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# The interactive effects of elevated temperature and nutrient concentrations on the physiological responses of *Ulva linza* Linnaeus (Ulvales, Chlorophyta)

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**Abstract**
We tested the interactive effects of increased temperature and nutrient (ammonium, $NH_4^+$) levels on physiological properties such as photosynthetic rates, $NH_4^+$ uptake rates, relative growth rates, chlorophyll fluorescence, and tissue nutrient contents in *Ulva linza* Linnaeus. The experiments were conducted at four temperatures (LT, low temperature (15 °C); MT, medium temperature (20 °C); CT, control temperature (25 °C); and HT, high temperature (30 °C)) and three $NH_4^+$ concentrations (LN, low nutrient (4 μM); MN, medium nutrient (60 μM); and HN, high nutrient (120 μM)). The interaction between temperature and $NH_4^+$ levels influenced the photosynthetic rates, $NH_4^+$ uptake rates, relative growth rates, photosynthetic efficiency, tissue nitrogen contents, and C:N ratios in algal tissues. Temperature strongly affected the photosynthetic rates, $NH_4^+$ uptake rates, and photosynthetic efficiency. Nutrient enrichment increased the photosynthetic rates, nutrient uptake rates, relative growth rates, photosynthetic efficiency, tissue nitrogen contents, and tissue C:N ratios. Our study results could help understand the physiological responses of *U. linza* under future ocean environmental conditions such as ocean warming and eutrophication.

**Keywords** Ammonium ($NH_4^+$) · Temperature · *Ulva linza* · Physiological responses

## Introduction

Atmospheric $CO_2$ concentration has increased from 280 ppm in the pre-industrial era to the current level of 400 ppm because of anthropogenic activities following the Industrial Revolution (IPCC 2014). Many studies predict $CO_2$ concentrations will double their current levels by the year 2100 (Roleda et al. 2012; IPCC 2014). According to the IPCC report (2014), global average temperature will increase by 0.6 to 2.0 °C, based on projections of the climate change model. If no effort is made to curb $CO_2$ emissions, then global average temperature will increase by 2.6 to 4.8 °C (IPCC 2014). In addition, seawater temperatures could increase from 1.9 to 5.8 °C by the end of the twenty-first century under elevated $CO_2$ conditions (IPCC 2014).

Seawater temperature is an important factor for macroalgae survival, growth, reproduction, morphology, and metabolism (Lüning and Neushul 1978; Davison 1991; Wernberg et al. 2010; Rothäusler et al. 2011; Martínez et al. 2012). Temperature change could influence the activity of enzymes including photosynthetic C and N assimilation, ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) activity, and nitrate reductase formation (Raven and Geider 1988; Davison 1991; Yoshida et al. 1999; Berges et al. 2002). Elevated seawater temperature can have a positive effect on some macroalgae (Fan et al. 2014; Zou and Gao 2014). However, ocean warming has negatively affected changing of biomass, productivity, growth, structure of community, and physiological performance of macroalgae (Raven and Geider 1988; Barry et al. 1995; Yesson et al. 2015; Ji et al. 2016; Kay et al. 2016). Macroalgae also experience high or low disruptive stresses in the form of cellular and subcellular damage (Davison and Pearson 1996; Eggert 2012). In addition, biogeographical distribution of macroalgae has moved from the tropical and temperate regions towards the poles because of ocean warming (Wernberg et al. 2011; Díez et al. 2012).

Eutrophication is also an acute environmental problem in the coastal areas that experience ocean warming (Lohman and

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Priscu 1992; Fei 2004). Elevated concentrations of nitrogen (N) and phosphorus (P) could increase macroalgal growth and biomass (Luo et al. 2012; Hurd et al. 2014; Li et al. 2016). Under natural conditions, nitrogen and phosphorus are factors limiting algal productivity (Dring and Dring 1991). Nitrogen is an important constituent of many compounds such as Rubisco, the main enzyme in photosynthesis (Dawes and Koch 1990); phosphorus has various chloroplast functions, including use in ATP generation, photosynthetic protein production, and enzyme phosphorylation (Zer and Ohad 2003). However, Carpenter (2008) reported that nitrogen and phosphorus could cause ocean eutrophication. Excessive nutrient input causes severe blooming of macroalgae such as *Ulva* spp., *Chaetomorpha* spp., *Cladophora* spp., and *Sargassum* spp., the latter two being responsible for what are known as green and golden seaweed tide, respectively (Taylor et al. 2001; Cohen and Fong 2006; Ye et al. 2011; Smetacek and Zingone 2013; Li et al. 2016). Bloom-forming species have a negative ecological impact because the decomposition of biomass in the water column decreases oxygen levels in the benthic environment (Lomstein et al. 2006; Wang et al. 2009). In addition, elevated nutrient concentrations have been shown to decrease biodiversity, affect marine habitats, and change ecosystem functioning (Yang et al. 2005; Liu et al. 2009; Mineur et al. 2015).

