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Seasonal interactive effects of pCO_2 and irradiance on the ecophysiology of brown macroalga *Fucus vesiculosus* L.

Running head: Seasonal changes outweigh CO_2 effects on *Fucus vesiculosus*

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Supplementary material of the article can be found at the end of the pdf document.

Keywords: Baltic Sea, climate change, *Fucus vesiculosus*, CO_2, ocean acidification, PAM fluorometry

Abstract

Stochastic upwelling of seawater in the Baltic Sea from the deep, anoxic bottoms may bring low-pH water rich in CO_2 close to the surface. Such events may become more frequent with climate change and ongoing ocean acidification (OA). Photoautotrophs, such as macroalgae, which are important foundation species, have been proposed to benefit from increased carbon availability due to reduced energetic cost in carbon acquisition. However, the exact effects of CO_2 fertilization may depend on the ambient light environment, as photosynthesis rates depend on available irradiance. In this experimental study, interacting effects of CO_2 addition and irradiance on the habitat-forming macroalga *Fucus vesiculosus* were investigated during two seasons – winter and summer – in the northern Baltic Sea.

Growth rates remained unaffected by CO_2 or irradiance during both seasons, suggesting that direct effects of elevated CO_2 on mature *F. vesiculosus* are small. Increases in CO_2 affected algal elemental ratios by increasing carbon and decreasing nitrogen content, with resulting changes in the C:N ratio, but only in winter.

In summer, chlorophyll *a* content increased under low irradiance. Increases in CO_2 caused a decline in light-harvesting efficiency (decrease in F_v/F_m and α) under high irradiance in summer, and conversely increased α under low irradiance. High irradiance caused increases in the maximum relative electron transport rate ($rETR_{max}$) in summer, but not in winter.

Differences between winter and summer indicate that *F. vesiculosus* responses to CO_2 and irradiance are season-specific. Increases in carbon content during winter could indicate slightly positive effects of CO_2 addition in the long run if the extra carbon gained may be capitalized in growth.

The results of this study suggest that increases in CO_2, either through upwelling or OA, may have positive effects on *F. vesiculosus*, but these effects are likely small.

1. Introduction

The Baltic Sea is one of the world's largest brackish water pools, characterized by low salinity and low dissolved inorganic carbon (DIC) content compared to ocean areas (Thomas and Schneider 1999, Snoeijs-Leijonmalm *et al*. 2017). The Baltic Sea has suffered heavily from anthropogenic eutrophication, which has caused hypoxia and anoxia in deep water, and subsequently led to the occurrence of spreading dead zones on the seafloor (Conley *et al*. 2011, Carstensen *et al*. 2014, van Helmond *et al*. 2018). Seawater upwelling from these areas may cause water low in O_2 and rich in CO_2 to also affect coastal littoral communities (Saderne *et al*. 2013). Coastal eutrophication has caused hypoxic conditions also in shallow coastal areas, as drifting macroalgal mats decompose, causing declines in O_2, elevated pCO_2 and declining pH (Bonsdorff *et al*. 1997, Sunda and Cai 2012).

Coastal ecosystems have highly variable ambient CO_2 concentrations, caused by seasonal changes in photosynthesis, respiration and upwelling (Thomas & Schneider 1999, Borges *et al.* 2006, Omstedt *et al.* 2009). Future seasonal pH fluctuations in the Baltic Sea are expected to increase in magnitude under the combined effects of eutrophication and climate change, with concomitant decreases in mean pH (Omstedt *et al.* 2012). Ocean acidification (OA) may be especially rapid in the Baltic Sea, as cold water readily absorbs CO_2, and the northern parts in particular have low water alkalinity (Müller *et al.* 2016), which intensifies the expected pH decreases by OA (Omstedt *et al.* 2010). Declining sea ice extent may concurrently increase light availability during, for

example, low pH periods in winter. On the other hand, intensifying eutrophication by climate change (Meier *et al.* 2012, Neumann *et al.* 2012) may cause diminished underwater light conditions. Hypoxia, a major consequence of eutrophication in the Baltic, may significantly enhance OA impacts, and stochastic seawater pulses with extremely high CO_2, originating from hypoxic bottom areas, may affect Baltic coastal biotic communities in the future (Melzner *et al.* 2013). Thus both the underwater light regime and CO_2 availability are expected to change in the future Baltic.

The DIC pool of seawater consists of three different fractions: carbonic acid (H_2CO_3); bicarbonate (HCO_3^-); and carbonate (CO_3^{2-}) (Fabry *et al.* 2008). Although seawater has a high supply of inorganic carbon compared with air, the majority of this DIC pool is in the form of HCO_3^-, which needs to be converted to CO_2 before it can be utilized by the Rubisco of primary producers as a carbon source (Kirk 2011).

CO_2 diffusion in seawater is an order of magnitude slower than in air (Falkowski & Raven 2007), meaning that when photosynthesis rates are high, carbon may be locally depleted in the immediate proximity of a photoautotroph. In highly productive shallow water habitats, free CO_2 may also be scarce, as it is taken up for photosynthesis (Middelboe & Hansen 2007). To avoid potential carbon limitation, many primary producers, such as macroalgae, have evolved carbon concentrating mechanisms (CCMs) that increase free CO_2 concentrations near the Rubisco binding site, allowing organisms to also utilize HCO_3^- as a carbon source (Raven & Hurd 2012), with the energetic cost associated with production and upkeep of CCMs (Raven *et al.* 2014).

Despite possessing CCMs, the photosynthesis of most macroalgal species studied does not appear to be saturated at present levels of inorganic carbon in the seawater (Koch *et al.* 2013). Climate change will increase CO_2 availability in seawater, as atmospheric dissolution of CO_2 increases (Orr *et al.* 2005). This has been suggested to confer energetic advantages to primary producers, as they could potentially downregulate CCM utilization (Koch *et al.* 2013). Increasing diffusive CO_2 usage as a carbon source would be more energy efficient in comparison to relying on active HCO_3^- transport as a carbon source for photosynthesis (Cornwall *et al.* 2012).

Different macroalgal species show variable responses to increased CO_2 availability, studied mainly in the context of OA. These may be caused by an interplay between various environmental factors and variable species-specific traits in carbon metabolism (Cornwall *et al.* 2012). Species with active CCMs, often residing under high irradiance, may benefit from CO_2 fertilization (Mercado & Gordillo 2011), as energy-consuming CCM may be downregulated under increased CO_2 concentrations, allowing improved carbon energetics (Raven *et al.* 2011). On the other hand, increased CO_2 may lower tolerance against high light intensities (Liu *et al.* 2012), as CCM acts as a sink for excessive energy (Wu *et al.* 2008). Indeed, marine primary production has been predicted to decrease under coupled high irradiance and high CO_2 predicted for the future, as primary producers downregulate photosynthetic machinery (Gao *et al.* 2012). It is thus likely that the exact effects of CO_2 availability on macroalgal physiology depend on irradiance (Verspagen *et al.* 2014, Celis-Plá *et al.* 2015, Kübler & Dudgeon 2015).

Fucus vesiculosus L. is the major habitat-forming macroalga in the northern Baltic Sea (Waern 1952, Kautsky *et al.* 1992), harbouring a rich community of associated floral and faunal species (Schagerström *et al.* 2014). In the Baltic, *F. vesiculosus* has suffered from eutrophication, and may be threatened by climate change (Vuorinen *et al.* 2015, Takolander *et al.* 2017a, Rugiu *et al.* 2018). *F. vesiculosus* has a highly efficient CCM (Giordano & Maberly 1989, Surif & Raven 1989); nevertheless, the photosynthesis of the Baltic Sea populations is carbon limited due to the low

carbon content of brackish water (Raven & Samuelsson 1988). Permanently submerged Baltic populations undergo substantial fluctuations in environmental conditions in different seasons, and the ecophysiology of *F. vesiculosus* also shows seasonal variability, with plants storing nitrogen and mannitol (carbon sink), which can be utilized under times of diminished external supply (Lehvo *et al.* 2001). Studies on OA effects (CO_2 fertilization) on *F. vesiculosus* have yielded mixed results, showing reduced growth (Gutow *et al.* 2014), no effect (Pajusalu *et al.* 2013) or increased juvenile survival in particular seasons (Al-Janabi *et al.* 2016b), reviewed in detail in Takolander *et al.* (2017b).

Interacting drivers may have synergistic effects (Wahl *et al.* 2011), and quantifying such responses on foundation species has high ecological importance. As the ecophysiology of *F. vesiculosus* shows seasonal variations, the responses to such interactions may differ per season (Al-Janabi *et al.* 2016b, Werner *et al.* 2016). OA effects on *F. vesiculosus* have been investigated in earlier studies, but the interactive effects of light and CO_2 remain unanswered, although these might explain some of the contrasting responses observed. In this study, we investigate the ecophysiological responses of *F. vesiculosus* to interacting effects of two environmental variables that are expected to change in the future (light and CO_2) during two seasons (winter and summer) in the northern Baltic Sea. We hypothesized that the effects of increasing CO_2 availability would have positive effects for *F. vesiculosus*, but only under low irradiance.

2. Methods

2.1. Experimental set-up and seawater parameters

The experiment was performed in two seasons – winter and summer – at Tvärminne Zoological Station (TZS), SW Finland. The winter experiment was conducted in November/December 2015 and the summer experiment in June 2016. The duration of the experiments was 18 days in winter and 22 days in summer.

The algae were collected with a rake or by snorkelling from nearby islands – Brännskär and Granbusken. Only vegetative tips free of epiphytes were sampled. The individuals collected were kept fully submerged and were rapidly transported to the laboratory, where they were placed in constant seawater flow-through for four days until the onset of the experiments with light conditions and light/dark rhythm as described later in this section. The experimental treatments consisted of two factors: OA treatment (three pCO_2 levels) and light treatment (two levels).

F. vesiculosus fronds (all fronds lacked vesicles, and thus they remained at the bottom of the jars) were placed in one-L glass jars. The jars were placed under two light regimes: 'high' and 'low' irradiance, provided by Philips TL-D Super 80 58W 830 fluorescent light bulbs, with 8:16 h (winter) and 16:8 h (summer) light/dark rhythm. Irradiance was monitored with HOBO Pendant UA-002-64 temperature and light loggers (Onset Computer Corporation), which were calibrated against a factory-calibrated light sensor (LI-COR LI-1500) using the exponential decay fit function suggested by Long *et al.* (2012). Irradiances measured at the water surface of the jars were 165 µmol photons m^2s^{-1} and 81 µmol photons m^2s^{-1} (winter) and 198 µmol photons m^2s^{-1} and 131 µmol photons m^2s^{-1} (summer) for high and low light treatments, respectively. These light levels correspond approximately to field light conditions at 3-m depth in June and at 0.5-m depth in January (Lindström 2000).

In the winter experiment, a single frond was placed in one jar, and in the summer experiment, three marked individuals were placed in each jar. Each light treatment contained 30 jars, with 10

replicate jars for each OA treatment level. Mean algal biomass per jar at the beginning of the experiment was 1.02 ± 0.3 g in winter and 2.35 ± 0.7 g in summer.

CO_2 treatments were administered by adjusting pH through bubbling gaseous CO_2 into seawater with Aqua Medic pH controllers (AB Aqua Medic GmbH). The adjustment was made in header tanks (volume = 100 L) into which seawater was pumped from the nearby bay, from a depth of 10 m. The seawater was run through a series of filters (100, 50 and 25 µm) prior to being used in the experiment. One header tank was used for each OA treatment. The bottom of each jar containing *F. vesiculosus* fronds received 80 mL/min flow-through of pH-adjusted water from a header tank through a 4-mm diameter silicon hose, which provided water motion, and replenished the water approximately every 12 minutes. At the beginning of the experiment, the pH levels were adjusted slowly over 24 h to avoid shock effects. Throughout the experiment, the pH levels in all jars were monitored by measuring them every second or third day (11 times in 22 days; Fig. S2) with a handheld pH meter (pH 1000 H, VWR, with pHenomenal 111 probe, 0.01 units accuracy and precision). Measurement accuracy was checked regularly against a bench-top pH metre (Jenway 3510, with Jenway 924030 Tris electrode). The bench-top pH meter has been annually intercalibrated in Proficiency tests by the Finnish Environmental Institute (Leivuori *et al*. 2018) using natural water samples. Both pH probes used in the experiment were calibrated with commercial NIST-traceable buffers (Merck Certipur®) using 3-point (pH 4, 7 and 10) calibration. The OA treatments had three pCO_2 levels: 'ambient' (236 µatm or 512 µatm, summer/winter), 'high' (1582 µatm or 2263 µatm, summer/winter) and 'extreme' (4673 µatm or 7074 µatm, summer/winter) (Table 1, values given in brackets are means across the two light treatments, calculated from all measurements for the duration of the experiment). The 'ambient' treatment consisted of unaltered filtered flow-through seawater.

