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Competition relations between selected microalgae and bloom-forming *Ulva prolifera*

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Research Article

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Abstract

Large-scale *Ulva prolifera* green tides have successively occurred for 16 years (2007–2022) in the Yellow Sea (YS), and the different life stages of *U. prolifera* play critical roles in regulating the occurrence and development of green tides. *U. prolifera* and microalgae have a similar niche in seawater, but their potential interactions are not yet clearly understood. In this study, we investigated the competition relationship between two microalgae and *U. prolifera* at five different development stages in controlled laboratory experiments. The results showed that one microalgae *Alexandrium tamarense*, can only inhibit *U. prolifera* gametes at the first settlement stage. Inversely, the germinated *U. prolifera* begin to show negative effects on microalgae in multiple ways at the subsequent four stages, and the growth inhibition rates among these stages ranged from 19 to 100%. The complex interactions may influence the formation of green tides. Meanwhile, the potential ecological consequences on phytoplankton, even the decreased occurrence of microalgal blooms in the YS need to be further evaluated.

Introduction

The excessive growth of some green macroalgae is termed 'green tide' (Fletcher, 1996). In recent years, coastal China has been widely threatened by green tides. The first report came from the *Ulva prolifera* green tides in the Yellow Sea (YS), which occurred consecutively for 16 years (2007–2022) and ranked first on scale in the world (Ye *et al.*, 2011; Liu and Zhou, 2018; Hao *et al.*, 2020; Wang *et al.*, 2022). Moreover, it was suggested that the drifting *U. prolifera* from the YS may expand to the East China Sea, resulting in the green tides in the Gouqi Island since 2011 (Zhang *et al.*, 2015a). In the Bohai Sea, *U. prolifera* has also been reported to cause summer green tides since 2015 (Song *et al.*, 2019). The unique features of *U. prolifera* green tides in the YS, including huge biomass, wide distribution, long-distance transport, have caused severe ecological consequences and wide concerns (Yu *et al.*, 2018; Zhang *et al.*, 2019; Song *et al.*, 2022; Zheng *et al.*, 2022).

The *U. prolifera* green tide in the YS is a trans-regional disaster. According to satellite monitoring and homology analysis, it was considered that *U. prolifera* green tides in the YS originated from Subei shoal of Jiangsu province and then floated to Qingdao Sea area. In every spring, small-scale free-floating green algae drifted northwards by seasonal monsoons and surface currents in mid-April to early May. During the migration process, the biomass of *U. prolifera* increased fast because of the appropriate temperature and nutrient enrichment. In June and July, a large amount of *U. prolifera* (biomass could reach 10^6–10^7 tonnes) drifted northern YS, covering a large area of the Shandong peninsula. After that, green tides declined gradually, with great *U. prolifera* sink down to the bottom of the YS in August (Keesing *et al.*, 2011; Liu *et al.*, 2013; Zhang *et al.*, 2019).

During the formation process of green tides in the YS, with invisible microscopic propagules as well as visible thalli occurring in *U. prolifera* life cycles (Liu *et al.*, 2013; Li *et al.*, 2014), *U. prolifera* has complicated life histories and multiple reproduction modes (Lin *et al.*, 2008; Liu *et al.*, 2015b). During their life cycles, various life forms at different development stages were observed including microscopic propagules, germlings, mature thalli and decayed thalli. A large quantity of microscopic propagules, including spores, gametes and zygotes are regarded as the simplest stage of *U. prolifera* (Clayton, 1992; Li *et al.*, 2014). They inevitably undergo two basic processes, settlement and germination, before developing into multicellular seedlings. With the growing of germinated seedlings, their branch gradually increased and developed into mature thalli. After releasing germ cells, thalli turned white and enter the decay stage. Every stage is critical for the dynamic adjustment of the whole macroalgal life histories, even the occurrence of green tides (Hoffmann and Santelices, 1991; Lotze *et al.*, 1999; Santelices *et al.*, 2002).

Green macroalgae including *Ulva* spp. and microalgae in seawater have a similar habitat, which gives them a chance to interact with each other. In view of the competition between microalgae and *U. prolifera*, it is well known that green macroalgal inhibit unicellular microalgae, and the effect of microalgae on macroalgae is rarely reported (Jensen, 1977; Wang *et al.*, 2013; Liu *et al.*, 2017). Most recent studies have pointed that *Ulva* thalli could inhibit the

growth and reproduction of microalgae in multiple ways, and reduce the diversity and stability of the phytoplankton community, even threaten the formation and succession of microalgal blooms (Tang and Gobler, 2011; Wang *et al.*, 2012; Sun *et al.*, 2016; Gao *et al.*, 2018). However, both Schonbeck and Norton (1979) and Huang and Boney (1983) believed that microalgae can also change the development process of macroalgae by affecting their early microscopic stage. Our previous research also found some microalgae could inhibit settlement of *U. prolifera* gametes, and subsequent development will be impacted (Liu *et al.*, 2017). Since every life stage is critical for the population dynamics of *U. prolifera*, any variation may eventually influence the population development and the magnitude of green tides. Moreover, *U. prolifera* is an opportunistic macroalgae, their growth characteristics gradually change during life stages (Clayton, 1992; Lotze *et al.*, 1999). So what are the competition relations between *U. prolifera* and microalgae at different life stages? What is the role of these interactions in the occurrence and influence of green tides in the YS? These questions are still poorly understood and need to be answered.

This is the first study to examine the interactions between microalgae and *U. prolifera* at different life stages. We used five separate co-culture assays to investigate the competition relationship between two microalgae and *U. prolifera* at settlement, germination, seedling, adult and decay stages under laboratory conditions. Two chosen microalgae *Alexandrium tamarense* and *Prorocentrum donghaiense* were all harmful strains, commonly occurring in the coastal waters of China (China Marine Environmental Bulletin, 2001–2021). *A. tamarense* was bloom-forming microalgae which have caused severe red tides in the YS, Bohai Sea and the East China Sea. It was known for producing paralytic shellfish toxins (PST) in many coastal regions. Although *P. donghaiense* only trigged large-scale red tides in the East China Sea every year, their special threat to marine environment made us interested in. The main objectives of this study were to provide evidence of any competitive relations between *U. prolifera* and microalgae, and to evaluate all potential factors affecting the formation and consequences of green tides in detail.

Materials and methods

Sample identification and preparation

The macroalgae *U. prolifera* were collected from Qingdao Huiquan Bay in July 2014. All strains were identified as *U. prolifera* by both morphological and molecular analyses. Thalli were washed several times with sterile seawater, gently cleaned with soft brushes and checked under a microscope to ensure that they were free of epiphytes. The treated thalli were controlled in a laboratory environment for several days prior to experiments.

