Future CO_2-induced seawater acidification mediates the physiological performance of a green alga *Ulva linza* in different photoperiods

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Abstract

19 Photoperiods have an important impact on macroalgae living in the intertidal zone. Ocean
20 acidification also influences the physiology of macroalgae. However, little is known about the
21 interaction between ocean acidification and photoperiod on macroalgae. In this study, a green
22 alga *Ulva linza* was cultured under three different photoperiods (L: D = 8:16, 12:12, 16:8) and
23 two different CO_2 levels (LC, 400 ppm; HC, 1000 ppm) to investigate their responses. The
24 results showed that relative growth rate of *U. linza* increased with extended light periods under
25 LC but decreased at HC when exposed to the longest light period of 16h compared to 12h.
26 Higher CO_2 levels enhanced the relative growth rate at a L: D of 8:16, had no effect at 12:12 but
27 reduced RGR at 16:8. At LC, the L: D of 16:8 significantly stimulated maximum quantum yield
28 (Yield). Higher CO_2 levels enhanced Yield at L: D of 12:12 and 8:16, had negative effect at 16:8.
29 Non-photochemical quenching (NPQ) increased with increasing light period. High CO_2 levels did
30 not affect respiration rate during shorter light periods but enhanced it at a light period of 16h.
31 Longer light periods had negative effects on Chl *a* and Chl *b* content, and high CO_2 level also
32 inhibited the synthesis of these pigments. Our data demonstrate the interactive effects of CO_2 and

33   photoperiod on the physiological characteristics of the green tide macroalga *Ulva linza* and

34   indicate that future ocean acidification may hinder the stimulatory effect of long light periods on

35   growth of *Ulva* species.

Introduction

37   Due to the activities of humans, the concentration of atmospheric CO_2 has increased to 400 ppm

38   from 278 ppm during the pre-industrial revolution (Gattuso et al., 2015). It is estimated that the

39   oceans, as a CO_2 sink, have taken up approximately 48% of the fossil-fuel and cement-

40   manufacturing emissions (Sabine et al., 2004). The concentration of atmospheric CO_2 has been

41   predicted to reach almost 1000 ppm by the end of 21st century (Gattuso et al., 2015). This would

42   result in the decline of surface ocean pH by 0.3-0.4 units, an increase of hydrogen ion

43   concentration and bicarbonate concentration in seawater, which is termed ocean acidification

44   (OA) (Orr et al., 2005; Koch et al., 2013 Albright et al., 2016). OA will be maintained for

45   hundreds of years, and thus could cause a huge change in marine ecosystems (Kroeker et al.,

46   2013; Wittmann & Pörtner, 2013).

47   Marine phytoplankton accounts for 50% of global primary production (Beardall et al., 2009)

48   and macroalgae accounts for 10% of marine primary productivity (Smith, 1981; Charpy-

49   Roubaud & Sournia, 1990; Heinrich et al., 2012). Macroalgae have evolved distinct strategies to

50   capture inorganic carbon (Ci) for photosynthesis: (1) by the diffusive uptake of CO_2 (Giordano &

51   Raven, 2005; Raven et al., 2005), (2) the active transport of HCO_3^-/CO_2 (termed carbon

52   concentrating mechanisms (CCMs) (Maberly et al., 1992; Raven, 2003; Giordano et al., 2005).

53   The elevated CO_2 might enhance the growth and photosynthesis of algae that depend on CO_2

54   diffusion or have weak CCMs (Kübler et al., 1999; Gao et al., 2016a). Therefore, the

55   physiological influences of OA on macroalgae might be species-specific. For example, OA has a

56   positive influences on the growth of macroalgae, such as the red algae *Pyropia yezoensis,*

57   *Pyropia haitanensis* and *Gracilaria lemaneiformis* (Gao et al., 1991; Xu & Gao, 2015; Chen et

58   al., 2018), green algae *Ulva prolifera*, *Ulva lactuca, Caulerpa taxifolia* (Xu & Gao, 2012; Chen

59   et al., 2015; Roth-Schulze et al., 2018) and brown algae *Hizikia fusiforme, Ecklonia radiata*,

60   *Sargassum muticum* (Zou, 2005; Britton et al., 2016; Xu et al., 2017). However, research has

61   shown that high CO_2 levels did not have a detectable effect on the relative growth rate of several

62   species, for example, *Sargassum henslowianum*, *Ulva rigida*, *Ulva australis*, the giant kelp

63   *Macrocystis pyrifera* and *Ulva australis* (Chen & Zou, 2014; Rautenberger et al., 2015;

64   Fernández et al., 2015; Reidenbach et al., 2017). Furthermore, increasing seawater CO_2

65   concentration reduced the growth of *Gracilaria tenuistipitata*, *Porphyra leucosticte, Fucus*

66   *vesiculosus, Halimeda opuntia* and *Ulva linza* (García-Sánchez et al., 1994; Mercado et al., 1999;

67   Gutow et al., 2014; Johnson et al., 2014; Gao et al., 2018). In addition, the various effects of

68 ocean acidification may be also modulated by other environmental factors, such as temperature,
69 nutrients and photoperiods (Gao et al., 2017a; Gao et al., 2019; Li et al., 2018).
70   Photoperiod is a key factor regulating seasonal responses of algae (Dring, 1998).
71 Furthermore, daylength can influence algal affinity for dissolved inorganic carbon and cellular
72 carbon demand, thus imposing an effect on CCMs regulation (Rost et al., 2006). Thus, the
73 photoperiod may be critical for algae cultured under elevated CO_2 levels. Meanwhile, the
74 interactive effects of OA and increased photoperiods could enhance the growth of *U. prolifera*,
75 thus increase the opportunities for occurrence of green tides (Li et al., 2018). Elevated CO_2 levels
76 enhanced the photosynthetic rate, but longer illumination periods reduced the photosynthetic
77 efficiency in *E. huxleyi* (Nielsen, 1997; Rost et al., 2006). Therefore, at HC, the responses of
78 algae to different photoperiods appear to be genera- and species-specific.
79   Green macroalgal bloom has occurred each year in the Yellow Sea (YS) since 2007, termed
80 green tides (Xu et al., 2016). Green tides generate aesthetic problems and toxic gas when thalli
81 decompose (Gao et al., 2016b). *Ulva* is the dominant genus contributing majority of green tides
82 and *U. linza* is one of species causing green tides in the Yellow Sea (Fletcher, 1996; Kang et al.,
83 2014; Gao et al., 2017a, b). However, little is known about the interactive effects of ocean
84 acidification and photoperiod on *U. linza*. Previous study showed that the effect of ocean
85 acidification on diatoms was related to light intensity (Gao et al., 2012). In this study, based on
86 previous studies, we hypothesized the effect of ocean acidification on *U. linza* may dependent on
87 photoperiod. To test our hypothesis, the physiological responses of the green macroalga *Ulva*
88 *linza* under two different CO_2 levels and three different photoperiods were examined.