The pH levels in the 'ambient' and 'high' treatments (Table 1) lie within the current observed variability of pH levels (1963–2016 mean 8.03, minimum 7.07, maximum 8.95) in the Gulf of Finland open sea area (ICES 2014, Fig S7). Although the CO_2 treatment levels in the "high" and 'extreme' treatment are high compared to annual mean pCO_2 seawater values (Fig. S5), stochastic upwelling has been observed to already have exposed coastal macrophyte habitats to pCO_2 > 2500 µatm in a eutrophied fjord in Denmark (Saderne *et al.* 2013) with salinity of 20, thus having higher alkalinity levels than the northern Baltic. Recent simulations in salinities of 20 and 35 predict peak pCO_2 levels of 4500 µatm and 3400 µatm (temperature = 10°C) with a doubling in the sea surface CO_2 concentration, when oxygen is depleted by heterotrophic respiration (Melzner *et al.* 2013). Eutrophication is expected to increase concurrently with climate change in the Baltic (Meier *et al.* 2012, Neumann *et al.* 2012), which leads to increased pH oscillations due to increased respiration and photosynthesis (Omstedt *et al.* 2012), and declining oxygen conditions, which intensifies acidification (Burnett 1997, Melzner *et al.* 2013). Finally, strong upwelling events are frequent in the study area (Haapala 1994), which supports the relevance of also examining the effects of extreme CO_2 scenarios on coastal biota (Melzner *et al.* 2013). Macronutrients (NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}) in the water flowing into the header tanks were monitored throughout the experiment. As the flow-through rate was high, and algal biomass in each jar was low, we decided to measure the nutrient levels from the inflowing water. Nutrient analyses were performed by TZS's laboratory, according to Koroleff (1983a, 1983b).

Table 1. Seawater parameters during the two experiments.

Season		**Winter**			**Summer**	
pCO_2 treatment	Ambient	High	Extreme	Ambient	High	Extreme
pH	7.97 ± 0.07	7.34 ± 0.03	6.84 ± 0.02	8.22 ± 0.19	7.36 ± 0.16	6.99 ± 0.13
Salinity	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	5.09 ± 0.14	5.09 ± 0.15	5.09 ± 0.14
Temperature (°C)	8.88 ± 0.28	9.06 ± 0.33	9.07 ± 0.33	13.49 ± 0.85	13.54 ± 1.02	13.62 ± 0.83
NH_4^+ (µg l^{-1})	3.1 ± 1.0	3.1 ± 1.0	3.1 ± 1.0	8.5 ± 2.2	8.5 ± 2.2	8.5 ± 2.2
NO_2^- + NO_3^- (µg l^{-1})	85.9 ± 12.3	85.9 ± 12.3	85.9 ± 12.3	26.3 ± 7.5	26.3 ± 7.5	26.3 ± 7.5
PO_4^{3-} (µg l^{-1})	21.7 ± 1.0	21.7 ± 1.0	21.7 ± 1.0	11.9 ± 1.3	11.9 ± 1.3	11.9 ± 1.3
Alkalinity (µmol kg^{-1})	1653 ± 42	1640 ± 42	1613 ± 50	1584 ± 49	1584 ± 35	1610 ± 39
DIC (µmol kg^{-1})	1644 ± 37	1752 ± 44	1986 ± 69	1523 ± 47	1647 ± 60	1828 ± 44
HCO_3^- (µmol kg^{-1})	1586 ± 35	1624 ± 41	1609 ± 50	1453 ± 45	1562 ± 34	1602 ± 39
CO_2 (µmol kg^{-1})	27 ± 2	121 ± 2	375 ± 19	11	75 ± 2	223 ± 5
CO_3^{2-} (µmol kg^{-1})	31 ± 4	7	2	60 ± 2	10	4
pCO_2 (µatm)	510 ± 50	2260 ± 41	7039 ± 296	236 ± 7	1582 ± 35	4681 ± 113

Note: Values are means across the duration of the experiment (i.e. one mean value was calculated from all daily measurements) ± standard deviation. For NH_4^+, NO_2^- & NO_3^- and PO_4^{3-} values are the same across all pCO_2 treatments as they were measured from the inflowing seawater.

Temperature and salinity were measured every two or three days with a conductivity meter (EC-300, VWR). Salinity and temperature followed fluctuations in ambient seawater. Salinity increased slightly during both experiments (from 6.0 to 6.2 and from 4.9 to 5.2, winter/summer, Fig. S1a, b), whereas temperature decreased slightly (from 9.7 to 9.0 °C and from 14.7 to 13.4 °C, winter/summer, Fig. S1c, d). In addition, temperature and light were monitored every 15 min with HOBO temperature/light loggers.

The winter and summer seasons showed differences when comparing the seawater parameters, most notably salinity, temperature and ambient pH (Table 1, Fig. S1, S2). Due to complications with balancing the flow-through system, pH values in the 'extreme' pCO_2 treatment deviated somewhat between summer and winter (Table 1, Fig. S2). Differences between summer and winter in the 'ambient' treatment were caused by natural seasonal fluctuations in the pH of the seawater.

Total DIC was measured twice during the winter experiment (days four and 14), and once during the summer experiment (day 12). In the winter experiment, three replicate samples were collected from each jar, while five replicate samples were collected from each header tank in the summer experiment. The seawater for the alkalinity and DIC measurements was collected with a syringe into glass bottles with airtight seals, which had been rinsed with ion-exchanged water.

DIC content of the samples was measured with a carbon analyser (Elektro-Dynamo URAS-3E). According to the method developed by Salonen (1981), 0.3 mL of the DIC sample was injected into the bubbling chamber of the analyser, converted to CO_2 by continuous addition of acid, and led to an infrared gas analyser using pure gaseous nitrogen as the carrier gas and $NaHCO_3$ solution as the standard.

To check the accuracy of the DIC measurements, total alkalinity was measured twice during the winter experiment by titrating 50 mL seawater to pH 4.5 with 20 mM HCl (SFS 3005 1981). This titration corresponds to ISO standard NS 4754: ISO 9963-1:1994, and has 2 µmol / L accuracy. The alkalinity samples were allowed to equilibrate to room temperature in darkness. After this, 50 mL of sample were transferred with a glass pipette into a 100-mL open glass beaker with a magnetic stirrer. Twenty mM HCl was injected into the beaker with a table-top titrator (Schott Titronic Basic), and the volume of HCl was recorded with 0.01 mL precision. pH was monitored with a Jenway 3510 pH meter.

Seawater carbon chemistry (alkalinity and partitioning of DIC into CO_2, CO_3^{2-} and HCO_3^-) was calculated from temperature, salinity, DIC and pH data using the R package 'seacarb' (Gattuso *et al.* 2015). The alkalinity values calculated in this way were compared against titrated alkalinity values and were accurate (mean difference 3 µmol kg^{-1}, Fig. S3).

2.2. Growth rate, carbon and nitrogen content

Fresh weight (FW) was measured with mg precision. Prior to the measurements, individuals were carefully blotted with a paper cloth to remove any extra water and were kept constantly submerged prior to and after the measurement. Relative growth rate (RGR) was calculated from FW according to Lüning (1990) referred to in Olischläger *et al.* (2013) by the equation

$$RGR = \frac{100*\ln(N_t \div N_0)}{t}, \quad (1)$$

where RGR is the daily growth (% FW), N_t is the FW at day t, N_0 is the initial FW, and t is the time interval in days.

Carbon and nitrogen content (% dry weight, DW) was analysed with a LECO TruSpec Micro CHNS analyser from dried (24 h 60°C) samples. After drying, the samples were ground with a mortar and pestle.

2.3. Chlorophyll content

For chlorophyll *α*, a small piece (29 ± 12 mg FW) of frozen algae was ground in the dark with a mortar and pestle. Chlorophyll was extracted in the dark over 4 h with 10 mL of 94% ethanol. Absorbance spectra of the extract were analysed with a Shimadzu UV-2550 Spectrophotometer with 1-nm precision. Chlorophyll *a* content was calculated from absorption spectra with equations from Ritchie (2008). Chlorophyll content (in mg) was normalized to algal fresh weight (g).

2.4.. Fluorescence measurements

Several chlorophyll fluorescence parameters were measured to quantify the responses of photosynthetic machinery to the treatments. All fluorescence measurements were conducted using a Diving-PAM pulse-amplitude modulated fluorometer (Walz GmbH). Maximum quantum efficiency of photosystem II (PS II) photochemistry, F_v/F_m, was measured at the end of the experiment after 12 h of dark adaptation, to ensure that all non-photochemical quenching components were relaxed (Maxwell and Johnson 2000). Measurements were made by first attaching a dark leaf clip onto the thallus tip, then attaching the fibre optics of the Diving-PAM to the clip to maintain a steady distance between the fibre optics and the clip. After this, F_0 was measured with a short burst (0.2 s) of 680 nm measuring light, followed by a saturating (>10 000

µmol m^2s^{-1}) light burst to saturate photosynthesis, and F_m was measured. F_v/F_m was calculated with the equation

$$F_v/F_m = \frac{F_m - F_0}{F_m} \quad . \qquad (2)$$

The Diving-PAM measuring light intensity and the distance between the sample and the tip of the fibreoptics were adjusted so that the F_0 reading fell between 300 and 500 (arbitrary units, as recommended by the manufacturer). The efficiency of light-limited photosynthetic energy capture (α), maximum relative electron transport rate through PS II ($_rETR_{max}$) and onset of light saturation of photosynthesis (E_k) were measured with rapid light curve (RLC) protocol (Ralph & Gademann 2005). The halogen lamp of the Diving-PAM was used as an actinic light source. The internal light library of the Diving-PAM was calibrated against a factory-calibrated light sensor (LI-COR LI-1500). Light intensity was increased from 0 µmol m^2s^{-1} to 681 µmol m^2s^{-1} in eight 20-s increments. As we did not measure absorbance of the tips, we give ETR values as relative electron transport rate (rETR) (Ralph & Gademann 2005). rETR in each light intensity was calculated by the equation

$$rETR = \frac{\Delta F}{F_{m'}} * PAR * 0.5 \, , \qquad (3)$$

where $\Delta F/F_{m'}$ is the effective quantum yield under actinic light, PAR is the photon flux density of photosynthetically active radiation and 0.5 is the factor accounting for assumed equal partitioning of photons between photosystems I and II (Genty *et al.* 1989). rETR versus PAR RLCs were fitted to a model by Platt *et al.* (1981) with R package 'phytotools' (Silsbe & Malkin 2015) and fit method 'PORT', and α, $rETR_{max}$ and E_k were solved from the equation.

Fresh weight was measured at the beginning and end of the experiments. All fluorescence measurements were taken at the end of the experiment, after which each specimen was split into three Eppendorf tubes and frozen (-80ºC) for carbon, nitrogen and chlorophyll analysis.

2.5. Statistical analyses

For the summer experiment, the effects of pCO_2 treatment and light on all variables measured were analysed with generalized mixed models using the "nlme" package (Pinheiro *et al.* 2015) in R. As three individuals were kept in a single jar, jar id was included as a random intercept in all the models. For the winter experiment, an ANOVA fit with generalized least squares (GLS) was applied. pCO_2 and light were analysed as factorial covariates with three or two levels, respectively. We followed a backwards-stepwise model selection strategy suggested by Zuur *et al.* (2009), starting with a full model with pCO_2 and light interaction and random intercept for the jar id (summer experiment). Models were fit with GLS using restricted maximum likelihood estimation (REML). If heterogeneity was observed when plotting model residuals against covariates, variance structures allowing for heterogeneity (Zuur *et al.* 2009) were applied (Supplementary Information section S3, Table S1), and/or the response variable was log-transformed (growth rate and E_k, summer and chlorophyll *c* content, winter). After this, the significance of the fixed terms was analysed by fitting a new model with GLS using maximum likelihood (ML) and comparing nested models using likelihood ratio tests. We used a p-value of 0.05 as the threshold for accepting a variable into the model. A similar model selection protocol was applied for the winter and summer data, with the exception of random factors in the summer experiment. When analysing F_v/F_m results in the summer experiment, jar horizontal position within the light treatment was also included as a second random effect in the model (see Supplementary Information section 3.2 for full

explanation). After all the significant terms were identified, validity of the model assumptions was checked by visually comparing the normalized residuals against fitted values and all covariates.

To investigate the magnitude of seasonal versus treatment effects, we examined the standardized regression coefficients for the main effects of season, light and pCO_2 on all measured variables. Because the measured variables were on different scales, all response variables were standardized to have a mean of 0 and a standard deviation of 1. For this analysis, the three summer measurements within a single jar were pooled. Absolute values of standardized main effects of the factor season (time of experiment) were compared with those of the light treatment (one level, Fig. 4) and the two pCO_2 treatments (two levels, Fig. 4) using Welch's ANOVA, followed by Games-Howell post hoc tests, as the standardized regression coefficients of the various main effects examined had heterogeneous variances. All numerical analyses were conducted in the R software environment version 3.2.0 (R Core Team 2015).

