



Seasonal Photophysiological Performance of Adult Western Baltic *Fucus vesiculosus* (Phaeophyceae) Under Ocean Warming and Acidification

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Shallow coastal marine ecosystems are exposed to intensive warming events in the last decade, threatening keystone macroalgal species such as the bladder wrack (*Fucus vesiculosus*, Phaeophyceae) in the Baltic Sea. Herein, we experimentally tested in four consecutive benthic mesocosm experiments, if the single and combined impact of elevated seawater temperature ($\Delta + 5^{\circ}\text{C}$) and pCO_2 (1100 ppm) under natural irradiance conditions seasonally affected the photophysiological performance (i.e., oxygen production, *in vivo* chlorophyll *a* fluorescence, energy dissipation pathways and chlorophyll concentration) of Baltic Sea *Fucus*. Photosynthesis was highest in spring/early summer when water temperature and solar irradiance increases naturally, and was lowest in winter (December to January/February). Temperature had a stronger effect than pCO_2 on photosynthetic performance of *Fucus* in all seasons. In contrast to the expectation that warmer winter conditions might be beneficial, elevated temperature conditions and sub-optimal low winter light conditions decreased photophysiological performance of *Fucus*. In summer, western Baltic Sea *Fucus* already lives close to its upper thermal tolerance limit and future warming of the Baltic Sea during summer may probably become deleterious for this species. However, our results indicate that over most of the year a combination of future ocean warming and increased pCO_2 will have slightly positive effects for *Fucus* photophysiological performance.

Keywords: bladder wrack, chlorophyll fluorescence, mesocosm, multi-factorial change, photoacclimation, photosynthesis, seasonal pattern

Abbreviations: α , light utilization efficiency; CCM, carbon-concentrating mechanism; F_v/F_m , maximum quantum yield of PSII; I_k , saturation irradiance for photosynthesis; KOB, Kiel Outdoor Benthocosm; NO, non-regulated non-photochemical quenching, NPQ, regulated non-photochemical quenching, rETR_{max} , relative maximum electron transport rate; PAR, photosynthetically active radiation; ROS, reactive oxygen species.

INTRODUCTION

Recent hot weather conditions in Central Europe, such as the weeks of record-breaking air temperatures during the summer of 2003 and 2018 (Schär et al., 2004; Imbery et al., 2018), pose a significant threat to macrophyte keystone species and their associated organisms, especially in shallow waters (Roth et al., 2010; Winters et al., 2011; Gouvêa et al., 2017). In the Baltic Sea on hard bottoms the bladder wrack *Fucus vesiculosus* L. (hereafter *Fucus*) is the most common canopy-forming and biomass dominating brown macroalga (Kautsky et al., 1992; Torn et al., 2006; Rönnbäck et al., 2007). The complex habitat formed by *Fucus* provides various ecosystem goods and services such as carbon storage and preservation of biodiversity in the coastal zone (Bokn et al., 2002; Rönnbäck et al., 2007; Schagerström et al., 2014).

Photosynthesis, growth and hence biomass production of algae are limited by biotic and abiotic factors if they exceed thresholds for optimal organismal function. Especially, shallow subtidal habitats of the temperate regions like in the Baltic Sea are a highly variable environment where organisms are regularly exposed to strong fluctuations in temperature, pH, irradiance, salinity and nutrient availability. The Baltic *Fucus* tolerates highly variable environmental conditions, especially annual and seasonal fluctuations in pH (7.4–8.5) and temperature (<0–20/25°C) (Wahl et al., 2010; Wahl et al., 2011). For instance, the *Fucus* system seems to react very sensitive to environmental change, which is suggested by the sharp decline of Baltic *Fucus* populations over the past decades (Wahl et al., 2011; Wahl et al., 2015b; Takolander et al., 2017). The decline of perennial *Fucus* populations is associated with the shift to a predominance of annual filamentous algae (Torn et al., 2006).

In temperate regions, high environmental stress occurs, for example, during summer on clear, calm days (Helmuth et al., 2002) as these conditions lead to harsh thermal stress, particularly in shallow coastal waters (Davison and Pearson, 1996; Collén and Davison, 2001). The ongoing warming trend and its intensification of seasonal fluctuations is stated to be a severe further challenge for *Fucus* (Jueterbock et al., 2013). Within the natural distribution range of *Fucus* 20°C is considered as the highest water temperature occurring over longer than weekly periods (Lüning, 1990). In the shallow coastal zone of the Baltic Sea the summer sea surface temperature is already close to this limit and is predicted to rise by 3–6°C till the end of the century (Elken et al., 2015). In addition to decadal-scale, gradual warming trends, short-term extreme warming events (e.g., “marine heat-waves” *sensu* Hobday et al., 2016) have increased in the Baltic Sea (HELCOM, 2013). In the western Baltic Sea shallow water temperatures may reach >30°C for short periods during summer (Wahl et al., 2010). Thus, *Fucus* communities populating shallow coastal areas may be one of the first to experience the effects of global warming and heat-waves.

As seaweeds have the capacity to optimize photosynthesis over a wide range of temperatures, they are able to physiologically acclimatize to temperature changes within hours and days (Davison et al., 1991; Kübler and Davison, 1995; Eggert et al., 2006). The potential for seasonal thermal acclimation

of photosynthesis has been described for different seaweed species (Mathieson and Norall, 1975; Davison et al., 1991; Pftzing et al., 2000). Whereas seasonal changes in temperature are usually anticipated and compensated by acclimation processes and/or physiological plasticity of individual organisms (Davison and Pearson, 1996; Kingsolver and Huey, 1998), the sporadic occurrence of extreme temperature conditions can seriously increase mortality (Roth et al., 2010; Winters et al., 2011). Additionally, physiological repair mechanisms may be weakened or the accumulation of harmful intermediates such as reactive oxygen species (ROS) may be induced by stressful, but sub-lethal conditions (Weidner and Ziemens, 1975; Davison and Pearson, 1996).

Photosynthetic activity is a sensitive indicator for thermal tolerance in seaweeds, because the photosynthetic apparatus is particularly sensitive to high temperature stress (Berry and Björkman, 1980; Dutta et al., 2009). The oxygen-evolving complex photosystem II (PSII) can be strongly affected by temperature, as high temperatures negatively influence the stability of PSII (e.g., damage and rapid turnover of the D1 protein) and by impairment of recovery processes due to ROS formation (Berry and Björkman, 1980; Allakhverdiev et al., 2008). The extent of PS II damage due to, for example, heat stress depends on the balance between damage and repair processes during such conditions. This balance provides the basis for acclimation and photosynthetic recovery processes (Adir et al., 2003; Mohanty et al., 2007; Murata et al., 2007). In *Fucus*, optimal temperature for photosynthesis has a broad range varying between 5 and 25°C in eastern Baltic Sea, North Sea or Atlantic populations, respectively (Niemeck and Mathieson, 1978; Russell, 1987; Nygård and Dring, 2008). For *Fucus* from the western Baltic Sea optimal temperature for highest photosynthetic performance under controlled laboratory experiments ranged between 20 and 26°C (Graiff et al., 2015b). However, *Fucus* was unable to photophysiologicaly acclimatize to temperatures higher than 26°C over 3 weeks of incubation (Graiff et al., 2015b).

Future CO₂-induced acidification of the surface waters of the Baltic Sea is difficult to predict (Müller et al., 2016). However, oceanographic models for the Baltic Proper project a long-term decrease in surface pH (Omstedt et al., 2012). It was demonstrated that increased pCO₂ conditions and/or dissolved inorganic carbon (DIC) enhanced photosynthetic rates of *Fucus* species and other non-calcifying seaweeds (Gordillo et al., 2001; Nygård and Dring, 2008; Wu et al., 2008; Saderne, 2012; Koch et al., 2013; Olischläger et al., 2013, 2017). The assumed explanation for a positive CO₂ effect on non-calcifying seaweeds is the down-regulation of energy-consuming carbon-concentrating mechanisms (CCMs) by facilitating their access to carbon (in the form of CO₂) under these conditions (Beardall and Giordano, 2002; Wu et al., 2008). The outcome of ocean acidification effects on photosynthesis seems to be dependent on the interaction with other environmental factors, such as sub-optimal temperatures (Fu et al., 2007; Feng et al., 2008; Olischläger and Wiencke, 2013; Sarker et al., 2013), limited nutrient availability (Gordillo et al., 2001; Xu et al., 2010) and light conditions (Rokitta and Rost, 2012; Sarker et al., 2013).

Due to anthropogenic global change, ocean warming and acidification may singly or interactively affect photophysiological performance of *Fucus* during the different seasons. *Fucus* was exposed to single and combined treatments of elevated seawater temperature ($\Delta + 5^{\circ}\text{C}$) and pCO_2 (1100 ppm) in all four seasons as predicted for shallow coastal Baltic habitats for the year 2100 (Elken et al., 2015; Schneider et al., 2015; Müller et al., 2016). These scenarios, including natural fluctuations forced by diurnal and seasonal changes, atmospheric and hydrographic conditions, were simulated using benthic mesocosms [Kiel Outdoor Benthocosms (KOBs), Wahl et al., 2015a]. We hypothesized that warming and increased pCO_2 (singly and combined) would have positive effects on the photophysiology of *Fucus* (i.e., oxygen production, *in vivo* chlorophyll *a* fluorescence and chlorophyll concentration) during winter, under low irradiances.

