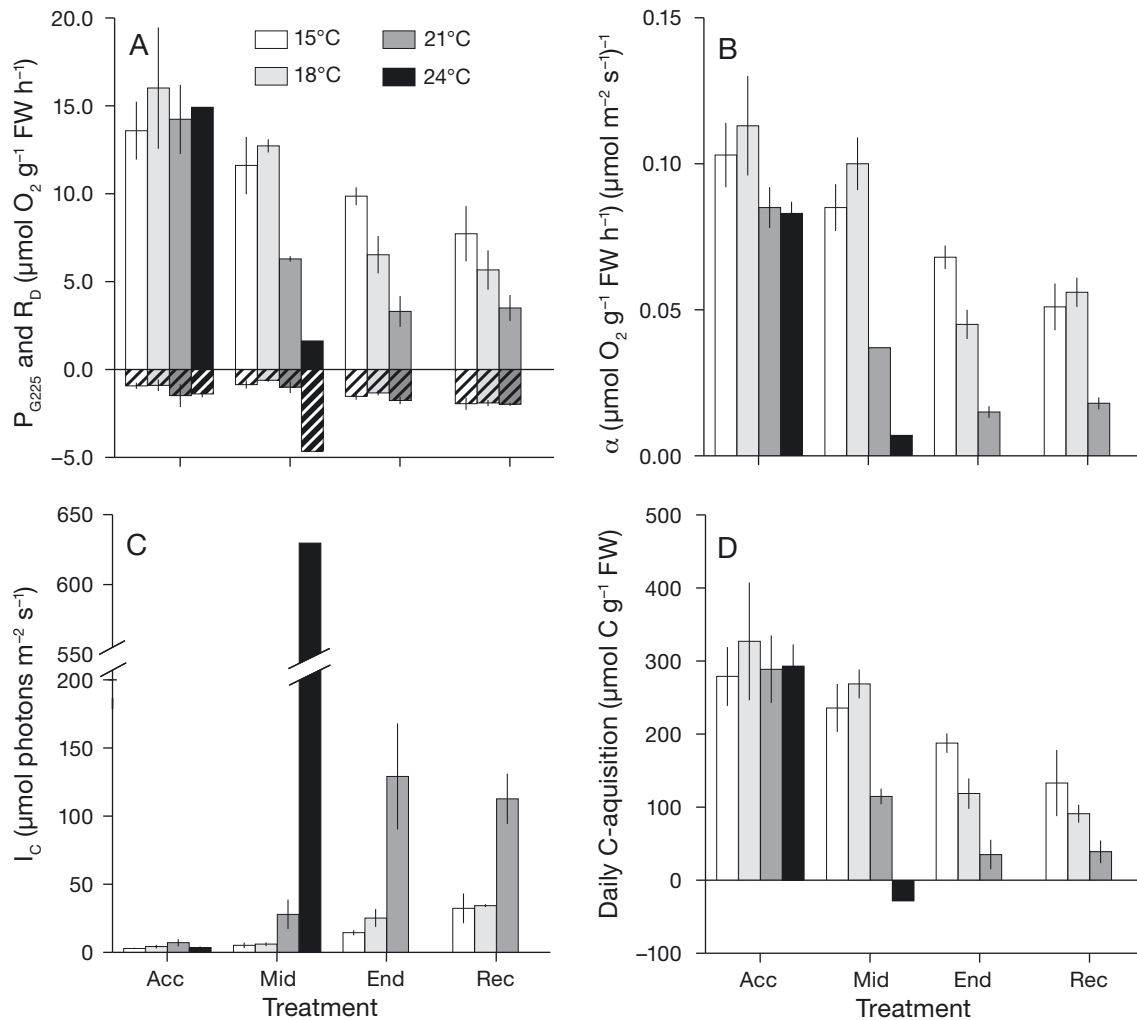
Fig. 4. Photosynthetic performance in sugar kelp exposed to a control treatment (15°C) and 3 simulated heatwave (HW) scenarios (18, 21 and 24°C). (A) Gross photosynthetic rate measured in high light (P_{G225}, top bars) and dark respiration (R_D, hatched bars). (B) Light utilization efficiency (α). (C) Light compensation point (I_C). (D) Daily net C acquisition. Acc: acclimation period (15°C); Mid: midway through HW; End: end of HW; Rec: recovery period (15°C). Means ± SD (n = 3)

C acquisition was similar in algae exposed to 15 and 18°C during the HW but was 81% lower in algae exposed to 21°C at the end of the HW. The daily net acquisition of C during recovery was similar to that observed by the end of the HW, being, respectively, 30 and 73% lower in algae previously exposed to 18 and 21°C than in those continuously exposed to 15°C.