3. Results

3.1. Growth rate, carbon and nitrogen content

Neither pCO_2 nor light significantly affected growth rate. pCO_2 and light had similar effects on the carbon and nitrogen content and carbon:nitrogen (C:N) ratio of the algae, and the observed effects showed differences between the two seasons investigated (Table 2, Fig. 1). Increasing pCO_2 and light significantly increased carbon content and the C:N ratio, and caused declines in nitrogen content, but, with the exception of carbon content, these were only observed during winter. The significant effect of pCO_2 to carbon content in summer was rather weak, with "high" treatment having 0.20% lower and "extreme" treatment 0.46% higher carbon content than the "ambient" treatment.

Table 2. Statistical analysis (Mixed model or ANOVA) results for growth rate, carbon and nitrogen content and C:N ratio measurements.

	Growth rate		**Carbon content (% DW)**		**Nitrogen content (% DW)**		**C:N ratio**	
Summer	L	*p*	F	*p*	F	*p*	F	*p*
pCO_2*Light	1.902	0.386	2.202	0.113	0.834	0.435	0.948	0.389
pCO_2	1.638	0.440	**4.596**	**0.011***	2.052	0.131	2.074	0.128
Light	0.214	0.643	2.947	0.087	2.074	0.128	0.688	0.408
Winter	F	*p*	F	*p*	F	*p*	L	*p*
pCO_2*Light	0.958	0.389	0.435	0.649	0.389	0.679	1.906	0.385
pCO_2	0.182	0.833	**7.537**	**0.001****	**10.247**	**< 0.001*****	**22.007**	**< 0.001*****
Light	1.538	0.219	**74.52**	**< 0.001*****	**14.352**	**< 0.001*****	**27.309**	**<0.001*****

*Note: DW = dry weight. Statistically significant ($p < 0.05$) effects are indicated in bold and with asterisks. Significance levels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. L indicates likelihood ratio (mixed model), and F an F-statistic (ANOVA), depending on the conducted analysis. Degrees of freedom are the number of treatment levels (three for CO_2 and two for light) -1.*

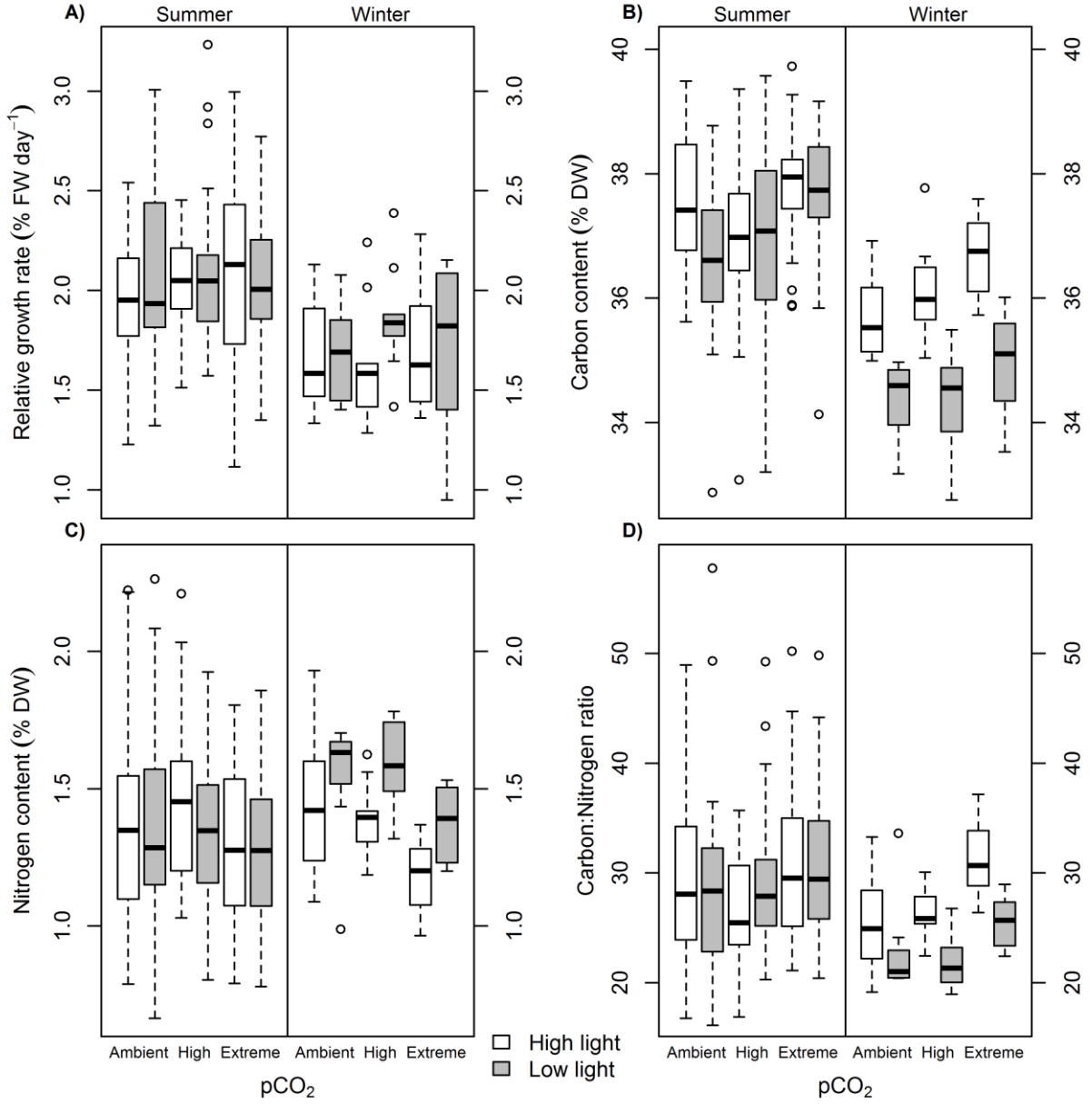

Figure 1. Effects of pCO_2, light and season on growth rate (A), carbon (B) and nitrogen content (C) and carbon:nitrogen ratio (D).

Nitrogen content was not affected by pCO_2 or light treatments in the summer, but increasing pCO_2 and light in winter caused significant declines in nitrogen content (Fig. 1C, Table 2). Likewise, the C:N ratio was unaffected by treatments during summer, but during winter increased light and CO_2 availability caused elevated C:N ratios (Fig. 1D, Table 2).

3.2. Chlorophyll content

Chlorophyll *a* content was relatively unaffected by pCO_2 and light, with only significant effects observed in summer, when low light caused higher chlorophyll content (Fig. 2, Table 3). This effect

was not observed in winter, but in winter the chlorophyll content was overall higher compared to summer (Fig. 2).

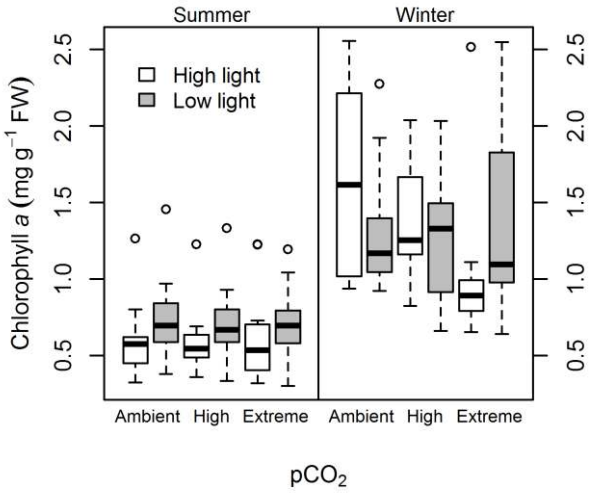

Figure 2. Effects of pCO_2, light and season on chlorophyll *a* content.

Table 3. Statistical analysis (ANOVA) results for chlorophyll *a* content.

	Chlorophyll *a*			
	Summer		**Winter**	
	F	*p*	F	*p*
pCO_2*Light	0.099	0.905	1.825	0.170
pCO_2	0.031	0.968	1.834	0.169
Light	**9.811**	**0.002****	0.128	0.721

*Note: DW = dry weight. Statistically significant ($p < 0.05$) effects are indicated by bold and asterisks. Significance levels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. F is an F-statistic (ANOVA). Degrees of freedom are the number of treatment levels (3 for CO_2 and 2 for light) -1.*

3.3. Fluorescence parameters

The mixed model indicated significant interaction between pCO_2 and light on maximum potential quantum yield (F_v/F_m) during summer (Fig. 3A, Table 4). Elevated pCO_2 caused F_v/F_m to decline, but only under high light treatment (Fig. 3A). pCO_2 and light both had significant effects on F_v/F_m during winter (Table 4), with 'extreme' pCO_2 treatment causing decreases in F_v/F_m, and individuals subjected to high light treatment having lower F_v/F_m values (Fig. 3A).

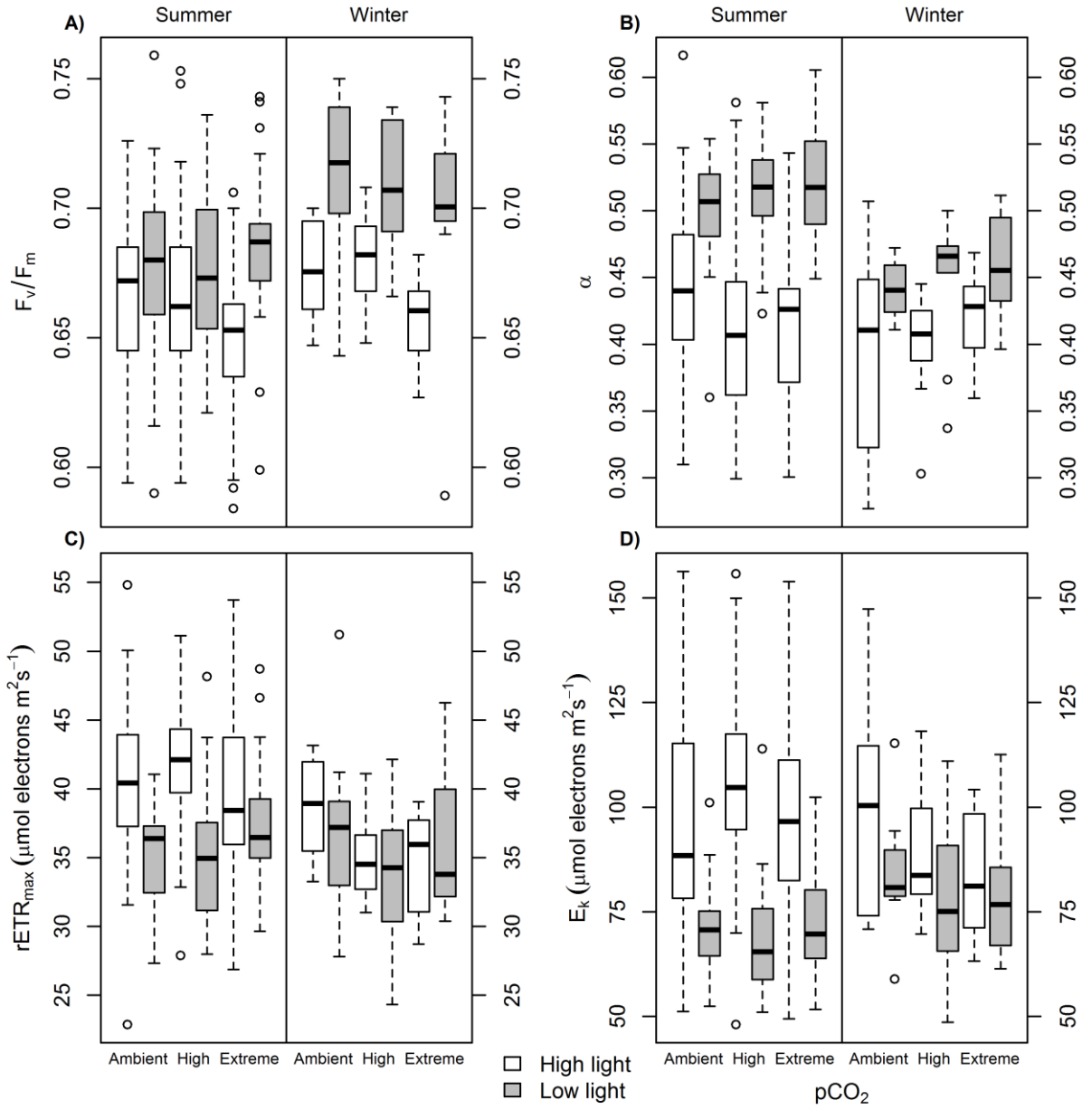

Figure 3. Effects of pCO_2, light and season on maximum potential quantum yield, F_v/F_m (A), light-limited photosynthetic efficiency, α (B), maximum relative electron transport rate, $rETR_{max}$ (C) and onset of light saturation, E_k (D).