MATERIALS AND METHODS

Collection of Algae and Sampling Site

Fucus vesiculosus L. specimens were sampled, in each season (spring: April 2, 2013; summer: July 2, 2013; autumn: 8 October; winter: January 14, 2014) from a depth of 0.2–1 m in the Kiel Fjord (Bülk), western Baltic Sea, Germany ($54^{\circ}27'\text{N}$; $10^{\circ}11,5'\text{E}$). In the atidal Kiel Fjord, *F. vesiculosus* forms almost monospecific dense stands on stones between 0.3 and 3 m depth. *Fucus* individuals still attached to stones were randomly collected from the field resulting in a variety of different sizes, growth forms and maturity levels. All collected individuals were taller than 15 cm and considered as “adults.” After sampling, the algae were directly placed in water-filled buckets and brought to the experimental site at the GEOMAR Helmholtz Centre for Ocean Research. For later identification all specimens were labeled.

Experimental Design and Treatments

The mesocosm experiments were performed in the KOB infrastructure at GEOMAR in the inner Kiel Fjord ($54^{\circ}20'\text{N}$; $10^{\circ}09'\text{E}$). In Wahl et al. (2015a) the technical details of this facility, the experimental setup and monitoring, are described in detail. The KOB system consists of six flow-through tanks (ca. 1800 L per day) divided into 12 completely autonomous experimental units with a water volume of 1470 L each and covered with gas-tight, translucent foils. The KOB facility is exposed to ambient light (irradiance and photoperiod) conditions year-round. Photosynthetically active radiation (PAR) data were obtained from the German Weather Service (DWD, for details see Graiff et al., 2020). Water conditions were kept as close as possible to the real ambient conditions of the Kiel Fjord, including their fluctuations. For this the experimental units were supplied with non-filtered seawater pumped from 1 m depth near the KOB facility and inside each tank a pump circulated the water continuously. Twenty individuals of *Fucus* growing on their natural substrata were established in each experimental unit. The rock substratum of each *Fucus* was placed in a small plastic dish ($\text{Ø} = 14$ cm, $h = 4$ cm) attached to a grating at a water depth of 40 cm in the tank.

The single and combined effects of ocean warming and acidification on *Fucus* photophysiology were tested by crossing two temperatures (*in situ* Kiel Fjord temperature vs. elevated temperature by $+5^{\circ}\text{C}$) and two CO_2 levels (ca. 400 ppm vs. ca. 1100 ppm in the headspace above the mesocosm) in four seasonal experiments. Thus, four treatments were surveyed: (1) the ambient *in situ* Kiel Fjord temperature and CO_2 conditions (Ambient), (2) ambient temperature with elevated pCO_2 ($+\text{CO}_2$), (3) elevated temperature with ambient pCO_2 ($+\text{Temp}$), and (4) elevated temperature with elevated pCO_2 ($+\text{Temp} + \text{CO}_2$). Each treatment was replicated in three independent experimental units. The treatments were superimposed on the natural fluctuations of all environmental variables. Elevated levels of both factors were chosen according to climate change forecasts for shallow coastal Baltic habitats for the year 2100 (Elken et al., 2015; Schneider et al., 2015; Müller et al., 2016). Before the experiments, the *Fucus* individuals were acclimated to the KOB conditions for 2 days under ambient conditions. The temperature in the warming treatments was elevated by 2°C on the second day and by 3°C on the third day to reach a 5°C higher temperature compared to the natural Kiel Fjord temperature on the fourth day. Under computer control, CO_2 was injected from the second day onward into the headspace above the elevated pCO_2 treatment tanks in order to maintain the headspace pCO_2 close to 1100 ppm.

The investigation of seasonal variations of single and combined effects of simulated ocean warming and acidification on *Fucus* photophysiological performance was restricted by the need to maintain the technical equipment and sensors every 4 months. Thus, the study was divided into four consecutive experiments rather than 1 year-round study. The experiments ran from April 4, to June 19, 2013 (spring), from July 4, to September 17, 2013 (summer), from October 10, to December 18, 2013 (autumn), and from January 16, to April 1, 2014 (winter), each lasting for at least 10 weeks.

Manipulation of Temperature and pCO_2

In each experimental unit the water temperature was constantly logged by sensors and automatically adjusted by heat exchangers and internal heating elements to a temperature rise of 5°C . The Kiel Fjord water temperature is subjected to the typical temperate seasonal pattern. In spring and early summer (April to June) mean water temperature increased and reached maximum values (24.1 – 24.8°C) in July to August. During autumn and early winter (September to December) the water temperature declined and reached a minimum of 4.2°C in January. Subsequently, the temperature increased from February to March again (**Supplementary Table 1**).

The manipulation of the seawater pCO_2 was achieved by computer-controlled injection of pure CO_2 into the headspace atmosphere above each experimental unit and was maintained at approximately 1100 ppm CO_2 . Inside the tanks the pH was continuously monitored by sensors (gel-electrolyte filled glass electrode, GHL Advanced Technology, Kaiserslautern, Germany) and was additionally measured daily using hand-held pH-meter to permit *post hoc* correction of sensor drift (Seven Multi + InLab Expert Pro, Mettler Toledo GmbH, Giessen, Germany). Salinity

was continuously logged at the institute pier (<100 m distant) by GEOMAR, and dissolved inorganic nitrogen (DIN) data were obtained from the State Agency for Agriculture, Environment and Rural Areas Schleswig-Holstein (LLUR). Variations in total alkalinity (TA) and DIC were measured regularly and are presented in detail in Wahl et al. (2015a). The analyses followed the procedures recommended by Dickson et al. (2007). The water pCO₂ in the four different treatments was calculated from regular measurements of TA, DIC, pH, salinity, and temperature using the CO2SYS Excel Macro spreadsheet developed by Pierrot et al. (2006). Water motion was regularly induced by a wave generator and thereby promoted diffusion of CO₂ from the headspace into the water column.

In situ pH in the Kiel Fjord was measured close to the inlet of the flow-through. Kiel Fjord surface water pH was high (8.5) in spring (April to June) and low (7.7) in autumn (October to November). Seawater carbonate chemistry parameters (pH, pCO₂, TA, and DIC) differed among the four different treatments and seasons (Supplementary Table 2). The overall mean effect of head-space enrichment with CO₂ from ambient (380–450 ppm) to 1050–1100 ppm decreased the tank water pH by 0.18 ± 0.08 pH units (Supplementary Figure 1). Mean difference between the ambient and increased CO₂ treatments was 340 ppm CO₂ at ambient temperature and 460 ppm CO₂ at elevated temperature (M. Böttcher and V. Winde, pers. comm.). Nevertheless, the seasonal amplitude of pH and pCO₂ in the water of the KOBs was large and sometimes exceeded the treatment size. Additionally, the diurnal metabolic activity of *Fucus* altered the *in situ* seasonal fluctuations. All abiotic variables assessed in the KOB experiments are available at the PANGAEA data platform¹.

Photophysiological Responses

Photosynthetic performance was measured with two different methods, one based on *in vivo* chlorophyll *a* fluorescence measurements of photosystem II (PSII), the other one based on oxygen production. For each experiment and treatment, three *F. vesiculosus* specimens 15–25 cm long with 91 ± 30 total apices and apparently equal vigor were chosen, each individually growing on a stone (10–15 cm in diameter) from a single holdfast. These individuals were visually free of macroscopic epiphytes and epizoobenthos.

For the *in vivo* chlorophyll fluorescence measurements a portable pulse-amplitude-modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) was used. Before measurement, the tips were carefully cleaned from visible epiphytes with seawater. The vegetative tip of the longest thalli per *Fucus* individual was measured at the beginning (spring: April 4, 2013; summer: July 4, 2013; autumn: October 10, 2013; winter: January 16, 2014) and constituted the “initial state.” Every 2–4 weeks during the experiments (spring: 2 and 30 May 2013; summer: July 18, and August 15, 2013; autumn: 7 and 21 November 2013; winter: February 13 and March 13, 2014) and at the end of each experiment (spring: June 19, 2013; summer: September 17, 2013; autumn: December 18, 2013; winter: April 1, 2014) this assessment was repeated at *in situ* KOB conditions.

The determination of the potential maximum quantum yield (F_v/F_m) was performed according to Hanelt (1998). The protocol was modified as follows: after 5 min of dark adaptation and a 5-s far red light pulse, the minimal fluorescence F_0 was recorded with a pulsed measuring light (650 nm, 0.3 μmol photons m⁻² s⁻¹), followed by short pulses of completely saturating white light (0.4–0.8 s, 1,000–5,000 μmol photons m⁻² s⁻¹) to record F_m ($F_v = F_m - F_0$). The maximum quantum yield indicates the possible photosynthetic efficiency or stress level of the seaweed.