89

Materials & Methods

90

Thalli collection and culture conditions

91

92 *U. linza* was collected from the coastal water of Gaogong peninsula (119.3°E, 34.5°N),
93 Lianyungang, Jiangsu Province, China. Gaogong peninsula is a public place and no approval is
94 required for collecting naturally growing *Ulva* species in China because *Ulva* species cause
95 green tides in coastal waters of the Yellow Sea. *U. linza* was identified by morphological
96 characters (Ma et al., 2009). The samples were transferred to the laboratory in a portable cooler
97 (4-6°C) box within one hour. Healthy thalli (first observed by color and then checked with
98 maximum quantum yield of PSII) were selected and cleaned with filtered natural seawater to
99 remove sediments, visible epiphytes and attached animals. Thalli were pre-cultured in a 500 ml
100 flask in an illuminated incubator (GXZ-500B, Ningbo, China) at 20 °C, with the illumination
101 intensity set at 150 μmol photons m^{-2} s^{-1} (12L:12D). Sterilized filtered seawater (salinity 30,

102   supplied with 8 μM NaH_2PO_4 and 60 μM $NaNO_3$) was used as culture medium and the medium

103   was bubbled with air before being renewed every two days.

104   For these experiments, approximately three 1 cm long thalli were placed into a 550 ml flask

105   filled with 500 ml sterile seawater aerated ambient outdoor air (400 ppm, LC) or with CO_2-

106   enriched air (1000 ppm, HC) and randomly cultured in three separate incubators with different

107   light levels (8L:16D, 12L:12D, 16L:8D) and continuous aeration at 20 ℃ with 150 μmol photons

108    $m^{-2}s^{-1}$. Different CO_2 levels (400 ppm, LC; 1000 ppm, HC) were achieved by bubbling ambient

109   air or CO_2-enriched air via a CO_2 plant incubator (HP 1000 G-D, Ruihua Instruments, Wuhan,

110   China).

111   To maintain the pH_{NBS} at about 8.12 (LC) and 7.78 (HC) under different photoperiods, the

112   increased algal biomass were removed constantly and the medium was changed every two days

113   and daily variations in pH were maintained at less than 0.05. The experiment lasted 9 days, the

114   physiological factors were taken on the last 3 days.

115

Estimate of carbonate system parameters

117   The seawater pH was monitored with a pH meter (pH 700, Eutech Instruments, Singapore) and

118   total alkalinity (TA) was calculated by titrations (Gao et al., 2019). Other parameters of the

119   carbonate system were obtained with CO2SYS software (Pierrot et al., 2006), the equilibrium

120   constants K_1 and K_2 for carbonic acid dissociation (Roy et al., 1993).

Measurement of growth

122   The length of *U. linza* was recorded every 2 days. The relative growth rate (RGR) of thalli

123   were calculated across the 6-day period before other physiological parameters were measured

124   during the following 3 days. RGR was calculated as follows: RGR = ln (W_t / W_0) /t, where W_t is

125   the length after t days culture, W_0 is the initial length.

Chlorophyll fluorescence measurements

127   Chlorophyll *a* fluorescence in *U. linza* was measured with a portable PAM (Pulse-amplitude-

128   modulation; AquaPen-P AP-P 100, Chech). Algae were dark adapted for 15 min before the

129   experiment. The parameters were calculated according to the following equations:

130   rETR = PAR × Y(II) × 0.84 × 0.5 (Schreiber, 2004; Zhang et al., 2017),

131   where rETR is the relative electron transport rate; PAR is the actinic light; Y(II) is the maximum

132   quantum yield of PSII.

133   NPQ= Fm -Fm'/1 (Bilger & Schreiber, 1986),

134   where NPQ is the non-photochemical quenching; Fm is the maximum fluorescence value of *U.*

135 *linza* when they were adapted in the dark for 15 min; Fm' is the maximum fluorescence value of
136 *U. linza* under actinic light conditions.

Respiration rate measurements

138 The respiration rate of *U. linza* was measured using a Clark-type oxygen electrode (YSI Model
139 5300, Yellow Springs Instrument Co., USA). The thallus was cut into 1 cm long segments with
140 scissors and the thalli were placed in culture conditions for at least 1h to decrease the effects of
141 cutting damage. Approximately 0.01g fresh weight of thalli were placed in the reaction chamber
142 with 8 ml medium. Temperature was controlled at 20 ℃ with a circulating water bath. The
143 decrease of the oxygen content in the seawater in darkness with seven minutes was defined as the
144 respiration rate.

Measurement of photosynthetic pigments

146 Chlorophyll *a* and *b* were extracted from thalli (about 10 mg FW) with 5 ml methanol at 4 ℃ for
147 24h in the dark. The absorption values were obtained at 652 nm, 663 nm and 665 nm using an
148 ultraviolet spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience, Sweden). The
149 contents of the Chl *a* and Chl *b* were estimated using the method of Porra (1989).

Data analysis

151 All the data are shown as mean ± SD. Origin 9.0 and SPSS 18.0 were used to analyze data. Two-
152 way ANOVA was used to assess the interactive effects of CO_2 levels and photoperiods on
153 relative growth rate, chlorophyll fluorescence parameters, respiration rate and pigment content of
154 *U. linza*. One-way ANOVA was used to analyze differences under the same conditions. Tukey
155 HSD was conducted for *post hoc* investigation. Confidence intervals were set at 95%.

Results

157 Both the elevated CO_2 levels and photoperiod altered carbonate parameters in seawater, and they
158 both had an interactive effect (Tables 1 and 2). The elevated CO_2 decreased pH and CO_3^{2-},
159 increased pCO_2, DIC, HCO_3^- and CO_2 in the seawater. Increased photoperiod did not affect
160 carbonate parameters at LC but the longest photoperiod increased DIC, HCO_3^- and TA compared
161 to shortest photoperiod.