Responses in α showed similar patterns, with α declining during the summer with increasing pCO_2, but only under high irradiance (Fig. 3B). Although pCO_2 and light interaction was non-significant ($p = 0.059$), dropping the interaction caused residual patterns (S10), which indicate that the interaction probably should have been included in the final model. Similarly to F_v/F_m values, individuals exposed to high irradiance during winter had lower values of α, but pCO_2 had no effect (Table 4, Fig. 3B).

High light treatment caused increases in maximum electron transport rate during the summer, but not in winter. pCO_2 treatment had no effect on $rETR_{max}$ in summer, but high pCO_2 caused decreases in $rETR_{max}$ in winter (Table 4, Fig. 3C).

F. vesiculosus plants acclimated to irradiance treatments during the experiment, which was seen in high light-treated plants having higher compensation points for photosynthesis (E_k) in both winter and summer (Table 4, Fig. 3D). pCO_2 treatment did not affect E_k.

Table 4. Statistical analyses (Mixed model or ANOVA) results for the chlorophyll fluorescence parameters measured.

	F_v/F_m		α		$rETR_{max}$		E_k	
Summer	L	*p*	L	*p*	L	p	L	*p*
pCO_2*Light	**7.235**	**0.026***	**7.079**	**0.029***	2.906	0.233	2.780	0.249
pCO_2					0.567	0.753	0.539	0.763
Light					**23.107**	**<0.001*****	**68.715**	**<0.001*****
Winter	L	*p*	L	*p*	L	*p*	F	*p*
pCO_2*Light	0.527	0.768	0.176	0.915	1.095	0.578	0.615	0.544
pCO_2	**8.812**	**0.012***	2.332	0.311	**8.33**	**0.015***	4.415	0.125
Light	**20.566**	**<0.001*****	**12.228**	**<0.001*****	0.248	0.617	**5.344**	**0.020***

*Note: In the case of F_v/F_m and α, missing rows for single terms indicate that model selection ceased on a significant interaction term before single-term effects were analysed. Statistically significant ($p < 0.05$) effects are indicated in bold and with asterisks. Significance levels: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. L indicates likelihood ratio (mixed model), and F an F-statistic (ANOVA), depending on analysis conducted. Degrees of freedom are the number of treatment levels (three for CO_2 and two for light) -1.*

3.4. Differences between the two seasons

Experiment timing had a substantial effect on all the measured parameters. The seasonal effect (winter-summer) was greater than that of "high" pCO_2 treatment ($t = 3.81$, $df = 8.22$, $p < 0.021$), and the difference between "extreme" pCO_2 treatment and season was marginally significant ($t = 2.96$, $df = 9.97$, $p = 0.059$). We observed no significant difference between the relative magnitude (standardized main effects) of light treatment and season, nor pCO_2 treatments and light (Fig. 4, table S2).

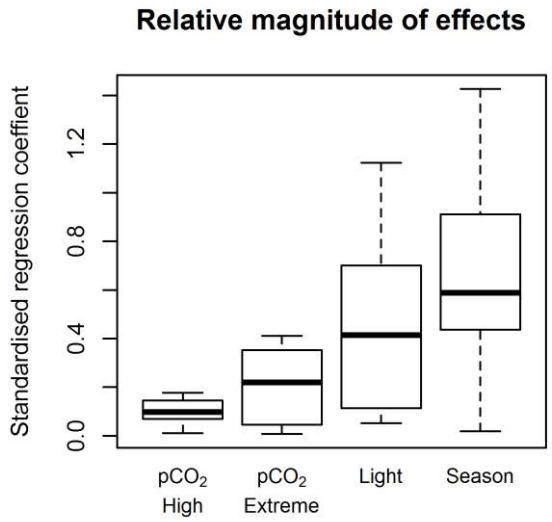

Figure 4. Standardized regression coefficients of main effects indicating relative effect of each variable.

4. Discussion

Growth rate was not affected by either light or CO_2. Increasing CO_2 availability caused increasing carbon content in the algae during both seasons, but the effect was substantially stronger in winter. CO_2 increase also caused a decline in nitrogen content and an elevated C:N ratio, but only in winter. Light had a similar effect in the same direction. In chlorophyll *a* and light-use efficiency (F_v/F_m and α), these effects were in the same direction: increasing light decreased chlorophyll content in summer, and decreased light-use efficiency in both seasons. Increasing pCO_2 availability caused a decline in light-use efficiency, except in summer under high irradiance.

4.1. Effects of light and CO_2 on growth rate and carbon and nitrogen content

Although growth rate remained unaffected by pCO_2 or light, algae in high pCO_2 treatments were able to obtain an equal or greater share of inorganic carbon, especially during winter. As the sugar alcohol mannitol is the main carbon sink in *F. vesiculosus* (Bidwell & Ghosh 1962, Lehvo *et al.* 2001), an increase in inorganic carbon content may indicate increases in the mannitol pool. Although growth rates were not directly stimulated by pCO_2 treatments, an increase in mannitol storage could imply that high pCO_2 would have positive effects on growth rates in the longer run, because the energy stored into mannitol could later be utilized in growth (Lehvo *et al.* 2001). In our study, increasing pCO_2 generally increased C content, which suggests that the algae could either elevate the carbon fixation rate or reduce respiratory losses of carbon under high pCO_2. Nitrogen content decreased under high pCO_2 in winter, and these together caused increases in the winter C:N ratio. In previous studies, OA (elevated pCO_2) has been observed to reduce nitrogen uptake in macroalgae by decreasing the activity of nitrate reductase (Hofmann *et al.* 2013). Nitrogen uptake is an active, energy-consuming process and thus its downregulation may arise if high pCO_2 reduces the energetic cost of carbon acquisition. The reason may be that when pCO_2 is elevated, plants need to invest less in photosynthetic energy capture, especially under abundant

irradiance (as the decline in F_v/F_m and α indicate here), and thus have a reduced need for the upkeep of photosynthetic machinery as, in particular, the chlorophyll molecules are one of the main sinks of nitrogen in aquatic photoautotrophs (Kirk 2011). Interestingly, pCO_2 treatment affected nitrogen content only during winter, but not in summer. *F. vesiculosus* takes up and stores nitrogen in winter, when seawater nitrogen content is high, to be utilized during rapid spring growth (Lehvo *et al.* 2001). If exposure to high pCO_2 seawater alters nitrogen uptake and storage in the alga during winter, this may decrease the internal nitrogen pool size. In turn, this may potentially result in reduced growth during the main growth season in spring and summer, when seawater nitrogen concentrations are low, and internal nitrogen pools of the alga become depleted.

Changes in C:N ratio under OA have been observed in phytoplankton (Riebesell *et al.* 2007) and macroalgae (Celis-Plá *et al.* 2015). In our study, the pCO_2 treatments had significant effects on C:N ratios only during winter, not in summer, indicating that the response of *F. vesiculosus* carbon metabolism on OA may depend on season. High C:N ratios have been found to correlate with phenol synthesis in *F. vesiculosus* (Ilvessalo & Tuomi 1989). Polyphenolic compounds, such as phlorotannins, are important defences against herbivory in *F. vesiculosus* (Koivikko *et al.* 2005, Jormalainen & Ramsay 2009). If phenol production is stimulated by elevated C:N ratios, this may also alter herbivory patterns in *F. vesiculosus*, thus potentially affecting the strength of trophic interactions in the littoral ecosystem. However, this remains unconfirmed in our study, as we did not measure algal phenol content. Our results, obtained with extreme pCO_2 treatments, contrast somewhat with those of Gutow *et al.* (2014), who observed declining C:N ratios and growth of *F. vesiculosus* under OA in a study conducted in a wholly marine ecosystem, albeit with ecologically more realistic pCO_2 treatments. It is possible that the responses in the relatively low-carbon Baltic and the Atlantic *F. vesiculosus* populations may be somewhat different. As our treatment levels for pCO_2 exceed those applied by Gutow *et al.* (2014), it is also possible that a threshold exists under which pCO_2 stimulates changes in the C:N ratio.

4.2. Effects on chlorophyll content and chlorophyll fluorescence parameters

Chlorophyll *a* content was only affected by light and only during summer. The low light-treated algae had higher chlorophyll *a* content, a pattern which has also been observed in the field (Rohde *et al.* 2008). This is caused by increases in size and changes in the number of photosynthetic units, especially in ratios of PS I to PS II, with PS I numbers declining in high irradiance (Kirk 2011). pCO_2 treatment did not affect chlorophyll *a* content in either season. Although pCO_2 had no significant effect in winter, dropping pCO_2 from the model caused residual patterns in validation graphs when residuals were plotted against the pCO_2 treatment (Fig. S8), which indicates that dismissing the pCO_2 effect as non-significant should be considered with caution.

Our study identified interacting effects between pCO_2 and light, with declining light-use efficiency (decrease in F_v/F_m and α) emerging under high pCO_2 under high irradiances. Under low irradiance, α and F_v/F_m remained high in both seasons, likely as the plants had to maintain relatively high light-harvesting capacities to supply dark reactions with sufficient reductant levels for carbon fixation. Under high irradiance, conversely, light-use efficiency generally declined, especially during summer, which was expected, as there was no pressure for efficient light utilization under high irradiance. However, the decline in F_v/F_m and α under high pCO_2 and high irradiance suggests that the light energy used for carbon fixation also declined under high pCO_2. This supports the idea that the algae could have downregulated CCM usage and supplied their carbon fixation

increasingly with free CO_2, but as we did not analyse carbon isotope ratios in algal tissue this remains speculative. In Eelgrass *Zostera marina*, increasing carbon (CO_2) availability reduces photosynthetic light requirements, and allows the plants to sustain their growth with lower accumulated daily irradiance (Zimmerman *et al.* 1997). Saturation of irradiance requirement, a similar process, is a plausible explanation for the decline in F_v/F_m and α under high pCO_2 and high irradiance observed here.

4.3. Concluding remarks

The observed responses to experimental treatments showed strong differences between the two time points sampled, which suggest seasonal variation in many physiological traits of *F. vesiculosus*, especially nitrogen and carbon content and growth rate (Lehvo *et al.* 2001). Responses to pCO_2 treatments were rather small compared to seasonal changes in all parameters measured. As CO_2 in our experiment was administered by manipulating the pH of seawater, the changing alkalinity, salinity and temperature between the two experiments caused the pCO_2 levels to vary between corresponding pH treatments in summer and winter (Fig. S4), with the winter experiment having higher pCO_2 levels for each corresponding pCO_2 treatment level when compared with summer (Table 1). As temperature strongly affects the physiology of *F. vesiculosus* (Kraufvelin *et al.* 2012, Al-Janabi *et al.* 2016a), differences in seawater temperature between the two seasons may partly explain the observed dissimilarities. It is also worthy to note that with two three-week experiments, the evaluation of seasonal responses relies on two snapshots in time, and thus may not provide a thorough picture of seasonal changes in the physiology of *F. vesiculosus*, although, with C and N content in particular our results are in general agreement with previous studies conducted in the same region (Lehvo *et al.* 2001). The responses observed in such relatively short experiments may also differ from the effects of longer exposure to the variables tested. Also, if the duration of CO_2 exposure is short, not all of the effects observed here may be realized *in situ*, as alterations in carbon physiology in macroalgae may occur over a time course of days to weeks (Hofmann *et al.* 2013), whereas alterations in irradiance conditions may cause physiological responses in minutes or hours (Falkowski and Raven 2007, Kirk 2011).

In summary, CO_2 and light had substantial effects on *F. vesiculosus* physiology, most notably in C and N content and photosynthetic parameters. The directions of pCO_2 and light effects were often in the same direction. The physiology of *F. vesiculosus* shows strong seasonality in the northern Baltic, and the potential effects of high pCO_2 vary in time. However, compared with the seasonal changes in all measured parameters, the effects of CO_2 were small in magnitude, at least on the mature vegetative thalli investigated in this study. In contrast, light availability had substantial effect on many of the parameters measured. Light and pCO_2 had similar effects (to the same direction) on carbon and nitrogen content, especially in winter, which suggests that during the winter experiment the algae were light- and carbon-limited, possibly because the internal carbon pools had been depleted by energetic requirements to sustain growth.

In our study, *F. vesiculosus* growth rates were not affected by either pCO_2 or light treatments. As growth rate is dependent on a multitude of physiological processes, we consider it to be the strongest performance proxy. Based on the results of our study, we conclude that stochastic upwelling of pCO_2-rich water may potentially have some beneficial effects (such as increased carbon accumulation in winter), but the full effects on algal fitness appear rather small. Consequently, CO_2 enrichment from other sources, such as anthropogenic ocean acidification, likely has only minor effects on *F. vesiculosus*.