Two microalgae were selected for studying the interactions with *U. prolifera*. Axenic strains of *A. tamarense* was isolated from the South China Sea and provided by Jinan University. *P. donghaiense* was isolated from the East China Sea and provided by the Second Institute of Oceanography. The detailed descriptions of the classification status, particle sizes, bloom densities and toxin-producing features are presented in Table 1. Two strains were cultured to the exponential phase before inoculation in the following experiments.

Microalgae and *U. prolifera* were cultured in f/2 medium, and maintained at 20 ± 1°C, under an irradiance of 50 µmol photons m^{-2} s^{-1}, with a light:dark cycle of 14:10. Natural seawater from Huiquan Bay, Qingdao was sand-filtered prior to utilization. Prior to each experiment, the seawater was filtered through a 0.45 µm membrane and autoclaved to prepare the culture medium. The salinity of the seawater was 30 ± 1. The average concentrations of NO_3^--N and PO_4^{3-}-P in the seawater were 24.9 ± 2.5 and 0.5 ± 0.08 µmol l^{-1}, respectively.

Obtaining different life forms of U. prolifera *at five development stages*

According to our laboratory observed results (Liu *et al.*, 2015a, 2017), the whole life cycle of *U. prolifera* was divided into five different stages, including the settlement stage (from free-swimming zoids to settled on substratum), germination stage (from settled unicellular zoids to two germinating cells), seedling stage (multicellular germling length of 1 mm or more), adult stage (mature thalli had abundant branches and length reached more than 5 cm) and decay stage (mature thalli turned white after releasing zoids).

To obtain different life forms of *U. prolifera* for the experiments, released gametes were collected to culture until they developed into different life forms. Gamete formation of *U. prolifera* was induced by a modified punching method (Dan *et al.*, 2002). The thalli were cut into 1–2 cm small fragments, and incubated in a Petri dish containing f/2 medium. The medium was replaced every day for 2–3 days until the gametes were released. The initial concentration of *U. prolifera* gametes used in the experiments was set as 1.0×10^6 cells l^{-1}, same as the microalgae.

Interactions between microalgae and U. prolifera *at five different stages*

We conducted time-course experiments of *U. prolifera* in five different stages. At the first day of these five stages, exponentially growing microalgae were inoculated into the beakers and used as treatment groups. Meanwhile, monocultures of *U. prolifera* and microalgae were used as controls, respectively. Before the experiments, one coverslip (10×10 mm^2) was placed at the bottom of each beaker to facilitate the counting of attached, germinated gamete numbers and the growth rate of *U. prolifera* thalli. Each experiment was conducted separately in three beakers, with triplicate samples taken from each beaker for the counts. Only at the settlement stage, the experiments were under dark conditions for 1 day to ensure gametes' settlement randomly. For the other four stages, experiments were conducted under light conditions for 7 days. To avoid nutrition limitation, 40 µl of f/2 nutrition solution was added at the beginning of the experiment. The concentration of f/2 nutrition solution was 882 µmol l^{-1} NO_3^--N and 36.2 µmol l^{-1} PO_4^{3-}-P.

At the settlement stage, numbers of attached gametes and growth inhibition rate of microalgae were used for statistical analysis. After 24 h, attached gametes on the coverslips were removed and counted in 20 fields under a microscope (400× magnification). The fields were taken at approximately 1 mm intervals across the surface of each coverslip from left to right, and the averages were used to calculate the number of settled gametes. Meanwhile, 200 µl of samples in each beaker were pipetted, fixed in Lugol's solution and the density of microalgae counted under a microscope. The growth inhibition rate (%) = $(1 - N/N_0) \times 100\%$ (where N_0, the cell density of microalgae in the control groups; and N, the cell density of microalgae in the treatment groups).

At the germination stage, the germination rate of *U. prolifera* and growth inhibition rate of microalgae in each beaker were used for statistical analysis. Every day, the coverslip in each beaker was taken out to count the germination rates (a gamete that divided into two cells was considered the germination standard) and then put back. Meanwhile, 200 µl of samples were pipetted, fixed in Lugol's solution and counted under a microscope. All beakers were shaken twice every day to avoid the microalgae

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Table 1. List of microalgal species used in the experiment

Microalgal species	Taxonomy: Phylum and Class	Morphology size (μm)	Toxicity characteristic	Bloom density (cells l^{-1})	Culture medium
A. tamarense	Alveolata: Dinophyceae	27.0 × 30.0	PST	10^4–10^5	f/2
P. donghaiense	Alveolata: Dinophyceae	12.0 × 14.0	/	10^5–10^7	f/2

PST, paralytic shellfish toxins.

growth adhering to the wall. All samples were preserved in Lugol's solutions for calculating the microalgal cell densities. The germination rate (%) = $N/N_0 \times 100\%$ (where N_0, the total number of gametes in one field; and N, the germinated gamete numbers in one field).

At the seedling, adult and decay stages, the growth rate of *U. prolifera* and growth inhibition rate of microalgae were used for statistical analysis. The growth rate (%) = $(\ln N_t - \ln N_0)/t \times 100\%$ (where N_t is the wet weight of *U. prolifera* at the end of the experiment; N_0 is the wet weight of *U. prolifera* at the beginning of the experiment and T is the experimental time).

In addition, the nutrients, dissolved oxygen (DO) and pH were measured after every experiment. All samples for nutrient analyses were filtered on GF/F filters, and then an auto analyzer (Quattro, Germany) was used to test the concentrations of NO_3^--N, NH_4^+-N and PO_4^{3-}-P. The DO and pH of incubated samples were measured with a portable dissolved oxygen meter (JENCO 901, America) and a handheld pH meter (pH211, Italy).

Statistical analysis

The data were analysed using origin 8.5 and SPSS 16.0 software. The data were calculated as the means ± standard deviations (SD) from the different replicated beakers per treatment ($n = 3$). Statistical differences between the treatments and controls were considered significant at the 0.05 level.

Two statistical methods were used in our experiments. First, the data from settled gametes, germination and growth rates of *U. prolifera* were analysed using one-way analysis of variance (ANOVA) after testing for normal distribution and homogeneity of variance. For some data, heterogeneity of variances between groups was detected and not corrected by transformation, which were then analysed using non-parametric tests (Kruskal–Wallis one-way ANOVA). If significant differences of the overall ANOVA were found, the post-hoc test (Tukey's test or Dunn's test) were performed to test among the experimental groups. The data from the growth of microalgae at five different stages were examined using independent-samples t test because there were only two experimental groups in each microalgal species.