*Ulva* spp. are green tide forming species with traits of opportunistic species, which is found near the coastal areas. Opportunistic macroalgae have higher growth and nutrient uptake under optimal environmental conditions than do other macroalgae (Taylor et al. 2001; Nelson et al. 2008). Many studies have been conducted using *Ulva* spp. under various environmental conditions such as different temperatures, $CO_2$ levels, nutrient concentrations, light intensities, and/or salinities (Figueroa et al. 2014a, b; Stengel et al. 2014; Cui et al. 2015; Kang et al. 2016; Gao et al. 2016a, b; Kang and Chung 2017). Previous studies indicated that the physiological responses of *Ulva* spp. change differently depending on the species. For example, *U. australis* was shown to display different physiological responses under elevated $CO_2$/pH and nutrient concentrations (Kang and Chung 2017; Reidenbach et al. 2017). Cui et al. (2015) indicated that *U. prolifera*, *U. compressa*, *U. flexuosa*, and *U. linza* were influenced differently under various temperatures and light intensities. Therefore, research needs to identify specific information on the physiology of *Ulva* species. Our study focused on *U. linza* Linnaeus (Ulvales, Chlorophyta), which several researches have studied under different environmental conditions (Kim et al. 2011; Luo et al. 2012; Kang et al. 2016), but not under combinations of temperature and ammonium ($NH_4^+$) concentrations.

In this study, we used $NH_4^+$ as the nitrogen form. *Ulva* spp. grow faster under $NH_4^+$ than under nitrate conditions ($NO_3^-$) (Ale et al. 2011; Li et al. 2019). In addition, $NH_4^+$ is the preferred nitrogen form for macroalgae because, in comparison with $NO_3^-$, less energy is required to assimilate its nitrogen (McGlathery et al. 1996; Pedersen and Borum 1996; Runcie et al. 2003). The utilization of $NO_3^-$ by macroalgae involves the reduction of $NH_4^+$ by nitrate reductase activity (NRA) (Syrett 1981; Teichberg et al. 2007).

The objective of this study was to examine the physiological activities of *U. linza* under elevated temperature and nutrient concentrations. In addition, we tried to determine the interactions between ocean warming and eutrophication in physiological responses of this alga. Therefore, we measured the oxygen evolution rates during photosynthesis, rates of nutrient uptake, rates of relative growth, chlorophyll fluorescence, and nutrient contents in tissues of this alga.

## Materials and methods

The samples of *U. linza* were collected from Cheongsapo, South Korea (35°09′N, 129°11′E) in September 2017. At this sampling site temperature was 25.80 ± 0.50 °C, salinity was 33.50 ± 0.2 ‰, and pH was 8.10 ± 0.12. Temperature and salinity were measured with a YSI Pro 2030 meter (YSI, USA) and pH values were measured with a YSI Pro 10 meter (YSI, USA). Samples were transported to the laboratory and washed several times with 0.20 μm filtered seawater to remove all epiphytes. After washing, the samples were kept in a culture room in filtered seawater at 20 °C with 80 μmol photons $m^{-2}$ $s^{-1}$ under a 12:12 light:dark cycle. The samples of *U. linza* were acclimated 3 days before the experiments. For each treatment, samples (1 g) were placed in 500 mL of filtered seawater. The multi-factorial design experiment was set up with four temperature conditions (LT; low temperature (15 °C), MT; medium temperature (20 °C), CT; control temperature (25 °C), and HT; high temperature (30 °C)) and three $NH_4^+$ concentrations (LN; low nutrient (4 μM), MN; medium nutrient (60 μM) and HN; high nutrient (120 μM)). Of the four temperature treatments, 15 and 20 °C were used for the optimal growth condition (Taylor et al. 2001). The temperature 25 °C was used to reflect the summer seawater temperature in the coastal areas of Cheongspo, Korea (KHOA 2017). The upper 30 °C condition represented the summer seawater temperature at Cheongsapo predicted to be recorded with increasing global temperature (4 °C), based on the IPCC report (2014). $NH_4^+$ nutrient levels followed Kang and Chung (2017). Other experimental conditions such as temperature, light intensity and light period followed the above acclimation conditions. Each experimental condition had four replicates. The temperature conditions were maintained in the incubator throughout the experiments. To support the $NH_4^+$ concentrations, we added $NH_4Cl$ to the filtered seawater. The medium was changed every two days to prevent nutrient depletion.