3.4. Chlorophyll fluorescence

F_v/F_m averaged 0.56 ± 0.03 (SD) at the end of the acclimation period (Fig. 5A, Table 1). Exposure to elevated temperature during the HW had a negative effect on F_v/F_m, which decreased by 12% in algae exposed to 18°C and by 29% in algae exposed to 21°C when compared to those kept at 15°C. F_v/F_m

was very low (ca. 0.35) in kelp exposed to 24°C just before that treatment was terminated. The negative effect of elevated temperature continued into the recovery period, where F_v/F_m in algae that had been exposed to 18 and 21°C during the HW were, respectively, 7 and 32% lower than in those kept at 15°C. $rETR_{156}$ averaged 18.2 ± 2.9 µmol m^{-2} s^{-1} at the end of the acclimation period, decreased slightly during the first part of the HW and then stabilized and remained constant at ca. 13 µmol m^{-2} s^{-1} during the rest of the experiment (Fig. 5B, Table 1). $rETR_{156}$ was not affected by temperature. NPQ_{156} (Fig. 5C, Table 1) averaged 0.077 ± 0.016 at the end of the acclimation period and was neither affected by time, nor by temperature, except in algae exposed to 24°C where NPQ_{156} dropped to 0.006 ± 0.008 just before this treatment was terminated.
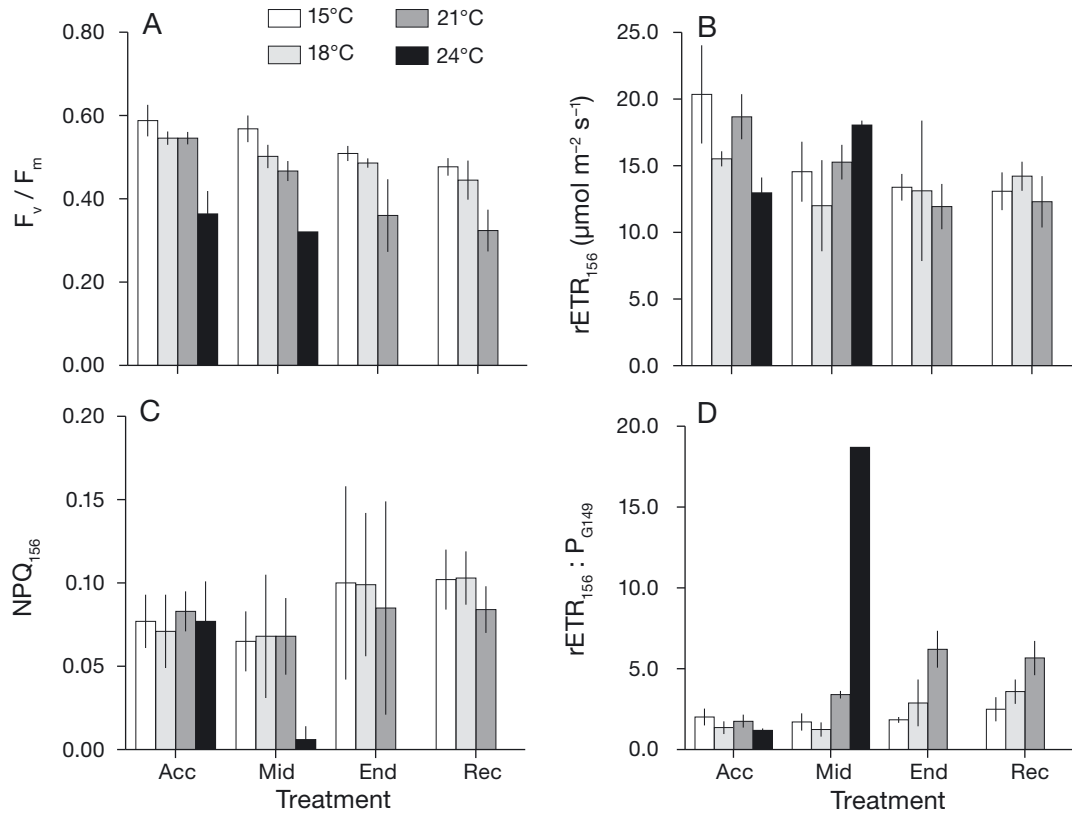
Fig. 5. Chlorophyll *a* fluorescence parameters in sugar kelp exposed to a control treatment (15°C) and 3 simulated heatwave (HW) scenarios (18, 21 and 24°C). (A) Maximum quantum yield (F_v/F_m). (B) Relative electron transport rate measured at 156 µmol photons m^{-2} s^{-1} ($rETR_{156}$). (C) Non-photochemical quenching measured at 156 µmol photons m^{-2} s^{-1} (NPQ_{156}). (D) Ratio between $rETR_{156}$ and gross photosynthetic rate measured at 149 µmol photons m^{-2} s^{-1} (P_{G149}). Acc: acclimation period (15°C); Mid: midway through HW; End: end of HW; Rec: recovery period (15°C). Means ± SD (n = 3)