162   The two-way ANOVA showed that elevated CO_2 and photoperiod had an interactive effect,
163 and both elevated CO_2 levels and the photoperiods had a significant effect on the RGR of *U.*
164 *linza* (Fig. 1 and Table 3). At LC, the RGR of adult *U. linza* increased with the extended light
165 periods, and the highest RGR occurred at a L: D of 16:8. The effect of CO_2 also varied with
166 photoperiod. Higher CO_2 levels enhanced RGR at L: D of 8:16, but had no effect at 12:12 and
167 reduced it at 16:8.

168 The Yield and NPQ were measured under different CO_2 levels and photoperiod conditions
169 (Fig. 2). Two-way ANOVA showed that elevated CO_2 and photoperiod had an interactive effect
170 on Yield (Table 4). Higher CO_2 levels increased Yield when thalli were cultured under
171 photoperiods of 8:16 and 12:12 but reduced it under 16:8. Photoperiod had the main effect on
172 NPQ (Table 4). At LC, thalli cultured at L: D of 16:8 had higher NPQ compared to L: D of 8:16
173 while the difference between 8:16 and 12:12 was insignificant. At HC, NPQ increased with the
174 increase in photoperiod although the increase was not statistically significant. The elevated CO_2
175 had neutral effect on NPQ of *U. linza*.

176 Maximum rETR (rETRmax), efficiency of electron transport (α), and saturating irradiance (I_k)
177 were calculated from the rapid light curves (Fig. 3, Table 5). Photoperiod and elevated CO_2
178 levels had an interactive effect, and elevated CO_2 levels had a main effect on light-saturated
179 electron transport rate (rETRmax) (Table 6). Higher CO_2 levels increased rETRmax during the
180 8:16 and 12:12 photoperiods, but did not affect it at 16:8. A similar pattern was also found for α.
181 In contrast to rETRmax and α, CO_2 did not affect I_k while photoperiod had the main effect on it.
182 At LC, I_k increased when L: D rose from 8:16 to 12:12 but did not change with the further
183 increase in photoperiod. At HC, I_k did not change when L: D rose from 8:16 to 12:12 but was
184 enhanced when L: D increased to 16:8 (Table 5).

185 In addition to photosynthetic parameters, the effects of pCO_2 and photoperiod on the
186 respiration rate of adult *U. linza* were also investigated (Fig. 4). Photoperiod and elevated CO_2
187 levels had an interactive effect, and both photoperiod and elevated CO_2 levels had the primary
188 effect on the respiration rate of *U. linza* (Table 7). Higher CO_2 levels did not affect the
189 respiration rate at photoperiods of 8:16 or 12:12 but increased it by 56.39% at a photoperiod of
190 16:8.

191 Changes in photosynthetic pigments of *U. linza* grown under various conditions are shown in
192 Fig. 5. Two-way ANOVA showed that CO_2 and photoperiod had an interactive effect, and both
193 CO_2 levels and the photoperiods had the main effect on the Chl *a* content of *U. linza* (Table 8).
194 Prolonged light periods reduced the synthesis of Chl *a* in thalli although the difference between
195 the photoperiods of 8:16 and 12:12 at HC was not statistically significant. Higher CO_2 levels
196 reduced Chl *a* at the photoperiods of 8:16 and 12:12 but did not affect it at a L: D of 12:12. The
197 same trend was found for Chl *b*. The Chl *a/b* ratios were all greater than 1.0, suggesting a higher
198 synthesis of Chl *a* than Chl *b* under all culture conditions. Photoperiod and elevated CO_2 levels
199 had an interactive effect on the Chl *a/b* ratio (Table 8); the higher CO_2 levels increased the ratio
200 at a photoperiod of 12:12 but not at the other photoperiods.

Discussion

202 In the present study, at LC, extended photoperiods had a positive effect on the relative growth
203 rate of adult *U. linza,* similar to previous studies on *Laminaria sacharina, Porphyra umbilicalis*

204 and *Ulva prolifera* (Fortes & Lüning,1980; Green & Neefus, 2016; Li et al., 2018). Carbon

205 isotope fractionation experiments suggested that extended photoperiods could enhance growth by

206 influencing inorganic carbon capture and fixation rate in algae (Rost et al., 2003). This

207 hypothesis is supported by the present study where extended photoperiods increased the

208 maximum quantum yield in PS II. On the other hand, the highest growth rates of *Compsopogon*

209 *coeruleus* were obtained in shorter light periods (L: D = 8:16) (Zucchi & Necchi, 2001), the

210 highest growth rates of *Porphyra umbilicalis* was found under neutral (L: D = 12:12) and longer

211 (L: D = 16:8) light periods using 110 μmol photons $m^{-2}s^{-1}$ (Green & Neefus, 2016), and the

212 relative growth rate of *Chlorella vulgaris* cultured at L: D = 20:4 was lower than the RGR of

213 alga cultured at L: D = 16:8 (Kendirlioglu et al., 2015). Therefore, the effects of photoperiod on

214 algae appear to be species-specific.

215 Although *Ulva* has efficient mechanisms for CO_2 concentration, the growth of *Ulva* can be

216 enhanced by elevated CO_2 (Young et al., 2016; Gao et al., 2016a, 2017). However, in this study,

217 we found that the effects of elevated CO_2 levels on the relative growth rate of *U. linza* depended

218 on photoperiod. CO_2 enhanced the growth of adult *U. linza* under light/dark conditions of 8:16,

219 had no effect on growth of *U. linza* under a L: D of 12:12, and reduced the relative growth rate

220 under a L: D of 16:8. High CO_2 levels can down-regulate algal CO_2 concentration mechanisms

221 (CCMs), meaning that energy would be saved, and thus enhancing the relative growth rate of

222 algae (Gao et al., 2012, 2016a; Raven et al., 2017). This is supported by decreased pigment

223 synthesis in thalli at higher CO_2 levels. However, higher CO_2 levels did not affect the growth rate

224 of *U. linza* at medium photoperiods. The neutral effects of CO_2 on the growth of *U. rigida*

225 (Rautenberger et al., 2015) and *U. linza* (Gao et al., 2018) were also reported. We speculate that

226 this is a compromise between the positive effects of elevated CO_2 and negative effects of

227 decreased pH. The negative effect of decreased pH on growth was documented for the brown

228 alga *Sargassum muticum* (Xu et al., 2017). Algae might need to consume additional energy to act

229 against acid-based perturbation caused by decreased pH, leading to reduced growth (Xu et al.,

230 2017). This is supported by an enhanced respiration rate at the higher CO_2 levels in this study.