The photosynthetic rates (μmol $O_2$ $g^{-1}$ FW $h^{-1}$) and $NH_4^+$ uptake rates (μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$) were measured 12 h after the beginning of the experiment. The photosynthetic rates (μmol $O_2$ $g^{-1}$ FW $h^{-1}$) were measured with a Clark-type microelectrode oxygen sensor (Unisense, Denmark). The oxygen sensor was calibrated by mixing a solution of sodium ascorbate ($C_6H_7NaO_6$) and sodium hydroxide (NaOH), and it detected photosynthetic rates in less than 1 s.

The $NH_4^+$ uptake rates (μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$) were determined based on the average amount that disappeared from the culture medium over the incubation period of 12 h. The measurement method followed Parsons et al. (1984). The following equation was used to calculate the $NH_4^+$ uptake rate:

$$V = \left(\left(S_i - S_f\right) \times \mathrm{vol}\right)/(W \times T)$$

where $S_i$ is the initial concentration of $NH_4^+$, $S_f$ is the final concentration of $NH_4^+$ after $T$ hours of incubation, vol is the volume of the culture medium, and $W$ is the fresh weight of each sample.

The growth, chlorophyll fluorescence and carbon (C) and nitrogen (N) contents in algal tissue were measured after 14 days of the experiment. *Ulva linza* growth was determined at the end of the experiment. The relative growth rates (% $day^{-1}$) were calculated as follows:

$$\mathrm{RGR} = ((\ln W_2 - \ln W_1)/T) \times 100$$

where $W_1$ is the initial fresh weight, $W_2$ is the final fresh weight after 14 days, and $T$ is the cultivation period (14 days).

Chlorophyll fluorescence was measured with a pulse amplitude modulation fluorometer (Diving-PAM, Walz, Germany) at the end of the experiment. The maximum quantum yield of photosystem II was measured as follows:

$$F_y/F_m = (F_m - F_o)/F_m$$

where $F_v/F_m$ is the photosynthetic efficiency, as measured using saturating pulse under dark-adaptation, $F_m$ is the maximum fluorescence after dark-adaptation, and $F_o$ is the minimum fluorescence after dark-adaptation. Samples were placed in the leaf-clip holders and kept in the dark for 15 min before measuring chlorophyll fluorescence.

Carbon and nitrogen contents in algal tissue were analyzed at the end of the experiment, using samples of *U. linza*. The samples were dried at 60 °C for 48 h and then ground to a powder. Carbon and nitrogen contents (%) in the tissue were analyzed using an elemental analyzer (Vario-Micro Cube, Elementar Analysensysteme GmbH, Germany). In addition, we calculated C:N ratio on a molar basis.

A two-way analysis of variance (ANOVA) was conducted on all experimental data. Before the statistical analysis was performed, all data were tested for normality and homogeneity. Tukey's tests were used to compare the treatments. A $p$ value of 0.05 represented significant difference among treatments. All statistical analyses were performed with the SPSS program version 23.0 (IBM, USA).

## Results

Photosynthetic rates (μmol $O_2$ $g^{-1}$ FW $h^{-1}$) were affected by temperature and $NH_4^+$ conditions. In addition, the samples of *U. linza* were influenced by the interactive effects of temperature and $NH_4^+$ concentrations (Table 1). After the 12-h experimental period, the photosynthetic rates (μmol $O_2$ $g^{-1}$ FW $h^{-1}$) ranged from 38.10 ± 3.76 to 89.38 ± 8.04 μmol $O_2$ $g^{-1}$ FW $h^{-1}$ (Fig. 1). The minimum value was found at LTLN and the maximum value was observed at MTHN. When the