Results

Effects of microalgae on the settlement, germination and growth of U. prolifera

Figure 1 shows the different effects of two microalgal species on the settlement of *U. prolifera* gametes. Compared with the controls, the tested microalgae *A. tamarense* significantly decreased the settled number of gametes (one-way ANOVA, $P < 0.05$), while *P. donghaiense* had no significant effects on the settlement of gametes (one-way ANOVA, $P > 0.05$).

Gametes in these tested groups were germinated completely within 7 days, and the final germination rates of gametes co-cultured with *A. tamarense* and *P. donghaiense* were not significantly different (Figure 2). Although the germination process

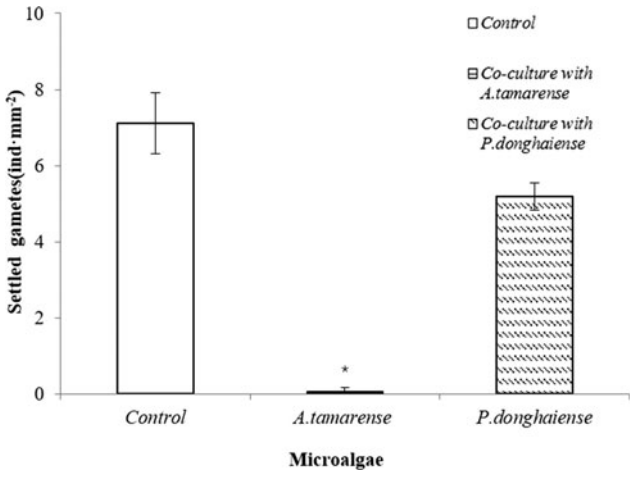

Figure 1. Effects of *A. tamarense* and *P. donghaiense* on the settlement of *U. prolifera* gametes. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

of gametes seems slightly accelerated in the groups co-cultured with two microalgae from 2 to 5 days, the differences between control and treatments were not significant (one-way ANOVA, $P > 0.05$).

Figure 3 shows the growth of *U. prolifera* co-cultured with two microalgae at three different stages. The growth rates of co-culture *U. prolifera* were less than that of monocultures, but there was no significant difference at these three stages (one-way ANOVA, $P > 0.05$). In the co-culture groups of *A. tamarense*, the growth rates of *U. prolifera* were 94, 98 and 81% at the seedling, adult and decay stages on the 7th day, compared with the control groups. For *P. donghaiense*, the growth rates of *U. prolifera* were 97, 93 and 81%, also had no significant growth inhibition relative to the controls.

For the monocultures of *U. prolifera* control groups, there were significant differences for *U. prolifera* incubated at these three stages (including seedling, adult and decay stages) (one-way ANOVA, $P < 0.05$). In the control groups, the growth rate of *U. prolifera* at the seedling stage reached 82%, significantly higher than that for the adult stage (10%). In addition, the growth rate of *U. prolifera* at the decay stage was less than 0, for the decomposition state of thalli.

Effects of U. prolifera on the growth of microalgae at five different stages

Figure 4 shows the growth of *A. tamarense* and *P. donghaiense* co-cultured with *U. prolifera* gametes at the settlement stage. Compared with the control groups, no significant inhibitory or lethal effects of gametes were observed on these two microalgae strains at this stage (t test, $P > 0.05$). The results suggested that *U. prolifera* gametes had no significant inhibitory effects on microalgae.

The effects of *U. prolifera* on the growth of *A. tamarense* and *P. donghaiense* at the other stages are shown in Figure 5. Among the four stages of *U. prolifera*, all treatment groups showed

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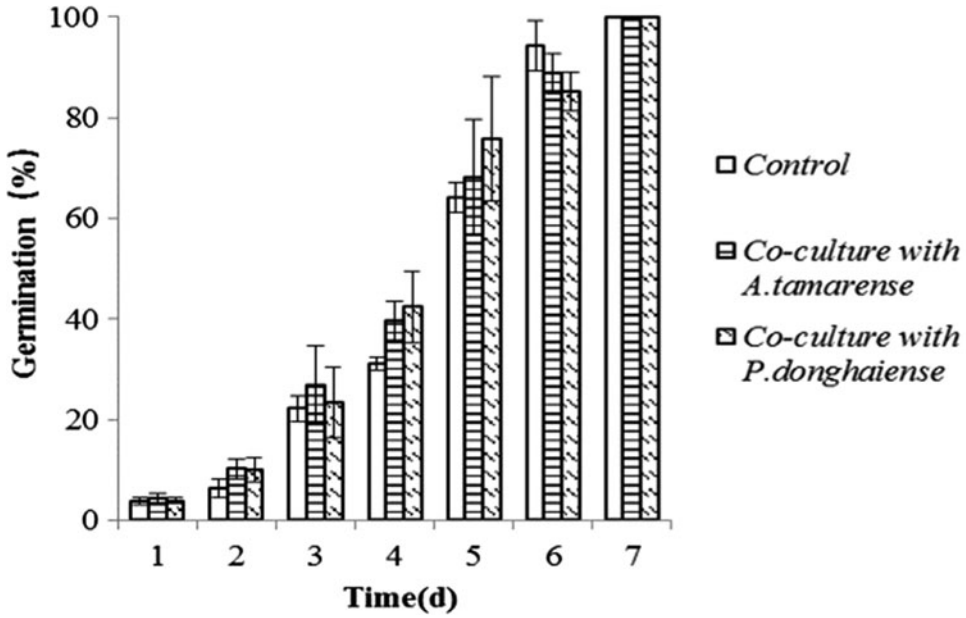

Figure 2. Effects of *A. tamarense* and *P. donghaiense* on the germination of *U. prolifera* gametes. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

significant inhibitory activity against tested two red tide microalgae, as the strongest inhibition was observed on the decay stage. For *A. tamarense*, their growths were significantly inhibited when co-cultured with *U. prolifera*, and the growth inhibition rates reached 19, 30, 78 and 93%, respectively, at the germination, seedling, adult and decay stages (Figure 5a, c, e, g) (*t* test, $P < 0.05$). For *P. donghaiense*, similar negative effects were displayed at these four stages, while growth inhibition exhibited by *U. prolifera* reached 28, 31, 61 and 100%, respectively (Figure 5b, d, f, h). The cell densities of treatment groups were significantly lower than control groups (*t* test, $P < 0.05$).

