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Interactions between selected microalgae and microscopic propagules of *Ulva prolifera*

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Large-scale green tides of Ulva prolifera *occur repeatedly in the Yellow Sea, and the microscopic propagules of* U. prolifera *play a critical role during the development of green tides.* Ulva prolifera *propagules and microalgae are both present in seawater and share similar niches, but their potential interactions are poorly understood. Nine species of microalgae were selected to study their interactions with the propagules of* U. prolifera *(gametes) in laboratory. The results showed that settlement of gametes could be inhibited by some microalgae, such as* Alexandrium tamarense, Prorocentrum lima *and* Karenia mikimotoi, *at the cell density of blooming (10^2–10^3 cells ml^{-1}). Inversely, the germlings germinated from* U. prolifera *gametes had negative effects on the microalgae, the inhibition rate ranged from 28 to 66%. Our results demonstrated the complex interactions between microalgae and propagules of green algae, which may influence the formation of green tides and their ecological consequences in the Yellow Sea.*

Keywords: *Ulva prolifera*, microscopic propagules, green tide, microalgal bloom, interaction

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INTRODUCTION

In some eutrophic coastal waters, successions of dominant primary producers from perennial macrophytes to opportunistic epiphytes, free-floating macroalgae, and then microalgae with increasing phases of eutrophication have been reported (Schramm, 1999). The excessive proliferation of free-floating green algae is generally termed as 'green tide' (Fletcher, 1996), while that of microalgae is known as 'red tide'. The successions of major primary producers, particularly the occurrence of green and red tides in eutrophic coastal waters, have been attributed to complex interactions among biotic and abiotic factors (Schramm, 1999), and those interactions between microalgae and seaweed have received much attention in ecological studies of harmful algal blooms (Smith & Horne, 1988; Sfrifo & Pavoni, 1994; Alamsjah *et al.*, 2005).

Large-scale green tides of *Ulva prolifera* occurred consecutively after 2007 in the Yellow Sea (YS), China, and attracted extensive attention (Ding & Luan, 2009; Leliaert *et al.*, 2009; Shimada *et al.*, 2010; Liu *et al.*, 2013). *Ulva prolifera* has multiple reproduction modes and complex life histories (Hiraoka *et al.*, 2003; Liu *et al.*, 2015b), with both haploid and diploid macroscopic, as well as microscopic phases occuring in its life cycles. The microscopic forms of *U. prolifera*, such as spores and gametes produced by thalli, are collectively known as propagules (Chapman, 1986; Hoffmann & Santelices, 1991; Santelices *et al.*, 2002; Zhang *et al.*, 2010). These propagules will undergo two basic processes, settlement and germination, before developing into multi-cellular thalli (Clayton, 1992; Fletcher & Callow, 1992; Schories & Reise, 1993; Gao *et al.*, 2010). Therefore, the microscopic propagules are important in controlling the population dynamics of macroalgae (Lotze *et al.*, 1999).

The amount of microscopic propagules of bloom-forming *Ulva prolifera* in the YS is huge and cannot be ignored. Zhang *et al.* (2011) reported that one floating haploid thallus of *U. prolifera* could produce about 2.31×10^7 gametes during a bloom season. Wang *et al.* (unpublished data) utilized fluorescence quantitative PCR to analyse the temporal-spatial distribution of *Ulva* microscopic propagules during the green tide in 2012. They found that the abundance of propagules increased rapidly from 0.30 to 10^2 cells ml^{-1} in May, and the maximum density of propagules could reach 10^4 cells $\cdot$ ml^{-1} in mid-May. The rapid increase of microscopic propagules in the YS, as addressed by Zhang *et al.* (2015) and Liu *et al.* (2015b), was mainly due to the proliferation of free-floating green algae. In Subei Shoal, where green tides initially developed, microscopic propagules of green algae presented in seawater throughout the year and could serve as seed stock for the formation of green tides (Song *et al.*, 2015). These investigations suggest that the propagules play a critical role in regulating the occurrence and development of green tides in the YS (Liu *et al.*, 2012; Li *et al.*, 2014; Song *et al.*, 2015).