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SUPPLEMENTARY MATERIAL

Supplement to Takolander et al. 2019: "Seasonal interactive effects of pCO 2 and irradiance on the ecophysiology of brown macroalga Fucus vesiculosus L." EurJPhycol 54 (3): 380-392 10.1080/09670262.2019.1572226

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S 1. Seawater parameters during the experiment

Salinity levels remained relatively stable during both experiments (Fig. S1a, b), whereas temperature declined (Fig. S1b, c). Salinity levels remained the same across all treatments, temperature levels fluctuated between various pCO_2 levels (~0.2 ^{o}C, Fig. S1 c,d), which was probably caused by small differences in the flow rate from the header tanks, causing minor warming of the "High" and "Very high" pCO_2 treatments.

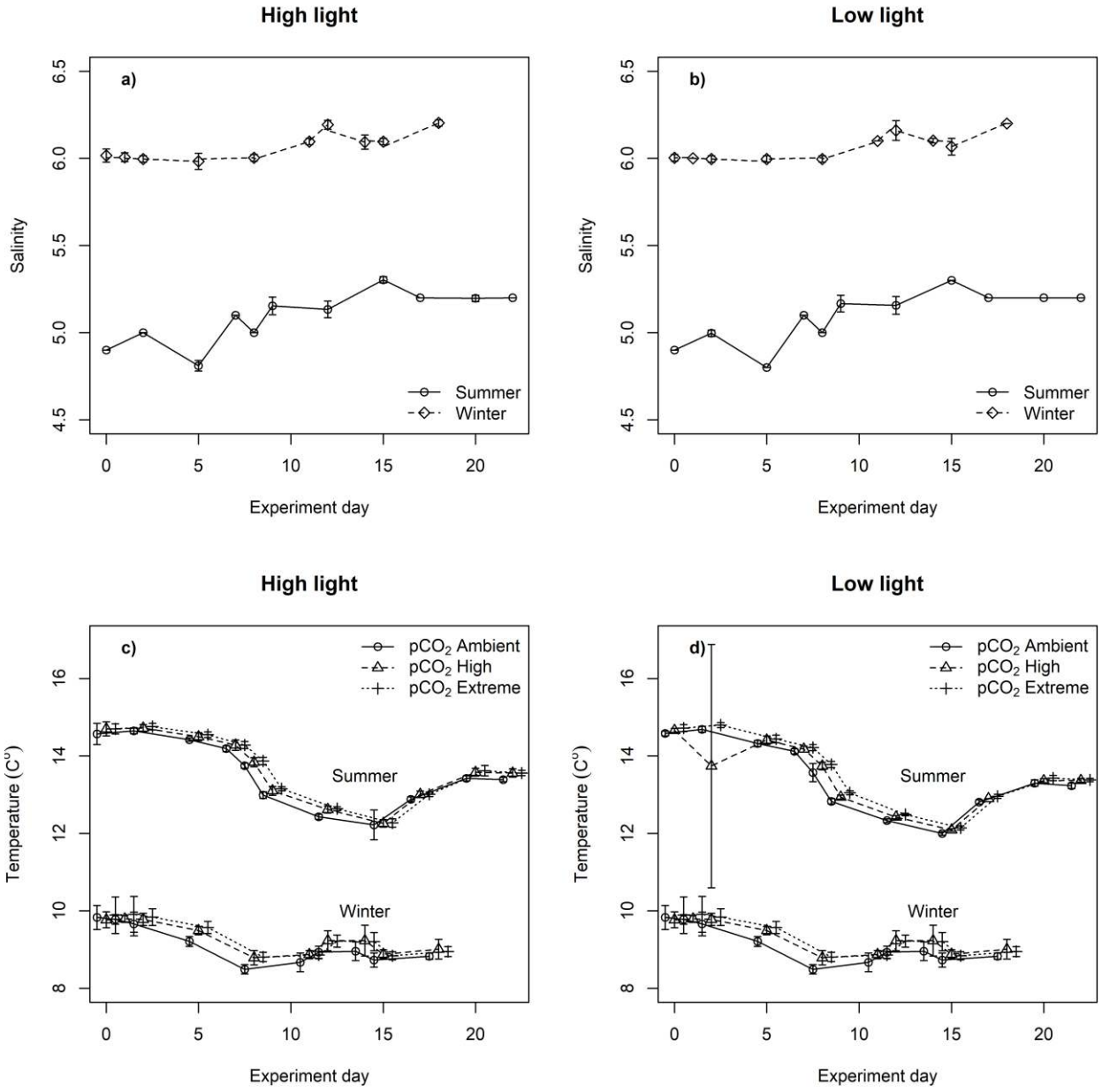

Fig. S1. Salinity and temperature during the two experiments. Values are means across all replicates ± standard deviation. For salinity, all pCO_2 treatments are pooled within "Summer" and "Winter" groups, as values were essentially identical. For temperature data, means are shown for each pCO_2 treatment. Values are shifted along the x-axis for visualization.

Heating from fluorescent light bulbs caused a very small (~0.1 ^{o}C) temperature difference between the “High” and “Low” light treatments (Fig. S1 c,d, Table 1 in the ms).

pH levels remained constant in the pCO_2 treatments during both experiments (Fig. S2a, b), and the standard deviation in pH across all replicates was ow, which indicates that the high flow-through rate (80 mL / min) in each experimental jar (V = 1l) was so high that the algal photosynthesis rates could not notably raise the seawater pH. The “Ambient” treatment had a decreasing pH trend during summer (Fig. S2c, d).

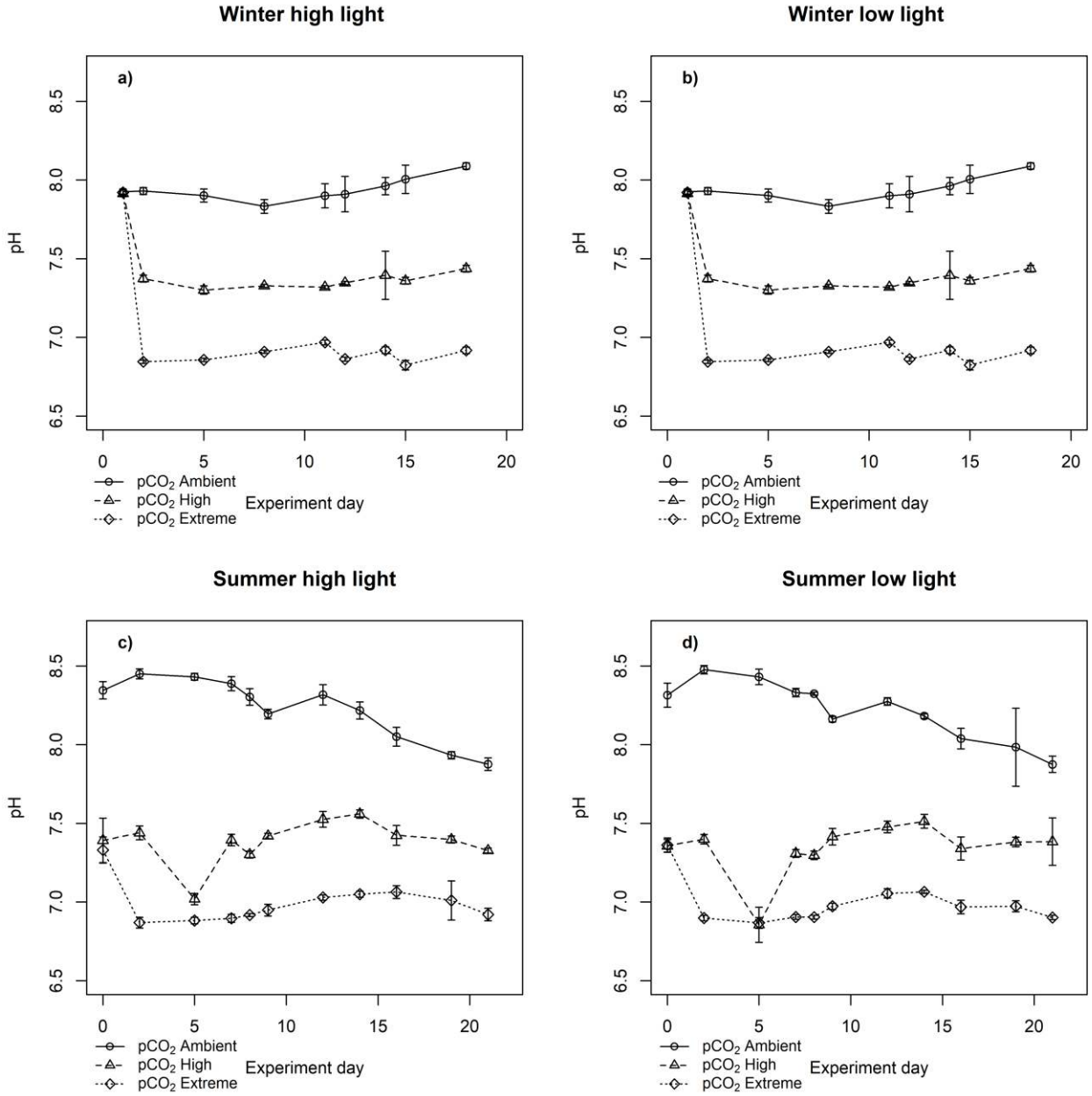

Fig. S2. pH levels during the two experiments for the two light treatments. Values are means across pCO_2 treatments ± standard deviation.

In the summer experiment, adjustment error of the pH computer caused pH levels in the "High" pCO_2 treatment to decrease below 7 (Fig. S2b). The adjustment was fixed immediately after observation (day five), after which the pH returned to the desired treatment level after afew hours. This is not visible in the data, however, as the next occasion pH was monitored on was day seven.

Alkalinity calculated from DIC measurements and pH, salinity and temperature values were compared against titrated alkalinity values during the winter experiment, and were similar (Fig. S3).

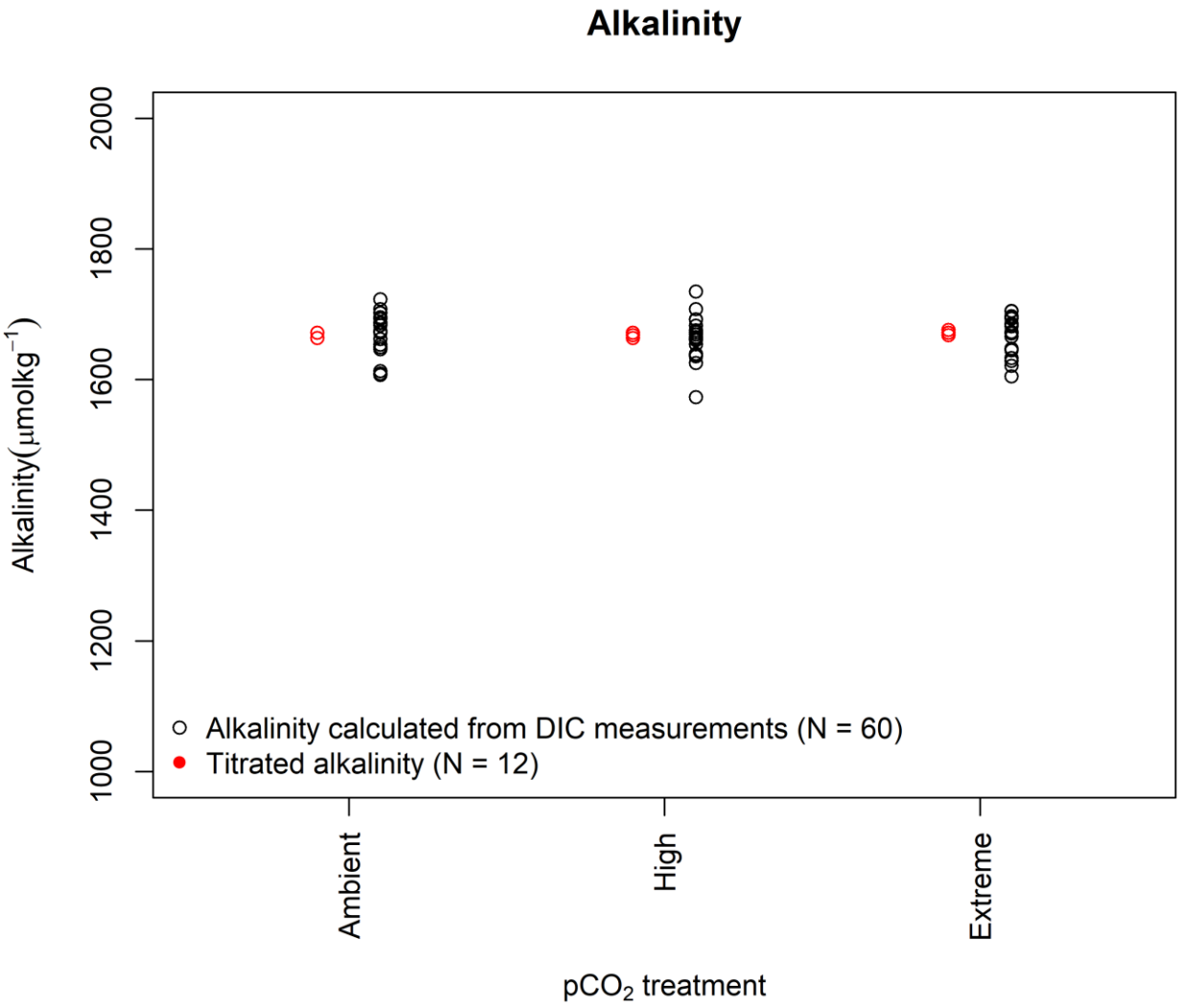

Fig. S3. Titrated and calculated alkalinity values during the winter experiment (day 14) for the three pCO_2 treatments.