3.5. Photosynthetic energy balance

The ratio between $rETR_{156}$ and gross photosynthesis at 149 µmol m^{-2} s^{-1} (P_{G149}) averaged 1.6 at the end of the acclimation period (Fig. 5D). The ratio did not change over time in algae from the 15°C treatment, but increased by, respectively, 57, 238 and 991 in algae exposed to 18, 21 and 24°C during the HW (Table 1). The ratio between $rETR_{156}$ and P_{G149} declined slightly in algae from the 21°C treatment during the recovery period, but was still 2 to 3 times higher than in those exposed to 15°C.

3.6. Pigment content

The average concentration of chl *a* decreased from 1.19 mg g^{-1} DW in the acclimation period to 0.45 mg g^{-1} DW at the end of the recovery period (Fig. 6A). The concentration of chl *a* tended to decline (p = 0.052, Table 1) with increasing temperature during the HW treatment where it decreased by 18 and 52% in algae exposed to 18 and 21°C, respectively, and by 70% in those exposed to 24°C when compared to the concentration in those exposed to 15°C. The concentration of chl *a* remained low in algae that had been exposed to elevated temperature during the recovery period. Absolute concentrations of chl *c*, fucoxanthin, β-carotene and xanthophylls largely followed the same pattern as chl *a* over time and across temperature treatments (not shown). The relative