To determine changes in the photosynthetic performance, photosynthesis vs. irradiance curves (PI-curve) were calculated. Algae were exposed to eleven increasing photon flux densities (PFD) of actinic red light (6, 15, 27, 36, 53, 73, 108, 154, 227, 332, and 490 μmol photons m⁻² s⁻¹, LED 650 nm), each for 2 min. The distance between the fiber optic and thallus surface was always kept constant at 1 mm. After each light step, a saturating white light pulse was applied to define the effective PSII quantum efficiency ($\Delta F/F_m'$) followed by a far-red pulse for 3 s to re-oxidize the electron transport chain (Ihnken et al., 2010). Accordingly, the relative electron transport rate (rETR) through PSII was calculated by multiplying $\Delta F/F_m'$ with the appropriate PFD values (rETR = $\Delta F/F_m' \times \text{PFD}$) (Genty et al., 1989; Schreiber et al., 1995). PI-curves with rETR as a function of PFD were fitted after Webb et al. (1974) due to the absence of photoinhibition. From each particular curve, the maximum relative electron transport rates (rETR_{max}) as well as the initial slope alpha (α) were calculated. The light saturation coefficient of the curves (I_k) was calculated as a quotient of rETR_{max} and α (Henley, 1993). In addition, the non-photochemical quenching (NPQ) for each PFD was recorded. For a quantitative description of NPQ as a function of PFD, NPQ vs. PFD curves were fitted after Serôdio and Lavaud (2011). Depending on this model NPQ_{max} as the maximum NPQ value of the NPQ vs. PFD curve was calculated. NPQ_{max} was used as estimator of the photoprotection capacity.

Similarly, complementary energy dissipation pathways of *Fucus* individuals were investigated by using PAM measurements. For this reason, the regulated non-photochemical quenching of light energy ($Y(\text{NPQ}) = F/F_m' - F/F_m$) was calculated for each *Fucus* individual. $Y(\text{NPQ})$ is used as a measure of the overall photoprotective capacity of the photosynthetic apparatus (e.g., Lavaud et al., 2007; Goss and Lepetit, 2015). In addition, non-regulated non-photochemical quenching ($Y(\text{NO}) = F/F_m$) was determined. This energy dissipation pathway reveals by increasing $Y(\text{NO})$ values changes at the PSII antenna complex, PSII damage or impacts on the thylakoid. The third energy dissipation pathway is via photochemistry and was calculated as the effective quantum yield $Y(\text{PSII}) = (F_m' - F)/F_m'$. The $Y(\text{PSII})$ was measured at ambient light. Importantly, the total energy is conserved whereby $Y(\text{PSII}) + Y(\text{NPQ}) + Y(\text{NO}) = 1$. Photoprotection mechanisms are applying if $Y(\text{NPQ})$ increases relative to $Y(\text{NO})$ and $Y(\text{PSII})$. Conversely, if $Y(\text{NO})$ increases at the expense of $Y(\text{PSII})$ and $Y(\text{NPQ})$, this suggests photoinhibition or permanent destruction of the photosynthetic apparatus

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(Klughammer and Schreiber, 2008). Therefore, it was hypothesized that *Fucus* individuals show greater Y(NPQ) and Y(NO) at the cost of photochemistry (Y(PSII)) under elevated temperatures, especially in the summer months, as a means of dissipating excessive energy.

As the *in vivo* chlorophyll *a* fluorescence measurements by the PAM do not allow quantifying respiration, PI-curves were also measured using oxygen optodes (PreSens GmbH, Regensburg, Germany) to detect oxygen consumption in the dark and oxygen formation under increasing PFDs. The oxygen optode, as a sensor device, optically measures oxygen concentration by means of a fluorescence dye. During preliminary experiments the saturating irradiance for *Fucus* vegetative apical tips was identified by recording PI-curves with oxygen production as a function of PFD at 10°C, 15°C, and 20 ± 0.1°C. The vegetative apical thallus tips (2 ± 0.1 cm) obtained from *Fucus* individuals grown in their native habitat in the Kiel Fjord (June 2013) were kept in dimmed light for wound healing overnight in ambient water. Closed cylindrical acryl glass chambers (25 mL) were used for measuring the production-light response of the vegetative tips and a water bath which was tempered by a thermostat provided constant temperature (DC10 K10; Thermo Haake GmbH, Karlsruhe, Germany). Each apical tip was fixed horizontally on a coarse plastic mesh in the chamber, and a magnetic stirrer assured good mixing of the chamber water. Before measurement, a two-point calibration (0 and 100% oxygen saturation) was carried out. Moreover, the medium of the samples in the chambers was enriched with 2 mM NaHCO₃ (final concentration) to avoid carbon limitation during the incubation and purged with nitrogen to start measurements at oxygen saturation levels between 50 and 70%. Respiration was recorded for at least 30 min before exposure to the increasing PFD levels. Incident light on the thallus surface was supplied from 150 W halogen lamps (HLX 64634, OSRAM GmbH, Bad Homburg, Germany) provided by flexible fiber optics (KL 1500, SCHOTT AG, Mainz, Germany). Subsequently, oxygen exchange rates were calculated over 10-min intervals after acclimation (5 min) to the respective PFD. The maximum photon flux density was set at 1400 μmol photon m⁻² s⁻¹, and variable photon fluxes (0, 26, 145, 273, 474, 682, 777, and 988 μmol photon m⁻² s⁻¹) were obtained by inserting neutral density filters (Hahne Lichttechnik, Düsseldorf, Germany) between the light source (150 W halogen lamps, HLX 64634, OSRAM GmbH, Bad Homburg, Germany) and the chamber. Respiration and photosynthetic production were measured as oxygen concentration changes by using planar SP-PSt3-PSUP-YOP-D5 oxygen sensor spots (PreSens GmbH, Regensburg, Germany) inside the chambers in combination with fiber optics connecting the outside of the chambers with a fiber optic oxygen meter (OXY-4 Mini-Sensor, PreSens GmbH, Regensburg, Germany) according to Warkentin et al. (2007). A least-squares regression was fit to each of the dissolved oxygen concentrations with respect to time, and the slope was used as the photosynthesis rate. PI-curves were calculated using the photosynthesis model of Walsby (1997). From these curves different photosynthetic parameters e.g., maximum photosynthesis (P_{max}), respiration (R), light utilization coefficient (α), the light compensation point

(I_c), and the light saturation point (I_k) were calculated. These curves were measured for *F. vesiculosus* to determine the PFD for light saturation of the photosynthesis in relation to fresh biomass, which showed no significant difference in I_k among the three temperatures tested.

After the determination of I_k, the PFD was kept constant at 200 μmol m⁻² s⁻¹ and the temperature at 15 ± 0.2°C in a constant climate room for measuring the oxygen production of *F. vesiculosus* at the end of each KOB experiment in every season. For these measurements ambient water with the ion and gas composition of the KOBs was used. Therefore, any production of oxygen by microalgae or consumption by microbial organisms in ambient water is accounted for in blanks. The samples were incubated for 30 min in the dark and subsequently for 10 min in light (200 μmol m⁻² s⁻¹; 150 W halogen lamps, HLX 64634, OSRAM GmbH, Bad Homburg, Germany). The net oxygen production was referred to the water volume of each acryl glass chamber and the fresh weight per sample.

Additionally, chlorophyll *a* and *c* (chl *a* and *c*) content was extracted with 100% ethanol from lyophilized, powdered samples of each individual (ca. 100 mg) and photometrically quantified according to Ritchie (2006) at the end of each KOB experiment.

Statistical Analyses

Before statistical analyses, all data were tested for normality with the Kolmogorov–Smirnov or Shapiro–Wilk's test and for homogeneity with the Levene's test or Fligner–Killeen test to comply with requirements. Differences in the maximum quantum yield, rETR_{max} and the complementary energy dissipation pathway parameters [Y(PSII), Y(NO), and Y(NPQ)] of *Fucus* were analyzed with repeated-measures analysis of variance (rm ANOVA), with the within-subject factor time (day) and the between-subject factors pCO₂ and temperature for each experiment separately. If the assumption of sphericity (Mauchly test) was not met, the univariate approach with Greenhouse–Geisser adjusted degrees of freedom and *p*-values for the *F*-test was applied. In order to evaluate the interactive effect of temperature and pCO₂ on all photosynthetic parameters (rETR_{max}, α, I_k, NPQ_{max}, oxygen production, and chlorophyll content) at the end of every KOB experiment, two-way ANOVAs were used with temperature and pCO₂ as fixed factors. However, the two-way ANOVAs did not show significant interactions, therefore one-way ANOVAs were carried out for each factor separately. Furthermore, to assess seasonal differences of the initial photophysiological status of *Fucus* in its native habitat one-way ANOVAs were applied. When the analyses revealed significant differences, pairwise comparisons between means were further explored using a *post hoc* Tukey's honest significant difference test. Differences between sampling dates of the different seasonal experiments cannot be compared because abiotic and biotic conditions were too different between the four consecutive experiments. Data were analyzed using SPSS Statistics 22 (IBM, Armonk, NY, United States) and the R software (Version 4.0.2, R Development Core Team, 2020).

RESULTS

Photophysiological Characteristics

The photosynthetic performance measured as *in vivo* chlorophyll *a* fluorescence, oxygen production and chlorophyll concentrations of *F. vesiculosus* vegetative apical tissue varied over the four seasons and the experimental treatment. At elevated temperatures in summer (>26°C) *Fucus* individuals died and thus results from this time point are missing.