Changes of environmental factors at five different stages

Changes of DO, pH and nutrients (NO_3^--N and PO_4^{3-}-P) in co-culture systems of *A. tamarense* and *P. donghaiense* at five stages are shown in Figure 6. For *A. tamarense* (Figure 6a), first, DO and pH had similar variation trends. The lowest DO concentration and

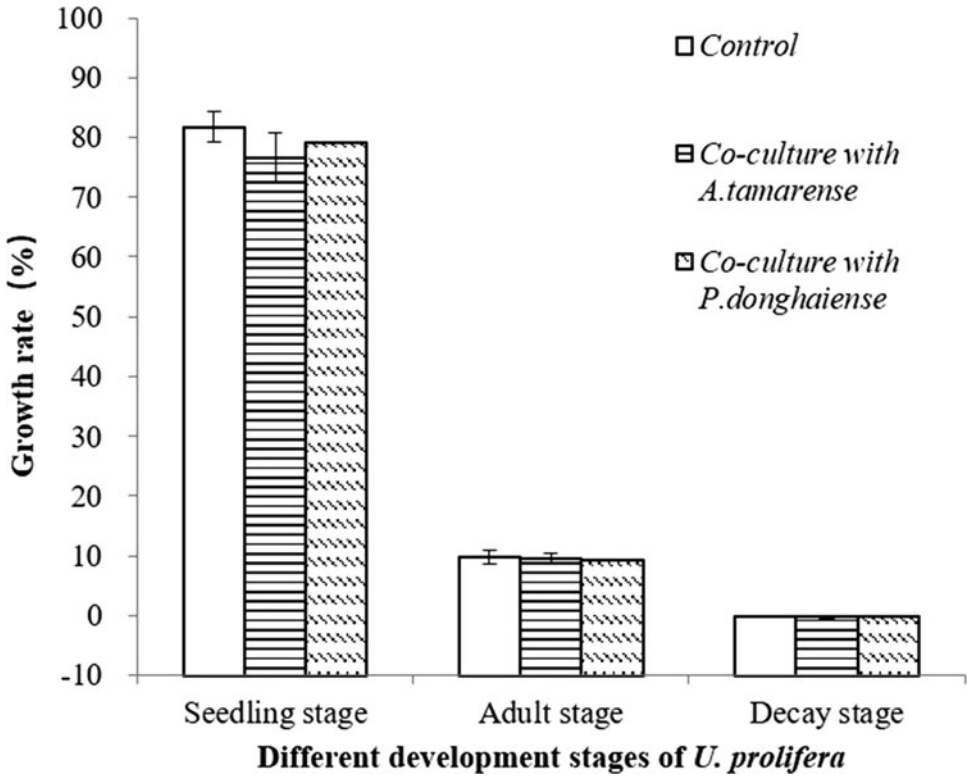

Figure 3. Effects of *A. tamarense* and *P. donghaiense* on the growth of *U. prolifera* thalli at the seedling, adult and decay stages. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

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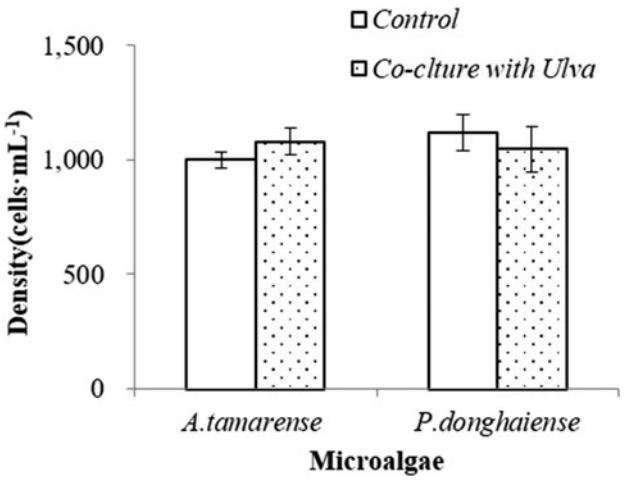

Figure 4. Growth-inhibition effects on microalgae by *U. prolifera* gametes at the settlement stage. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

pH in the seawater both appeared at the decay stage, which have been in the state of low oxygen and acidification. When compared to the normal seawater quality (DO: 8.5 mg l^{-1}, pH: 8.6), the DO and pH decreased by 54 and 10%, respectively. At the other four stages, the DO concentration was higher than the normal level, which is in the saturated state, and pH was also in the normal level. The results indicate that at the decay stage the seawater quality was greatly influenced by algae and even caused hypoxia and acidification. In addition, the nutrient concentrations (NO_3^--N and PO_4^{3-}-P) of *A. tamarense* co-culture systems all showed the declining trends at five different stages, compared to the initial nutrient levels. Especially at the adult stage, NO_3^--N and PO_4^{3-}-P decreased quickly in the co-culture groups, the uptake rate of NO_3^--N by *U. prolifera* and *A. tamarense* reached 96%, and PO_4^{3-}-P were almost depleted on day 7. At the first three stages and the last decay stage, there were still some nutrients that remained throughout the experiment, although the consumption rate of nutrients during the experiments varied with different stages. The uptake rates of NO_3^--N were 28, 52, 88 and 14% by day 7, and 35, 49, 91 and 20% for the PO_4^{3-}-P, respectively, have been removed. Among these four stages, the nutrient consumption was the highest at the seedling stage. The results imply that nutrients were the limiting factors for the growth of algae at the adult stage.

In the *P. donghaiense* treatment groups (Figure 6b), the changes of DO and pH were also consistent with *A. tamarense*. Only at the decay stage, the concentration of DO and pH decreased by 62 and 9%, obviously below the normal seawater levels. At the settlement, seedling, adult and decay stages, the DO and pH in the co-culture systems all present the normal states. Moreover, the declining trends of nutrient levels (NO_3^--N and PO_4^{3-}-P) were also similar to *A. tamarense*. Only at the adult stage, the nutrients were almost taken up completely by *U. prolifera* and the microalgae. The remaining NO_3^--N concentrations are extremely low (only 1%), and PO_4^{3-}-P was fully removed after the 7 day incubation. In other four stages, there are still different degrees of residual nutrients in all incubation groups, so nutrient was not the key factor to interrupt the growth of microalgae among these stages.

Discussion

Possible causes of the observed interactions between microalgae and green algae U. prolifera

This study was the first to analyse the competition relationship between microalgae and bloom-forming *U. prolifera* at five different growth stages through co-culture experiments. The results showed that the interactions were complex and different at five different stages (Table 2). Only at the settlement stage, one experimental microalgae *A. tamarense* showed significant inhibition on *U. prolifera* gametes, while the gametes had no apparent effects on microalgae. However, at the subsequent four stages, the microalgae did not affect the germination and growth of *U. prolifera*, while the thalli showed significant inhibitory effects on the growth of microalgae. The above results demonstrated that microalgae could only inhibit the naked *U. prolifera* propagules at the early settlement stage. Since *U. prolifera* propagules germinated, they began to show strong and non-selective inhibitory effects on microalgae.

