

Comparison of growth and nutrient uptake capacities of three dominant species of Qinhuangdao green tides

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Received 30 May 2022; accepted 13 September 2022

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Abstract

Since 2015, green tides have been blooming in offshore waters of Qinhuangdao, with serious impacts on the local ecological environment and tourism. *Ulva australis*, *Bryopsis plumosa*, and *U. prolifera* are the dominant species of Qinhuangdao green tides, following a sequential succession pattern. *Ulva prolifera* is the dominant species, with the highest biomass and the greatest influence on the local ecological environment. To study the reason of green tide dominant species succession and *U. prolifera* became the dominant species with the largest biomass, we compared and analyzed the growth and nutrient uptake capacity of the three algae. The results showed that temperature significantly affects the growth of the three species. Within the temperature range of the experimental setup, the optimum temperature for the growth of *U. australis*, *B. plumosa* and *U. prolifera* is 10 °C, 15 °C, and 20–25 °C, respectively. Combined with the temperature variation trend during green tide bloom development, we believe that temperature is the key environmental factor for the succession of the dominant species. *Ulva prolifera* has a higher growth rate than *U. australis* and *B. plumosa* under the same nitrate, ammonium, and phosphate levels. Significant differences in the maximum absorption rate (R_{max}) and R_{max}/K_s (the relationship between uptake rate and substrate concentration) values indicated that *U. prolifera* had an apparent competitive advantage over *U. australis* and *B. plumosa* regarding nutrient uptake. Therefore, the strong growth and nutrient uptake capacities of *U. prolifera* might be the main reason for becoming the dominant species with the largest biomass in Qinhuangdao green tides.

Key words: growth, nutrient uptake, dominant specie, green tide, Qinhuangdao

Citation: Han Hongbin, Wen Ruobing, Wang Hui, Zhao Sheng. 2023. Comparison of growth and nutrient uptake capacities of three dominant species of Qinhuangdao green tides. Acta Oceanologica Sinica, 42(4): 114–123, doi: 10.1007/s13131-022-2100-7

1 Introduction

Green macroalgae blooms, referred to as green tides, are formed primarily by the accumulation of unattached macroalgal biomass including *Ulva*, *Chaetomorpha*, *Cladophora*, and *Ulvaria* species (Fletcher, 1996; Nelson et al., 2003). During the past several decades, green tides have occurred regularly in coastal areas around the worldwide (Teichberg et al., 2010). International studies on the causes of green tide formation have shown that green tides usually occur in areas with relatively high eutrophication levels, and eutrophication is considered an important cause of green tides (Kamer et al., 2001; Charlier et al., 2007; Yabe et al., 2009; Teichberg et al., 2010). Compared with other algae, green tide algae, the species causing green tides, have stronger competitive advantages regarding nutrient uptake (Luo et al., 2012). In eutrophic waters, they can rapidly absorb large amounts of nutrients and maintain a high growth rate, resulting in the continuous increase in algae biomass and making it the dominant species (Luo et al., 2012; Wang et al., 2012). Green tide algae can also store excess nutrients to maintain a high growth rate under low-

nutrient conditions (Pedersen and Borum, 1997).

Since 2015, a novel green tide has been blooming in Qinhuangdao City (Hebei Province, China) on the western coast of the Bohai Sea (Han et al., 2019; Song et al., 2019b). The dominant species of green tides in offshore waters of Qinhuangdao include *Ulva australis*, *Bryopsis plumosa*, and *Ulva prolifera*, with obvious morphological differences (Song et al., 2019a). In previous publications, *Ulva pertusa* has been reported to be one of dominant species for the green tide in Qinhuangdao (Song et al., 2019a, 2019b; Han et al., 2019, 2022a). Since this species has been identified as a synonym of *U. australis* recently (Hughes et al., 2021), we replaced *U. pertusa* with *U. australis* in this text. Of these, *U. australis* is an economic macroalgae widely distributed along the coastal areas of the western North Pacific (Tseng, 1983). It has a slightly yellow color, with wrinkles at the edge and irregular pores on the surface. The species *Bryopsis plumosa* is a green macroalgae distributed worldwide and a well-known nutrient pollution indicator (Lapointe et al., 2010). *Bryopsis plumosa* is dark green in color and has a main branch and several irregular branches. In

Foundation item: The Fund of Key Laboratory of Ecological Prewarning, Protection and Restoration of Bohai Sea, Ministry of Natural Resources under contract No. 2022107; the Qingdao Postdoctoral Applied Research Project under contract No. QDBSH202001.

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addition, *U. prolifera* is also the causative species in the world's largest green macroalgae blooms in the Yellow Sea, China (Liu et al., 2010; Wang et al., 2015). It is bright green in color and tubular, with more branches (Zhang et al., 2013; Song et al., 2019b). There is an obvious succession phenomenon during the occurrence of Qinhuangdao green tides (Han et al., 2019; Song et al., 2019a, 2019b). According to the different dominant species, the occurrence of green tides can be divided into three stages (Song et al., 2019a). The first stage lasts from late April to mid-May, during which the biomass levels are low and *U. australis* is the main species. In the second stage, biomass increases gradually from mid-May to mid-June, and the dominant species is *B. plumosa*. The third stage occurs from mid-June to late September, when green algal biomass is highest and the duration is longest. The species *U. prolifera* is the dominant species in this stage (Han et al., 2019; Song et al., 2019a). However, it is still unclear which environmental factors drive the succession of dominant species of green tides in Qinhuangdao and which physiological characteristics of *U. prolifera* are involved in the competition with the other two species, making it the dominant species with the largest biomass.

We speculated that the different adaptation strategies of *U. australis*, *B. plumosa*, and *U. prolifera* to environmental factors are responsible for dominant species succession. In this work, we investigated the effects of temperature, irradiance, and nutrients on the growth rates of *U. australis*, *B. plumosa*, and *U. prolifera* and compared their nutrient uptake capacities. By this investigation, it would be possible to answer the following two questions: (1) was the succession of dominant species of green tide in Qinhuangdao related to the dominance of growth of the three dominant species over a range of temperature and light? (2) was the growth and nutrient uptake capacity of *U. prolifera* higher than that of *U. australis* and *B. plumosa*?

2 Materials and methods

2.1 Algal material

During green tides outbreak, vast numbers of suspended macroalgae accumulate primarily on the Jinmenghaiwan Bathing Beach of Qinhuangdao, which is the main area affected by green tides in Qinhuangdao. Macroalgae were sampled from the Jinmenghaiwan Bathing Beach (39°53'42.93"N, 119°32'34.49"E) in July 2019. Morphological characteristics and internal transcribed spacer (ITS) sequences and 5S ribosomal DNA (5S rDNA) spacer sequences were chosen for species identification.