**Table 1** Results of two-way ANOVA derived from physiological responses (photosynthetic rate (μmol $O_2$ $g^{-1}$ FW $h^{-1}$), $NH_4^+$ uptake rate (μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$), relative growth rate (% $day^{-1}$), photosynthetic efficiency ($F_v/F_m$), tissue carbon content (%), tissue nitrogen content (%), and C:N molar ratio) of *Ulva linza*

| Source | DF | MS | *F* value | *p* value |
|---|---|---|---|---|
| Photosynthetic rate | | | | |
| Temperature | 3 | 72.21 | 4.50 | 0.01 |
| $NH_4^+$ concentration | 2 | 5157.19 | 321.37 | < 0.01 |
| Tx$NH_4^+$ | 6 | 46.18 | 2.88 | 0.03 |
| $NH_4^+$ uptake rate | | | | |
| Temperature | 3 | 0.88 | 39.56 | <0.01 |
| $NH_4^+$ concentration | 2 | 85.87 | 3848.25 | <0.01 |
| Tx$NH_4^+$ | 6 | 0.33 | 14.64 | <0.01 |
| Relative growth rate | | | | |
| Temperature | 3 | 0.14 | 1.63 | 0.18 |
| $NH_4^+$ concentration | 2 | 18.78 | 218.16 | < 0.01 |
| Tx$NH_4^+$ | 6 | 0.41 | 4.73 | 0.01 |
| Photosynthetic efficiency | | | | |
| Temperature | 3 | < 0.05 | 2.87 | 0.03 |
| $NH_4^+$ concentration | 2 | < 0.05 | 7.21 | < 0.01 |
| Tx$NH_4^+$ | 6 | < 0.05 | 11.22 | < 0.01 |
| Tissue carbon content | | | | |
| Temperature | 3 | 0.24 | 1.29 | 0.30 |
| $NH_4^+$ concentration | 2 | 1.23 | 1.99 | 0.16 |
| Tx$NH_4^+$ | 6 | <0.05 | 0.57 | 0.75 |
| Tissue nitrogen content | | | | |
| Temperature | 3 | 0.54 | 1.32 | 0.29 |
| $NH_4^+$ concentration | 2 | 0.03 | 7.20 | <0.01 |
| Tx$NH_4^+$ | 6 | <0.05 | 8.14 | <0.01 |
| C:N molar ratio | | | | |
| Temperature | 3 | 29.70 | 2.63 | 0.08 |
| $NH_4^+$ concentration | 2 | 2.02 | 7.21 | < 0.01 |
| Tx$NH_4^+$ | 6 | 1.78 | 6.37 | < 0.01 |

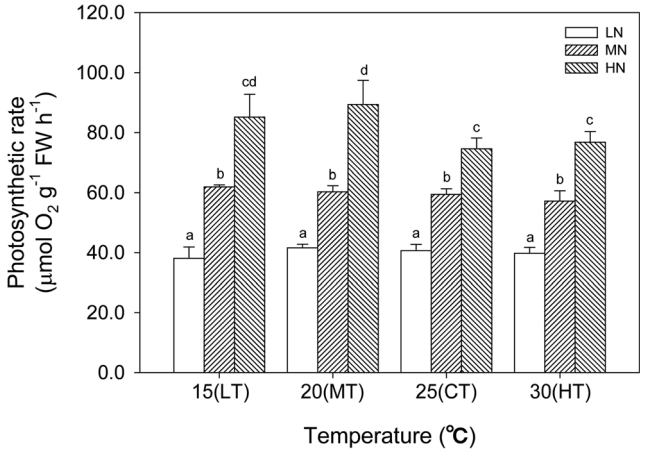


**Fig. 1** Photosynthetic rate (μmol $O_2$ $g^{-1}$ FW $h^{-1}$) of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

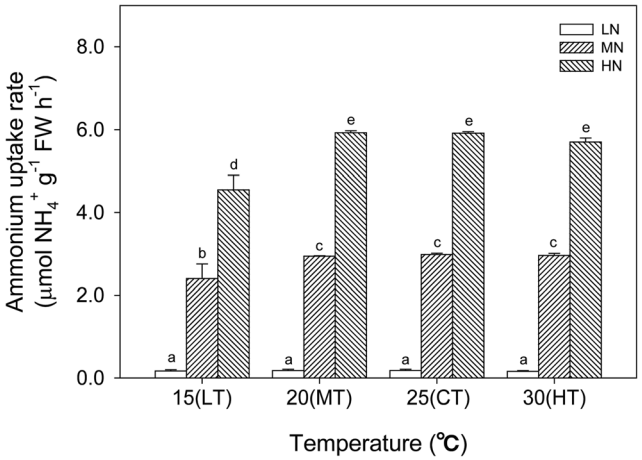


**Fig. 2** Rates of $NH_4^+$ uptake (μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$) of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

temperature remained constant, the photosynthetic rates increased significantly with increasing $NH_4^+$ concentrations ($p < 0.05$). In the case of the HN, the MT treatment had significantly higher photosynthetic rates than did the treatments at CT and HT ($p < 0.05$).