231 The phenomenon of an increased respiration rate of algae under elevated CO_2 concentrations was

232 found in *Hizikia fusiformis* (Zou et al., 2005), the microalgae *Phaeodactylum tricornutum* (Wu et

233 al., 2010) and *Emiliania huxleyi* (Jin et al., 2015).

234 Furthermore, the higher CO_2 level reduced the growth rate of *U. linza* under the longest

235 photoperiod in the present study. This may be due to the combination of down-regulated CCMs

236 and excess light energy. The operation of CCMs is an energy-consuming process and the down-

237 regulation of CCMs can result in additional energy (Raven et al., 2017). Higher light can usually

238 reduce algal photosynthetic activity (Singh and Singh, 2015; Gao et al., 2016a). The energy

239 saved due to down-regulation of CCMs at HC combined with high light intensity could

240 synergistically damage the algal photosystem and photosynthetic rate (Gao et al., 2012, 2016a).
241 Although light intensity did not change among different CO_2 treatments, the lengthened
242 photoperiod may have similar effect to increased light intensity. This argument is supported by
243 increased NPQ at longer photoperiods as NPQ is photo-protective process to dissipate excess
244 light energy. Higher CO_2 levels also reduced maximum quantum yield and stimulated the
245 respiration rate of *U. linza* during the longest photoperiod in this study, leading to the decrease in
246 growth. Enhanced respiration rate is a signal that organisms are fighting against stress and
247 damage (Xu et al., 2017).
248   In addition to growth, the interactive effect of CO_2 and photoperiod was also found in
249 photosynthetic parameters. For instance, elevated CO_2 increased Yield, rETRmax and α at
250 shorter photoperiods (L: D of 8:16 and 12:12) but did not affect rETRmax and reduced Yield at
251 longest photoperiod (L: D of 16:8). These findings indicate the close connection between growth
252 and photosynthesis in terms of responding to the combination of CO_2 and photoperiod. It is
253 worth noting that the interaction of CO_2 and photoperiod on growth of *U. linza* in this study is
254 different from the findings in *U. prolifera* reported by Li et al (2018). Elevated CO_2 increased
255 growth of *U. prolifera* at all three photoperiods (L: D of 12:12, 10:14 and 16:8). The different
256 results may be due to differential physiological property between *U. linza* and *U. prolifera*. It has
257 been documented that *U. prolifera* has a higher tolerance to high light intensity compared to *U.
258 linza* (Cui et al., 2015). The strong capacity in dealing with high light intensity could contribute
259 to the effect of elevated CO_2 and prolonged photoperiod on growth *U. linza* was still positive.
260 Integrating our findings with Li et al's (2018) study, the interaction of CO_2 and photoperiod on
261 *Ulva* species would be species dependent.
262

Conclusions

264 This work is the first attempt to clarify the interaction between light/dark and elevated CO_2
265 levels on the physiological responses of *Ulva linza*. We found that the effect of OA on *U. linza*
266 depended on photoperiod. Outbreaks of green tides during spring and summer in China occur
267 when the photoperiod is reaching its peak. Our findings indicate future OA may hinder the
268 occurrence of green tides dominated by *U. linza* in combination with extended photoperiods.
269 More environmental factors, such as temperature and nutrient levels, need to be investigated to
270 obtain a more comprehensive understanding on development of green tides in future oceans.

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Figure legend

Fig. 1. Relative growth rate (RGR) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8). Vertical bars are mean ± SD for triplicate samples. Different lowercase letters denote significant difference among the photoperiod levels at LC and different capital letters denote significant difference among the photoperiod levels at HC. Significant differences between two CO_2 levels under the same photoperiod are indicated by short lines.

Fig. 2. Yield (A) and NPQ (B) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8). Vertical bars are mean ± SD for triplicate samples. Different lowercase letters denote significant difference among the photoperiod levels at LC and different capital letters denote significant difference among the photoperiod levels at HC. Significant differences between two CO_2 levels under the same photoperiod are indicated by short lines.

Fig. 3. Rapid light curves (RLC) of *U. linza* thalli grown at ambient CO_2 levels (400 ppm, LC, A; 1000 ppm, HC, B) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Fig. 4. Respiration rate of *U. linza* thalli grown at different CO_2 levels (1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8). Vertical bars are mean ± SD for triplicate samples. Different lowercase letters denote significant difference among the photoperiod levels at LC and different capital letters denote significant difference among the photoperiod levels at HC. Significant differences between two CO_2 levels under the same photoperiod are indicated by short lines.

Fig. 5. Content of the pigments Chl *a* (A) and Chl *b* (B), and the Chl *a*/*b* ratio (C) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8). Vertical bars are mean ± SD for triplicate samples. Different lowercase letters denote significant difference among the photoperiod levels at LC and different capital letters denote significant difference among the photoperiod levels at HC. Significant differences between two CO_2 levels under the same photoperiod are indicated by short lines.

Figure 1

Relative growth rate.