These samples were cleaned thrice using sterilized seawater on site, dried with absorbent paper, stored in liquid nitrogen, then transported to the laboratory within 48 h. In order to minimize the effect of natural environmental conditions on algal physiological traits, each algal sample was cultured for 3–5 d using air stones before the experiment. The condition was kept at 16°C at an irradiance of 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ in a 12 h light:12 h dark cycle.

2.2 Competitive growth of the three algae species under different temperature and light conditions

To test the effects of temperature and light on the growth of *U. australis*, *B. plumosa*, and *U. prolifera*, we selected the temperatures of 10°C, 15°C, 20°C, and 25°C, with the light intensities of 50 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, and 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$. Nutrients were supplemented according to f/2 medium, and the experiment was carried out in a glass flask with 200 mL of f/2 medium, which was added every 5 d during the culture period with 12 h light: 12 h dark cycle. The initial biomass of

each algae was 0.2 g. The experiment was divided into two groups, single culture and co-culture. In the single culture, *U. australis*, *B. plumosa*, and *U. prolifera* were cultured separately under different temperature and light conditions. According to the succession rule of dominant species, co-culture groups were defined as follows: (1) *U. australis* and *B. plumosa* at 10°C; (2) *U. australis* and *B. plumosa* at 20°C; (3) *B. plumosa* and *U. prolifera* at 20°C; and (4) *B. plumosa* and *U. prolifera* at 25°C. The light intensities for each group were 50 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, and 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$. Each group consisted of three replicates. The algae were weighed after 10 d, and the relative growth rate (RGR) was calculated as follows: $\text{RGR} = (\ln w_2 - \ln w_1) / t$, where w_1 denotes the initial fresh mass and w_2 the fresh mass after t d (Cui et al., 2015). We selected the RGR as the evaluation indicator of the effect.

2.3 Effects of NO_3^- , NH_4^+ and PO_4^{3-} on algal growth rates

The experimental period for investigating the effects of nitrogen and phosphorus on the growth of *U. prolifera*, *U. pertulva*, and *B. plumosa* was 10 d. Nitrogen available for macroalgal uptake in coastal waters can be in the form of nitrate and ammonium, we used two different nitrogen sources, namely nitrate (NaNO_3) and ammonium (NH_4Cl). To prevent phosphate restriction during the experiment, 30 $\mu\text{mol}/\text{L}$ phosphate (KH_2PO_4) was added to all experiments. In the nitrogen source experiment, the concentrations of nitrogen (NO_3^- or NH_4^+) were 5 $\mu\text{mol}/\text{L}$, 10 $\mu\text{mol}/\text{L}$, 20 $\mu\text{mol}/\text{L}$, 50 $\mu\text{mol}/\text{L}$, and 100 $\mu\text{mol}/\text{L}$. NaNO_3 , NH_4Cl , and KH_2PO_4 were applied to adjust the nutrient concentrations. In the phosphate experiment, the concentrations of phosphate were 5 $\mu\text{mol}/\text{L}$, 10 $\mu\text{mol}/\text{L}$, 20 $\mu\text{mol}/\text{L}$, 30 $\mu\text{mol}/\text{L}$, and 40 $\mu\text{mol}/\text{L}$. To prevent nitrogen restriction, 50 $\mu\text{mol}/\text{L}$ NO_3^- and 50 $\mu\text{mol}/\text{L}$ NH_4^+ were added to each group of experiments. Briefly, we placed 0.2 g of algae into a 1 L glass bottle which contained 500 mL of sterile artificial sea water and added the corresponding nutrients. We selected the RGR as the evaluation indicator of the effect. The experiment was carried out at 20°C and 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ with a 12 h light: 12 h dark cycle. Each group consisted of three replicates. Previous studies have shown that the third stage with *U. prolifera* as the dominant species, which has the largest biomass, the longest duration, and the greatest impact on the environment, begins in mid-June. According to our field survey, the average temperature and light intensity in the sea area in mid-June were about 20°C and 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, therefore, in order to simulate the environmental conditions in the field to the greatest extent, above environmental conditions were designed to explore whether *U. prolifera* may become the dominant species with the largest biomass.

2.4 Effects of NO_3^- , NH_4^+ and PO_4^{3-} on nutrient uptake

The nitrogen (NO_3^- and NH_4^+) uptake experiment was carried out in 1-L sterile glass bottles, whereas for the phosphate uptake experiment, 1-L sterile plastic bottles were used. Prior to use, all bottles were cleaned with 10% hydrochloric acid. In the nitrogen uptake experiment, the concentrations of NO_3^- or NH_4^+ were 5 $\mu\text{mol}/\text{L}$, 10 $\mu\text{mol}/\text{L}$, 20 $\mu\text{mol}/\text{L}$, 50 $\mu\text{mol}/\text{L}$, and 100 $\mu\text{mol}/\text{L}$. To avoid phosphorus restriction, 30 $\mu\text{mol}/\text{L}$ PO_4^{3-} was added. In the phosphorus uptake experiment, the concentrations of PO_4^{3-} were 5 $\mu\text{mol}/\text{L}$, 10 $\mu\text{mol}/\text{L}$, 20 $\mu\text{mol}/\text{L}$, 30 $\mu\text{mol}/\text{L}$, and 40 $\mu\text{mol}/\text{L}$. To prevent nitrogen restriction, 50 $\mu\text{mol}/\text{L}$ NO_3^- and 50 $\mu\text{mol}/\text{L}$ NH_4^+ were added. The initial biomass for this part of experiment is 0.2 g. Nitrogen starvation incubation was performed for about 3 d before the start of the experiment. In all experiments, 2 mL seawater samples were collected after 1 h, 2 h, 3 h, and 4 h, and the nutri-

ent concentration was analyzed using an automatic nutrient analyzer (AA3, BRAN LUEBBE). The experiment was carried out at 20°C and 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ with a 12 h light: 12 h dark cycle. The algal material was oven-dried at 60°C for 72 h to determine the dry mass (D_M). Uptake rates (V) were calculated from changes in substrate concentrations during each sampling interval using the equation:

$$V = [(C_0 \times V_0) - (C_t \times V_t)] / (t \times D_M), \quad (1)$$

where C_0 denotes the initial nutrient concentration, C_t is the concentration after t d, V_0 denotes the initial amount of water, V_t is the amount of water after t d, t denotes the sampling interval time, and D_M represents the dry weight of algal biomass.