The rates of $NH_4^+$ uptake (μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$) were influenced by temperature, $NH_4^+$ levels, and the combined effects of temperature and $NH_4^+$ levels (Table 1). The minimum $NH_4^+$ uptake rate was 0.16 ± 0.02 μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$ at HTLN; the maximum rate was 5.93 ± 0.05 μmol $NH_4^+$ $g^{-1}$ FW $h^{-1}$ at MTHN (Fig. 2). At LN, the $NH_4^+$ uptake rates were not significantly different under any temperature conditions ($p > 0.05$). However, the rate was significantly different between treatments at LT and over LT under LN condition ($p < 0.05$).

The relative growth rates were measured after 14 days of incubation. The relative growth rates (% $day^{-1}$) were affected by $NH_4^+$ and the combined effects of temperature and $NH_4^+$ combinations (Table 1). The relative growth rates ranged from 5.36 ± 0.38 to 8.07 ± 0.12% $day^{-1}$ (Fig. 3). The minimum relative growth rate was observed at LTLN; the maximum value was observed at MTHN. When the temperature was the same, relative growth rates under MN and HN were significantly higher than that under LN ($p < 0.05$), but the values were not significantly different between the MN and HN ($p > 0.05$).

Photosynthetic efficiency ($F_v/F_m$), as measured by chlorophyll fluorescence after 2 weeks of the experiment, was influenced by temperature, $NH_4^+$, and the interactive effects of temperature and $NH_4^+$ (Table 1). $F_v/F_m$ ranged from 0.61 ± 0.01 to 0.76 ± 0.02 (Fig. 4). $F_v/F_m$ was lowest at CTLN and highest at MTHN. In addition, $F_v/F_m$ values increased significantly in elevated $NH_4^+$ concentrations at all temperatures ($p < 0.05$), but were not significantly different between MN and HN ($p > 0.05$). At the HN condition, the $F_v/F_m$ values were significantly different between CT and HT ($p < 0.05$).

The carbon content in algal tissues (%) was not affected by any culture treatments (Table 1). The tissue carbon contents ranged from 24.40 ± 0.39 to 24.94 ± 0.08% (Fig. 5). Carbon content was lowest at LTMN and highest at MTHN, but there was no significant difference among the culture conditions ($p > 0.05$).

The tissue nitrogen contents (%) were influenced by $NH_4^+$ levels and the combined effects of temperature and $NH_4^+$ level (Table 1). The tissue nitrogen contents ranged from 1.52 ± 0.11 to 2.20 ± 0.04% (Fig. 6). Nitrogen content was lowest at MTLN and highest at MTHN. When the temperature remained constant, the tissue nitrogen content increased under elevated $NH_4^+$ levels ($p < 0.05$). When $NH_4^+$ levels were the