It is well known that the interactions between macroalgae and microalgae are common in the marine environment. Multiple mechanisms were responsible for these negative interactions, mainly including resource competition, allelopathy and alteration of seawater environment (Tang and Gobler, 2011; Wang *et al.*, 2012, 2013; Sun *et al.*, 2016). In our study, an interesting finding is that the competition mechanisms between microalgae and *U. prolifera* at different stages were different.

At the settlement stage, resource competition was first excluded to account for the interactions, because enough nutrients were added before the experiments to avoid the possible nutrient competition. Moreover, other environmental factors (e.g. light, temperature, DO and pH) would not be the limiting factor. Because the experiment at this stage was performed under dark conditions and temperature was controlled in our laboratory. Additionally, DO and pH were still well suited for the growth of algae when they were measured after the experiments. Through the settlement experiment, we found that microalgae *A. tamarense* significantly impaired *Ulva* gametes' settlement. Although harmful algal bloom species *A. tamarense* could produce PST, the PST did not play a major role in affecting gametes, because there are no differences between the inhibitory effects on gametes by the PST-producing *A. tamarense* and the non-PST-producing *Alexandrium affine* under the same experimental conditions. When non-toxic *A. affine* (at the density of 1.0×10^6 cells l^{-1}) was co-cultured with the gametes, the settled number of gametes was less than 1 cell mm^{-2}, very close to the co-culture with toxic *A. tamarense* (0 cell mm^{-2}) (Liu *et al.*, 2015a, 2017). Other chemicals like bioactive compounds may be involved in the interactions. However, we did not test the specific compounds produced by *A. tamarense* in inhibiting the gametes, for which further elucidation is needed.

The initial inhibitory effects of *U. prolifera* on microalgae started from the early germination stage. Resource competition was also unlikely to cause this phenomenon. Nutrients, DO and pH were also measured throughout the experiments and were unlikely to become limiting factors. Light competition was also excluded, because the propagules of *U. prolifera* would hardly reach extreme high densities to cause the shading effects (Amsler *et al.*, 1992). All containers were shaken twice every day to ensure all phytoplankton were suspended in the culture medium to fully utilize the light as much as possible. It seemed that certain metabolites, produced by microscopic propagules of *U. prolifera*, may be the main factor inhibiting the microalgae. The metabolites were generally thought to cause negative impacts on microalgae by changing the microenvironment (Jensen, 1977; Huang and Boney, 1983).

At the seedling stage, *U. prolifera* also showed significant inhibition on two microalgae, even the toxin-producing species. Chemicals produced by *U. prolifera* seedlings, instead of resource competition, were responsible for this phenomenon, because the environmental factors like nutrients, DO, pH and light were also suitable for the growth of algae at this stage. In addition, we analysed the relationship between the biomass of *U. prolifera*

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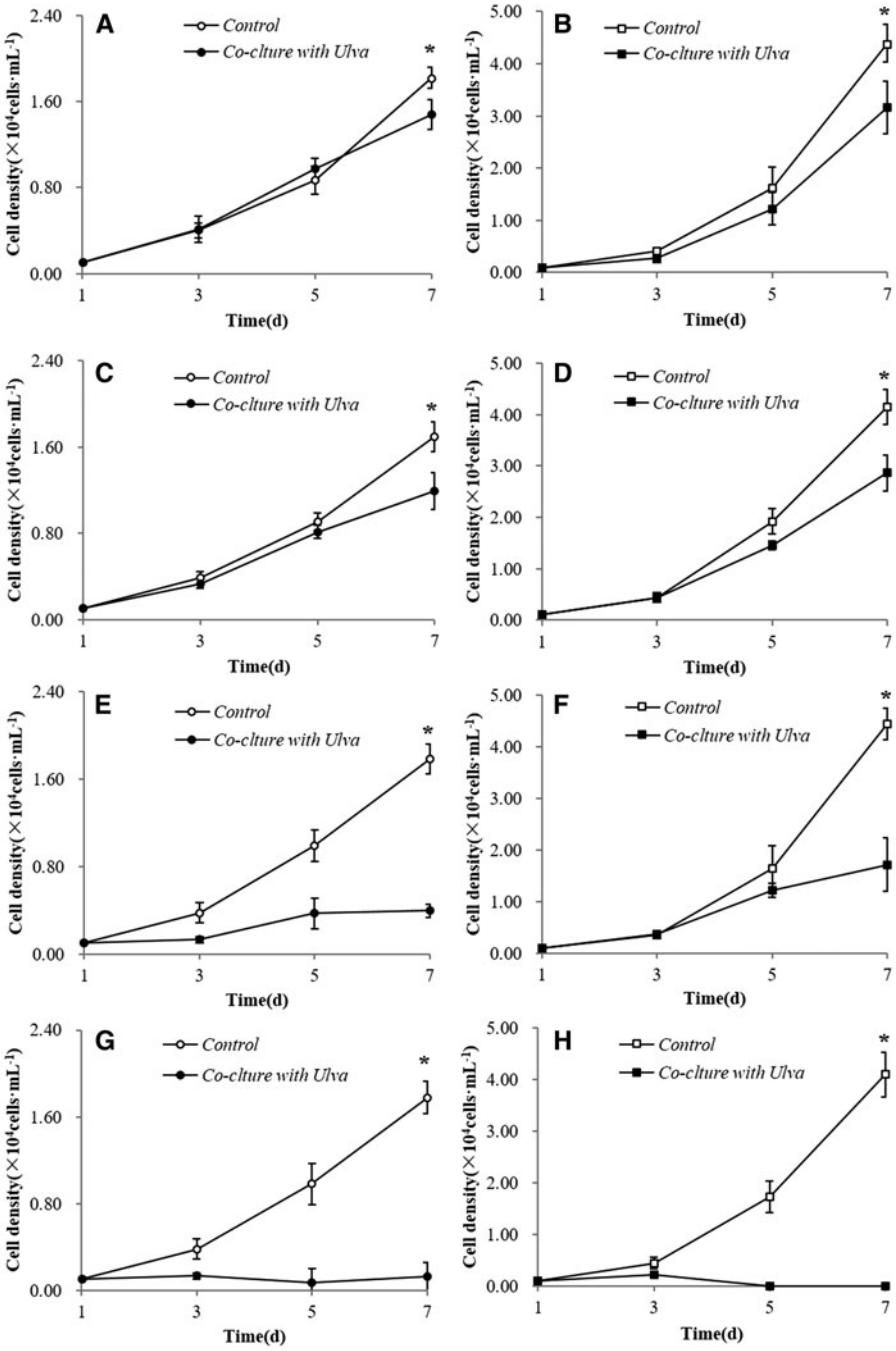