The Michaelis-Menten equation is widely used to describe the growth and nutrient uptake of macroalgae. The competitive ability of macroalgae to obtain nutrients can be determined using the kinetic parameter R_{max} (maximum absorption rate). The parameter R_{max} is obtained from a hyperbolic plot of absorption rates at a series of concentrations and is used to measure the ability of macroalgae to absorb nutrients at different concentrations. The R_{max}/K_s ratio describes the relationship between uptake rate and substrate concentration, with higher values suggesting competitive advantages and higher affinity.

2.5 Data analysis

Tests of homogeneity of variance were conducted, and separ-

ate two-way analysis of variance (ANOVA) was performed. Differences were considered significant at $P < 0.05$. Data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The data of the uptake rate experiment were analyzed using multivariate variance. Tukey's test was performed for all parameters.

3 Results

3.1 Molecular identification of macroalgae

The Neighbor-Joining Tree based on the ITS gene showed that the 15 green macroalgal samples belonged to 3 clades (Fig. 1). All of the K samples grouped together in the *U. pertusa/australis* clade. All of the Y samples grouped together in the *B. plumosa* clade. All of the H samples showed the highest affiliation with the *U. prolifera/linza* clade. However, according to the phylogeny of the 5S spacer region, the *U. prolifera/linza* clade in the phylogenetic tree based on 5S sequence was divided clearly into two groups (*U. prolifera* and *U. linza*, Fig. 2). All of the H samples grouped in the *U. prolifera* clade. The sequences of 15 samples have been submitted to NCBI, and the sequence numbers are shown in Table 1.

3.2 Effects of temperature and light on the growth of dominant green tide species

Within the temperature and light ranges of the experimental setup, the optimum combination of temperature and light for *U. australis* was 10°C and 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$, resulting in a growth rate

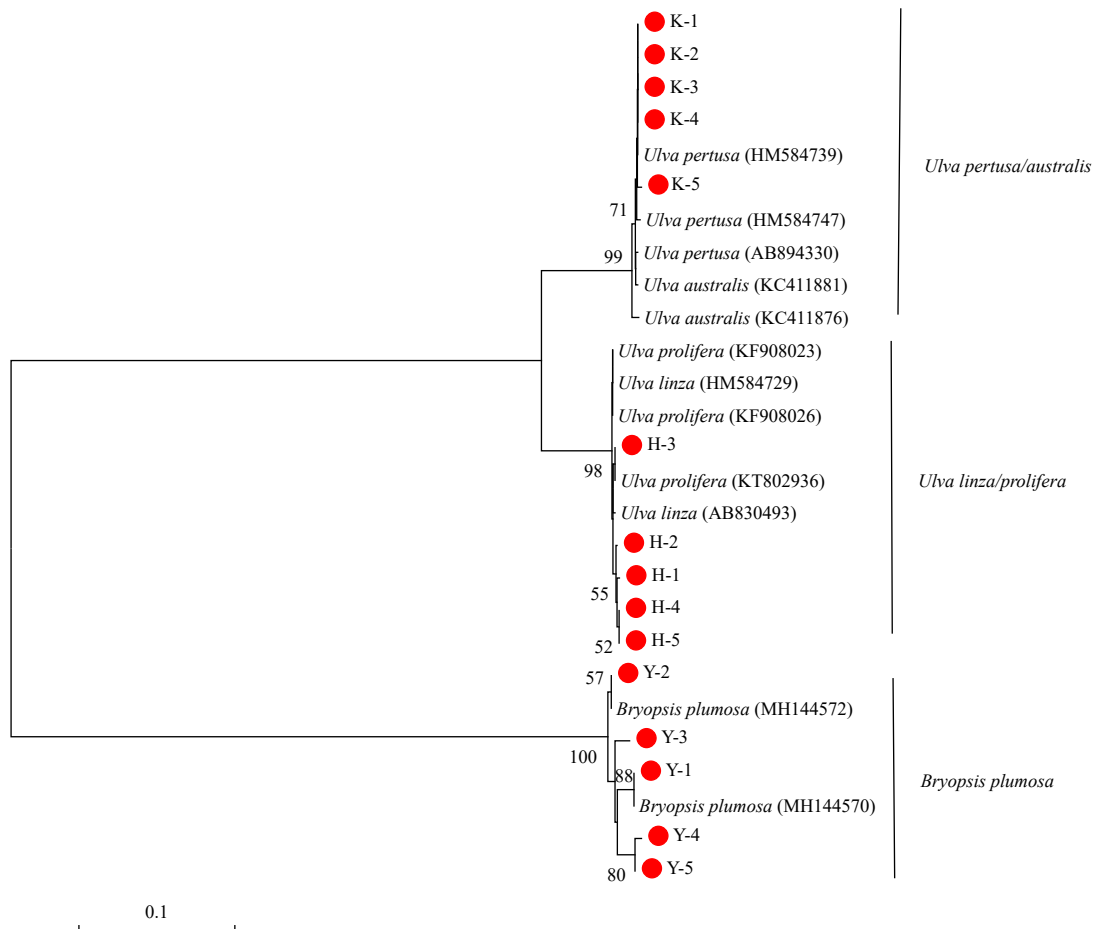


Fig. 1. Phylogenetic tree determined by analysis of internal transcribed spacer sequences of macroalgae. Numbers at the nodes were bootstrapping support values larger than 50% after 1 000 replicates in Neighbor-Joining analyses.

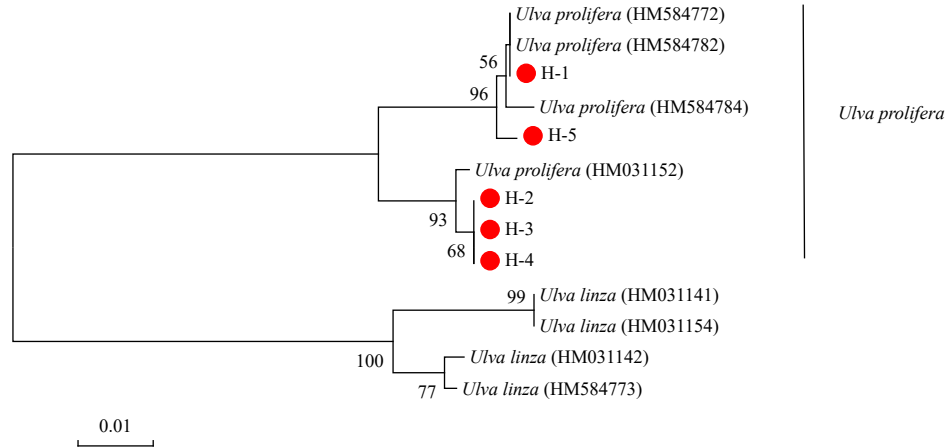


Fig. 2. Phylogenetic tree determined by analysis of 5S sequences of macroalgae. Numbers at the nodes were bootstrapping support values larger than 50% after 1 000 replicates in Neighbor-Joining analyses.