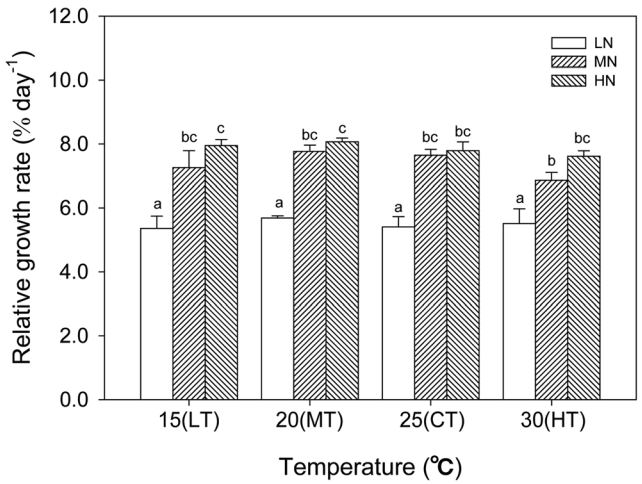


**Fig. 3** Relative growth rate (% $day^{-1}$) of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

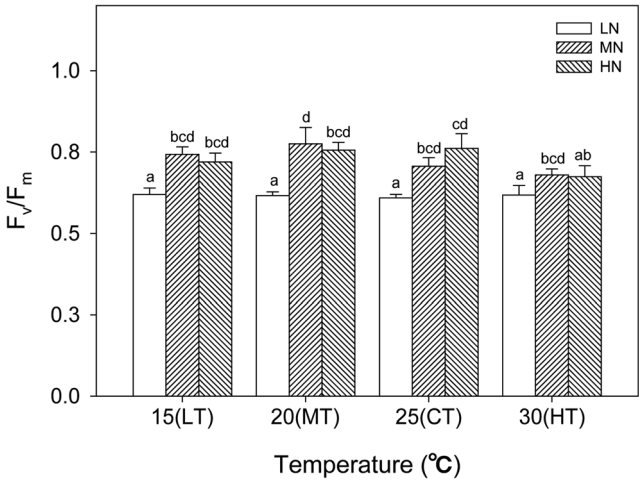


**Fig. 4** Photosynthetic efficiency ($F_v/F_m$) of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

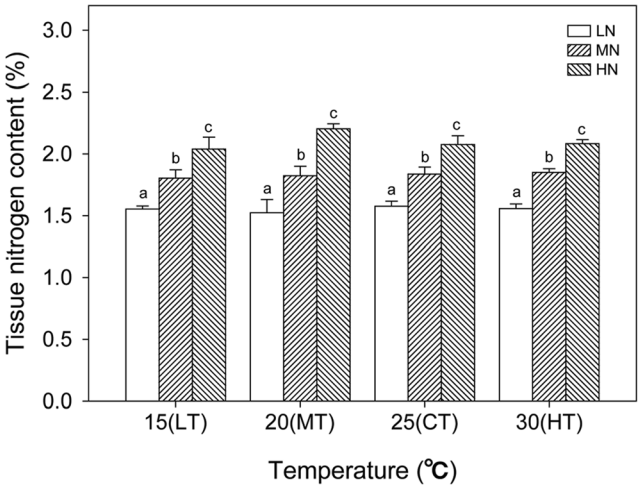


**Fig. 6** Nitrogen content (%) in tissue of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

same, tissue nitrogen contents were not significantly different among temperature conditions ($p > 0.05$).

The C:N ratios in tissue were affected by $NH_4^+$ and the interactive effects of temperature and $NH_4^+$ level (Table 1). The tissue C:N ratios ranged from 11.32 ± 0.24 to 16.15 ± 0.92 (Fig. 7). The ratio was lowest at MTHN and highest at MTLN. At the MT condition, the ratios were significantly different under elevated $NH_4^+$ conditions ($p < 0.05$). At other temperatures, there were not significantly different between MN and HN ($p > 0.05$). In the case of HN, the values were significantly different between LT and MT conditions ($p < 0.05$).

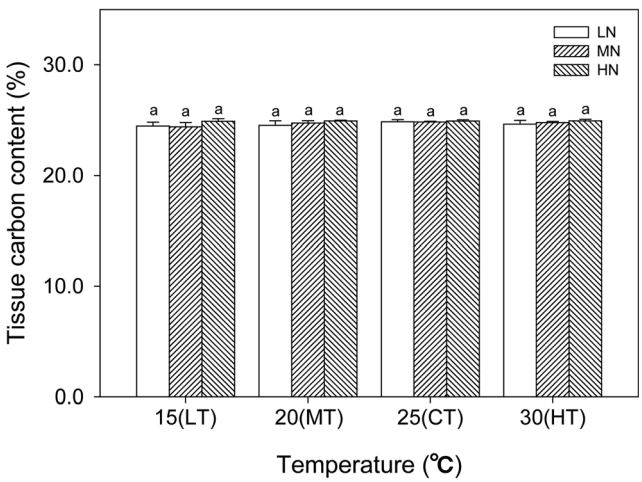


**Fig. 5** Carbon content (%) in tissue of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

## Discussion

Previous studies indicated that the photosynthetic rates of *Ulva* spp. increased under elevated temperature or nutrient concentrations (Zou and Gao 2014; Li et al. 2016; Kang and Chung 2017). In our study, the photosynthetic rate was highest at MTHN. Photosynthesis in *Ulva* sp. was highest at 20 to 25 °C (Murase et al. 1994). Also, the elevated nutrient concentrations increased photosynthetic rates in *Ulva* spp. (Kang et al. 2016; Kang and Chung 2017). Li et al. (2016) showed that the photosynthetic rates of *U. prolifera* were increased at elevated nitrogen and phosphorus levels. In case of the increased nutrient concentrations in the seawater, macroalgae could easily take up nutrients because of higher nutrient availability (Yu and Yang 2008). The higher availability of nutrients could increase photosynthesis in *U. linza* because this