Microscopic propagules of *Ulva* spp. and microalgae floating in seawater have a similar habitat, which gives them a chance to interact with each other. It is well known that thalli of green algae can inhibit the growth of unicellular microalgae (Jin & Dong, 2003; Wang *et al.*, 2013). Many previous studies have indicated that green algae can adversely affect phytoplankton in general, and lead to changes in composition and primary productivity of the phytoplankton community, even the occurrences of microalgal blooms (Smith & Horne, 1988; Jin & Dong, 2003; Nan *et al.*, 2004; Alamsjah *et al.*, 2005, 2008; Wang *et al.*, 2006; Tang & Gobler, 2011; Wang *et al.*, 2012). There is little recent research on the

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affects of microalgae on germlings of macroalgae (Schonbeck & Norton, 1979; Huang & Boney, 1983). Since microscopic propagules are critical for the population dynamics of *U. prolifera*, any variation may eventually influence the subsequent population development and the magnitude of green tides. Therefore, it is necessary to look into the interactions between microalgae and microscopic propagules of macroalgae to examine all potential factors affecting the formation and consequences of green tides in detail.

In this study, we selected nine species of microalgae, including some toxic or harmful species commonly occurring in the coastal waters of China, to study the potential interactions between these microalgae and the gametes of bloom-forming *Ulva prolifera* isolated from the YS, through two co-culture experiments under laboratory conditions.

MATERIALS AND METHODS

Sample identification and preparation

Thalli of *Ulva prolifera* were collected from Huiquan Bay, Qingdao in July 2011. All samples were identified as *U. prolifera* using morphological and molecular methods as described in Zhang *et al.* (2015). 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 maintained with 1 mg l^{-1} GeO_2 to control the growth of diatoms and other silicified algae in the laboratory.

Nine species of microalgae representing five different phyla were selected to study the interactions with gametes of *Ulva prolifera*. They were provided by the Algal Culture Center of the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). Detailed information concerning the microalgae is listed in Table 1.

Microalgae and *Ulva prolifera* were cultured in flasks containing specific medium (Guillard, 1975; Guillard & Hargraves, 1993) (Table 1), and maintained at 19 ± 1°C. Irradiance was 50 μmol photons m^{-2} s^{-1}, and the light:dark cycle was 14:10. Natural seawater for preparation of culture medium was collected from Huiquan Bay, Qingdao, and sand-filtered prior to use. The seawater was further filtered through a 0.45 μm membrane and autoclaved to prepare the culture medium. Salinity of the seawater was 30 ± 1.

Obtaining gametes of *Ulva prolifera*

Thalli of *Ulva prolifera* cultured in the laboratory showed alternative phases of parthenosporophytes and gametophytes as described in Liu *et al.* (2015b). The gametophytes produced biflagellate unfertilized gametes, which could develop parthenogenetically into sporophytes. The sporophytes then produced tetraflagellate spores, which would develop into gametophytes. Gametes and spores could be easily distinguished by their size, numbers of flagella and phototactic responses. The gametes with two flagella were obviously smaller than spores that had four flagella. Besides, the gametes showed positive phototaxis, while the spores showed negative phototaxis.

To prepare gametes for the experiments, spores produced by a single thallus were cultured until they developed into gametophytes. A modified punching method was then

Table 1. List of microalgal species used in the experiment.

Microalgal species	Taxonomy: Phylum and Class	Morphology size (μm)	Toxicity characteristic	Maximum bloom density (cell l^{-1})	Culture medium	Source
Alexandrium tamarense	Alveolata: Dinophyceae	27.0 × 30.0	PST	10^5	f/2-si	East Sea, China
Prorocentrum lima	Alveolata: Dinophyceae	32.0 × 48.0	DST	10^5	f/2-si	CCMP1966, USA
Karenia mikimotoi	Alveolata: Dinophyceae	22.0 × 29.0	Haemolytic toxins	10^6	f/2-si	South Sea, China
Skeletonema costatum	Ochrophyta: Bacillariophyceae	5.0 × 32.0	/	10^7	f/2	East Sea, China
Prorocentrum donghaiense	Alveolata: Dinophyceae	12.0 × 14.0	/	10^7	f/2-si	Bohai Sea, China
Phaeodactylum tricornutum	Ochrophyta: Bacillariophyceae	3.0 × 9.0	/	/	f/2	IOCAS, China
Chlorella sp.	Chlorophyta: Trebouxiophyceae	3.0	/	/	f/2-si	IOCAS, China
Isochrysis galbana	Haptophyta: Prymnesiophyceae	5.0	/	/	f/2-si	Yellow Sea, China
Aureococcus anophagefferens	Ochrophyta: Pelagophyceae	1.5	/	10^9	L1	CCMP1984, USA

PST, Paralytic shellfish toxins; DST, Diarrhetic shellfish toxins; CCMP, Provasoli-Guillard National Center for Marine Algae and Microbiota; IOCAS, Institute of Oceanology, Chinese Academy of Sciences.