Table 1. Accession numbers of the three dominant species of Qinhuangdao green tides

Sample numbers	Primes	Molecular identification	Accession numbers
K-1; K-2; K-3; K-4; K-5	ITS	<i>Ulva australis</i>	ON263399; ON263400; ON263401; ON263402; ON263403
Y-1; Y-2; Y-3; Y-4; Y-5	ITS	<i>Bryopsis plumosa</i>	ON263404; ON263405; ON263406; ON263407; ON263408
H-1; H-2; H-3; H-4; H-5	ITS	<i>Ulva linza/prolifera</i>	ON263394; ON263395; ON263396; ON263397; ON263398
H-1; H-2; H-3; H-4; H-5	5S	<i>Ulva prolifera</i>	ON286724; ON286725; ON286726; ON286727; ON286728

Note: ITS is the abbreviation of internal transcribed spacer.

of about 12% per day (Fig. 3). The RGR of *U. australis* decreased with increasing temperature in the range of 15–25°C. The optimum temperature and light conditions for the growth of *B. plumosa* were 15°C and 150 μmol/(m²·s), obtaining RGR of 10.2% per day (Fig. 4). The temperature range of 20–25°C was most suitable for the growth of *U. prolifera*. The optimum temperature and light conditions for *U. prolifera* were 20°C and 200 μmol/(m²·s), resulting in RGR of 18.6% per day (Fig. 5).

ANOVA was performed to test the significant effects of light and temperature on the growth of *U. prolifera*, *U. australis*, and *B. plumosa*. The results showed that temperature had a significant effect on the growth of the three algae ($P < 0.01$), while light had no significant effect on the growth of the three seaweeds relative to temperature ($P > 0.05$). So, temperature exerted a stronger influence on the RGR of all the three species. For example, at a

light condition of 150 μmol/(m²·s), the RGR of *U. prolifera* varied from 4.2% to 16.8% at a temperature of 10–25°C. In comparison, the variation of RGR at different light levels was relatively small at a certain temperature. RGR of *U. australis*, *B. plumosa*, and *U. prolifera* were generally highest at 15°C, 20°C, and 25°C, respectively, and decreased towards both lower and higher temperatures.

All co-culture experiments were conducted under a light range of 50–200 μmol/(m²·s). At the temperature of 10°C, the growth rate of *U. australis* was significantly higher than that of *B. plumosa* ($P < 0.05$). The growth rate of *B. plumosa* was significantly higher than that of *U. australis* at the temperature of 15°C ($P < 0.05$). At 20°C and 25°C, the growth rate of *U. prolifera* was significantly higher than that of *B. plumosa* ($P < 0.05$) (Fig. 6).

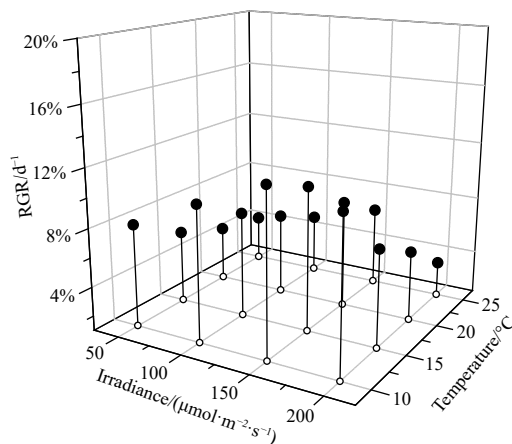


Fig. 3. Effects of temperature and light on the growth of *Ulva australis*. RGR: relative growth rate.

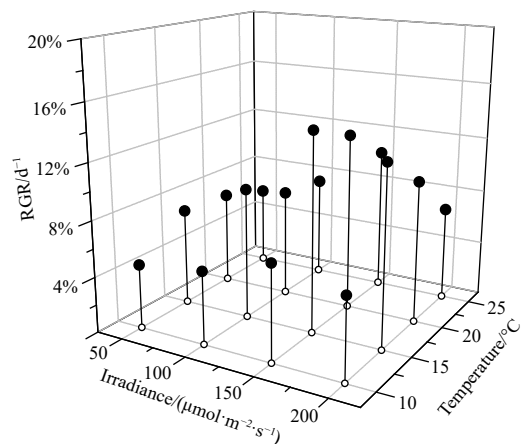


Fig. 4. Effects of temperature and light on the growth of *Bryopsis plumosa*. RGR: relative growth rate.

3.3 Effect of nutrients on the RGR of dominant green tide species

At the same concentrations of NO_3^- , NH_4^+ , or PO_4^{3-} , the growth rate of *U. prolifera* was significantly higher than those of *B. plumosa* and *U. australis* ($P < 0.05$). Compared with NO_3^- and NH_4^+ , changes in PO_4^{3-} concentration had no significant effect on the growth rates of all three species ($P > 0.05$) (Figs 7–9).

3.4 Nutrient uptake by dominant green tide species

The uptake rates of NO_3^- , NH_4^+ , and PO_4^{3-} were accelerated with increasing nutrient levels. In the 4-h experiment, the nutrient uptake rates of the three species decreased gradually over time. In the same period, *U. prolifera* had stronger uptake rates of

NO_3^- , NH_4^+ , and PO_4^{3-} than *B. plumosa* and *U. australis* ($P < 0.05$) ($P > 0.05$) (Figs 10–12).

Bryopsis plumosa, *U. australis*, and *U. prolifera* showed saturated NO_3^- , NH_4^+ , and PO_4^{3-} uptake kinetics. In the 4-h experiment, The R_{\max} and R_{\max}/K_s values of *U. prolifera* were higher compared with those of *U. australis* and *B. plumosa*. Compared with the uptake of NO_3^- , the uptake of NH_4^+ resulted in higher R_{\max} values for all species. Compared with phosphate uptake, the three species showed higher R_{\max} values for nitrogen uptake (NO_3^- and NH_4^+) (Tables 2–4).