Relative growth rate (RGR) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

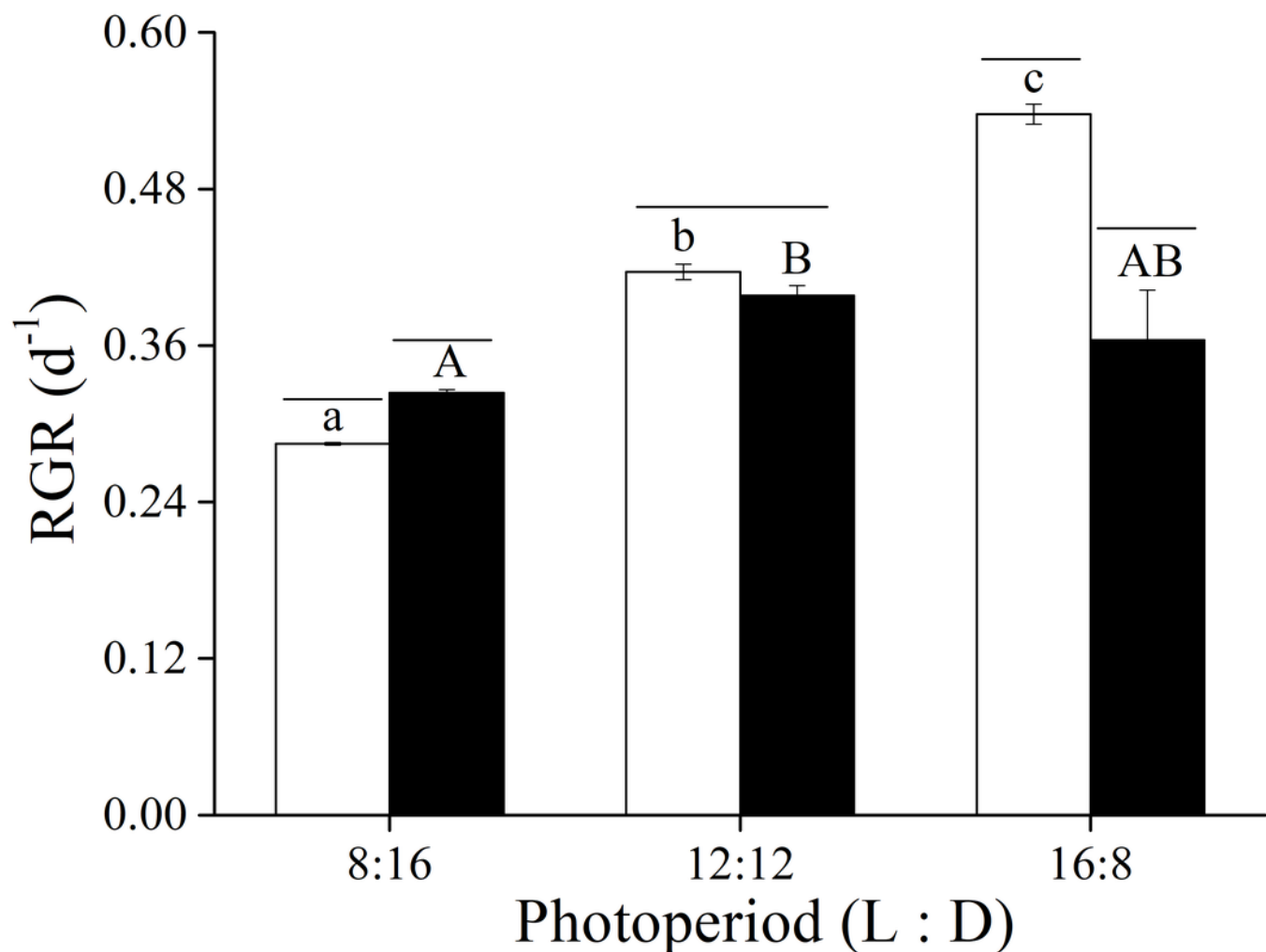



Figure 2

Yield and NPQ.

Yield (A) and NPQ (B) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

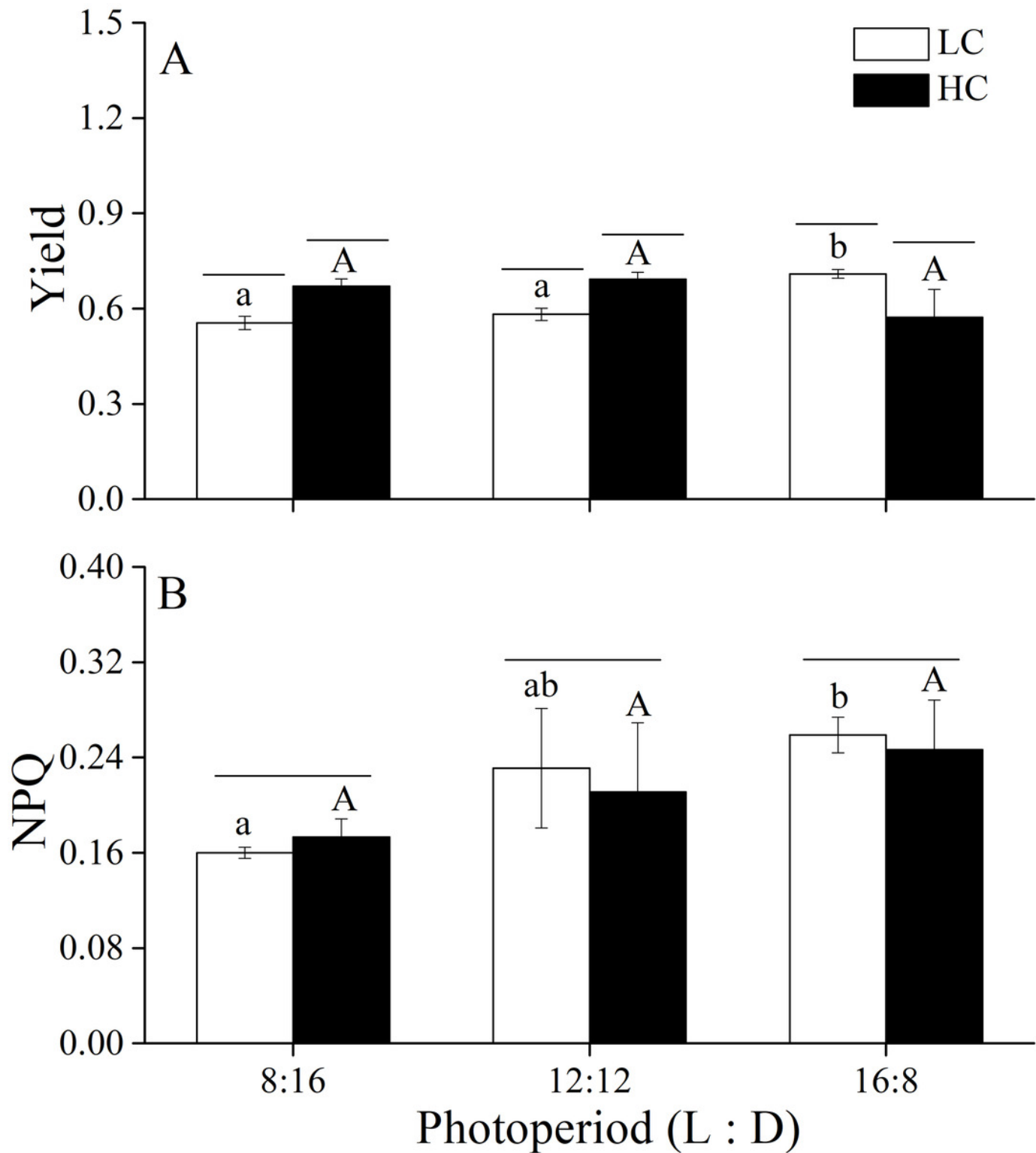



Figure 3

Rapid light curves.