In vivo chl *a* Fluorescence Parameters (PI-Curves and Non-photochemical Quenching)

Seasonal variations in the light utilization coefficient (α), relative maximal electron transport rates ($rETR_{max}$), and saturation irradiance for photosynthesis (I_k) were evident for *Fucus* grown in its native habitat (Table 1 and Supplementary Table 3). The light utilization coefficient of vegetative *Fucus* tips was significantly lower in spring (0.68 electrons photons⁻¹) compared to the other seasons which ranged between 0.96, 1.01, and 0.93 electrons photons⁻¹ in July, October, and January, respectively (Supplementary Table 3). In addition, $rETR_{max}$

of field grown *Fucus* in spring and autumn varied around 55 $\mu\text{mol e m}^{-2} \text{s}^{-1}$ which was almost half of summer and winter where values varied around 100 $\mu\text{mol e m}^{-2} \text{s}^{-1}$ (Table 1). Light saturation coefficient of *Fucus* was lowest in autumn with 56 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ compared to the other seasons, which ranged between 79, 101, and 112 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Table 1 and Supplementary Table 3). Maximum non-photochemical quenching (NPQ_{max}) of *Fucus* in its native habitat varied significantly with season (Supplementary Table 3). In spring and winter mean NPQ_{max} of *Fucus* was approx. 4.4 and thereby was significantly lower than in summer and autumn where values varied around 6.1 (Table 1).

The relative change of *Fucus* $rETR_{max}$ during the four different experiments revealed seasonal acclimation of photosynthetic performance (Figure 1A and Table 2). *Fucus* photosynthesis grown in the KOBs showed a differential temperature optimum and/or tolerance during the different seasons. *Fucus* revealed increasing $rETR_{max}$ during the spring experiment, when there were adequate temperatures (8–16°C) for photosynthetic activity (adequate temperature range: 10–24°C, Graiff et al., 2015b) even under elevated temperature conditions (11–21°C). Although $rETR_{max}$ of *Fucus* under combined warming and acidification was slightly enhanced (26%) at the end of the spring experiment

TABLE 1 | Photophysiological responses of initial *Fucus vesiculosus* individuals growing in its native habitat ($n = 12$) and at the end of the experiments in the Kiel Outdoor Benthocosms ($n = 3$), with manipulated temperature and $p\text{CO}_2$ conditions over different seasons.

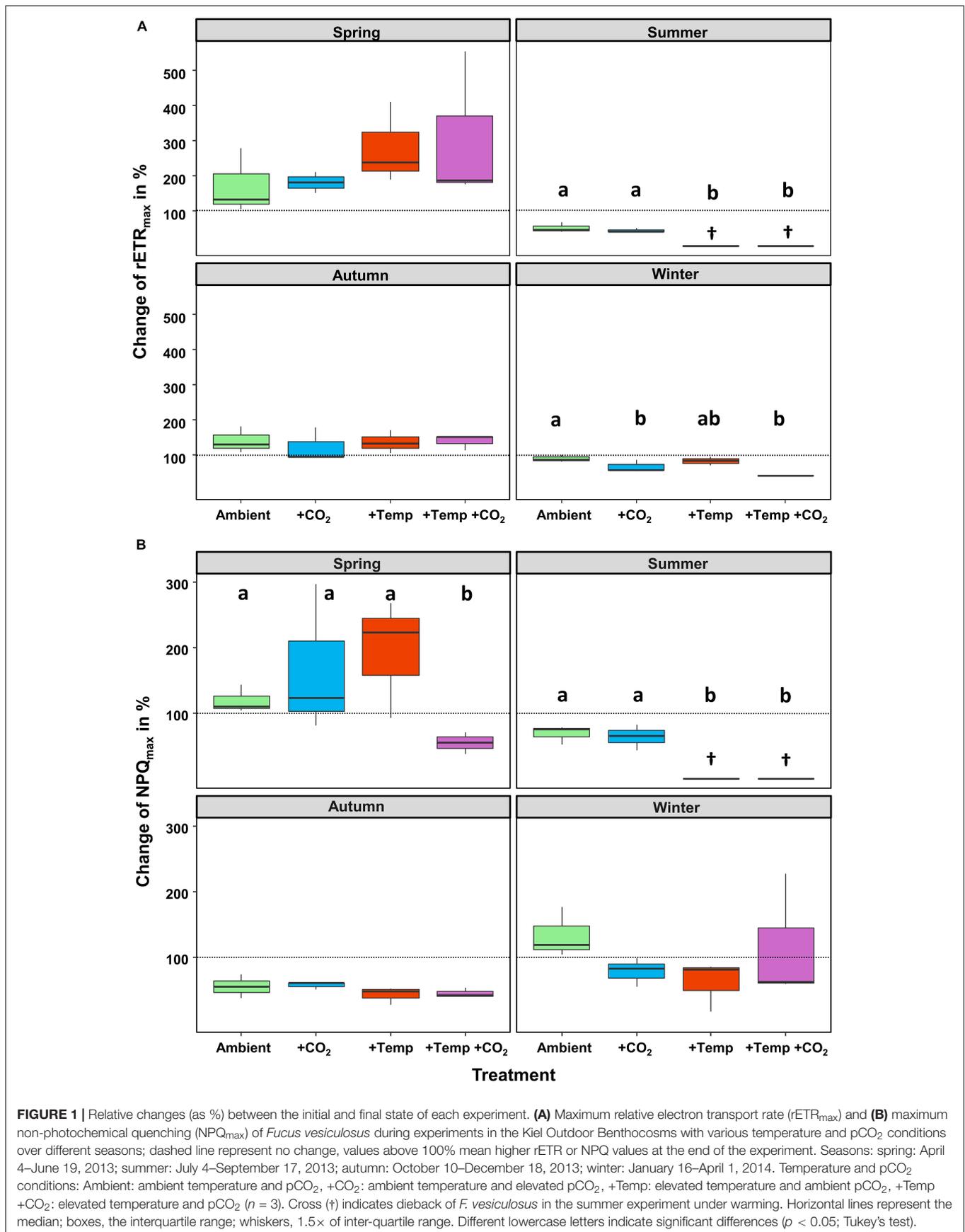
		PI-curve parameters				Non-photochemical quenching			
		α (e photons ⁻¹)		$rETR_{max}$ ($\mu\text{mol e m}^{-2} \text{s}^{-1}$)		I_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)		NPQ_{max}	
Spring	Initial	0.68 ± 0.09	A	54.06 ± 14.52	A	79.23 ± 18.88	A	4.36 ± 1.96	A
	Ambient	0.85 ± 0.73	a	102.86 ± 34.20	a	124.10 ± 52.74	a	6.19 ± 1.26	b
	+CO ₂	0.91 ± 0.11	a	94.42 ± 20.03	a	105.12 ± 31.01	a	5.27 ± 0.34	b
	+Temp	0.92 ± 0.05	a	121.11 ± 34.72	a	132.53 ± 41.65	a	5.38 ± 0.87	b
	+Temp +CO ₂	0.85 ± 0.09	a	143.27 ± 54.76	a	173.25 ± 85.27	a	2.19 ± 0.23	a
Summer	Initial	0.96 ± 0.07	BC	95.70 ± 23.93	B	100.59 ± 27.29	AB	6.10 ± 1.29	B
	Ambient	1.01 ± 0.05	b	49.83 ± 8.94	b	49.29 ± 9.57	b	4.26 ± 0.55	b
	+CO ₂	1.01 ± 0.03	b	49.93 ± 11.24	b	49.48 ± 9.85	b	4.50 ± 0.91	b
	+Temp	†	a	†	a	†	a	†	a
	+Temp +CO ₂	†	a	†	a	†	a	†	a
Autumn	Initial	1.01 ± 0.05	B	56.40 ± 10.66	A	56.09 ± 12.34	C	6.05 ± 0.66	B
	Ambient	0.86 ± 0.08	a	77.65 ± 25.77	a	97.32 ± 38.29	a	2.88 ± 0.97	a
	+CO ₂	0.94 ± 0.07	a	65.83 ± 18.85	a	71.26 ± 26.06	a	3.42 ± 0.17	a
	+Temp	0.82 ± 0.07	a	70.34 ± 10.26	a	86.63 ± 19.38	a	2.77 ± 0.73	a
	+Temp +CO ₂	0.79 ± 0.09	a	81.62 ± 15.70	a	103.94 ± 14.28	a	2.81 ± 0.45	a
Winter	Initial	0.93 ± 0.04	C	103.71 ± 16.58	B	111.50 ± 18.36	B	4.43 ± 1.12	A
	Ambient	0.89 ± 0.05	a	86.65 ± 8.02	b	96.86 ± 3.47	b	5.12 ± 0.72	a
	+CO ₂	0.87 ± 0.03	a	67.48 ± 11.11	ab	77.43 ± 13.91	ab	3.77 ± 0.91	a
	+Temp	0.79 ± 0.01	a	70.20 ± 12.82	ab	89.44 ± 18.02	b	2.67 ± 1.66	a
	+Temp +CO ₂	0.88 ± 0.11	a	50.62 ± 1.79	a	57.92 ± 6.82 a	a	3.66 ± 0.46	a

Seasons: spring: April 4–June 19, 2013; summer: July 4–September 17, 2013; autumn: October 10–December 18, 2013; winter: January 16–April 1, 2014.

Temperature and $p\text{CO}_2$ conditions: Ambient: ambient temperature and $p\text{CO}_2$, +CO₂: ambient temperature and elevated $p\text{CO}_2$, +Temp: elevated temperature and ambient $p\text{CO}_2$, +Temp +CO₂: elevated temperature and $p\text{CO}_2$. Values are means ± SD (standard deviation).

Different uppercase (comparison of initial values) and lowercase (comparison of values at the end of each experiment between treatments) letters indicate significant differences ($p < 0.05$; Tukey's test).