4 Discussion

4.1 Temperature as a key environmental factor for the succession of dominant species of Qinhuangdao green tides

Eutrophication, especially the increase in inorganic nitrogen, largely drives macroalgae blooms (Fong et al., 1996). Studies have shown that river pollution in Qinhuangdao is becoming more and more serious, and the eutrophication of coastal areas in Qinhuangdao has shown an increasing trend in recent years (Wang et al., 2013). Fong et al. (1996) assume that other factors (temperature and light) also play an important role in the growth of macroalgae when the nutrient supply is sufficient. Fan et al. (2015) showed that temperature is a key environmental factor for the succession of dominant species of raft-fouling green macroalgae. In addition, the phenomenon of species succession during macroalgae blooms in other coastal waters was also controlled by temperature. Yoshida et al. (2015) showed that in the Hiroshima Bay green tide, Japan, *Ulva* spp. is the dominant species in summer and autumn, whereas in winter, *U. australis*, a cold-water species, becomes the dominant species.

The strain of *Ulva prolifera* in the Bohai Sea green tides is not

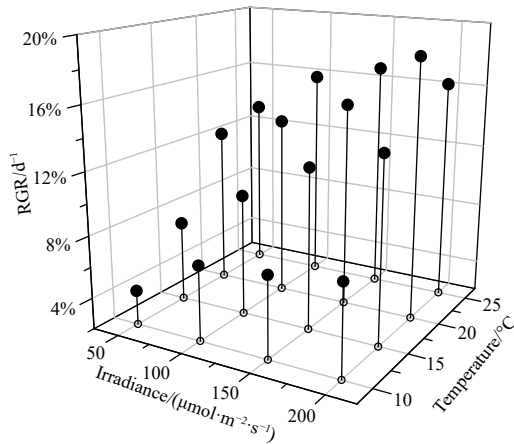


Fig. 5. Effects of temperature and light on the growth of *Ulva prolifera*. RGR: relative growth rate.

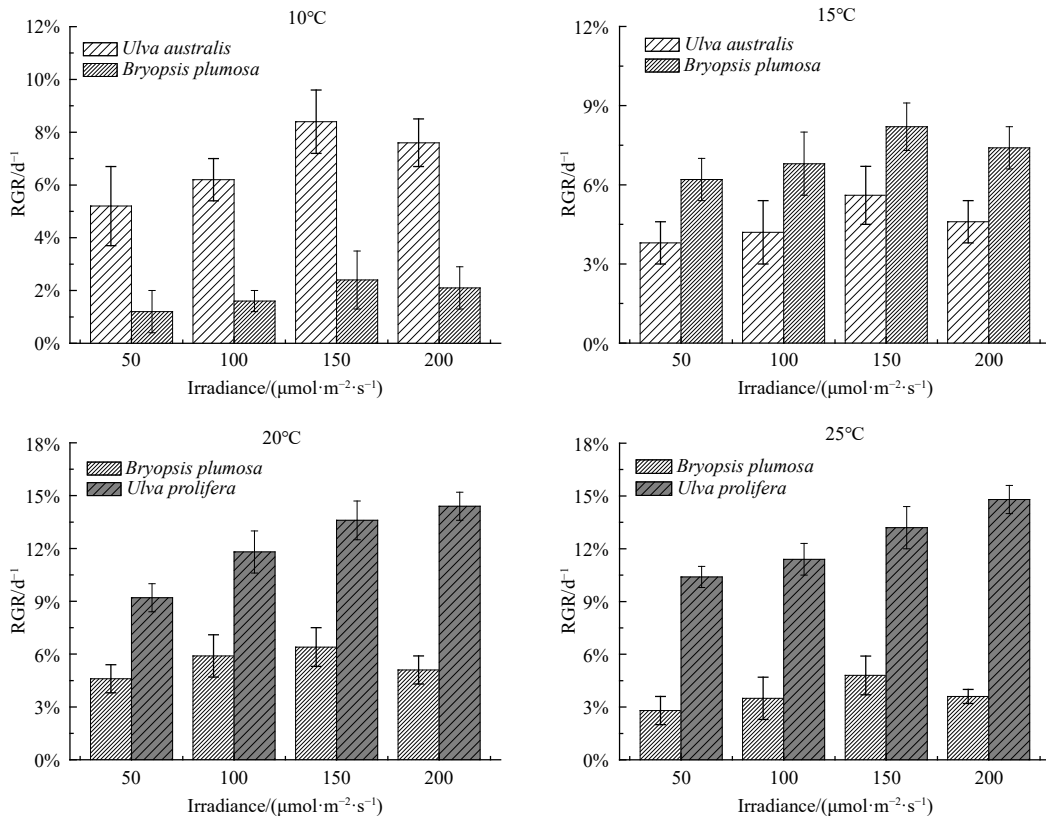


Fig. 6. The relative growth rate (RGR) of co-culture two algae under different temperature and light conditions.

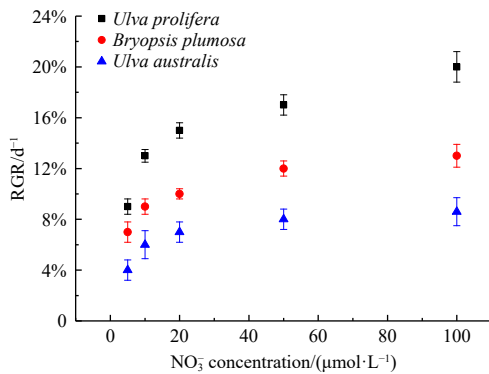


Fig. 7. The relative growth rate (RGR) of three species of green tide algae at different nitrate concentrations.

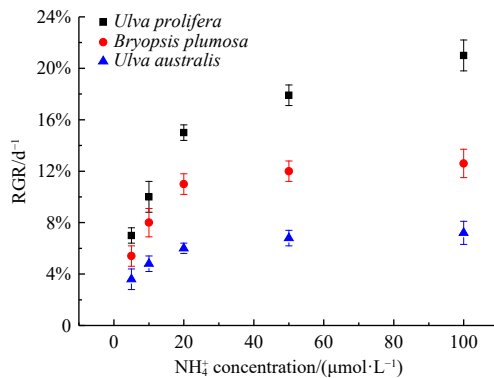


Fig. 8. The relative growth rate (RGR) of three species of green tide algae at different ammonium salt concentrations.

the same as that in the Yellow Sea green tides. The green tide in Qinhuangdao is not correlated with the green tide Yellow Sea and has a different origin and development mode (Han et al., 2022a). In previous study, the relationship between green tides in Qinhuangdao and the Yellow Sea (Rudong and Qingdao) was evaluated by genetic analyses of *U. prolifera* based on SNP markers. In total, 12 945 SNP markers were obtained for all samples. The *U. prolifera* populations in Rudong and Qingdao were closely clustered, while samples from Qinhuangdao clustered separately. These results showed genetic differentiation was lower among floating *U. prolifera* populations in Rudong and Qingdao than in Qinhuangdao. Physiological experiments showed that the net buoyancy of floating *U. prolifera* were higher in Qingdao and Rudong compare to Qinhuangdao under the same light conditions (Han et al., 2022a). Previous studies have shown that the attached macroalgae on seaweed beds in the coastal waters of Jinnenghaiwan Bathing Beach are the major original source of the green tide blooms in the Bohai Sea (Song et al., 2019).