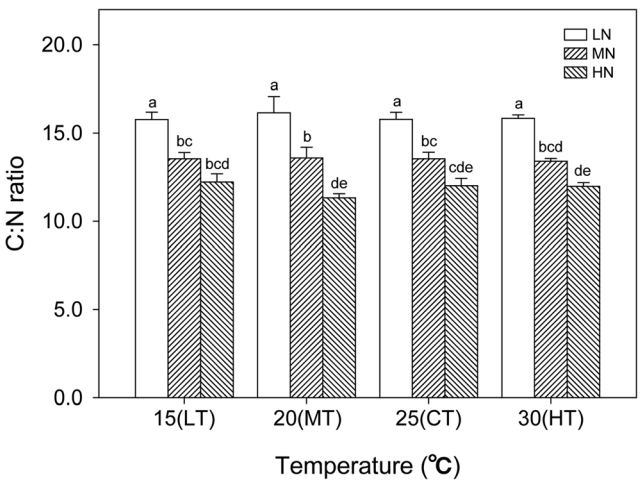


**Fig. 7** C:N molar ratio in tissue of *Ulva linza* under different temperature and ammonium treatments. Significant differences among the treatments are indicated by different letters ($p < 0.05$). Data are means±SD ($n = 4$)

species is an opportunistic and bloom-forming species found near coastal areas. In addition, Zou and Gao (2014) indicated that photosynthetic rates increase under elevated temperatures at high nutrient concentrations.

The rate of $NH_4^+$ uptakes were influenced by temperature, $NH_4^+$ treatment, and the combined effects of temperature and $NH_4^+$ treatments. Temperature is an important factor in the nutrient uptake of algae (Gao et al. 2018). Fan et al. (2014) indicated that macroalgae could have different nutrient requirements under various temperature conditions. Our result shows that the $NH_4^+$ uptake rate was highest at MTHN. Fan et al. (2014) also showed that the nutrient uptake rate of *U. prolifera* was highest at 20 °C. In addition, the rate increased at higher temperatures (25 and 30 °C) when compared to control the temperature condition (15 °C) at 60 and 120 μM $NH_4^+$ (Fan et al. 2014). The $NH_4^+$ uptake rates of *Ulva* spp. increased under elevated $NH_4^+$ concentrations. Many researches have indicated that increased nutrient concentrations could elevate the nutrient uptake rate of *Ulva* spp. (Luo et al. 2012; Kang and Chung 2017; Reidenbach et al. 2017). Seawater has high nutrient concentrations; so, macroalgae could more easily and efficiently perform nutrient uptake in seawater than in other environments (Yu and Yang 2008; Runcie et al. 2003). In addition, the morphological and opportunistic characteristics of *U. linza* could increase nutrient uptake rates under elevated nutrient concentrations (Littler 1980; Wallentinus 1984). The high nutrient uptake rate could affect the metabolism of macroalgae because they generate Rubisco and ATP using nitrogen and phosphorus, respectively (Dawes and Koch 1990; Zer and Ohad 2003).

The relative growth rates of our samples were not affected by elevated temperature conditions. However, several studies have indicated that temperature affects macroalgal growth (Mantri et al. 2011; Gao et al. 2016a, b, 2017; Chen et al. 2018). Temperature is an important factor affecting the physiology of algal metabolism and growth (Zou and Gao 2013, 2014). Mantri et al. (2011) found that seaweed growth could accelerate immediately under elevated temperatures because of increased metabolism. Taylor et al. (2001) found that *Ulva* species could grow over a broad temperature range (10–30 °C). The growth rates of *Ulva* spp. were highest at 15–20 °C and decreased over 20 °C (Taylor et al. 2001). Cui et al. (2015) also observed that *U. linza* grew the fastest at 20 °C. However, our results suggest that relative growth rates did not significantly decrease over 20 °C under constant $NH_4^+$ concentrations. Therefore, there may be several environmental conditions influencing *U. linza* growth. In contrast, the relative growth rate of *Ulva* sp. has been reported to increase under various nutrient concentrations (Zou and Gao 2014; Li et al. 2016; Kang and Chung 2017; Ober and Thornber 2017; Reidenbach et al. 2017). Kang et al. (2016) mentioned that the growth of *U. linza* increased more under elevated nutrient concentrations at a low salinity (10 ‰) condition than under a high salinity one (30 ‰). In addition, the relative growth rates of *U. linza* were not only affected by temperature but also by the interaction between temperature and $NH_4^+$. Therefore, in our culture conditions, the $NH_4^+$ level was the main factor affecting growth in *U. linza*. Lotze and Worm (2002) have reported that elevated temperature and nutrient levels have a combined effect on the growth of green algae.