concentration of chl *c* (i.e. the chl *c*:chl *a* ratio; Fig. 6B) remained constant over time and across temperature treatments (Table 1), whereas the relative concentration of fucoxanthin increased over time, but was unaffected by temperature (Fig. 6C, Table 1). The relative concentrations of β-carotene and total xanthophylls (Fig. 6D,E) increased over time and with increasing temperature (Table 1), a pattern that remained through the recovery period. The de-epoxidation ratio (i.e. the ratio between zea- and violaxanthin; Fig. 6F) (Harker et al. 1999) increased with increasing temperature and became especially high in algae exposed to 21 and 24°C during the HW. The de-epoxidation ratio remained
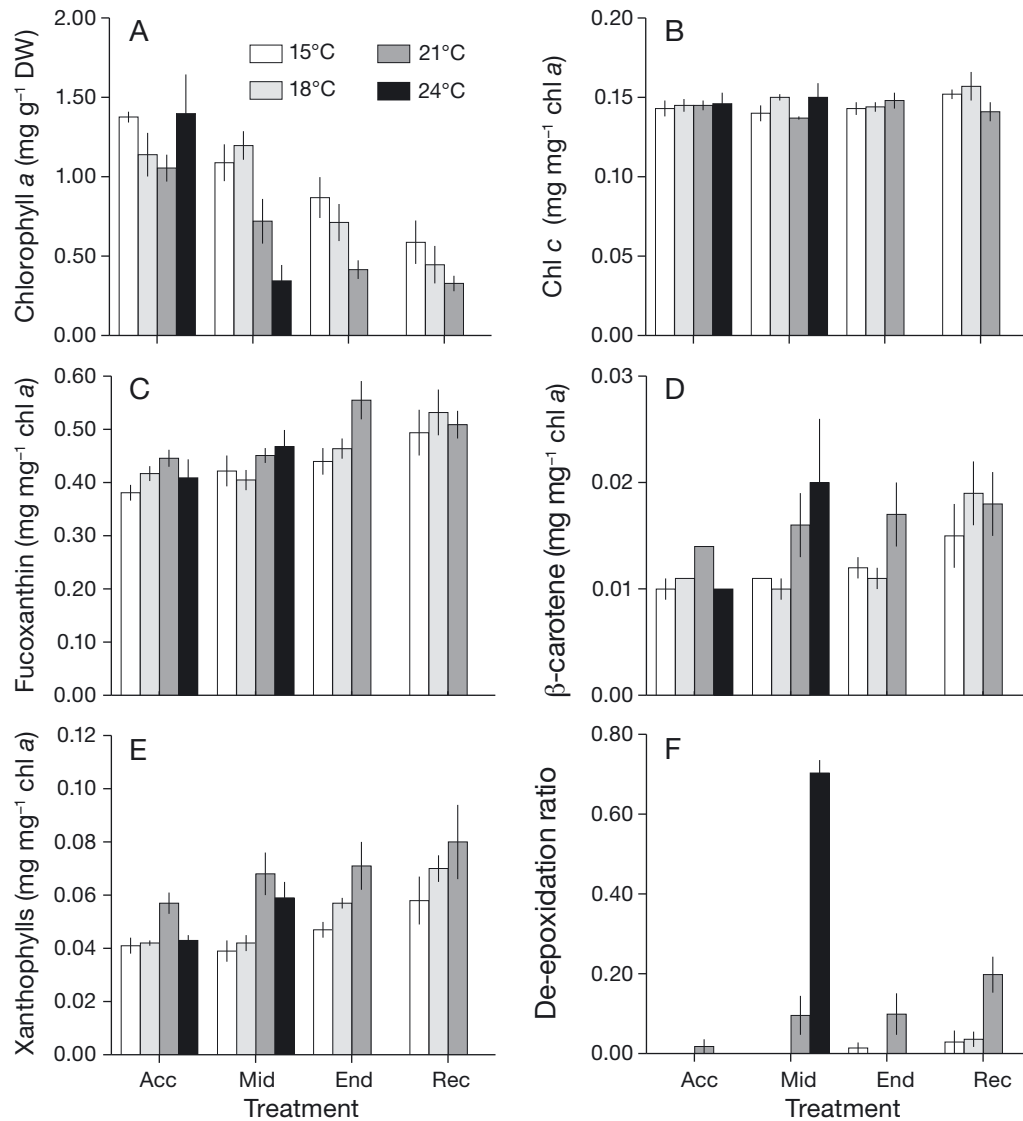
Fig. 6. Pigment concentrations and composition in sugar kelp exposed to a control treatment (15°C) and 3 simulated heatwave (HW) scenarios (18, 21 and 24°C). (A) Chlorophyll *a* (chl *a*) concentration. (B) Ratio between chl *c* and chl *a*. (C) Ratio between fucoxanthin and chl *a*. (D) Ratio between β-carotene and chl *a*. (E) Ratio between xanthophylls and chl *a*. (F) Ratio between zea- and violaxanthin (de-epoxidation ratio). Acc: acclimation period (15°C); Mid: midway through HW; End: end of HW; Rec: recovery period (15°C). Means ± SD (n = 3)

high during recovery in algae previously exposed to 21°C.