Caused by seasonally fluctuating salinity, temperature and alkalinity, parameters of the carbonate system deviated slightly between winter and summer experiments (Fig. S4), with generally the winter experiment having higher concentrations of pCO_2, HCO_3^- and total dissolved inorganic carbon (DIC, Fig. S4).

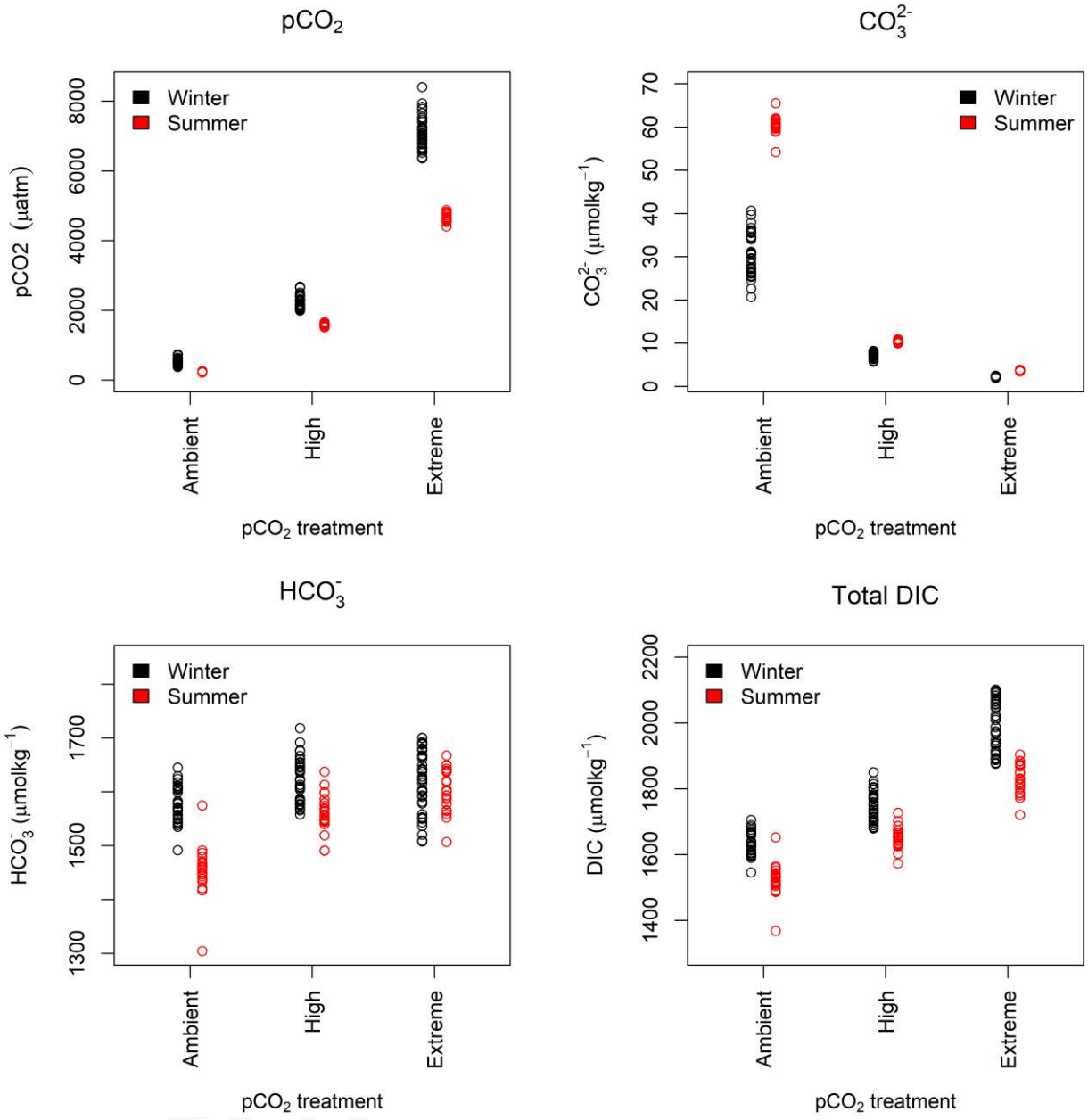

Fig. S4. pCO_2, CO_3, HCO_3 and total dissolved inorganic carbon (DIC) in the seawater during both experiments.

S 2. Variations in the environmental parameters in the study area

As part of another project, we conducted a field campaign in 2017 around coastal sites near (< 10 km) Tvärminne Zoological Station, and measured variability in seawater parameters at five different locations: Ångbåtsbryggan, Spikarna, Björnholmen, Danskog and Ekö. All sites were shallow (< 3 m) and had dense *Fucus* vegetation. Spikarna is a rocky island located far out in the archipelago, Ångbåtsbryggan is a shallow strait in the vicinity of TZS, the other three sites are shallow *Fucus* beds in the inner archipelago. We measured temperature and salinity with a hand-held conductivity meter (EC-300, VWR). Alkalinity and pH were measured from five replicate

samples collected into airtight glass bottles. Bottles were kept in dark and transported to the laboratory, where pH was measured with a pH meter (Jenway 3510). Alkalinity was measured by titrating 50 ml of water sample with HCl to pH 4.5, as explained in the main manuscript. pCO_2 and total DIC concentration were calculated with the "seacarb" package from pH, alkalinity, temperature and salinity values. Our sampling revealed large natural variability in pH, temperature and pCO_2, whereas seasonal changes in DIC concentration were more modest (Fig. S5).

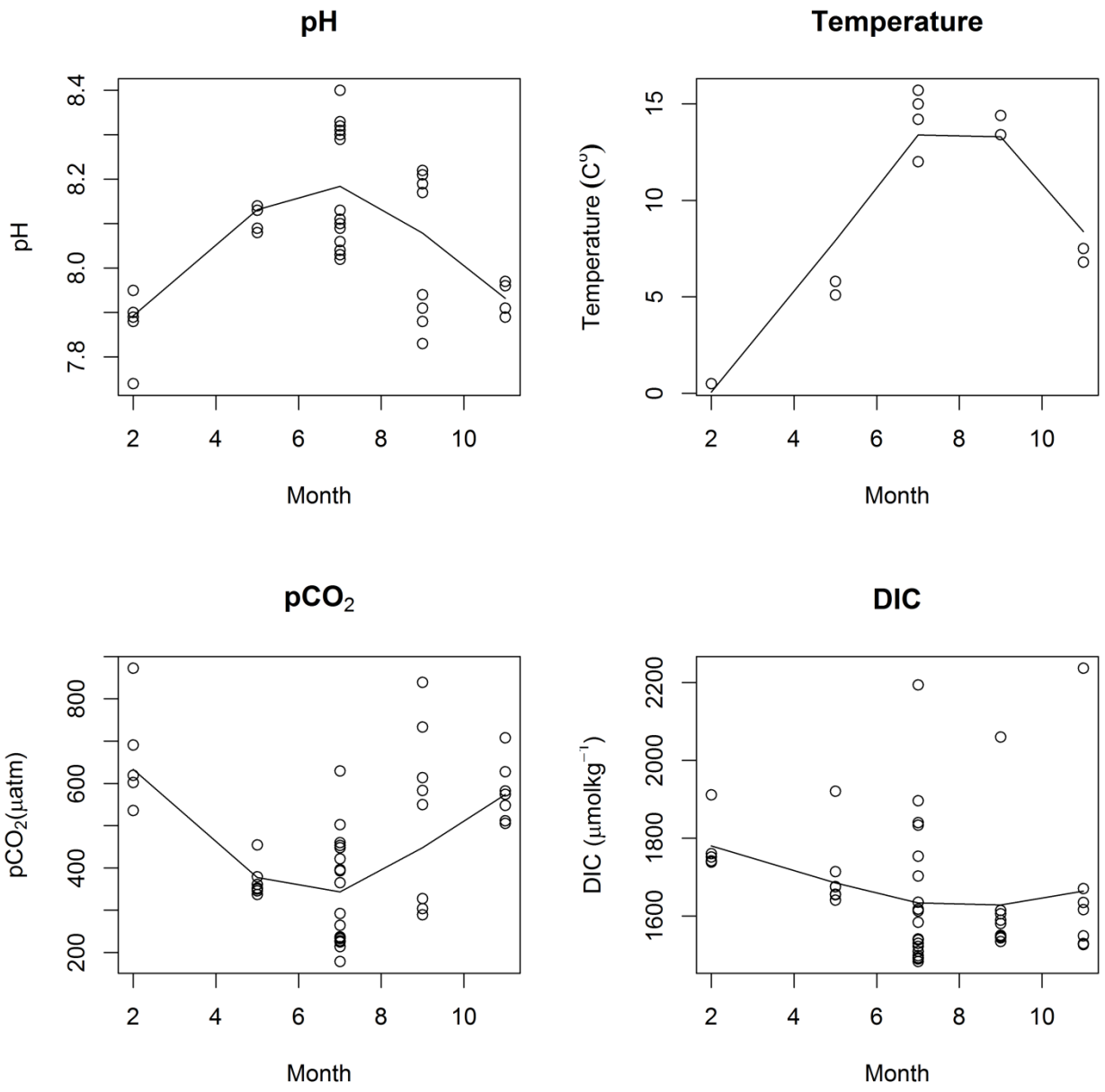

Figure S5. pH and temperature and calculated pCO_2 and DIC values at five coastal sites near TZS with dense *Fucus* vegetation measured in 2017. Trendline added for visualization.

To quantify the natural variability in the carbonate system in larger scale in our study area, we downloaded monitoring data from the ICES data portal

(http://ocean.ices.dk/Helcom/Helcom.aspx?Mode=1) (ICES 2014). We queried for data entries in the surface water (< 20 m depth) that had recorded values of temperature, salinity, pH and alkalinity. This yielded 297 observations between 1958 and 2009 from nine HELCOM monitoring stations along the Finnish coastal zone, the majority of which were located in the Gulf of Finland near our study site, Tvärminne Zoological Station (Fig. S6). We calculated total DIC, pCO_2 and HCO_3^- values from these data using R package "seacarb" (Gattuso et al. 2015). As alkalinity was seldom recorded in the data entries, the number of observations was low. To more properly quantify seasonal patterns in temperature and pH, we also ran separate queries on these variables independently, and recovered 20 983 temperature recordings and 8 197 pH recordings, which show the high natural variability in these variables in the open sea surrounding the study area (Fig. S7).