Schreiber and Bilger (1993) state that chlorophyll fluorescence studies are a powerful tool for analyzing photosynthesis. $F_v/F_m$ is a particularly valuable measure of photosynthetic efficiency (Hanelt et al. 1995). Many studies have been conducted on photosynthetic efficiency under different temperatures or nutrient levels (Padilla-Gamino and Carpenter 2007; Zou and Gao 2014; Kang and Kim 2016). The photosynthetic efficiency of our samples was affected by temperature. Kang and Kim (2016) reported that warming temperatures do not affect the photosynthetic efficiency of *U. australis*. Padilla-Gamino and Carpenter (2007) show that the photosynthetic efficiency of *Asparagopsis taxiformis* decreased under elevated temperature. However, the photosynthetic efficiency in their study possibly changed seasonally because of acclimatization to natural seawater temperatures in different seasons. Therefore, we need to determine the pattern of photosynthetic efficiency of *U. linza* in different seasons. The photosynthetic efficiency was also affected by $NH_4^+$ levels. Photosynthetic efficiency increases with increasing nitrogen concentrations in macroalgae (Dawes and Koch 1990). In the case of *U. australis*, photosynthetic efficiency increased at higher $NH_4^+$ concentrations (Kang and Chung 2017; Reidenbach et al. 2017). However, Kang et al. (2016) found that the photosynthetic efficiency of *U. linza* was not affected by elevated nitrate concentrations. Therefore, the photosynthetic efficiency of *U. linza* changes with various nitrogen sources and concentrations.

The tissue carbon and nitrogen contents and C:N ratios of *U. linza* were not affected by different temperatures. A similar result was also was found in *U. australis* (Kang and Kim 2016). Our results, however, show that $NH_4^+$ concentration affects tissue nitrogen content and C:N ratio. The C:N ratio in tissues is a good index of the physiological status of macroalgae and can be used as an indicator of macroalgae status (Vergara et al. 1993; Kang et al. 2011). When nutrients were abundant, the C:N ratio of *U. australis* decreased (Kang and Chung 2017; Reidenbach et al. 2017; Ober and Thornber 2017). Ober and Thornber (2017) stated that C:N ratios decrease under higher nutrient treatments, and this, in turn, increases the tissue quality of samples. Under sufficient nutrient concentrations, macroalgae could possibly assimilate more nutrients compared with the control nutrient level (Reidenbach et al. 2017). Gómez-Pinchetti et al. (1998) indicated that macroalgae might store the nitrogen in their tissue under abundant nutrient conditions. We found that tissue nitrogen content and C:N ratio were significantly affected by the

interaction between temperature and $NH_4^+$ concentrations. Although temperature and $NH_4^+$ concentration had a significant interactive effect on the C:N ratio of *U. linza*, $NH_4^+$ concentration is the main driver of C:N ratio because the temperatures were not affected any culture treatments.

In conclusion, the *U. linza* was positively affected by increased temperatures and nutrient concentrations. The photosynthetic rates, $NH_4^+$ uptake rates, and photosynthetic efficiency were affected by temperature, $NH_4^+$ concentration, and the interaction between temperature and $NH_4^+$ concentration. The relative growth rates, tissue nitrogen contents, and C:N ratios were affected by $NH_4^+$ concentration and the combined effects of temperature and $NH_4^+$ concentration. The tissue carbon contents, however, were not affected by any culture condition. According to our study, the physiological responses of *U. linza* could increase under future ocean conditions. This phenomenon could be a serious problem near the coastal areas by inducing the formation of green tides. On the contrary, this species could have a bioremediation capacity because of its fast growth and high nutrient uptake rate. Therefore, both characteristics of *U. linza* have to be considered when judging whether it represents a harmful macroalga or a potential solution to coastal environmental problems.

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