We found that *U. australis*, *B. plumosa*, and *U. prolifera* could tolerate a wide range of temperature and light levels and maintained growth rate of 3.4%–12.2% per day, 4.6%–14.2% per day, and 3.2%–18.6% per day, respectively, at the tested temperature and light ranges. The high RGR indicated the great ecological advance for this species under the favored environmental conditions (Xiao et al., 2016). Compared to the relatively smaller variation as a result of the light conditions, the growth rates of these three algae were more significantly influenced by temperature. According to the results, within the temperature and light range of the experimental setup, the optimum growth temperatures of

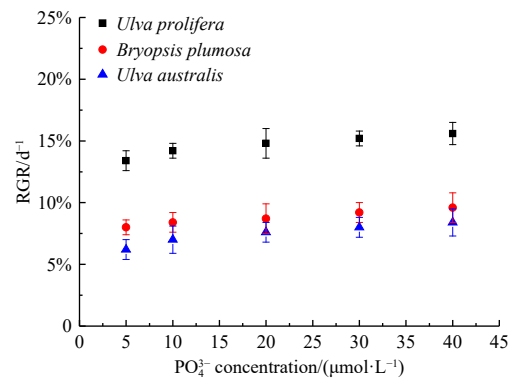


Fig. 9. The relative growth rate (RGR) of three species of green tide algae at different phosphate concentrations.

U. australis, *B. plumosa*, and *U. prolifera* were 10°C, 15°C, and 20–25°C, respectively. According to the data released by the Department of Natural Resources of Hebei Province on the seawater temperature of Jinnenghaiwan Bathing Beach from April to September 2018–2020 (Fig. 13), the sea water temperature is low in April (about 10°C), which is conducive to the growth of *U. australis*, making it the dominant species in the first stage. In May, the sea water temperature is about 15°C, which is conducive to the growth of *B. plumosa*. In the co-culture experiment with *B. plumosa* and *U. australis*, *B. plumosa* gained competitive advantages due to its high growth rate and became the dominant species in the second stage. The indoor experiment showed that the growth rate of *U. prolifera* was highest at 20–25°C. After June, the sea water temperature exceeds 20°C, which is suitable for the growth of *U. prolifera*. This species also showed obvious growth advantages in the co-culture with *B. plumosa* and became the dominant species in the third stage. Therefore, we believe that the sea water temperature is the key environmental factor for the succession of dominant species of green tides in the Qinhuangdao coastal area.

4.2 Growth and nutrient uptake capacity of the three green tide algae

According to the previous study, the content of NO₃-N in the water column of Bohai Bay accounted for more than half of the total nitrogen, 53.08%, while the content of NH₄-N also accounted for 23.57%, and these two forms of nitrogen accounted for 76.65% of the total nitrogen, indicating that the nitrogen nutrients in the water column mainly existed in the form of inorganic nitrogen. Both PO₄-P are the main forms of phosphorus, with PO₄-P content in the water column accounting for 76.46% of the total phosphorus (Song et al., 2019). This region is phosphate limited with nitrogen to phosphorus ratio above 20 (Zhang et al., 2018). The main occurrence area of green tide in Qinhuangdao is the sea area adjacent to Jinnenghaiwan Bathing Beach (Han et al., 2022b), which is adjacent to Tang River Estuary and rich in nutrient. According to the preliminary field survey, the approximate ranges of nitrate, ammonium and phosphate in the green tide occurrence area from April to September were 4.08–9.20, 2.15–4.47 and 0.12–0.47, respectively (unpublished data).

In this study, compared with *U. australis* and *B. plumosa*, *U. prolifera* had higher growth and nutrient uptake capacities. The growth rate is mainly determined by the ability of nutrient uptake from adjacent waters (Taylor and Rees, 1999; Ale et al., 2011), indicating that *U. prolifera* can use and store nutrients

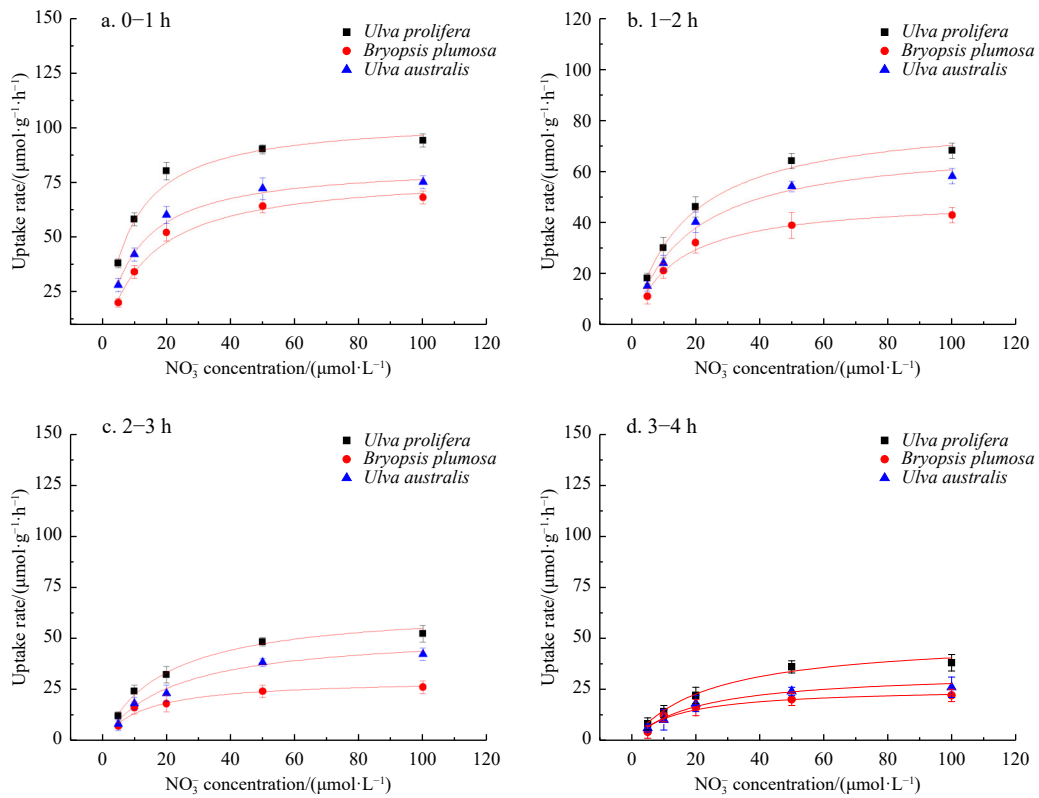


Fig. 10. Comparison of the nitrate uptake capacity of *Ulva australis*, *Bryopsis plumosa*, and *Ulva prolifera*.