Cross (†) indicates dieback of *F. vesiculosus* in the summer experiment under warming.



compared to the other treatments, this enhancement was not significant (Table 1). In the course of the summer experiment, an unexpected natural heat-wave in the Kiel Fjord produced peak temperatures of 27–30°C over a period of 30 days in the experimental warming treatments (Supplementary Table 4). This period of high water temperatures resulted in a dieback of the *Fucus* individuals under increased temperature conditions. During summer, $rETR_{max}$ of *Fucus* decreased drastically from 70 to 15 $\mu\text{mol e m}^{-2} \text{s}^{-1}$ with the increasing water temperatures and PAR (Supplementary Table 5). In the autumn experiment, $rETR_{max}$ was low under all treatments revealing no influence of experimental warming (Table 2). During the winter experiment, $rETR_{max}$ of *Fucus* was slightly higher under ambient conditions, but declined under $p\text{CO}_2$ enriched conditions (Figure 1A and Table 1).

Thus, after growing in the KOBs for 3 months at the different experimental conditions during spring, autumn and winter α of the *Fucus* individuals did not significantly differ between the applied treatments (Table 1, for statistics see Supplementary Table 6). Correspondingly, $rETR_{max}$ and I_k were not significantly different between the treatments at the end of the spring and autumn experiment (Supplementary Table 6). In contrast, NPQ_{max} of *Fucus* was significantly decreased by 60% under combined warming and acidification in the spring experiment (Figure 1B and Supplementary Table 7). At the end of the winter experiment, combined warming and acidification decreased $rETR_{max}$ and I_k significantly by 40% (Table 1 and Supplementary Table 6). In addition, elevated temperature levels decreased NPQ_{max} during the winter experiment, but this reduction was not significant (Figure 1B and Supplementary Table 7).

Maximum Quantum Yield

During the spring experiment, the maximum quantum yield (F_v/F_m) of vegetative *Fucus* apices increased until the end of the season under all treatments (Figure 2 and Table 2). In the summer experiment, the F_v/F_m of *Fucus* under warming remained at a high level after 2 weeks of incubation in the KOBs (Supplementary Figure 2). However, in the subsequent weeks warming reduced the F_v/F_m of *Fucus* significantly by 40% until mid-August (Supplementary Figure 2 and Table 2). When the Fjord water temperature exhibited a natural heat-wave in the following weeks, water temperatures in the warming treatment reached maximum levels which clearly exceeded the thermal tolerance of *Fucus* (Graiff et al., 2015b). Thus, the increasing destructive influence of warming on the photochemical apparatus of *Fucus* was also indicated by significantly reduced F_v/F_m values among the measurement dates (Supplementary Figure 2 and Table 2). There was neither a stimulating nor a detrimental effect of increased $p\text{CO}_2$ on F_v/F_m of *Fucus* during the summer experiment (Figure 2). During autumn, the F_v/F_m of *Fucus* slightly decreased until the end of the experiment under all treatments (Figure 2). In the winter experiment, F_v/F_m of *Fucus* was significantly reduced among measurement dates and under warming (Supplementary Figure 2 and Table 2). However, this effect of warming on *Fucus* was slightly mitigated at increased $p\text{CO}_2$ conditions during the winter experiment, which kept the F_v/F_m at the control level (Figure 2 and Table 3).

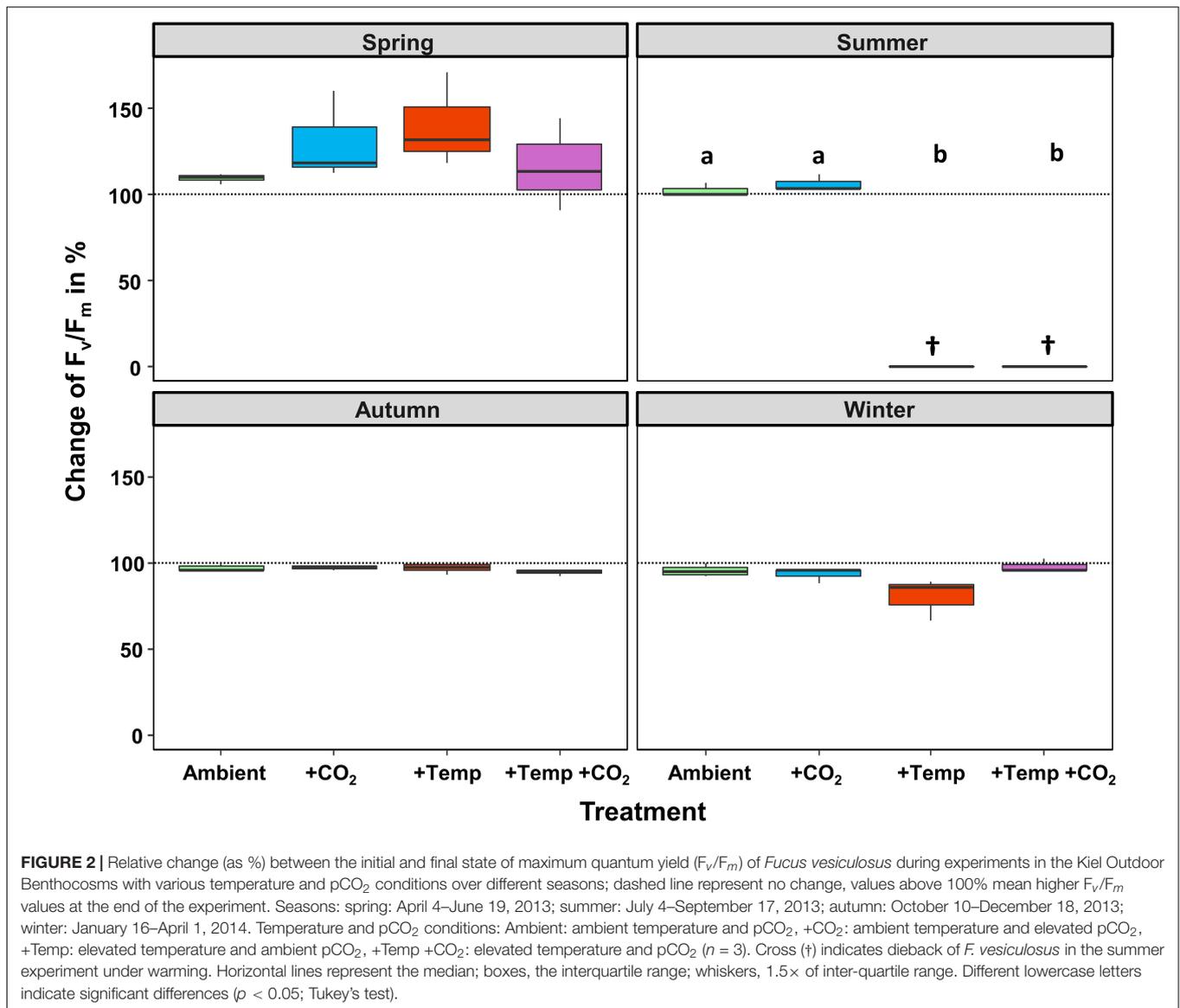
TABLE 2 | Results of repeated-measures ANOVA for effects of temperature, CO_2 and time during each experiment on maximum relative electron transport rates ($rETR_{max}$) and maximum quantum yields (F_v/F_m) in *Fucus vesiculosus* in different seasons.

Source of variation	$rETR_{max}$			F_v/F_m		
	DF	F-value	p-value	DF	F-value	p-value
(a) Spring						
Temperature	1	0.171	0.690	1	0.223	0.650
CO_2	1	1.131	0.319	1	0.206	0.662
Time	1.571	7.815	0.009	3	12.607	<0.001
Temp \times CO_2	1	4.402	0.069	1	0.650	0.444
Temp \times Time	1.571	1.235	0.313	3	0.238	0.869
$\text{CO}_2 \times$ Time	1.571	0.279	0.709	3	0.206	0.891
Temp \times $\text{CO}_2 \times$ Time	1.571	0.236	0.741	3	1.650	0.204
(b) Summer						
Temperature	1	15.128	0.005	1	137.544	<0.001
CO_2	1	1.261	0.294	1	0.197	0.669
Time	1.633	30.687	<0.001	3	102.616	<0.001
Temp \times CO_2	1	0.251	0.630	1	0.031	0.864
Temp \times Time	1.633	3.007	0.092	3	132.608	<0.001
$\text{CO}_2 \times$ Time	1.633	0.454	0.606	3	0.583	0.632
Temp \times $\text{CO}_2 \times$ Time	1.633	0.568	0.546	3	0.160	0.922
(c) Autumn						
Temperature	1	0.087	0.776	1	0.934	0.362
CO_2	1	0.009	0.925	1	1.213	0.303
Time	3	8.692	<0.001	1.740	16.910	<0.001
Temp \times CO_2	1	1.122	0.320	1	0.160	0.700
Temp \times Time	3	0.644	0.594	1.740	2.176	0.154
$\text{CO}_2 \times$ Time	3	0.223	0.879	1.740	0.337	0.690
Temp \times $\text{CO}_2 \times$ Time	3	0.320	0.811	1.740	0.721	0.485
(d) Winter						
Temperature	1	28.623	0.001	1	3.519	0.089
CO_2	1	1.106	0.324	1	15.909	0.004
Time	3	21.012	<0.001	1.475	9.696	0.005
Temp \times CO_2	1	0.748	0.412	1	10.672	0.011
Temp \times Time	3	4.721	0.01	1.475	0.864	0.415
$\text{CO}_2 \times$ Time	3	9.262	<0.001	1.475	2.254	0.155
Temp \times $\text{CO}_2 \times$ Time	3	3.156	0.043	1.475	4.447	0.045

Seasons: spring: April 4–June 19, 2013; summer: July 4–September 17, 2013; autumn: October 10–December 18, 2013; winter: January 16–April 1, 2014. Bold type indicates p-values < 0.05.