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adopted to induce the formation of gametes (Dan *et al.*, 2002). The thalli of gametophytes were cut into small fragments (1–2 cm long), and incubated in a glass Petri dish (95 mm in diameter, 10 mm high) containing 20 ml f/2 medium. The medium was replaced every day for 2–3 days until the gametes were released.

Design of experiments

Two experiments were designed to study the interactions between microalgae and propagules of *Ulva prolifera*. Based on the abundance of these microalgae in natural seawater, the microalgal cell density in the experiments was set to be 1.0×10^2–1.0×10^4 cells ml^{-1}. For all the experiments, microalgae at the exponential phase were collected and used. Cell density of gametes used in the experiments was set to be 2.0×10^4 cells ml^{-1}, which is similar to the abundance of microscopic propagules (2.0×10^4 cells ml^{-1}) in the YS determined by the qPCR assay (Wang *et al.*, unpublished data).

EXPERIMENT I: INTERACTIONS BETWEEN MICROALGAE AND GAMETES OF *ULVA PROLIFERA* AT THE SETTLEMENT STAGE

To study the interactions between microalgae and gametes of *Ulva prolifera* at the settlement stage, the newly released gametes were collected and put into small beakers containing 40 ml of sterile seawater (final cell density at 2.0×10^4 cells ml^{-1}). For each species of microalgae, three levels of cell density were set up (1.0×10^2, 1.0×10^3 and 1.0×10^4 cells ml^{-1}). Monocultures of *U. prolifera* gametes and nine species of microalgae (cell density at 1.0×10^2, 1.0×10^3 and 1.0×10^4 cells ml^{-1} for each species, respectively) were used as controls. Every treatment or control had three replicates. To avoid nutrient limitation, 40 μl of f/2 or L1 stock solution was added at the beginning of the experiments, the concentrations of NO_3-N, PO_4-P in the f/2 or L1 stock solution were 882 and 36 mmol l^{-1}.

Part 1: Effects of microalgae on settlement of gametes. To test the effects of microalgae on gametes of *Ulva prolifera*, a coverslip (10 × 10 mm) was placed at the bottom of each beaker to facilitate the counting of attached gametes. The experiments were conducted under darkness in a stable environment for one day to ensure gametes randomly attached to the coverslip. After 24 h, the coverslips were removed and placed in sterilized seawater several times to remove unattached gametes. Attached gametes were then counted in 20 fields under a microscope (400 × magnification), and the average value of the 20 fields was used to represent the number of settled gametes.

Part 2: Effects of gametes on the growth of microalgae. To test the effects of gametes on the growth of microalgae, 200 μl of culture medium was collected from each group of treatments and controls after the experiments, and fixed with Lugol's solution. The cell density of microalgae was counted under a microscope, and the significance of variation was compared between each treatment and the control.

EXPERIMENT II: INTERACTIONS BETWEEN MICROALGAE AND GAMETES OF *ULVA PROLIFERA* AT THE GERMINATION STAGE

For this experiment, gametes of *Ulva prolifera* (2.0×10^4 cells ml^{-1}) were incubated in small beakers containing 40 ml of sterile seawater under darkness for one day to ensure settlement of gametes. Then the beakers were put back to the light condition for the germination experiment. Nine species of microalgae were then inoculated into the medium, and the cell density was adjusted to 1.0×10^4 cells ml^{-1}. Monocultures of *U. prolifera* gametes and nine species of microalgae were used as controls. Each group of treatments and controls was performed in three beakers. Every day, 200 μl of f/2 or L1 stock solution was added to avoid nutrient limitation.