Rapid light curves (RLC) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

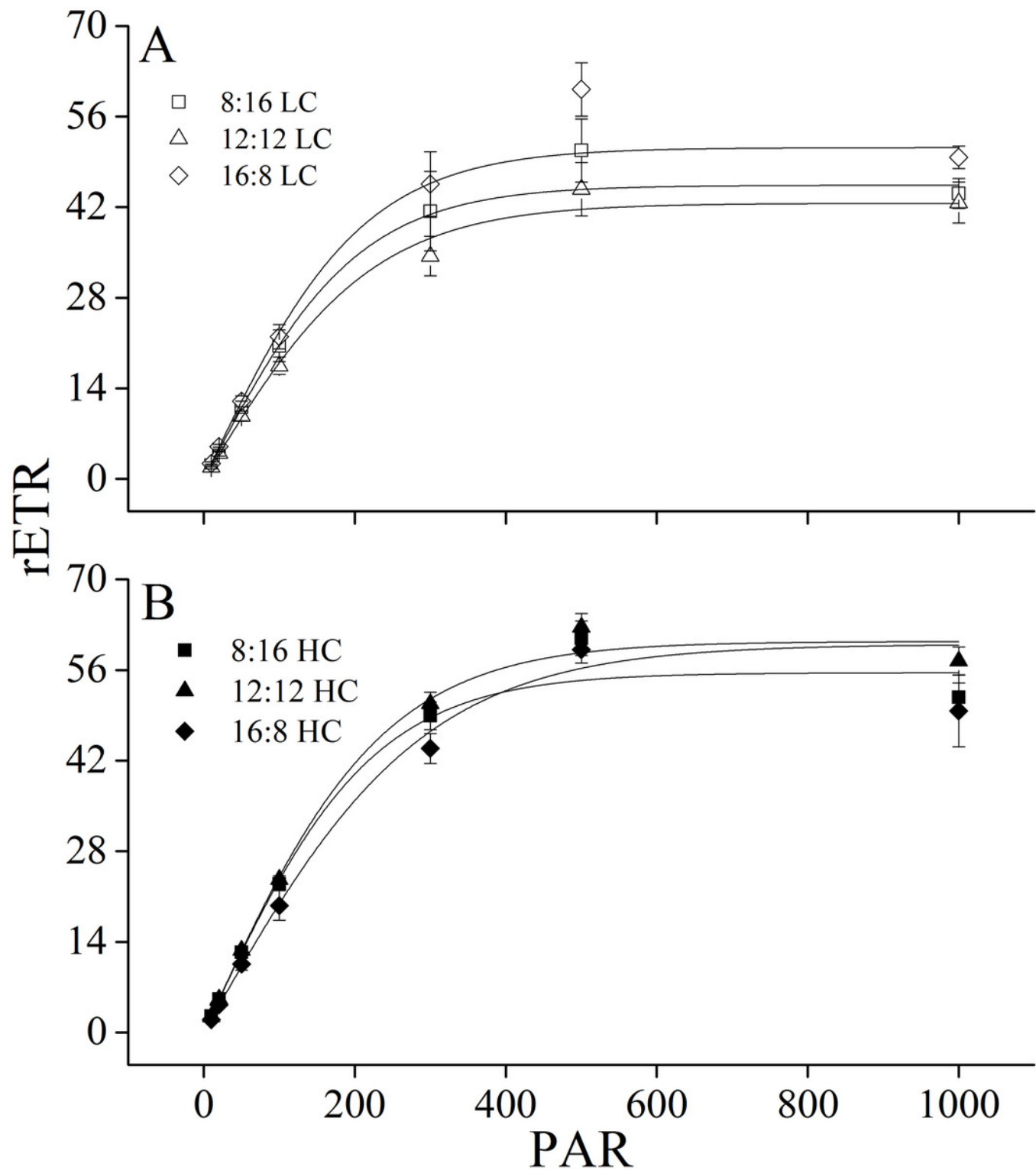



Figure 4

Respiration rate.