Energy Dissipation Pathways

Fucus apical tips showed variable energy distribution among the complementary energy dissipation pathways under natural ambient light: photochemistry Y(PSII), non-regulated non-photochemical quenching Y(NO) and regulated non-photochemical quenching Y(NPQ) at PS II centers during the different seasons and treatments (Figure 3). During spring Y(PSII) slightly and Y(NPQ) significantly increased as Y(NO) significantly decreased until the end of the experiment, emphasizing acclimation processes of *Fucus* (for statistics see Supplementary Tables 8–10). The regulated non-photochemical quenching Y(NPQ) was significantly reduced under warming in the spring experiment (Supplementary Table 10).



In summer, effective quantum yield of PSII (photosynthetic efficiency in the light) of *Fucus* was significantly decreased by warming and time (**Supplementary Table 8**). Also, regulated non-photochemical quenching $Y(\text{NPQ})$ was decreased by warming (**Supplementary Table 10**). However, under ambient summer conditions $Y(\text{NPQ})$ increased significantly until the end of the experiment (**Supplementary Table 10**), reflecting down-regulation of PS II as a protective mechanism against excess photon fluence rates. Non-regulated non-photochemical quenching $Y(\text{NO})$ was enhanced by warming and time (**Supplementary Table 9**). Thus, photoinhibition or permanent destruction of the photosynthetic apparatus was obvious under elevated temperature levels on day 42 (**Figure 3**, see black arrows).

Non-regulated non-photochemical quenching of *Fucus* increased (**Supplementary Table 9**) at the expense of regulated non-photochemical quenching (**Supplementary Table 10**), but

effective quantum yield was kept constant under all treatments during the autumn experiment (**Figure 3**). In winter $Y(\text{PSII})$ was significantly decreased by warming and time until the end of the experiment (**Supplementary Table 8**). Especially, under elevated temperature levels in winter non-regulated non-photochemical quenching $Y(\text{NO})$ increased significantly among the measurement dates (**Figure 3**, see black arrow and **Supplementary Table 9**), suggesting that both photochemical energy conversion and protective regulatory mechanisms were inefficient.

Net Oxygen Production

Net oxygen production of *Fucus* showed a clear seasonal pattern, with highest oxygen production in spring ($50\text{--}170 \mu\text{mol O}_2 \text{ g}^{-1} \text{ FM h}^{-1}$) compared to the other seasons which ranged between 29 and $105 \mu\text{mol O}_2 \text{ g}^{-1} \text{ FM h}^{-1}$ (**Figure 4**). In spring, increased CO_2 slightly enhanced oxygen production of

TABLE 3 | Results of (A) one-way ANOVAs and (B) Tukey's test for effects of manipulated temperature and CO₂ conditions on maximum quantum yields (F_v/F_m) in *Fucus vesiculosus* over different seasons.

(A)	Source of variation	DF	Mean squares	F-value	p-value
Fv/Fm	(a) Spring				
	Treatments	3	582.3	1.075	0.413
	Residual	8	541.9		
	(b) Summer				
	Treatments	3	10883	1012	<0.001
	Residual	8	11		
	(c) Autumn				
	Treatments	3	5.025	0.886	0.488
	Residual	8	5.670		
	(d) Winter				
	Treatments	3	189.5	3.715	0.061
	Residual	8	51.0		
(B)	Season	treatments	difference	p-value	
Fv/Fm	Summer	Ambient vs. +CO ₂	-3.74	0.534	
		Ambient vs. +Temp	102.42	<0.001	
		Ambient vs. +Temp +CO ₂	102.42	<0.001	
		+CO ₂ vs. +Temp	-106.16	<0.001	
		+CO ₂ vs. +Temp +CO ₂	-106.16	<0.001	
		+Temp vs. +Temp +CO ₂	>0.001	1.000	

Seasons: spring: April 4–June 19, 2013; summer: July 4–September 17, 2013; autumn: October 10–December 18, 2013; winter: January 16–April 1, 2014.

Temperature and pCO₂ conditions: Ambient: ambient temperature and pCO₂, +CO₂: ambient temperature and elevated pCO₂, +Temp: elevated temperature and ambient pCO₂, +Temp +CO₂: elevated temperature and pCO₂.

Bold type indicates p-values < 0.05.

Fucus under ambient as well as increased temperature conditions, however this tendency was not significant. During autumn and winter there were no differences between the treatments (Figure 4 and Supplementary Table 11).

Chlorophyll a and c

Chlorophyll *a* and *c* contents were high at the end of the spring experiment and decreased during summer to minimal values (Table 4). In the subsequent autumn experiment chlorophyll content of *Fucus* increased again and reached highest values at the end of the winter experiment. However, chl *a* and *c* content did not significantly differ between the treatments of each seasonal experiment (Table 4).

DISCUSSION

Photosynthetic performance and efficiency (measured as net oxygen production, rETR_{max} and F_v/F_m) of *Fucus* in the KOBs under ambient conditions was highest in spring/early summer when annual growth activity of the species commenced and water temperature as well as solar irradiance increased naturally, while it was lowest in winter (December to January/February). Temperature had a stronger effect than pCO₂ on photosynthetic performance of *Fucus* in all seasons. Photophysiological responses were generally

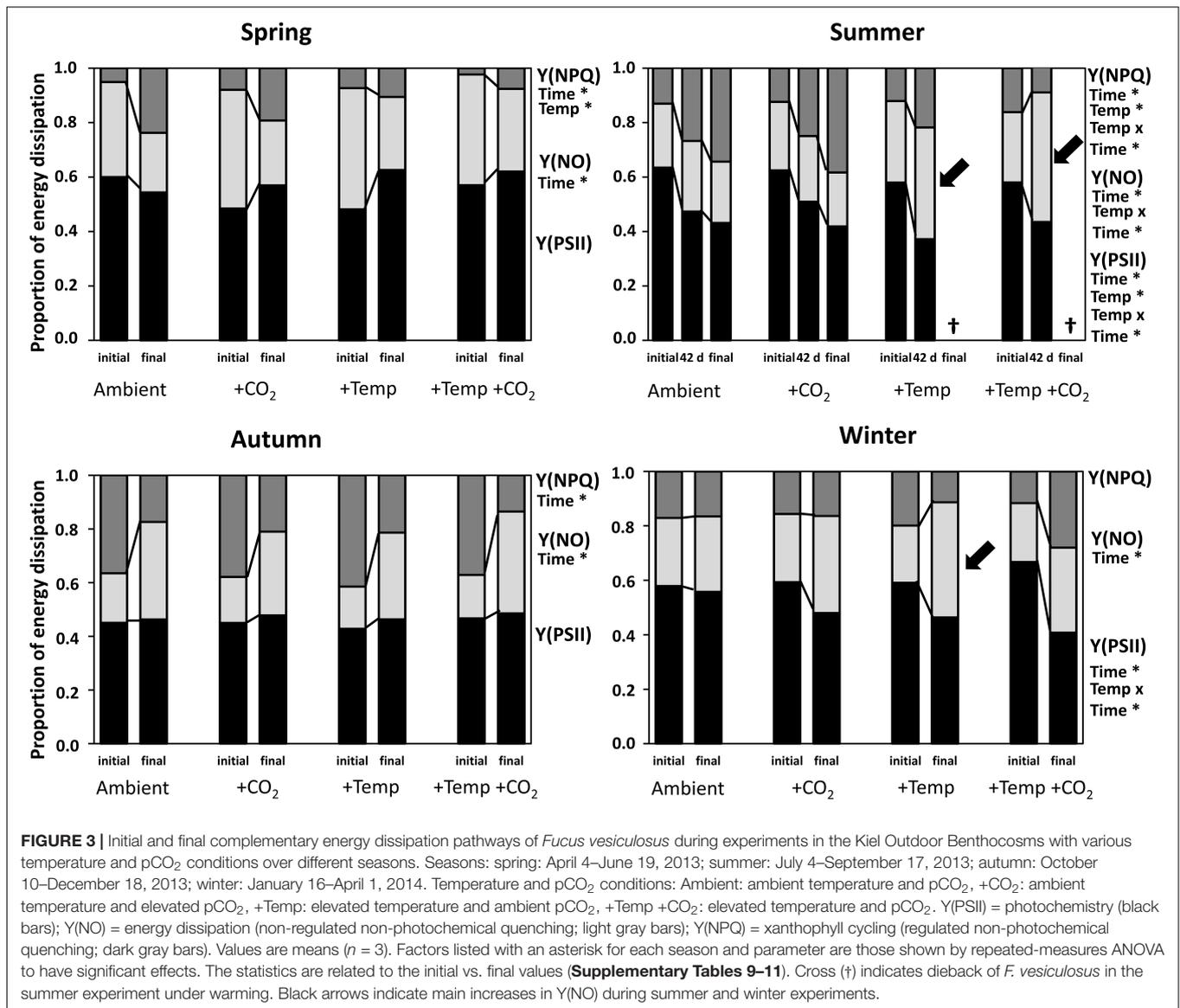
positive during the cooler spring months, but strongly negatively affected during summer (due to a marine heat-wave). Especially, future summer temperatures exceeded the thermal tolerance threshold of western Baltic Sea *Fucus* and had a deleterious impact overall. In contrast to the expectation that warmer winter conditions might be beneficial for *Fucus*, increased temperature conditions in combination with sub-optimal low winter light conditions decreased photophysiological performance. Overall, the results of this study suggest potential benefits of the combination of future ocean warming and increased pCO₂ over most of the year for *Fucus* photophysiological performance, but not during summer peak temperatures.