Part 1: Effects of microalgae on germination of gametes. To test the effects of microalgae on germination of gametes, a coverslip (10 × 10 mm) was placed at the bottom of each beaker prior to the experiment to observe the germination of gametes (a gamete that divided into at least two cells was considered as the standard of germination). Every day, the coverslip in each beaker was taken out to count the germination rates and then put back. Germinated gametes were counted for 20 fields under a microscope (400 × magnification), and the average germination rate was calculated according to the following formula: GR (germination rate, %) $= N/N_o \times 100\%$, where N_o is the total number of gametes observed in one field, and N is the number of germinated gametes in one field. The experiments lasted for 7 days. All beakers were shaken twice a day during the experiments to avoid adherence of microalgae to the beakers.

Part 2: Effects of germinated gametes on the growth of microalgae. To test the effects of germinated gametes on the growth of microalgae, 200 μl of seawater was collected from each beaker every day, and 200 μl of f/2 or L1 stock solution was added to ensure a constant volume and to avoid nutrient limitation. All samples were fixed with Lugol's solution prior to cell counting. The experiment lasted for 7 days and the growth inhibition rates on the 7th day were calculated with the following formula: GIR (growth inhibition rate, %) $= (1 - N/N_o) \times 100\%$, where N_o is the cell density of the control groups on the 7th day; and N is the cell density of the treatment groups on the 7th day.

Statistical analysis

Data were calculated as mean ± standard deviation from the three replicates (N = 3). Statistical differences were analysed with Origin 8.5 and SPSS 16.0, and the differences were considered significant when $P < 0.05$.

Two statistical methods were used in the experiments. First, the significance of difference among nine species of microalgae in affecting the settlement and germination of gametes were tested with one-way ANOVA, after testing for normal distribution and homogeneity of variance. For the data with heterogeneity of variances, non-parametric tests (Kruskal–Wallis one-way ANOVA) were used. If the difference was significant, a *post-hoc* test (Tukey's test or Dunn's test) was performed to test the difference among the experimental groups. For the effects of gametes on the growth of microalgae, *t*-test for independent samples was used to test the significance between the treatment and the control.

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RESULTS

Effects of microalgae on the settlement and germination of *Ulva prolifera* gametes

EFFECTS OF MICROALGAE ON THE SETTLEMENT OF *ULVA PROLIFERA* GAMETES

Effects of nine microalgal species on the settlement of *Ulva prolifera* gametes were shown in Figure 1. The tested microalgal species displayed significant differences in affecting the settlement of gametes (one-way ANOVA, $P < 0.05$). Six microalgal species, i.e. *Skeletonema costatum*, *Prorocentrum donghaiense*, *Phaeodactylum tricornutum*, *Chlorella* sp., *Isochrysis galbana* and *Aureococcus anophagefferens*, had no significant effects on the settlement of gametes at the three levels of cell density (Figure 1D–H). The other three microalgal species, i.e. *Alexandrium tamarense*, *Prorocentrum lima* and *Karenia mikimotoi*, significantly decreased the settled number of gametes compared with the control, and the inhibitory effect increased with cell density (Figure 1A–C). These microalgae will lead to adverse effects on the settlement of gametes of *U. prolifera* at the bloom density (Table 1).

EFFECTS OF MICROALGAE ON THE GERMINATION OF *ULVA PROLIFERA* GAMETES

Gametes in all tested groups of Experiment II germinated completely within 7 days, and there was no significant difference in the final germination rate of gametes co-cultured with nine species of microalgae (Figure 2). Although the germination process of gametes seems slightly accelerated in the groups co-cultured with *A. tamarense*, *P. lima*, *K. mikimotoi*, *S. costatum* and *P. donghaiense* from 2–6 day, the differences between control and treatments were not significant (one-way ANOVA, $P > 0.05$).

In addition, the total number of cells of germinated and un-germinated gametes were counted every day to reflect the increase of green algal biomass during the experiment. A rapid increase of *Ulva prolifera* biomass was observed over the 7-day experiment. The total biomass of *U. prolifera* increased about 3–4 times as reflected from the total cell number recorded every day. There was no significant difference among the treatments of germinated gametes co-cultured with nine species of microalgae (Figure 3) (one-way ANOVA, $P > 0.05$).