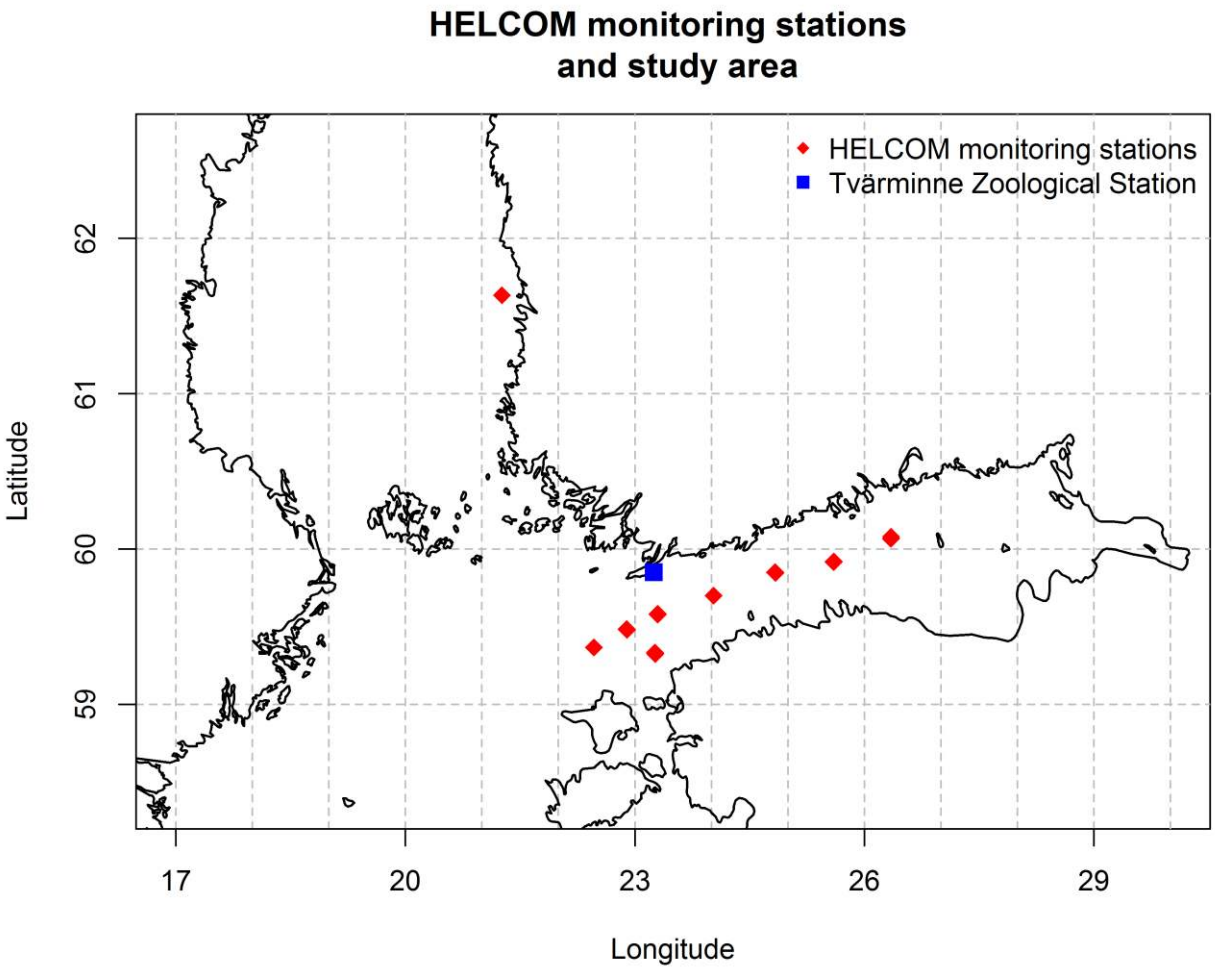

Fig. S6. Map of HELCOM monitoring stations from which data were downloaded, and Tvärminne Zoological Station.

Alkalinity, total DIC, pCO_2 and HCO_3^- showed high variability and distinct patterns throughout the year (Fig. S7). pCO_2 and pH showed complementary seasonal variations. Although the mean pH trend remained close to 8, occasional recordings of pH ~ 7 were also observed. In the future, the mean pH is expected to decline with ongoing OA, and pCO_2 is expected to increase (Omstedt et al.

2012), with concominant increases in seasonal variability as eutrophication will likely also intensify (BACC II Author Team 2015).

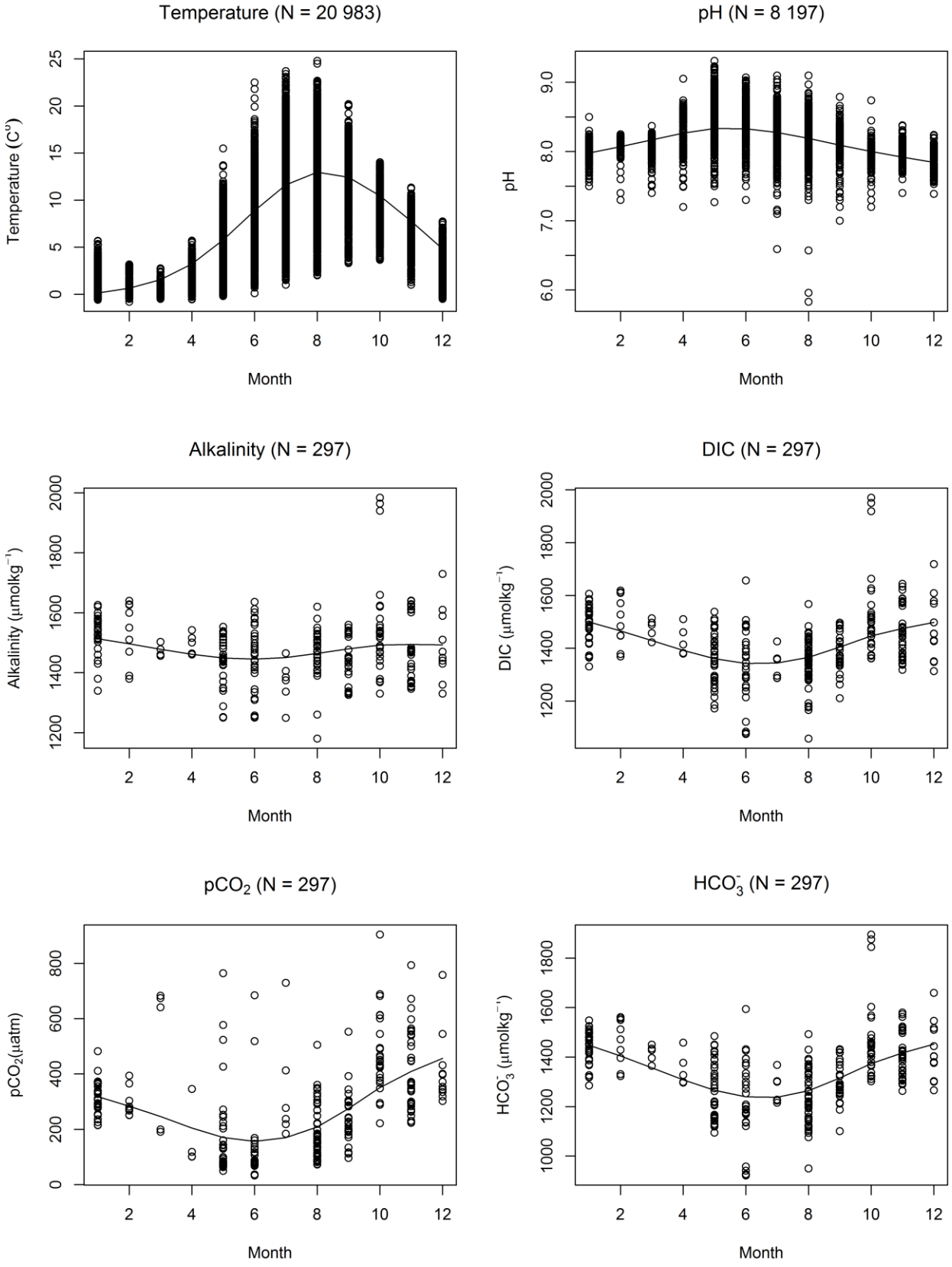

Fig S7. Values of temperature, pH, alkalinity, DIC, pCO_2 and HCO_3^- from the HELCOM monitoring stations (Fig. S6). Trendline added for visualization.

S3. Statistical analyses

In case residuals patterns were observed when plotting residuals of the full model against covariates, variance structures were applied. These allowed different variances per treatment level combinations. If such heterogeneity was observed, model improvement with addition of variance structures was investigated by comparing nested models fitted with REML estimation (a full model without var structures and a full model with var structures) with likelihood ratio tests (Table 1 in ms). Several variance structures were applied, where variance was either allowed to vary by pCO_2 treatment, light treatment or both. Variance structures were applied, if significant ($p < 0.05$) model improvement was detected (Table S1).

Table S1. Variables for which variance structures were applied in the analysis. L is the likelihood ratio of comparing a model with variance structures to a model without, df is the degrees of freedom used to define the variance structures. AIC improvement is the difference in AIC of model with var structures and the model without.

Variable	**Differing variances allowed by**	**L**	**df**	***p***	**AIC improvement**
log growth rate, summer	Light*pCO_2	11.160	5	0.048	-1.160
Nitrogen content, winter	pCO_2	9.066	2	0.010	-5.066
C:N ratio, winter	pCO_2	6.541	2	0.038	-2.541
alpha, summer	Light	6.222	1	0.012	-4.222
alpha, winter	pCO_2*Light	15.452	5	0.008	-5.452
rETRmax, summer	Light	5.625	1	0.017	-3.625
rETRmax, summer	Light	4.438	1	0.035	-2.438
log Ek, summer	Light	3.947	1	0.047	-1.947
F_v/F_m, winter	Light	8.000	1	0.004	-6.000

S 3.1. Chlorophyll analyses

None of the covariates had a significant effect on chlorophyll *a* content during the winter experiment (Table 3 in ms). However, although backwards model selection suggested dropping both pCO_2:Light interaction and pCO_2 effects from the model (Table 3 in ms), a residual plot from the final model (containing only light as a covariate) showed residual patterns when normalized residuals were plotted against pCO_2 treatment (Fig. S8), which indicates that pCO_2 should potentially have been included into the final model, and that the rather low number of replicates (N= 10 in the winter experiment) was too low to detect the potentially existing effect of pCO_2 on chlorophyll *a* in winter.

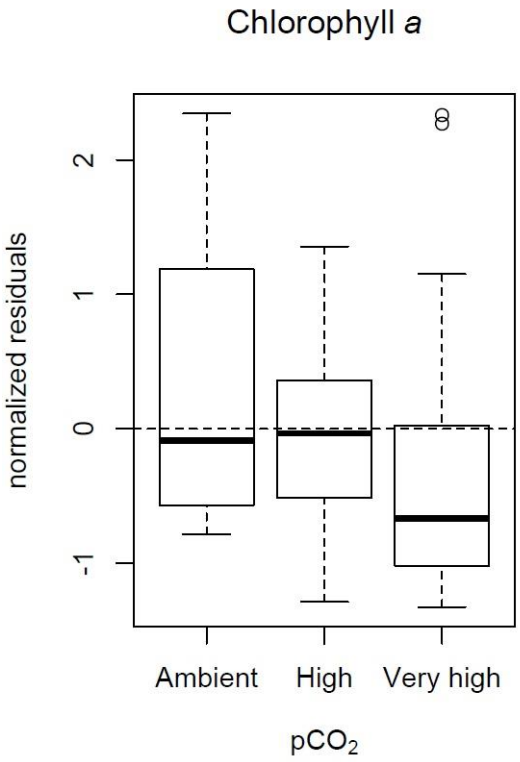

Fig. S8. Normalized residuals of final model (pCO_2 and Light dropped) in chl *a* analysis (winter experiment) plotted against pCO_2 treatment. The final model contains only the intercept, and shows clear residual patterns by pCO_2.

S 3.2. F_v/F_m analysis

When analysing the F_v/F_m data from the summer experiment, a residual pattern was identified when plotting residuals against fitted values (Fig. S9a), most notably residual values showing an increasing trend by the fitted values. This was identified to be probably caused by the experimental jars in the middle of the experiment having slightly lower F_v/F_m values. The most likely reason for this was that the light field provided by the fluorescent lamps was not totally homogeneous, and that the jars in the middle of the experiment likely received somewhat more light than the jars located at the sides of the experiment. In both experiments, 60 jars were used, which were split into 30 high and low light treatments. The 30 jars in each light treatment were placed under the fluorescent lights in three rows and 10 columns, and the pCO_2 treatments were fully crossed and randomized in such way that each column had one jar containing water from each of the three pCO_2 treatment level headers in random order.