Figure 5. Growth-inhibition effects on microalgae by *U. prolifera* at the germination, seedling, adult and decay stages. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

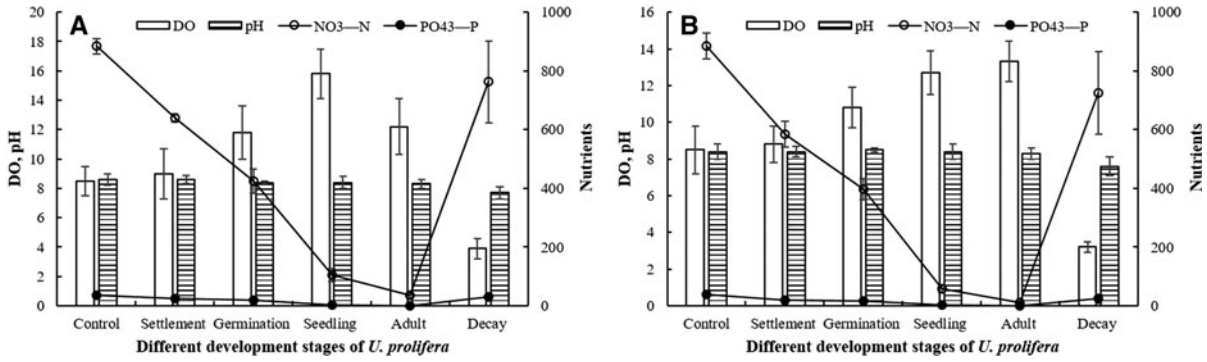

Figure 6. Changes of DO, pH and nutrients (NO_3^--N and PO_4^{3-}-P) in co-culture systems of *A. tamarense* and *P. donghaiense* at five stages. Data points are means ± SD ($n = 3$). *$P < 0.05$ as compared to control.

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Table 2. Competition relationship between microalgae and *U. prolifera* at five different development stages

	Competition relationship		Competition relationship	
Different development stages of *U. prolifera*	*U. prolifera*	*A. tamarense*	*U. prolifera*	*P. donghaiense*
Settlement	_	/	/	/
Germination	/	_	/	_
Seedling	/	_	/	_
Adult	/	_	/	_
Decay	/	_	/	_

'–' represents no effect; '/' represents negative effect.

and the growth inhibition rate of microalgae at the germination and seedling stages. The average length of *U. prolifera* was 0.5 and 50 mm, respectively, at these two stages, while the growth inhibition rate on *A. tamarense* reached 19 and 30%. The inhibition rates of microalgae only increased by 1.5 times, while the total biomass of *U. prolifera* increased by approximately 100 times. Therefore, the potential threats by *U. prolifera* propagules cannot be ignored and should be carefully considered.

At the adult stage, *U. prolifera* thalli displayed stronger inhibition on microalgae, while microalgae still showed no significant negative effect on *U. prolifera*. By analysing the environmental data, we found that the nutrient uptake characteristics of *U. prolifera* could be an important factor for reducing the microalgae density. It is well known that *U. prolifera* exhibit high rates of nutrient uptake; they can rapidly assimilate the organic and inorganic nutrients for growth. This characteristic provided a competitive advantage to *U. prolifera*, relative to other planktonic algae (Li *et al.*, 2016; Wang *et al.*, 2019; Zhang *et al.*, 2019). However, we compared the inhibitory effects of *A. tamarense* and *P. donghaiense* with daily addition of enough nutrients under the same experimental conditions, and found the growth of microalgae was just alleviated but still inhibited. Under the sufficient nutrient conditions, the growth inhibition rates of *A. tamarense* and *P. donghaiense* were 42 and 44%, obviously lower than the rates under limited nutrient conditions (78 and 61%) (Figure S1). Therefore, we conclude that except for nutrient competition, other mechanisms may be responsible for the adverse role. Recent research studies have shown that mature thalli of *U. prolifera* could produce some chemicals that reduce the growth of microalgae (Tang and Gobler, 2011; Wang *et al.*, 2013; Sun *et al.*, 2016; Gao *et al.*, 2018). Certain metabolites like polyunsaturated fatty acids were considered to cause negative effects on microalgae, and some other antialgal active substances from macroalgae *Ulva* were isolated and identified (Sun *et al.*, 2016). However, whether the mature thalli of *U. prolifera* secreted the same chemicals as the germlings and seedlings is still unclear. It is necessary to compare and analyse the antialgal activity substances at different stages of *U. prolifera* in future research.

At the decay stage, *U. prolifera* displayed the strongest inhibition on microalgae, and the tested red tide microalgae were nearly completely suppressed in the co-cultured environment. The bad seawater quality (hypoxia and acidification) caused by *U. prolifera* played the main adverse role in microalgae. The uncomfortable environment makes microalgae hardly grow even under the sufficient nutrient conditions. Moreover, our results revealed that the decomposition of *U. prolifera* release abundant NH_4^+, which is likely to inhibit the growth of microalgae species. The negative effect of ammonium cannot be ignored, which could cause regional eutrophication and threaten the microalgae (Nelson *et al.*, 2003; Wang *et al.*, 2012). In addition, substantial amounts of microbial organisms, mainly bacteria, are released after *U. prolifera* decomposition, which are well known to have negative effects on microalgae (Hardison *et al.*, 2010; Zhang *et al.*, 2015b).

Potential ecological influences of the interactions in the YS

Our findings may provide some theoretical references for the outbreak reasons of green tides and their following consequences on phytoplankton. The *Porphyra* culture rafts in Subei shoal are considered to be the initial origin of green tide (Keesing *et al.*, 2011; Liu *et al.*, 2013; Hao *et al.*, 2020). Due to the highly turbid seawater, the biomass of phytoplankton is low and few reports on microalgal blooms are available (Kang *et al.*, 2013). In addition, large amounts of attached green macroalgae on the rafts are observed, which also inhibit the growth of microalgae based on our research and other reports (Wang *et al.*, 2013; Sun *et al.*, 2016). Therefore, the inhibition effect of microalgae on the settlement of *U. prolifera* is unlikely to exist in Subei shoal, which is beneficial for the growth of *U. prolifera*. It is a protective mechanism for the early development of green tides, and even promotes the occurrence of green tides. If these 'seed banks' of micropropagules in Subei shoal are not inhibited by microalgae at the initial settlement stage, they will gradually show negative effects on microalgae through various ways. First, the germinated micropropagules in Subei shoal could inhibit or even kill microalgae by secreting certain metabolites. Elimination of competitors would accelerate the growth and development of *U. prolifera*, which is beneficial for developing into mature thalli. During the subsequent drift process, the biomass of *U. prolifera* increased rapidly under appropriate environmental conditions, including light, temperature and nutrients. The inhibition effects on microalgae can be further increased by blocking sunlight and absorbing large amounts of nutrients. These results are consistent with reports of dramatic decreases in chlorophyll-a concentrations during the blooming period of *U. prolifera* green tides (Xing *et al.*, 2015; Sun *et al.*, 2018). Even though the environmental conditions were not suitable for *U. prolifera* in August, decayed *U. prolifera* thalli still showed strong inhibitory effects on microalgae by altering the seawater environment, such as hypoxia and acidification (Wang *et al.*, 2012; Zhang *et al.*, 2019).