Effects of *Ulva prolifera* gametes on the growth of microalgae

The growth of microalgae co-cultured with gametes of *Ulva prolifera* at the settlement stage is shown in Figure 4. Compared with the controls, no significant inhibitory or lethal effects of gametes were observed on the microalgae at the three different levels of cell density (*t*-test, $P > 0.05$).

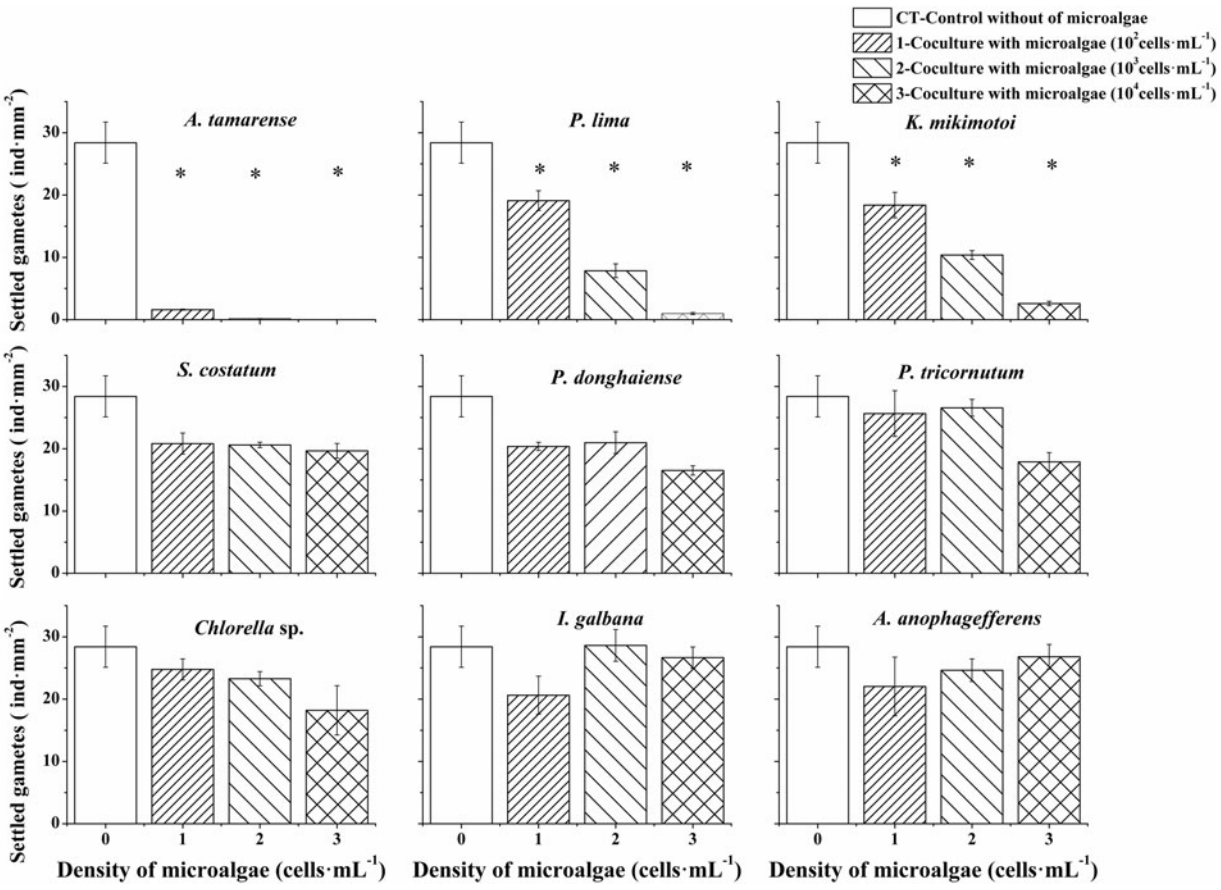


Fig. 1. Effects of microalgae on the settlement of *Ulva prolifera* gametes. Cell densities of microalgae were set at three levels: 1.0×10^2, 1.0×10^3, 1.0×10^4 cells ml^{-1}, respectively, and the concentration of *U. prolifera* gametes was 2.0×10^4 cells ml^{-1}. Data points are means ± SD (N = 3). $^*P < 0.05$ as compared with the control.