Respiration rate of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

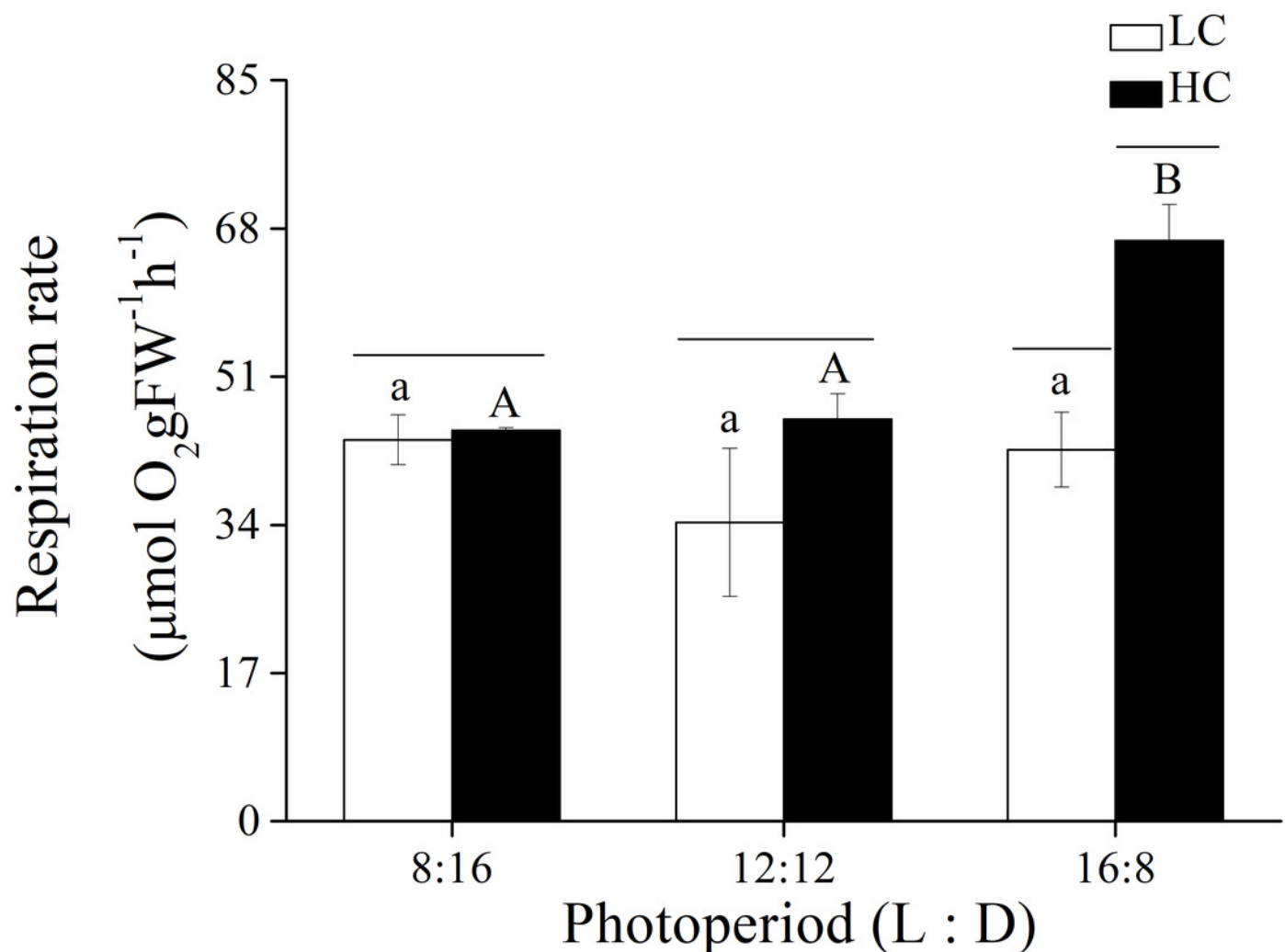
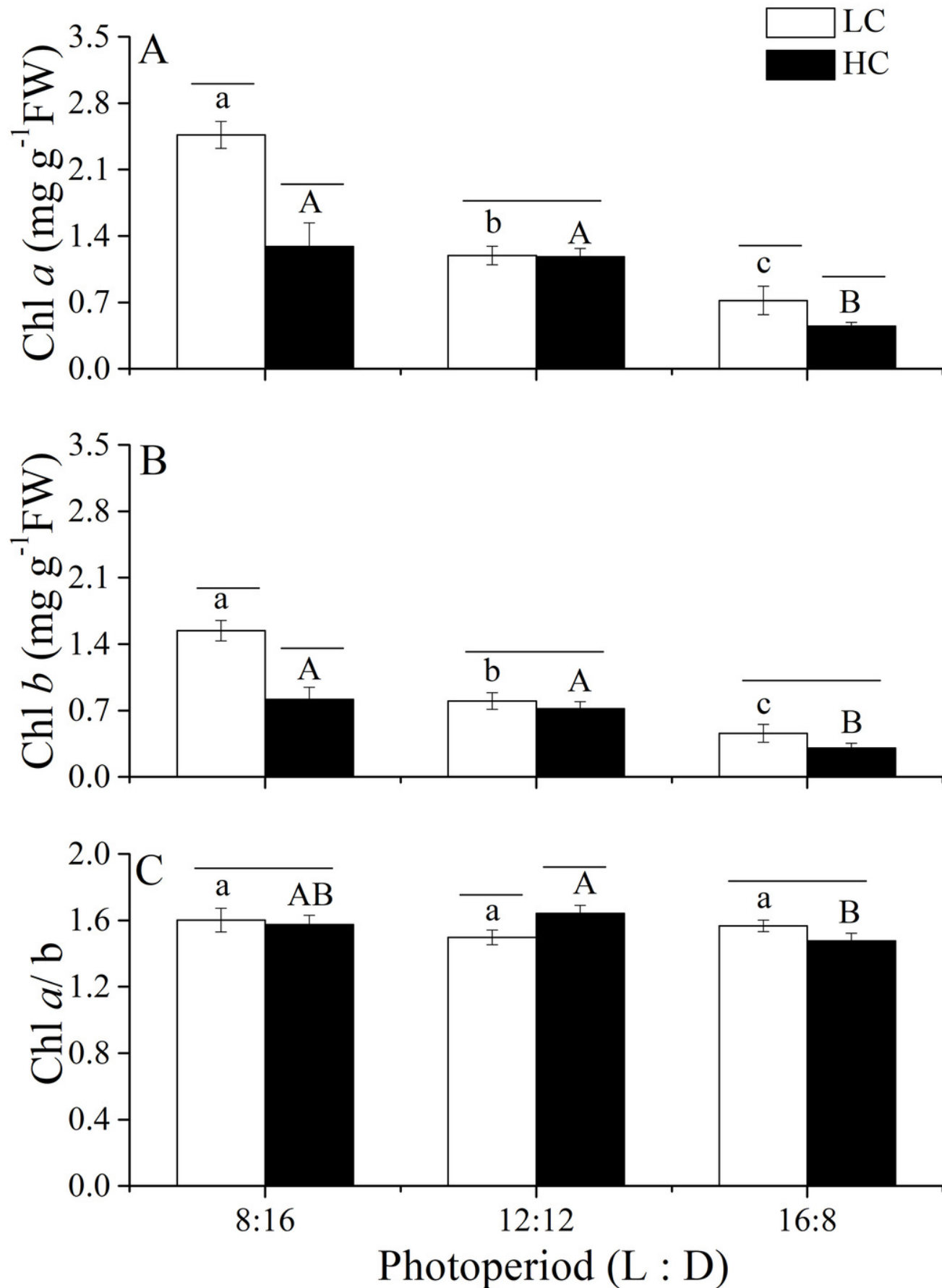



Figure 5

Pigment contents and the Chl *a/ b* ratio.

Pigment contents (Chl *a,* A; Chl *b,* B) and the Chl *a/ b* ratio (C) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Parameters of the seawater carbonate system in different cultures. DIC = dissolved inorganic carbon, TA =total alkalinity. Data are the mean ± SD (n = 3).

TA = total alkalinity. Data are the mean ± SD (n = 3).