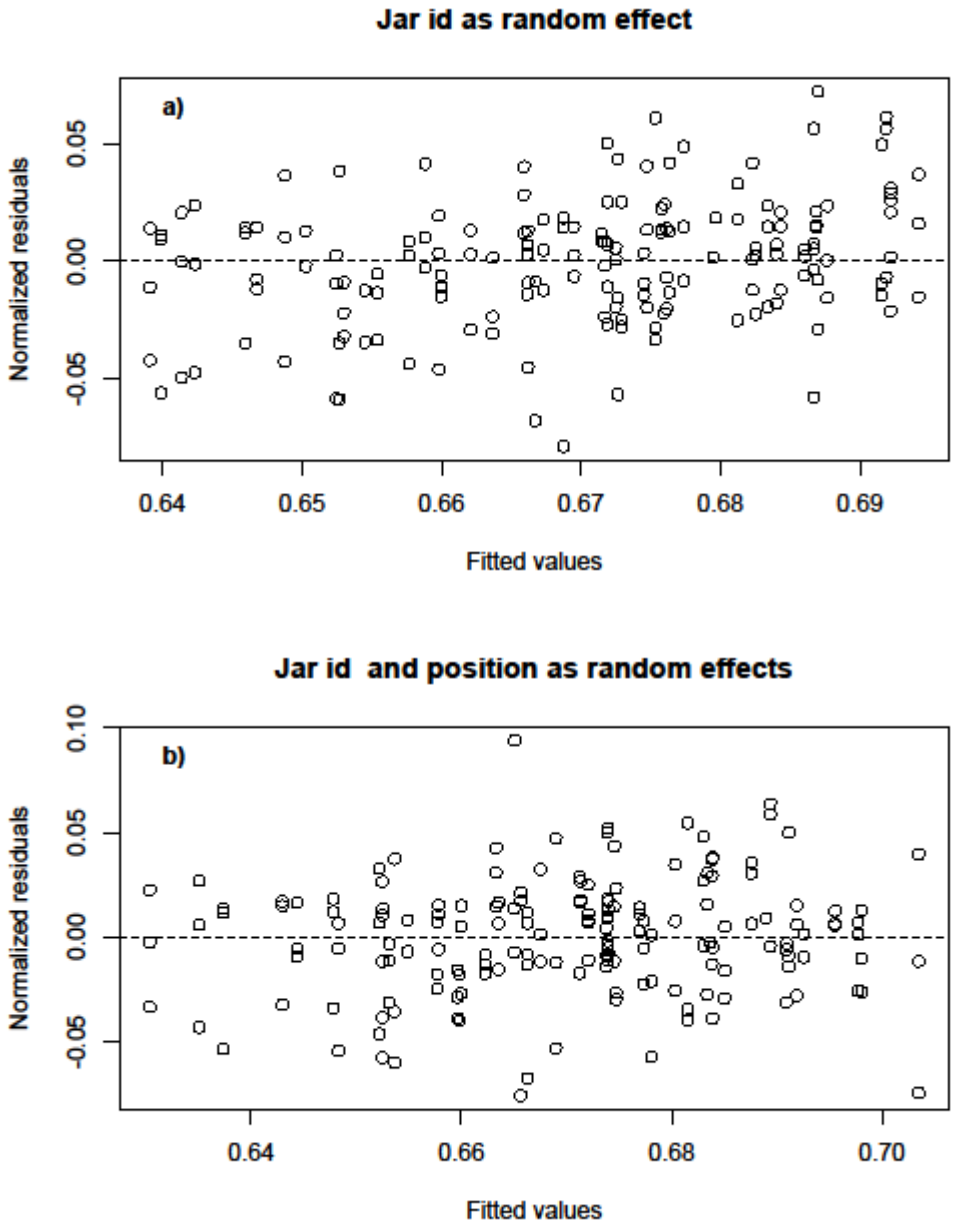

Fig. S9. Residuals of two candidate models for F_v/F_m analysis. Above (a)): a model fitted with pCO_2 and light interaction, plus random intercept for the experimental jar. Below (b)): a model fitted with pCO_2 and light interaction, plus random intercept for the experimental jar and position along the experiment.

As the full F_v/F_m model (model with pCO_2 and light interaction, and random intercept for the jar effect) showed a residual pattern, the effect of the non-homogenous light environment was included in the model as a second random effect. Column number (indicating horizontal position of the jar under fluorescent lights) was coded into a factorial covariate (10 levels) and modelled as a random intercept. This removed the residual pattern observed when plotting the normalized residuals against fitted values (Fig. S9b), and thus the model selection process was conducted with

the random structure described. The interaction term (Light*pCO_2) was significant (Table 4 in ms), and assumptions of the model were examined by plotting all covariates against fitted values.

S 3.3. Analysis of α

Full model in analysis of alpha in the summer experiment had minor residual patterns, most notably related to more negative residuals associated with smaller fitted values (Fig. S10).

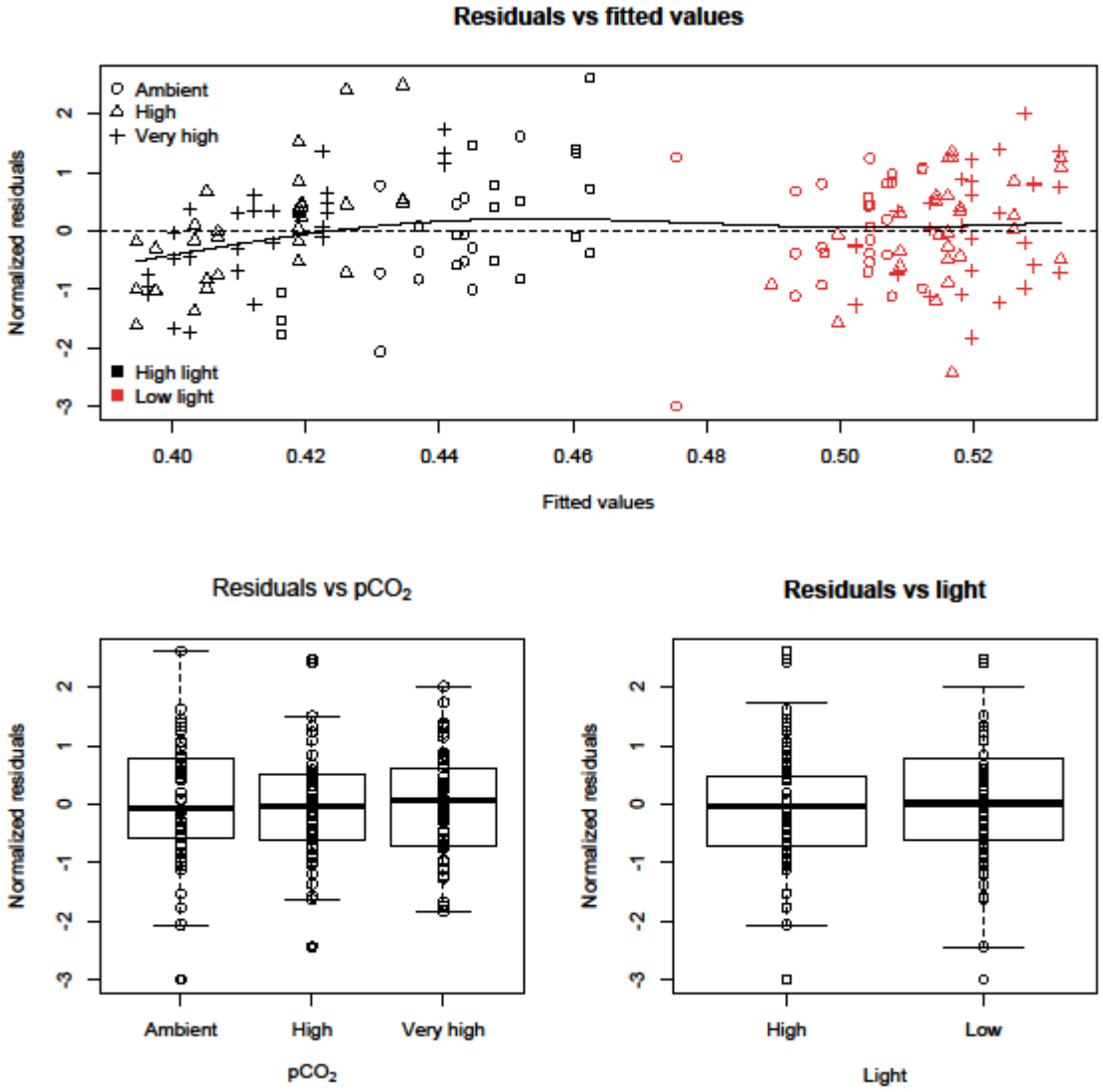

Fig. S10. Model validation graphs for the full model for alpha in the summer experiment (pCO_2*light interaction and various variances allowed for both levels of light). The symbol in the upper plot indicates pCO_2 treatment. Trendline added for visualization.

When testing the significance of the interaction with likelihood ratio tests, it turned out to be non-significant ($p = 0.059$). When the interaction was dropped, however, the residual patterns observed when plotting residuals against fitted values increased (Fig. S10), although no obvious residual patterns were observed when plotting residuals against covariates. This indicates that the

interaction should have been included in the model, despite the model selection process identified only light having a significant effect on alpha.

To remove the residual patterns, we also tried adding jar position as a random factor into the model as with the F_v/F_m analysis, and transforming the response variable in multiple ways, but these did not lead to any improvements in the model validation graphs.

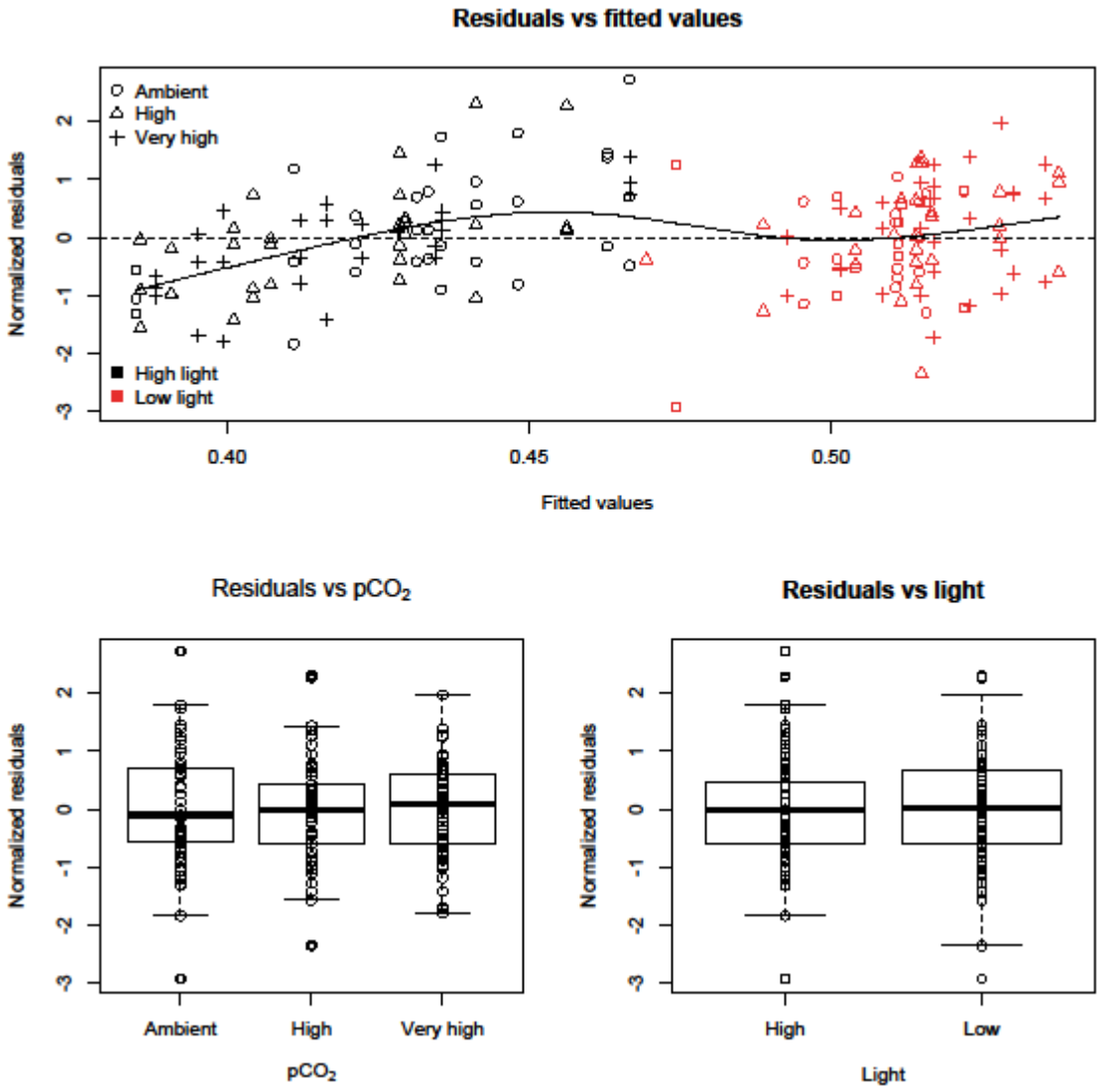

Fig. S11. Model validation graphs for a no-interaction model for alpha in the summer experiment (pCO_2 and light, interaction dropped and various variances allowed for both levels of light). A symbol in the upper plot indicates pCO_2 treatment. Trendline added for visualization.

S 3.4. Analysis of relative magnitude of effects

The relative magnitude of the effects of season, light and pCO_2 (2 levels) was analysed using Welch's ANOVA. The standardized regression coefficients of the main effects were compared. Standardization was used because the various variables studied were measured on very different scales. Differences between main effects (season, light, CO_2) were analysed with Games-Howell post hoc –test (Table S2).

Table S2. Results of Games-Howell post hoc –test of the differences of standardized main effects of different factors (Season, Light, pCO_2 high, PCO_2 extreme).

Comparison	Difference	95 % c.i.	t	df	p
pCO_2 high-pCO_2 extreme	0.11	-0.07 – 0.29	1.87	9.71	0.301
Light-pCO_2 high	0.35	-0.09 – 0.80	2.54	8.28	0.125
Season-pCO_2 high	0.60	0.10 – 1.10	3.81	8.22	0.021*
Light-pCO_2 extreme	0.25	-0.21 – 0.7	1.65	10.49	0.395
Season-pCO_2 extreme	0.49	-0.02 – 1	2.96	9.97	0.059.
Season-Light	0.24	-0.35 – 0.84	1.17	15.77	0.652

Significance levels: *: $p < 0.05$, .: marginally significant ($p \sim 0.05$).

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