Seasonal Photosynthetic Acclimation

Photosynthetic parameters indicate that *Fucus* metabolism under ambient conditions became up-regulated during spring and down-regulated during winter (December to January/February). Net oxygen production, rETR_{max} and chlorophyll content of *Fucus* declined from high values at the end of the spring experiment (June) to minimal values at the end of the summer experiment (September). As a consequence, *Fucus* individuals accumulated high quantities of mannitol as main photosynthetic product over spring until June and exhibited low quantities in December (Graiff et al., 2015a). *Fucus* had lowest rETR_{max} and light saturation coefficients (I_k) as well as a very low chlorophyll content in summer under high irradiances compared to all other seasons. Concordantly, Rothäusler et al. (2018) stated that *Fucus* needs low to moderate irradiances to reach maximum photosynthesis. For most seaweeds growing in the shallow subtidal, the available PAR during summer is often far exceeding values needed to saturate photosynthesis (Franklin and Forster, 1997). Excess irradiance may induce photo-oxidative damage via increased production of ROS, causing pigment bleaching and death in extreme cases (Müller et al., 2001). In order to avoid irradiance stress, seaweeds must acclimate to changes in irradiance by optimizing photosynthesis.

The different energy dissipation pathways including regulated non-photochemical quenching (NPQ) of *Fucus*, are subjected to a seasonal cycle, with spring/summer maxima and a minimum in December. NPQ is an important, rapidly inducible, photoregulation mechanism to optimize light utilization, serving to prevent or reduce chronic photoinhibition and maximize photosynthetic efficiency under high irradiances (Müller et al., 2001; Wilhelm and Selmar, 2011; Lavaud and Lepetit, 2013). NPQ is a proxy of xanthophyll cycling processes which dissipate excess irradiance energy as heat to protect the photosynthetic apparatus from over-excitation (Hanelt et al., 1993; Franklin and Forster, 1997; Lavaud et al., 2002a,b; Ruban et al., 2007; Lavaud and Lepetit, 2013). Therefore, a reduced rETR_{max} and NPQ_{max} during summer indicated that seasonal acclimation of *Fucus* photochemistry was limited, probably due to the natural heat-wave in the Kiel Fjord in combination with excess summer irradiance.

In winter during low-light conditions, a reversed acclimation pattern is expected in order to allow efficient light harvesting



at reduced irradiance levels. Photoacclimation can be obtained through either an alteration in the size or number of photosynthetic units (Richardson et al., 1983; Falkowski and LaRoche, 1991; Beer et al., 2014). Under low-light conditions like in winter, an increase in photosynthetic unit sizes is reflected by an increase in light utilization efficiency (α), but a decline in maximal photosynthesis (P_{\max} or $rETR_{\max}$) (Richardson et al., 1983; Beer et al., 2014). Higher light-harvesting capability in winter months was indicated for *Fucus* through lowered $rETR_{\max}$ and I_k but higher chlorophyll content compared to the other seasons under ambient conditions. In conclusion, seasonal acclimation of photochemistry was apparent in *Fucus*. Data indicate that photoacclimation under ambient conditions maximizes both photoprotection via NPQ during high-light at the end of spring and up-regulation of light-harvesting capability during low-light winter months (December to January). These photoacclimation processes reflect an important physiological

trait of *Fucus* to maintain photosynthesis over a seasonal cycle of highly fluctuating environmental conditions.

Photosynthesis Under Ocean Warming and Acidification

Relative maximum electron transport rate and F_v/F_m of *Fucus* increased within a temperature range of 13–24°C and thereby experimental warming was even beneficial for photosynthesis in spring as it was still in the range of optimum temperatures for photosynthesis. This positive effect of warm temperatures > 20°C on photosynthetic performance is attributed to its role in carbon fixation (Davison et al., 1991). Warm temperatures positively affect the availability of inorganic carbon (Surif and Raven, 1990) and the rate of carbon fixation by RuBisCO (Sukenik et al., 1987).

Similarly, in the first 2 weeks of the summer experiment (early July) $rETR_{\max}$ and F_v/F_m of *Fucus* remained at similarly high

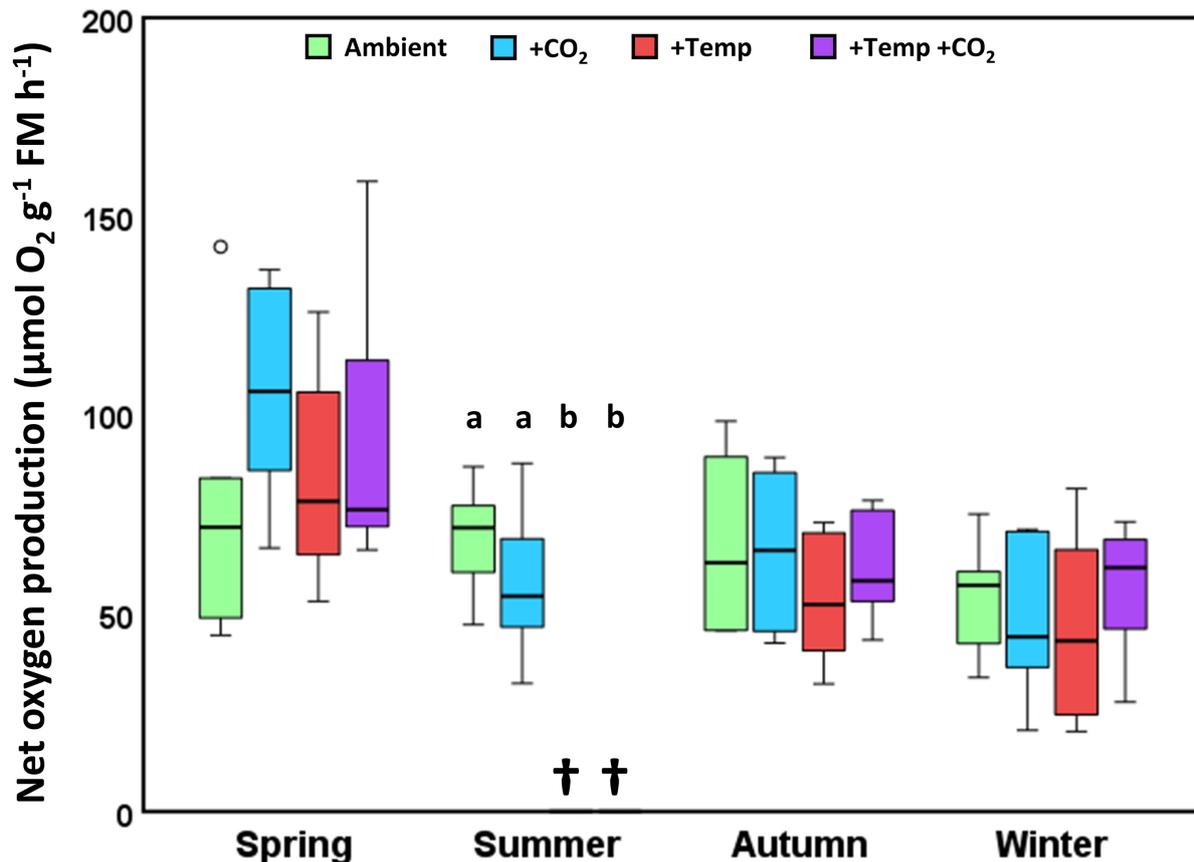


FIGURE 4 | Net oxygen production of *Fucus vesiculosus* individuals at the end of the experiments in the Kiel Outdoor Benthocosms (KOBs), with various temperature and pCO₂ conditions over different seasons. Experimental individuals were measured at 15°C and 200 µmol photons m⁻² s⁻¹ after 3 months of incubation in the KOBs. Seasons: spring: April 4–June 19, 2013; summer: July 4–September 17, 2013; autumn: October 10–December 18, 2013; winter: January 16–April 1, 2014. Temperature and pCO₂ conditions: Ambient: ambient temperature and pCO₂, +CO₂: ambient temperature and elevated pCO₂, +Temp: elevated temperature and ambient pCO₂, +Temp +CO₂: elevated temperature and pCO₂ (*n* = 3). Different lowercase letters indicate significant differences (*p* < 0.05; Tukey's test). Horizontal lines represent the median; boxes, the interquartile range; whiskers, 1.5× of inter-quartile range; circle, the outlier. Cross (†) indicates dieback of *F. vesiculosus* in the summer experiment under warming.

levels under the warming scenario as under ambient temperature conditions. In contrast, growth of *Fucus* under the same warming scenario stopped when temperatures increased above 24°C (Graiff et al., 2015a). This temperature was identified as a critical thermal threshold in the laboratory (Graiff et al., 2015b). Optimal temperature for photosynthetic performance (24°C) is higher and the optimal temperature range of photosynthesis much narrower compared to that for growth (15–20°C) in *Fucus* (Graiff et al., 2015b) as both physiological processes are not directly coupled (Kübler et al., 1991; Eggert and Wiencke, 2000; Eggert, 2012).