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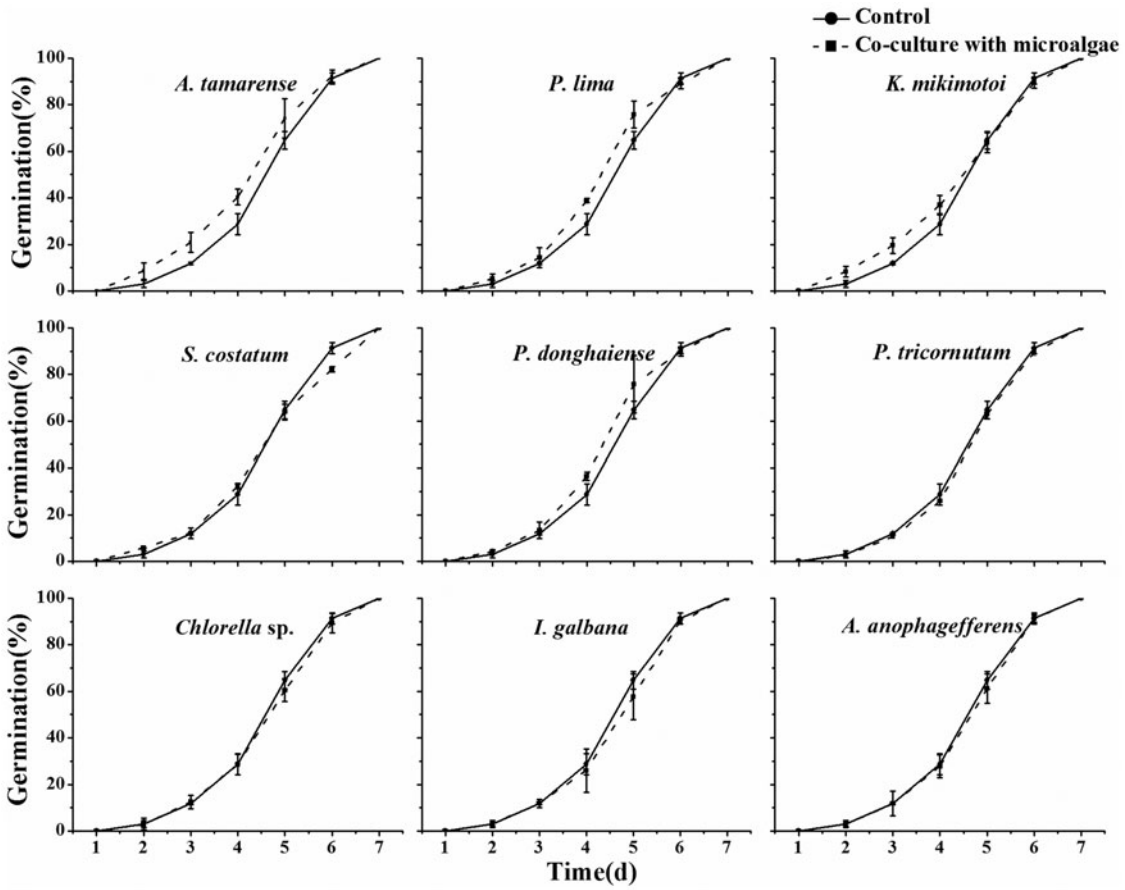


Fig. 2. Effects of microalgae on the germination of *Ulva prolifera* gametes. Cell density of microalgae was set at 1.0×10^3 cells ml^{-1}, and the concentration of *U. prolifera* gametes was 2.0×10^4 cells ml^{-1}. Data points are means ± SD (N = 3). $^*P < 0.05$ as compared with the control.