In addition, interactions between macroalgae and microalgae play an important role in the succession process of primary producers and even change the occurrence of harmful algal blooms (Smith and Horne, 1988; Sfrifo and Pavoni, 1994; Schramm, 1999). Our results are consistent with this conclusion. Large-scale *U. prolifera* green tides have successively occurred in the YS for 16 years (2007–2022), while the occurrence of red tides is relatively infrequent. The outbreak periods of these harmful algal blooms coincides well with the green tides in the YS (from May to August). According to China Marine Environmental Bulletin (2001–2021), the records of red tides decreased rapidly over the past few years, from nine times in

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2001–2005 to four times in 2008–2012. Our experimental results demonstrated *U. prolifera* can affect the growth of some red tide species in various ways at multiple development stages, which is likely to account for this phenomenon. Similar situations have also been reported that accumulated *Ulva* decreased the occurrence of microalgal blooms (Smith and Horne, 1988; Sfrifo and Pavoni, 1994; Qin *et al.*, 2011). Therefore, the negative impacts on microalgae by green tides in the YS, may have positive significance in the biological control of red tides in the future.

Conclusion

This paper discussed the competition relationships between *U. prolifera* and microalgae by five co-culture experiments, and also discussed the possible feedback between green tide and phytoplankton in the YS, China. The main findings are as follows:

(1) One harmful microalgae *A. tamarense*, only inhibited the naked *U. prolifera* gametes at the settlement stage. However, PST produced by *A. tamarense* did not play a major role.
(2) *U. prolifera* show strong and non-selective inhibitory effects on microalgae early from the germination stage in multiple ways. The negative impacts and influence mechanisms are different for each stage.
(3) The competition relationship may influence the formation of green tides and cause potential ecological influence on phytoplankton in the YS.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315423000310

Data availability. Data are available on request from the corresponding author.

Author contributions. Q. L., J. N. L., Z. J. K. and X. J. Z. conceived and planned the experiments. Q. L. and R. F. C. conducted experiments, analysed experimental and statistical data and wrote the manuscripts. J. N. L., Z. J. K. and X. J. Z. supervised and contributed to the final version of the manuscript.

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References

Amsler CD, Reed DC and Neushul M (1992) The microclimate inhabited by macroalgal propagules. *British Phycological Journal* **27**, 253–270.

China Marine Environmental Bulletin (2001–2021) Available at https://www.mee.gov.cn/hjzl/sthjzk/jagb/.

Clayton MN (1992) Propagules of marine macroalgae: structure and development. *British Phycological Journal* **27**, 219–232.

Dan A, Hiraoka M, Ohno M and Critchley AT (2002) Observations on the effect of salinity and photon fluence rate on the induction of sporulation and rhizoid formation in the green alga *Enteromorpha prolifera* (Müller) J. Agardh (Chlorophyta, Ulvales). *Fisheries Science* **68**, 1182–1188.

Fletcher RL (1996) The occurrence of 'green tide'. In Schramm W and Nienhuis PH (eds), *Marine Benthic Vegetation – Recent Changes and the Effects of Eutrophication*. Berlin: Springer Verlag, pp. 7–43.

Gao H, Zhou FF, Tang HJ, Shi XY and Su RG (2018) Allelopathy of extracts of *Ulva prolifera* on green tides in the Yellow Sea and the identification of the allelochemicals. *Acta Oceanologica Sinica* **40**, 11–20.

Hao Y, Qu TF, Guan C, Zhao XY, Hou CZ, Tang XX and Wang Y (2020) Competitive advantages of *Ulva prolifera* from *Pyropia* aquaculture rafts in Subei shoal and its implication for the green tide in the Yellow Sea. *Marine Pollution Bulletin* **157**, 111353.

Hardison AK, Canuel EA, Anderson IC and Veuger B (2010) Fate of macroalgae in benthic systems: carbon and nitrogen cycling within the microbial community. *Marine Ecology Progress Series* **414**, 41–55.

Hoffmann AJ and Santelices B (1991) Banks of algal microscopic forms: hypotheses on their functioning and comparisons with seed banks. *Marine Ecology Progress Series* **79**, 185–194.

Huang R and Boney AD (1983) Effects of diatom mucilage on the growth and morphology of marine algae. *Journal of Experimental Marine Biology and Ecology* **67**, 79–89.

Jensen A (1977) Chemical conditioning of seawater by algal growth and development. In Faulkner DJ and Fenical WH (eds), *Marine Natural Products Chemistry*. New York, NY: Plenum Press, pp. 329–344.

Kang W, Sun Y, Sun LF, Xu ZL and Que JL (2013) Distribution of phytoplankton in radial sand ridge area in north Jiangsu shoal. *Chinese Journal of Applied Environmental Biology* **19**, 727–733.

Keesing JK, Liu DY, Fearns P and Garcia R (2011) Inter- and intra-annual patterns of *Ulva prolifera* green tides in the Yellow Sea during 2007–2009, their origin and relationship to the expansion of coastal seaweed aquaculture in China. *Marine Pollution Bulletin* **62**, 1169–1182.

Li Y, Song W, Xiao J, Wang ZL, Fu MZ, Zhu MY, Li RX, Zhang XL and Wang XN (2014) Tempo-spatial distribution and species diversity of green algae micro-propagules in the Yellow Sea during the large-scale green tide development. *Harmful Algae* **39**, 40–47.

Li HM, Zhang YY, Han XR, Shi XY, Rivkin RB and Legendre L (2016) Growth responses of *Ulva prolifera* to inorganic and organic nutrients: implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports* **6**, 26498–26498.

Lin AP, Shen SD, Wang JW and Yan BL (2008) Reproduction diversity of *Enteromorpha prolifera*. *Journal of Integrative Plant Biology* **50**, 622–629.

Liu DY, Keesing JK, He PM, Wang ZL, Shi YJ and Wang YJ (2013) The world's largest macroalgal bloom in the Yellow Sea, China: formation and implications. *Estuarine, Coastal and Shelf Science* **129**, 2–10.