With rising temperatures, up to the thermal threshold of 26°C, *Fucus* exhibited increasing electron transport rates, followed by a rapid decline (Graiff et al., 2015b). A similar pattern was also found by Figueroa et al. (2019) for *Fucus serratus*. This sudden reduction in photosynthetic capacity at temperatures >26°C can be attributed to several factors such as temperature sensitivity of enzymes for carbon fixation, photophosphorylation and/or the thermal stability of PSII (Lynch and Thompson, 1984; Davison

et al., 1991; Wahid et al., 2007). The damaging influence of elevated temperature levels on the photochemical apparatus of *Fucus* became apparent by significantly reduced F_v/F_m and the increase of non-regulated non-photochemical quenching $Y(NO)$. When Kiel Fjord temperatures reached 27–30°C under the warming scenario during a natural heat-wave (late July to early August) (Supplementary Table 5) the thermal range exceeded the tolerance or lethal limit of *Fucus* (Graiff et al., 2015b) and this seaweed died. In conclusion, at temperatures exceeding 26°C *Fucus* from the western Baltic Sea was unable to acclimate.

Despite the fact that *Fucus* is already at risk to decline in abundance or vanish over summer in the shallow subtidal of the Baltic Sea, it may theoretically benefit from mild winter temperatures (Bartsch et al., 2012) as the duration of favorable temperatures for growth may increase (BACC II Author Team, 2015). In contrast to these expectations, during the winter experiment warming decreased F_v/F_m , and NPQ_{max}, and the mannitol content of *Fucus* was significantly lowered (Graiff et al., 2015a). This situation together with an increased

TABLE 4 | Chlorophyll *a* and *c* contents (mg chl g⁻¹ dry mass) of *Fucus vesiculosus* individuals at the end of the experiments in the Kiel Outdoor Benthocosms, with various temperature and pCO₂ conditions over different seasons.

		chl <i>a</i> (mg chl g ⁻¹ DM)	chl <i>c</i> (mg chl g ⁻¹ DM)
Spring	Ambient	3.11 ± 0.83	0.18 ± 0.09
	+CO ₂	3.52 ± 0.89	0.19 ± 0.05
	+Temp	2.96 ± 0.56	0.12 ± 0.06
	+Temp +CO ₂	3.03 ± 0.95	0.11 ± 0.10
Summer	Ambient	0.89 ± 0.57	0.03 ± 0.01
	+CO ₂	1.65 ± 0.78	0.08 ± 0.03
	+Temp	†	†
	+Temp +CO ₂	†	†
Autumn	Ambient	2.40 ± 0.23	0.06 ± 0.02
	+CO ₂	2.18 ± 0.34	0.05 ± 0.03
	+Temp	2.52 ± 0.56	0.16 ± 0.07
	+Temp +CO ₂	2.69 ± 0.66	0.25 ± 0.14
Winter	Ambient	4.66 ± 0.62	0.18 ± 0.06
	+CO ₂	3.71 ± 0.44	0.23 ± 0.06
	+Temp	3.97 ± 1.19	0.28 ± 0.11
	+Temp +CO ₂	3.72 ± 0.40	0.28 ± 0.04

Seasons: spring: April 4–June 19, 2013; summer: July 4–September 17, 2013; autumn: October 10–December 18, 2013; winter: January 16–April 1, 2014.

Temperature and pCO₂ conditions: Ambient: ambient temperature and pCO₂, +CO₂: ambient temperature and elevated pCO₂, +Temp: elevated temperature and ambient pCO₂, +Temp +CO₂: elevated temperature and pCO₂.

Median values ± MAD (median absolute deviation; *n* = 3). There were no significant differences among treatments within a sampling date (*p*-value > 0.05; one-way ANOVA).

Cross (†) indicates dieback of *F. vesiculosus* in the summer experiment under warming.

non-regulated non-photochemical quenching Y(NO) under increased temperature conditions in winter, clearly indicates a reduced functionality of the photosynthetic apparatus. This negative impact of elevated winter temperature on *Fucus* performance seems conflicting, as the simulated warming increased temperatures from ambient 4–7°C to 8–12°C (December to January) and thereby shifting toward the optimum temperature range of *Fucus* growth (Graiff et al., 2015b). There might be a mismatch between limited photosynthesis due to low-light conditions in winter and enhancement of general metabolic activity during warmer winters that might explain this imbalance (Rohde et al., 2008). This response may also indicate that *Fucus* has seasonally varying temperature optima for photosynthesis which is known e.g., for subtidal red algae (Mathieson and Norall, 1975). Seaweeds sampled during winter exhibited higher rates of apparent photosynthesis at low water temperature, lower temperature optima, and a reduced tolerance to high temperatures than specimens sampled during summer (Mathieson and Norall, 1975).

Net oxygen production and chlorophyll *a* fluorescence parameters obtained from apical tissue of *Fucus* were not significantly enhanced under acidification in the KOB experiments during all seasonal experiments. The lack of significant effects of acidification on *Fucus* photophysiological performance in the present study may be due to *F. vesiculosus* already being adapted to natural high and strongly fluctuating

pCO₂ and pH conditions in their native habitat in the Kiel Fjord (Thomsen et al., 2010; Saderne, 2012; Melzner et al., 2013). In the spring experiment, however, acidification slightly enhanced net oxygen production of *Fucus*, which is in accordance with Olischläger et al. (2012), who found a tendency of increased net photosynthesis of young vegetative sporophytes of *Laminaria hyperborea* at elevated pCO₂. The effect of stimulating photosynthesis and relative electron transport rates under increasing external DIC was described in different brown algae (Forster and Dring, 1992; Nygård and Dring, 2008; Johnson et al., 2012). Especially, the kelps *Laminaria digitata* and *Saccharina latissima* as well as Baltic *F. vesiculosus* reacted to moderately increased DIC with enhanced rates of photosynthesis and carbon acquisition (Schmid et al., 1996; Klenell et al., 2004; Nygård and Dring, 2008). Under elevated pCO₂, it is presumed, that photorespiration is reduced and less energy is needed for restoring the internal carbon storage after periods of high photosynthetic activity. Increased pCO₂ can also trigger changes in pigment composition and chlorophyll content which are a frequently observed responses in different algal species as part of an acclimation mechanism (García-Sánchez et al., 1994; Gordillo et al., 2003). However, *F. serratus* (Johnston and Raven, 1990) and *F. vesiculosus* (Takolander et al., 2019 and the present study) did not show any changes in their pigment concentration under increased pCO₂.

The present study reveals new details about the combined effects of ocean warming and acidification on the seasonal regulation of the photophysiological performance of *Fucus* (i.e., net oxygen production, chlorophyll *a* fluorescence parameters, energy dissipation pathways and chlorophyll content) which are otherwise not well studied and understood (Raven et al., 2011; Kübler and Dudgeon, 2015; Ji and Gao, 2020). It was predicted that under light saturation like in late spring and early summer, enhanced pCO₂ in combination with warming may have positive synergistic effects on seaweed performance (Kübler and Dudgeon, 2015), as previously reported for filamentous turf algae (Connell and Russell, 2010). In the KOBs photosynthetic performance of *Fucus* under warming was not synergistically enhanced by acidification in spring and summer which might be a consequence of photorespiration. The latter physiological process is interactively regulated by increasing pCO₂ and rising temperatures (Sage and Kubien, 2007). The pCO₂ treatments were quite variable in the KOBs due to natural pCO₂ fluctuations in the Kiel Fjord water; this may have masked physiological responses of *Fucus*, but better represent their ecological *in situ* response pattern.

CONCLUSION

Global warming will elevate maximum temperatures during all seasons, such that sub-lethal and lethal conditions may become more frequent and sustained, especially over summer (Meehl and Tebaldi, 2004; Vasseur et al., 2014). Understanding the mechanisms of tolerance and species acclimation to these extremes and also to prolonged sub-lethal conditions or altered optimum is crucial for predicting climate change impacts,

particularly for species growing close to their thermal limits (Collier et al., 2011; Brodie et al., 2014). *Fucus* in the Baltic already lives close to its upper thermal tolerance limit in summer and probably cannot cope with such elevated average temperatures and expected increasing heat-wave frequency in the shallow subtidal of the future Baltic Sea. The results of the current study and of Graiff et al. (2015a,b) as well as Wahl et al. (2019) indicate that future ocean warming effects on *Fucus* will be strongest during summer. The expected combined increase in warming and pCO₂ will have slightly positive effects in spring, autumn and winter for *Fucus* as revealed by photophysiological performance (this study), growth (Graiff et al., 2015a) and fertility (Graiff et al., 2017). This suggests that *Fucus* populations may have a better start into the next growing season under the tested global change conditions. Although frequency and intensity of summer heat-waves approaching lethal temperatures may probably be the limiting factor for overall *Fucus* population's persistence.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found below: PANGAEA <https://doi.org/10.1594/PANGAEA.842719>.

AUTHOR CONTRIBUTIONS

AG, UK, and IB designed the study. AG performed the experiments and wrote the manuscript, with UK, IB, and KG

providing substantial contributions. AG and KG analyzed the data. UK and IB provided funding and intellectual input into the analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.666493/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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