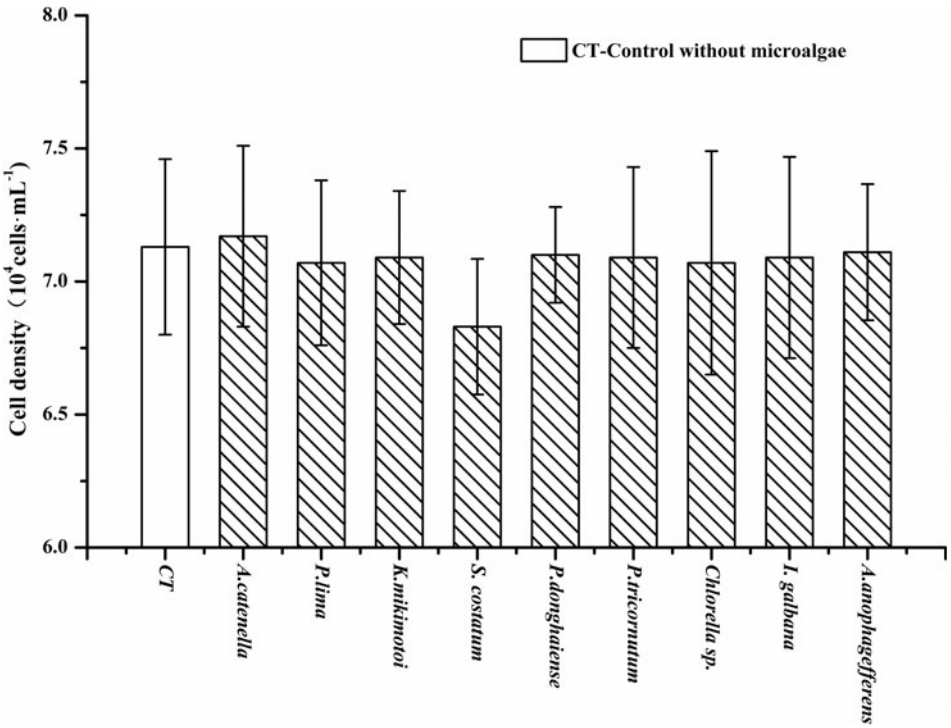


Fig. 3. The total number of germinated and un-germinated gametes of *Ulva prolifera* recorded on the 7th day of Experiment II. Data points are means ± SD (N = 3). * $P < 0.05$ as compared with the control.

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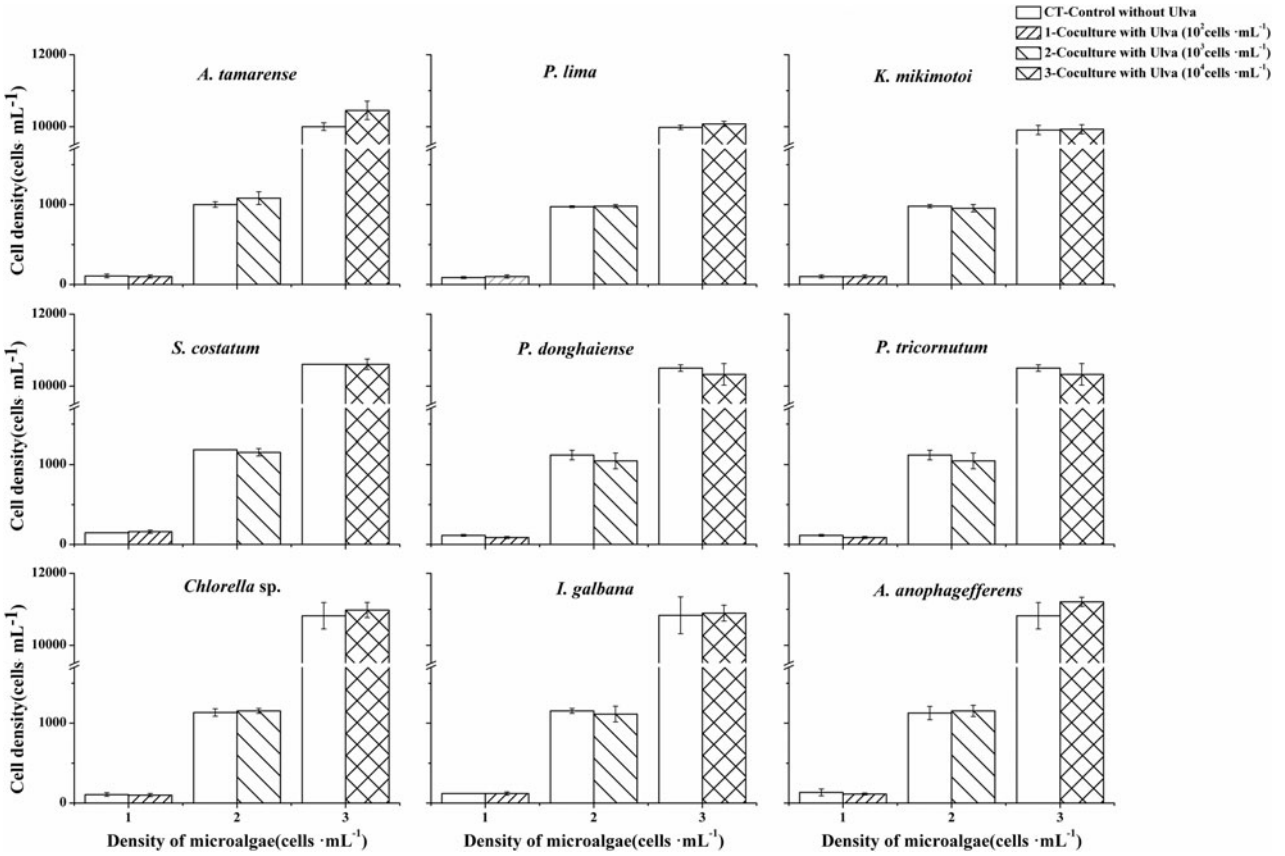