1 Table 1. Parameters of the seawater carbonate system in different cultures. DIC = dissolved inorganic carbon, TA = total alkalinity.
2 Data are the mean ± SD (n = 3).

	pH_{NBS}	pCO_2 (µatm)	DIC (µmol kg^{-1})	HCO$_3^-$ (µmol kg^{-1})	CO$_3^{2-}$ (µmol kg^{-1})	CO$_2$ (µmol kg^{-1})	TA (µmol kg^{-1})
8:16 LC	8.14 ± 0.02[a]	580.32 ± 35.74[a]	2597.04 ± 57[a]	2385.19 ± 56.15[a]	192.59 ± 7.23[a]	19.56 ± 1.19[a]	2850.78 ± 56.26[a]
12:12 LC	8.12 ± 0.01[a]	613.13 ± 11.21[a]	2594.32 ± 61.96[a]	2391.03 ± 55.85[a]	182.94 ± 6.07[a]	20.35 ± 0.37[a]	2834.31 ± 67.99[a]
16:8 LC	8.13 ± 0.01[a]	608.54 ± 14.83[a]	2638.26 ± 40.24[a]	2427.94 ± 35.09[a]	190.12 ± 6.73[a]	20.19 ± 0.49[a]	2886.86 ± 46.67[a]
8:16 HC	7.74 ± 0.03[b]	1614.77 ± 100.05[b]	2780.28 ± 12.05[b]	2641.71 ± 12.23[b]	84.99 ± 5.08[b]	53.58 ± 3.32[b]	2854.16 ± 13.32[a]
12:12 HC	7.79 ± 0.02[cd]	1445.67 ± 58.75[c]	2799.47 ± 14.61[b]	2655.70 ± 13.74[b]	95.80 ± 3.81[b]	47.97 ± 1.95[c]	2893.15 ± 17.18[a]
16:8 HC	7.75 ± 0.01[bd]	1652.45 ± 43.38[b]	2938.18 ± 47.63[c]	2790.86 ± 45.52[c]	92.48 ± 1.28[b]	54.83 ± 1.44[b]	3019.14 ± 46.95[b]

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod on pH, pCO_2, dissolved inorganic carbon (DIC), HCO_3^- , CO_3^{2-} ,CO_2 and total alkalinity (TA) in seawater.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means p-value.

1   Table 2. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on pH, pCO_2, dissolved inorganic

2   carbon (DIC), HCO_3^-, CO_3^{2-}, CO_2 and total alkalinity (TA) in seawater. CO_2*photoperiod means the interactive effects of CO_2 and

3   photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means p-value.

Source	df	pH		pCO_2		DIC		HCO_3^-		CO_3^{2-}		CO_2		TA	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Photoperiod	2	1.333	0.3	5.640	0.019	9.697	0.003	10.112	0.003	.353	0.710	5.632	0.019	8.638	0.005
CO_2	1	2268.750	<0.001	1498.294	<0.001	125.390	<0.001	236.530	<0.001	1453.669	<0.001	1497.372	<0.001	8.977	0.011
Photoperiod*CO_2	2	7.750	0.007	7.562	0.007	3.054	0.085	3.184	0.078	5.342	0.022	7.557	0.008	2.976	0.089
Error	12														

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on RGR of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

1 Table 3. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes
2 on RGR of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.
3 CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of
4 freedom and F means the value of F statistic, and Sig. means *p*-value.

Source	df	F	Sig.
Photoperiod	2	124.249	<0.001
CO_2	1	42.292	<0.001
Photoperiod* CO_2	2	65.959	<0.001
Error	12		

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on Yield and NPQ of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

1   Table 4. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes
2   on Yield and NPQ of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.
3   CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of
4   freedom and F means the value of F statistic, and Sig. means *p*-value.

Source	Yield			NPQ		
	df	F	Sig.	df	F	Sig.
Photoperiod	2	0.919	0.425	2	8.453	0.005
CO_2	1	2.549	0.136	1	0.132	0.722
Photoperiod* CO_2	2	19.786	<0.001	2	0.339	0.719
Error	12			12		

Parameters of rapid light curves (RLC) of *U. linza* thalli cultured at different CO_2 levels and photoperiod regimes.

Data are the mean ± SD (n ≧ 3). Different letters represent significant difference ($p < 0.05$) among different treatments.

1 Table 5. Parameters of rapid light curves (RLC) of *U. linza* thalli cultured at different CO_2 levels

2 and photoperiod regimes. Data are the mean ± SD (n ≧ 3). Different letters represent significant

3 difference ($p < 0.05$) among different treatments.

	rETRmax	α	I_k
8:16 LC	47.53 ± 3.63[a]	0.22 ± 0.04[a]	215.67 ± 19.89[a]
12:12 LC	43.87 ± 3.60[c]	0.18 ± 0.02[c]	248.87 ± 22.91[bd]
16:8 LC	54.83 ± 2.65[b]	0.24 ± 0.03[ab]	231.58 ± 21.37[adef]
8:16 HC	56.51 ± 2.34[b]	0.25 ± 0.01[b]	222.15 ± 13.41[ac]
12:12 HC	60.45 ± 1.38[d]	0.26 ± 0.01[b]	236.58 ± 12.63[bce]
16:8 HC	54.55 ± 3.77[b]	0.22 ± 0.01[a]	251.72 ± 11.69[bf]

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on maximum rETR (rETRmax), efficiency of electron transport (α), and saturating irradiance (I_k) of *Ulva linza.*

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

1 Table 6. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on maximum rETR (rETRmax),

2 efficiency of electron transport (α), and saturating irradiance (I_k) of *Ulva linza* cultured at different CO_2 levels and photoperiod

3 regimes. CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of

4 F statistic, and Sig. means *p*-value.

Source	ETRmax			α			I_k		
	Df	F	Sig.	df	F	Sig.	df	F	Sig.
Photoperiod	2	2.981	0.066	2	2.909	0.070	2	7.030	0.003
CO_2	1	69.993	<0.001	1	15.225	<0.001	1	0.664	0.421
Photoperiod* CO_2	2	23.445	<0.001	2	14.896	<0.001	2	2.576	0.093
Error	30			30			30		

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on respiration rate of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

1   Table 7. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes

2   on respiration rate of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

3   CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of

4   freedom and F means the value of F statistic, and Sig. means *p*-value.

Source	df	F	Sig.
Photoperiod	2	16.006	<0.001
CO_2	1	33.058	<0.001
Photoperiod* CO_2	2	9.566	0.003
Error	12		

Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on Chl *a*, Chl *b*, and Chl *a/ b* of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

1   Table 8. Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on Chl *a*, Chl *b*, and Chl *a/ b* of *Ulva*

2   *linza* cultured at different CO_2 levels and photoperiod regimes. CO_2*photoperiod means the interactive effects of CO_2 and photoperiod,

3   df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

Source	Chl *a*			Chl *b*			Chl *a/ b*		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
Photoperiod	2	121.283	<0.001	2	111.035	<0.001	2	2.625	0.113
CO_2	1	51.793	<0.001	1	53.849	<0.001	1	0.171	0.686
Photoperiod* CO_2	2	26.932	<0.001	2	21.719	<0.001	2	8.389	0.005
Error	12			12			12		