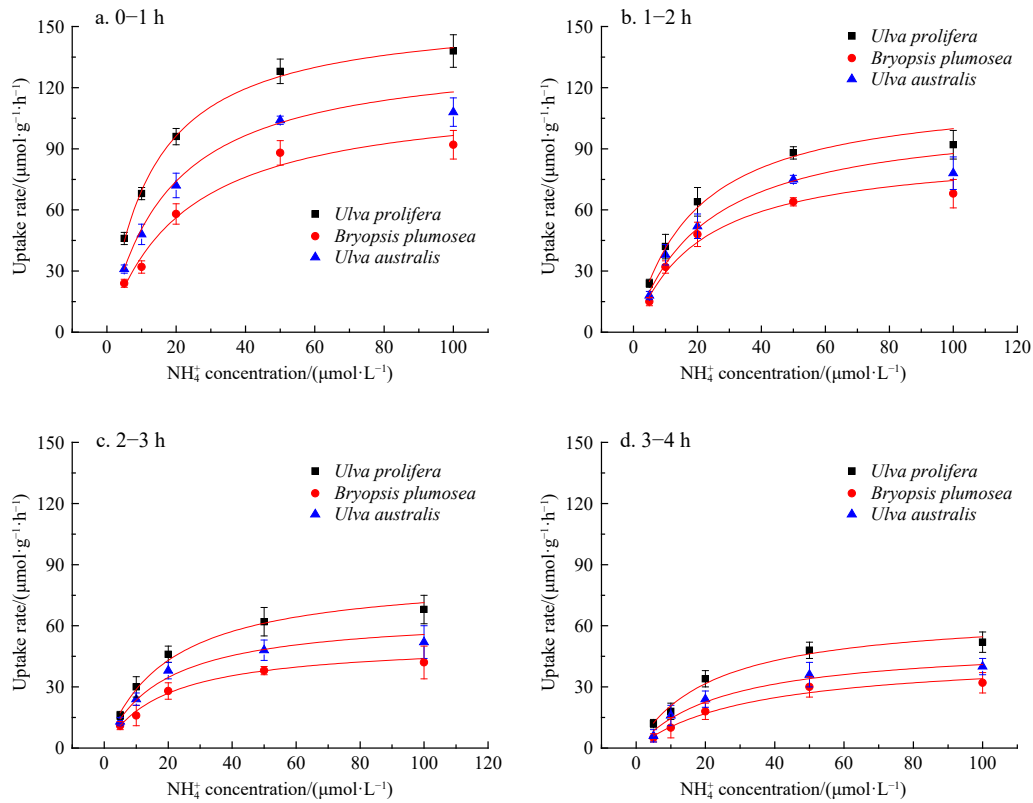


Fig. 11. Comparison of the ammonium uptake capacity of *Ulva australis*, *Bryopsis plumosa*, and *Ulva prolifera*.

more effectively for rapid growth. At high nitrogen concentrations, species with higher nutrient uptake rates have an advantage over species with lower rates (Pedersen and Borum, 1997).

The Jinmenghaiwan Bathing Beach is the main area affected by Qinhuangdao green tides, which is adjacent to the mouth of the Tang River and rich in nutrient salts (Wu et al., 2015). For nitrate,

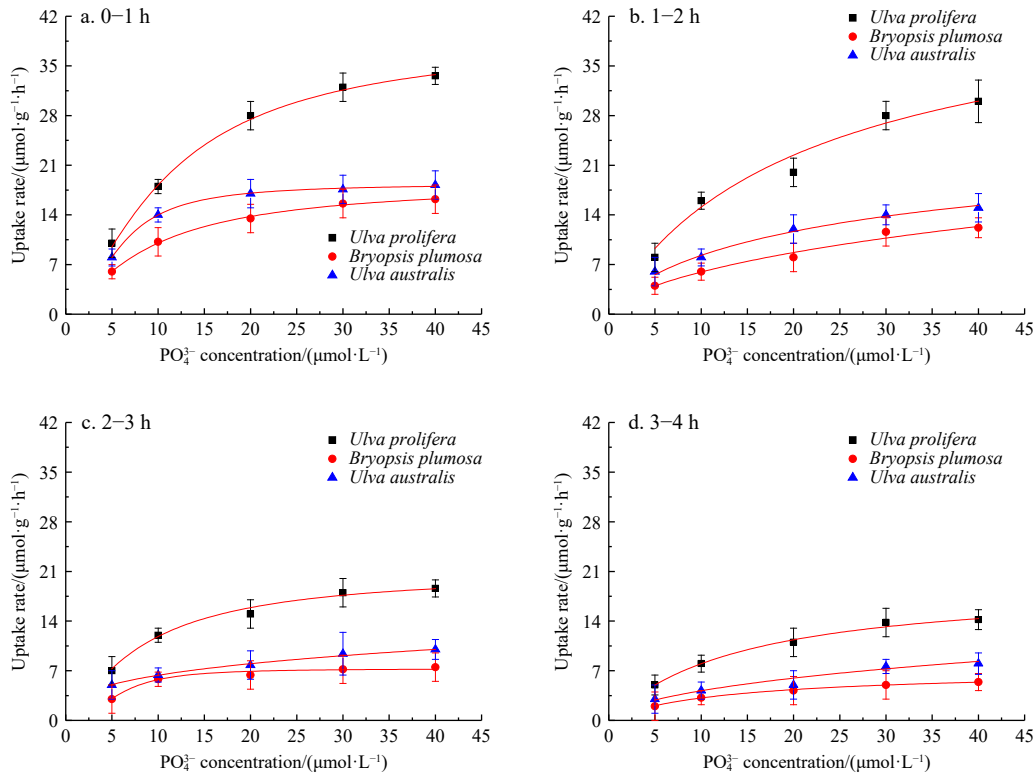


Fig. 12. Comparison of the phosphate uptake capacity of *Ulva australis*, *Bryopsis plumosa*, and *Ulva prolifera*.