Liu Q, Yan T, Yu RC, Zhang QC and Zhou MJ (2017) Interactions between selected microalgae and microscopic propagules of *Ulva prolifera*. *Journal of the Marine Biological Association of the United Kingdom* **98**, 1571–1580.

Liu Q, Yan T, Zhou MJ, Zhang QC and Lin JN (2015a) Interactions between microscopic propagules of *Ulva prolifera* and *Alexandrium*. *Oceanologia et Limnologia Sinica* **46**, 1312–1320.

Liu Q, Yu RC, Yan T, Zhang QC and Zhou MJ (2015b) Laboratory study on the life history of bloom-forming *Ulva prolifera* in the Yellow Sea. *Estuarine, Coastal and Shelf Science* **163**, 82–88.

Liu DY and Zhou MJ (2018) Green tides of the Yellow Sea: massive free-floating blooms of *Ulva prolifera*. In Glibert P, Berdalet E, Burford M, Pitcher G and Zhou M (eds), *Global Ecology and Oceanography of Harmful Algal Blooms*. Berlin: Springer Verlag, pp. 317–326.

Lotze HK, Schramm W, Schories D and Worm B (1999) Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha* spp. *Oecologia* **119**, 46–54.

Nelson TA, Lee DJ and Smith BC (2003) Are 'green tides' harmful algal blooms? Toxic properties of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). *Journal of Phycology* **39**, 874–879.

Qin YT, Ji HH, Song CY and Xu R (2011) Phytoplankton ecological characteristics in green tide area of southern Yellow Sea. *Marine Environmental Science* **30**, 394–397.

Santelices B, Aedo D and Hoffmann A (2002) Banks of microscopic forms and survival to darkness of propagules and microscopic stages of macroalgae. *Revista Chilena de Historia Natural* **75**, 547–555.

Schonbeck MW and Norton TA (1979) The effects of diatoms on the growth of *Fucus spiralis* germlings in culture. *Botanica Marina* **22**, 233–236.

Schramm W (1999) Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *Journal of Applied Phycology* **11**, 69–78.

Sfrifo A and Pavoni B (1994) Macroalgae and phytoplankton competition in the central Venice lagoon. *Environmental Technology* **15**, 1–14.

Smith DW and Horne AJ (1988) Experimental measurement of resource competition between planktonic microalgae and macroalgae (seaweeds) in mesocosms simulating the San Francisco Bay-Estuary, California. *Hydrobiologia* **159**, 259–268.

Song W, Han HB, Wang ZL and Li Y (2019) Molecular identification of the macroalgae that cause green tides in the Bohai Sea, China. *Aquatic Botany* **156**, 38–46.

Song MJ, Kong FZ, Li YF, Zhao J, Yu RC, Zhou MJ, Jiang P and Yan T (2022) A massive green tide in the Yellow Sea in 2021: field investigation and analysis. *International Journal of Environmental Research and Public Health* **19**, 11753.

Sun YY, Wang H, Guo GL, Pu YF, Yan BL and Wang CH (2016) Isolation, purification, and identification of antialgal substances in green alga *Ulva prolifera* for antialgal activity against the common harmful red tide microalgae. *Environmental Science and Pollution Research* **23**, 1449–1459.

Sun X, Wu MQ, Xing QG, Song XD, Zhao DH, Han QQ and Zhang GZ (2018) Spatio-temporal patterns of *Ulva prolifera* blooms and the corresponding influence on chlorophyll-a concentration in the southern Yellow Sea, China. *Science of the Total Environment* **640**, 807–820.

Tang YZ and Gobler CJ (2011) The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. *Harmful Algae* **10**, 480–488.

Wang CY, Su RG, Guo LD, Yang B, Zhang Y, Zhang L, Xu H, Shi WJ and Wei LS (2019) Nutrient absorption by *Ulva prolifera* and the growth mechanism leading to green-tides. *Estuarine Coastal and Shelf Science* **227**, 106329.

Wang RJ, Wang Y, Zhou J, Sun JH and Tang XX (2013) Algicidal activity of *Ulva pertusa* and *Ulva prolifera* on *Prorocentrum donghaiense* under laboratory conditions. *African Journal of Microbiology Research* **7**, 4389–4396.

Wang C, Yu RC and Zhou MJ (2012) Effects of the decomposing green macroalga *Ulva* (*Enteromorpha*) *prolifera* on the growth of four red-tide species. *Harmful Algae* **16**, 12–19.

Wang S, Zhao L, Wang YH, Zhang HY, Li F and Zhang YJ (2022) Distribution characteristics of green tides and its impact on environment in the Yellow Sea. *Marine Environmental Research* **181**, 105756.

Xing QG, Hu CM, Tang DL, Tian LQ, Tang SL, Wang XH, Lou MJ and Gao XL (2015) World's largest macroalgal blooms altered phytoplankton biomass in summer in the Yellow Sea: satellite observations. *Remote Sensing* **7**, 12297–12313.

Ye NH, Zhang XW, Mao YZ, Liang CW, Xu D, Zou J, Zhuang ZM and Wang QY (2011) 'Green tides' are overwhelming the coastline of our blue planet: taking the world's largest example. *Ecological Research* **26**, 477–485.

Yu RC, Sun S, Yan T and Zhou MJ (2018) Progresses and perspectives on green-tide studies in the Yellow Sea. *Journal of Oceanology and Limnology* **49**, 942–949.

Zhang YY, He PM, Li HM, Li G, Liu JH, Jiao FL, Zhang JH, Huo YZ, Shi XY and Su RG (2019) *Ulva prolifera* green-tide outbreaks and their environmental impact in the Yellow Sea, China. *National Science Review* **6**, 825–838.

Zhang JH, Liu CC, Yang LL, Song G, Ji X, Huo YZ, Yu KF, Xu R and He PM (2015a) The source of the *Ulva* blooms in the East China Sea by the combination of morphological, molecular and numerical analysis. *Estuarine, Coastal and Shelf Science* **164**, 418–424.

Zhang XL, Song YJ, Liu DY, Keesing JK and Gong J (2015b) Macroalgal blooms favor heterotrophic diazotrophic bacteria in nitrogen-rich and phosphorus-limited coastal surface waters in the Yellow Sea. *Estuarine, Coastal and Shelf Science* **163**, 75–81.

Zheng LX, Wu MQ, Cui YT, Tian L, Yang PS, Zhao LJ, Xue MY and Liu JY (2022) What causes the great green tide disaster in the South Yellow Sea of China in 2021? *Ecological Indicators* **140**, 108988.

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