3.7. Carbon, nitrogen and mannitol content

The average C content (across all temperature treatments) increased from 29.7% of DW during acclimation to 33.2% of DW at the end of the recovery period while temperature had no effect on the C content (Fig. 7A, Table 1). The average N content (Fig. 7B) decreased from 0.66% of DW during the acclimation period to 0.44% of DW at the end of the recovery period, whereas temperature had no effect on the N content in algae exposed to 15, 18 and 21°C (Table 1). The highest N content was found in algae exposed to 24°C just before this treatment was terminated. The average concentration of mannitol (Fig. 7C, Table 1) decreased from 8.8% of DW during acclimation to 4.9% of DW at the end of the recovery period, but was not affected by temperature (Table 1). The lowest level of mannitol (4% DW) was found in kelps exposed to 24°C just before this treatment was terminated.
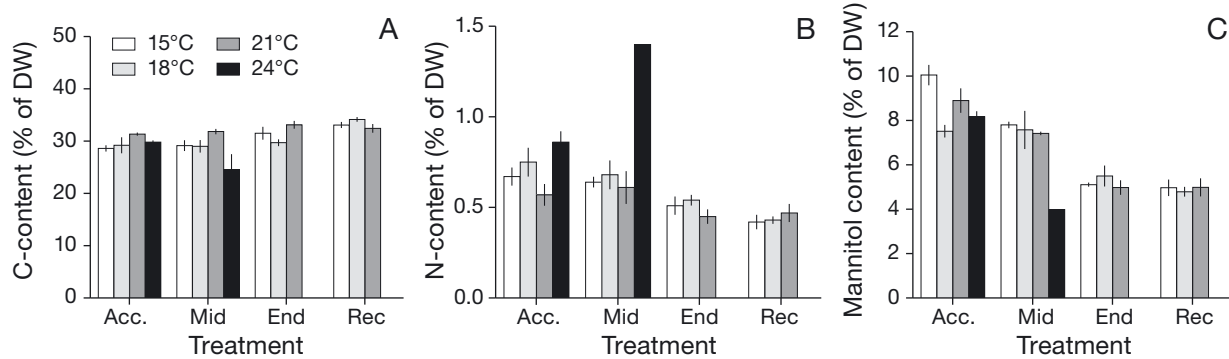
Fig. 7. Concentration of (A) carbon, (B) nitrogen and (C) mannitol in sugar kelp exposed to a control treatment (15°C) and 3 simulated heatwave (HW) scenarios (18, 21 and 24°C). Acc.: acclimation period (15°C); Mid: midway through HW; End: end of HW; Rec: recovery period (15°C). Means ± SD (n = 3)

4. DISCUSSION

Global average SST has increased at a rate of 0.1°C per decade over the last century (Burrows et al. 2011) and is expected to increase by 2–4°C in the North Sea and the Baltic Sea within the 21st century (Hulme et al. 2002, Döscher & Meier 2004, Sheppard 2004). Even small increases in mean SST lead to a higher frequency and increased intensity of MHWs, as natural variation superimposes onto the rising temperature trend (Lima & Wethey 2012, Hobday et al. 2016). The climatological data from Limfjorden support the trend found in other studies and show that the frequency, duration and intensity of MHWs have increased in the outer Baltic (including the Kattegat and Skagerak areas) over the last 30–40 yr (Meehl & Tebaldi 2004, Oliver et al. 2018). Sugar kelp was once the most abundant kelp species in the outer Baltic area, but its abundance has declined dramatically over the last 2 to 3 decades (Karlsson 2007, Moy & Christie 2012), parallel to a general increase in summer SST and increased frequency of MHW events in Northern Europe (Müller et al. 2009).

We found that sugar kelp could grow (albeit at reduced rates) and survive exposure to 18 and 21°C during an experimentally induced HW lasting for 3 wk, whereas 91% of the algae died after exposure to 24°C for 1 wk. This is not surprising, given that several studies have shown that the optimum temperature for growth in sugar kelp ranges between 10 and 15°C (Fortes & Lüning 1980, Bolton & Lüning 1982, Davison & Davison 1987) and that the upper thermal limit for survival ranges between 20 and 23°C, depending on origin and experimental conditions (e.g. Bolton & Lüning 1982, Lüning 1984, Gerard & Du Bois 1988, Gerard 1997). The most important finding in our study is, however, that the performance of sugar kelp exposed to super-optimal but sub-lethal temperatures (here 18 and 21°C) remained low during the subsequent recovery period where temperature was lowered to a supposedly non-stressful level of 15°C. This suggests that the algae suffered from irreversible or partly irreversible heat-related damages that may leave them susceptible to other stressors, such as intense light, eutrophication and water motion (Wernberg et al. 2010, Heinrich et al. 2012, Moy & Christie 2012, Simonson et al. 2015) even when the temperature returns to non-stressful levels after a HW event.

Exposure to high but sub-lethal temperature may have both acute reversible and long-lasting effects on performance. Acute reversible effects are generally caused by direct temperature effects on enzyme activities and membrane fluidity that may affect metabolism, including photosynthesis (Hasanuzzaman et al. 2013), and reduce C acquisition, but these effects are usually transient and will disappear as soon as temperature is lowered again. In contrast, long-term heat-related effects are typically caused by increased production of reactive oxygen species (ROS) that may damage large molecules and thus impact pigment levels, enzyme systems, membrane lipids and DNA (Lesser 2006). Such stress leads to elevated allocation of energy to protection and repair processes that will affect long-term performance beyond the time where the organism is exposed to elevated temperature (Davison 1991).