Table 2. Kinetic parameters of nutrient uptake at different concentrations for *Ulva prolifera*

Nutrition	Time	<i>Ulva prolifera</i>			
		$R_{max}/(\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1})$	$K_s/(\mu\text{mol}\cdot\text{L}^{-1})$	R_{max}/K_s	r^2
NO_3^-	0–1 h	104.31	8.19	12.74	0.98
	1–2 h	81.84	16.69	4.90	0.98
	2–3 h	65.34	19.48	3.35	0.98
	3–4 h	50.31	24.48	2.06	0.97
NH_4^+	0–1 h	157.35	12.68	12.41	0.99
	1–2 h	118.62	18.80	6.31	0.99
	2–3 h	85.41	19.71	4.33	0.98
	3–4 h	66.70	22.20	3.00	0.98
PO_4^{3-}	0–1 h	48.41	16.72	3.90	0.98
	1–2 h	44.25	19.27	2.30	0.94
	2–3 h	23.32	9.98	2.34	0.98
	3–4 h	19.57	14.53	1.35	0.99

Table 3. Kinetic parameters of nutrient uptake at different concentrations for *Bryopsis plumosa*

Nutrition	Time	<i>Bryopsis plumosa</i>			
		$R_{max}/(\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1})$	$K_s/(\mu\text{mol}\cdot\text{L}^{-1})$	R_{max}/K_s	r^2
NO_3^-	0–1 h	79.43	13.4	5.93	0.97
	1–2 h	49.48	13.92	3.55	0.96
	2–3 h	29.83	12.68	2.35	0.95
	3–4 h	25.72	14.58	1.76	0.93
NH_4^+	0–1 h	117.59	21.81	5.39	0.96
	1–2 h	89.83	20.68	4.34	0.98
	2–3 h	52.12	18.76	2.78	0.99
	3–4 h	45.47	33.69	1.35	0.97
PO_4^{3-}	0–1 h	22.48	7.21	3.12	0.92
	1–2 h	20.79	14.99	1.39	0.98
	2–3 h	10.17	5.73	1.77	0.98
	3–4 h	11.89	18.56	0.64	0.93

Table 4. Kinetic parameters of nutrient uptake at different concentrations for *Ulva australis*

Nutrition	Time	<i>Ulva australis</i>			
		$R_{max}/(\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1})$	$K_s/(\mu\text{mol}\cdot\text{L}^{-1})$	R_{max}/K_s	r^2
NO_3^-	0–1 h	83.42	9.46	8.82	0.98
	1–2 h	71.31	17.82	4.00	0.97
	2–3 h	52.96	21.82	2.43	0.98
	3–4 h	33.56	20.75	1.82	0.98
NH_4^+	0–1 h	138.38	17.36	7.97	0.99
	1–2 h	107.06	22.10	4.84	0.98
	2–3 h	65.80	18.01	3.65	0.97
	3–4 h	51.45	25.58	2.01	0.97
PO_4^{3-}	0–1 h	21.82	12.63	1.73	0.99
	1–2 h	19.16	21.69	0.88	0.97
	2–3 h	8.75	6.04	1.45	0.84
	3–4 h	7.01	12.17	0.58	0.99

ammonium, and phosphate uptake, *U. prolifera* had higher R_{max} values than *U. australis* and *B. plumosa*, indicating that this species has a higher uptake rate at the same nutrient concentration. The R_{max}/K_s ratio describes the relationship between uptake rate and substrate concentration, with higher values indicating competitive advantages and higher affinity. The significant difference in the R_{max}/K_s values of the three dominant species of Qinquangdao green tides indicates that *U. prolifera* had obvious competitive advantages in taking up and storing nitrogen and phosphate from neighboring waters compared with *U. australis* and *B. plumosa*. A high nutrient absorption ability greatly contributes to the rapid growth rate and ecological competitive advantage of this species over the other co-occurring species, especially in eutrophic waters (Luo et al., 2012; Wang et al., 2012). This study also explains why the biomass of the third stage of the green tide, with *U. prolifera* as the dominant species, was the highest, with the greatest impact on the local ecological environment.

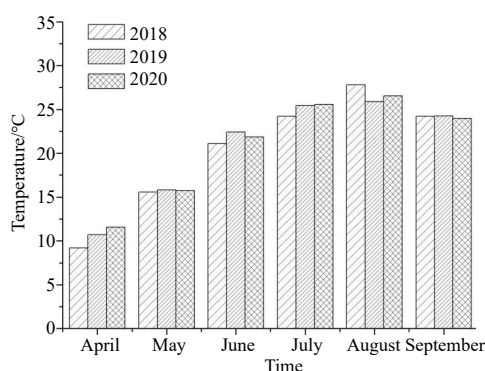


Fig. 13. Seawater temperature of Jinmenghaiwan Bathing Beach from April to September 2018–2020 (<http://zrzy.hebei.gov.cn/heb/gongk/gkml/yjgl/hyhz/>).

The maximum absorption rate of the three algae for NH_4^+ was higher than that to NO_3^- . For example, in the interval of 0–1 h, the R_{\max} values of *U. prolifera*, *U. australis*, and *B. plumosa* of NO_3^- were 104.31 $\mu\text{mol}/(\text{g}\cdot\text{h})$, 83.42 $\mu\text{mol}/(\text{g}\cdot\text{h})$, and 79.43 $\mu\text{mol}/(\text{g}\cdot\text{h})$, respectively, whereas those of NH_4^+ were 157.35 $\mu\text{mol}/(\text{g}\cdot\text{h})$, 138.38 $\mu\text{mol}/(\text{g}\cdot\text{h})$, and 117.59 $\mu\text{mol}/(\text{g}\cdot\text{h})$, respectively. This is because the algae absorb ammonia nitrogen and convert it directly into glutamate, which is used for algal growth. After the uptake of nitrate salts, they must be reduced to ammonia nitrogen or low-valence ammonium salt before being absorbed and used by the algae. Since NO_3^- is reduced to NH_4^+ by nitrate enzyme before assimilation (Hurd et al., 1995; Abreu et al., 2011), the higher affinity to NH_4^+ might be related to the less energy required for the assimilation of this nutrient.

In conclusion, *U. australis*, *B. plumosa*, and *U. prolifera*, the three dominant species of the green tide in Qinhuangdao, showed significant interspecific differences in physiological characteristics such as growth and nutrient uptake capacities. Temperature has a significant effect on the growth of the three species and is the key environmental factor for the dominant species succession of green tides in Qinhuangdao. *Ulva prolifera* has a higher growth rate than *U. australis* and *B. plumosa* at the same nitrate, ammonium, and phosphate concentrations, with obvious competitive advantages in nutrient uptake capacity. Therefore, the strong growth and nutrient uptake capacities of *U. prolifera* might be the main reason why *U. prolifera* becomes the dominant species, with the highest biomass and the greatest influence on the local ecological environment.

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