Reduced growth and survival in sugar kelp exposed to elevated temperature has previously been related to impaired C balance (Gerard & Du Bois 1988, Gerard 1997). Growth relies fundamentally on C acquisition and thus on photosynthetic performance. Photosynthesis is one of the most heat-sensitive metabolic processes (Crafts-Brandner & Salvucci 2002), and the photosynthetic performance of sugar kelp was clearly affected by elevated temperature as evidenced by

significant reductions in F_v/F_m, P_{G225} and α with increasing temperature. In contrast, R_D remained unaffected by elevated temperature (except at 24°C), which suggests that R_D in sugar kelp is relatively heat resistant or that sugar kelp is able to acclimate R_D to a wide range of temperatures (Davison 1987). Temperature-related changes in photosynthetic performance (especially in α) led to a significant increase in I_C and, thus, reductions in daily net C acquisition, which was significantly reduced during exposure to elevated temperature and remained low during the subsequent recovery period. Growth rate was strongly correlated to C acquisition (R = 0.848), thus underlining the close relationship between growth and net C acquisition and hence between growth and photosynthetic performance. Long-term reductions in C acquisition may lead to depletion of C reserves and has previously been related to increased mortality in sugar kelp during heat exposure (Gerard 1997). However, the daily net uptake of C remained positive, despite significant reductions in algae exposed to 18 and 21°C, and the C reserves (here mannitol) and mortality remained unaffected by the temperature treatments. Reduced C uptake in these algae can thus explain reductions in growth rate, but the reduction was not severe enough to deplete the C reserves and cause increased mortality at 18 and 21°C within the time frame of the experiment.

Photosynthetic capacity depends largely on pigment concentrations (Hurd et al. 2014). Exposure to elevated temperature increases the turnover of plant pigments, which often leads to reduced pigment levels (Ashraf & Harris 2013, Hasanuzzaman et al. 2013). The concentration of chl *a* and other pigments decreased with increasing temperature, and reductions in photosynthetic performance were strongly correlated with changes in chl *a* concentration (P_{G225}: R = 0.828; α: R = 0.784), thus indicating that parts of the observed reductions in photosynthetic performance, and hence in growth, during the HW treatment were driven by heat-related reductions in chl *a* content.

Specific growth rate declined but remained positive at elevated temperature during the HW treatment (except at 24°C) and continued to decline during the recovery period, where rates became negative, indicating a net loss of biomass. In contrast, frond elongation rate remained positive during the recovery period, which shows that the meristems were intact and produced new frond tissue while old tissue, which had been exposed to elevated temperature, was lost at a higher rate due to heat-related damages. Both photosynthetic performance (P_{G225} and α) and chl *a* concentrations remained low during the

recovery period, showing no signs of improvement in response to reduced temperature. Algae exposed to 18 and 21°C during the HW therefore seemed to suffer from heat-related damages, from which they could not recover within 2 wk upon return to a more suitable temperature.

Increased production of ROS is one of the most universal consequences of environmental stress in plants and algae (Collen & Davison 1999). Most ROS are produced in the chloroplasts by over-excitation of pigments when light absorption exceeds the capacity for photochemistry (Asada 2006), and photosynthetic organisms therefore need to maintain balance between energy absorption and photosynthetic efficiency (Adams et al. 2008). Photosynthesis measured from O_2 evolution is a direct measure of apparent photosynthesis, while rETR is a proxy for the transport of absorbed energy to PSII (Maxwell & Johnson 2000, Figueroa et al. 2003). The ratio between rETR and P_G is therefore a proxy for the amount of absorbed energy that is being used in photosynthesis, with increasing ratios indicating a higher production of ROS (Fryer et al. 1998, Figueroa et al. 2003). The ratio of rETR to P_G increased 2-, 4- and 10-fold in algae exposed to 18, 21 and 24°C, respectively, which indicates that the production of ROS increased significantly with increasing temperature. This suggestion is supported by the enhanced expression of genes promoting ROS scavengers in sugar kelp exposed to elevated temperature (Heinrich et al. 2012).

Algae can mitigate excessive light capture by reducing the amount of accessory pigments relative to chl *a* (Hurd et al. 2014). The absolute concentrations of accessory pigments (chl *c*, fucoxanthin and β-carotene) decreased over time, but the relative concentration (i.e. relative to chl *a*) of the 2 most abundant accessory pigments (chl *c* and fucoxanthin) remained constant over time and across temperature treatments, while the relative concentration of β-carotene increased significantly with increasing temperature, likely due to its additional role as an antioxidant in Photosystem II. Sugar kelp thus seems unable to reduce its light-harvesting capacity significantly during heat stress. Photo-protection can also take place through controlled heat dissipation (i.e. non-photochemical quenching, NPQ), which is correlated to the amount of xanthophyll cycle pigments and to the de-epoxidation ratio of zea- to violaxanthin (Harker et al. 1999, Magney et al. 2017). The relative concentration of xanthophylls increased with increasing temperature, and the de-epoxidation ratio increased substantially in algae exposed to 21 and 24°C during the HW. These changes should theoreti-

cally result in higher NPQ, but NPQ_{156} did not vary significantly across temperature treatments, except at 24°C where it dropped to a very low level. Prolonged exposure to high temperature may thus have a negative rather than a positive effect on NPQ as also shown by Andersen et al. (2013). These results suggest that sugar kelp has a limited capacity to alleviate the effects of excess energy absorption through photoacclimation and heat dissipation when exposed to elevated temperature, which will lead to an increased production of ROS.

Most end points (e.g. growth rate, photosynthetic performance and pigment concentrations) decreased over time regardless of temperature treatment. Reduced performance over time may potentially have been induced by the long-day conditions applied, by (high) light stress or by N-starvation. The 16:8 h light:dark cycle applied was chosen to mimic *in situ* conditions in summer where high temperatures prevail. However, long-day conditions induce arrested growth in many kelp species (Schaffelke & Lüning 1994), which could explain the observed reduction in growth over the course of the experiment. Whether long-day conditions affect the sensitivity of sugar kelp to elevated temperature remains unknown at present, and we can therefore not judge whether day-length conditions influenced our results. High light intensity may harm algae through photoinhibition and photodamage, including severely reduced pigment levels and, thus, lead to reduced performance. The light intensity used in our study (141 µmol m^{-2} s^{-1}) was chosen to mirror *in situ* summer conditions for Northern European kelp populations where the light intensity at 2–5 m depth typically reaches 500–1000 µmol m^{-2} s^{-1} at mid-day (Pedersen et al. 2014). The chosen light level was higher than that in many other laboratory studies on sugar kelp (e.g. Bolton & Lüning 1982, Davison & Davison 1987, Machalek et al. 1996, Heinrich et al. 2012, Simonson et al. 2015) but still below the saturating irradiance for photosynthesis and growth in sugar kelp (e.g. Lüning 1979, Gerard 1988) and far from the light levels that induce reduced growth (Fortes & Lüning 1980). We therefore consider it unlikely that (high) light stress can explain the observed decline in performance over time. The most likely reason for the observed reduction in performance over time is N starvation. N starvation substantially reduces algal performance (Hurd et al. 2014), and the N content in our algae was low (ca. 0.4–0.6 % of DW) when compared to that reported in other studies (Davison & Davison 1987, Davison et al. 2007, Andersen et al. 2013, Boderskov et al. 2016). N-starvation affects the content of pigments, enzymes and proteins, including stress proteins, and may therefore increase the sensitivity to heat stress (Gerard 1997). The heat response observed in the present study may thus have been intensified by N limitation (Gerard 1997), but we argue nevertheless that the experimental conditions mirror those *in situ* since heat stress typically occurs in mid- to late summer where nutrient concentrations in the water are low and coastal macroalgae suffer the strongest N limitation (Pedersen & Borum 1996, 1997, Pedersen et al. 2010).

In conclusion, we found that exposure to temperatures at and above 18°C for more than 3 wk had a significant negative long-term effect on sugar kelp even after the return to a more suitable temperature. Average SST in summer is expected to increase by several degrees in the North Sea and the Baltic Sea within the 21st century, which will likely cause more frequent and extreme MHW events (Oliver et al. 2018). Whether sugar kelp can persist under such conditions depends on its ability to quickly adapt to higher temperatures (Harley et al. 2012). Our study investigated the heat response of individuals from one population, but different genetic strains may possess higher heat tolerance and stronger capacity for thermal acclimation than others (Clark et al. 2013), and we suggest that future studies should evaluate genotypic variations in heat response within and between populations of sugar kelp across a broader geographical scale in order to predict how climate changes will affect this species.

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