Fig. 4. Effects of gametes on the growth of microalgae at the settlement stage. The experiment lasted for 1 day. Cell density of microalgae were set at three levels of 1.0×10^2, 1.0×10^3, 1.0×10^4 cells ml^{-1}, respectively, and the concentration of *Ulva prolifera* gametes was 2.0×10^4 cells ml^{-1}. The data are presented as the means ± SD (N = 3). * $P < 0.05$ as compared with the control.

The results suggest that gametes of *U. prolifera* had no significant inhibitory effects on microalgae.

The effects of germlings germinated from gametes on the growth of microalgae are shown in Figure 5. Compared with the controls, the growths of microalgae co-cultured with germlings of *U. prolifera* were significantly inhibited. On the 7th day, the growth inhibition rates of the nine species of microalgae were 28%, 43%, 27%, 41%, 66%, 40% and 45% for *A. tamarense*, *K. mikimotoi*, *S. costatum*, *P. donghaiense*, *P. tricornutum*, *Chlorella* sp. and *A. anophagefferens*, respectively (*t*-test, $P < 0.05$).

DISCUSSION

Complex interactions between microalgae and propagules of green algae

In the current study, the complex interactions between propagules of bloom-forming *Ulva prolifera* in the YS and microalgae were demonstrated for the first time through two co-culture experiments. Three species, i.e. *Alexandrium tamarense*, *Prorocentrum lima* and *Karenia mikimotoi*, showed significant inhibitory effects on the settlement of gametes, while the gametes had no apparent effects on the growth of microalgae. At the germination stage, however, the microalgae did not affect the germination of gametes, while the germlings showed significant inhibitory effects on the growth of microalgae. It seems that the complex interactions between propagules of *U. prolifera* and microalgae do not result from resource competition. During the experiments, enough nutrients were supplemented into the culture medium so that nutrients would not be a limiting factor. The impacts were unlikely to be caused by other environmental factors such as light, because the settlement experiment (Experiment I) was performed in darkness. During the germination experiment (Experiment II), the density of microalgae (1.0×10^4 cells ml^{-1}) was not high, and there would be little shading effect. Amsler *et al.* (1992) pointed out that propagules of macroalgae would hardly reach a high enough density to compete for light. Other abiotic factors (e.g. temperature and pH) were also measured throughout the experiments and were unlikely to become limiting factors. As mentioned above, it seems that resource competition is unlikely to account for the interactions in our study.

An interesting finding in this study is that three species with significant inhibitory effects on the settlement of *Ulva prolifera* gametes were species which can produce toxins, such as paralytic shellfish toxins (PST), diarrhetic shellfish toxins (DST) and haemolytic toxins (Table 1). Among the three species, *A. tamarense* showed most significant inhibition on the settlement of gametes. However, we compared the inhibitory effects of the PST-producing *A. tamarense* with a non-PST-producing dinoflagellate *Alexandrium affine* under the same experimental conditions, and found this non-toxic *Alexandrium* strain also inhibited the settlement of *U. prolifera*. When *A. affine* (at the density of 1.0×10^2 cells ml^{-1})

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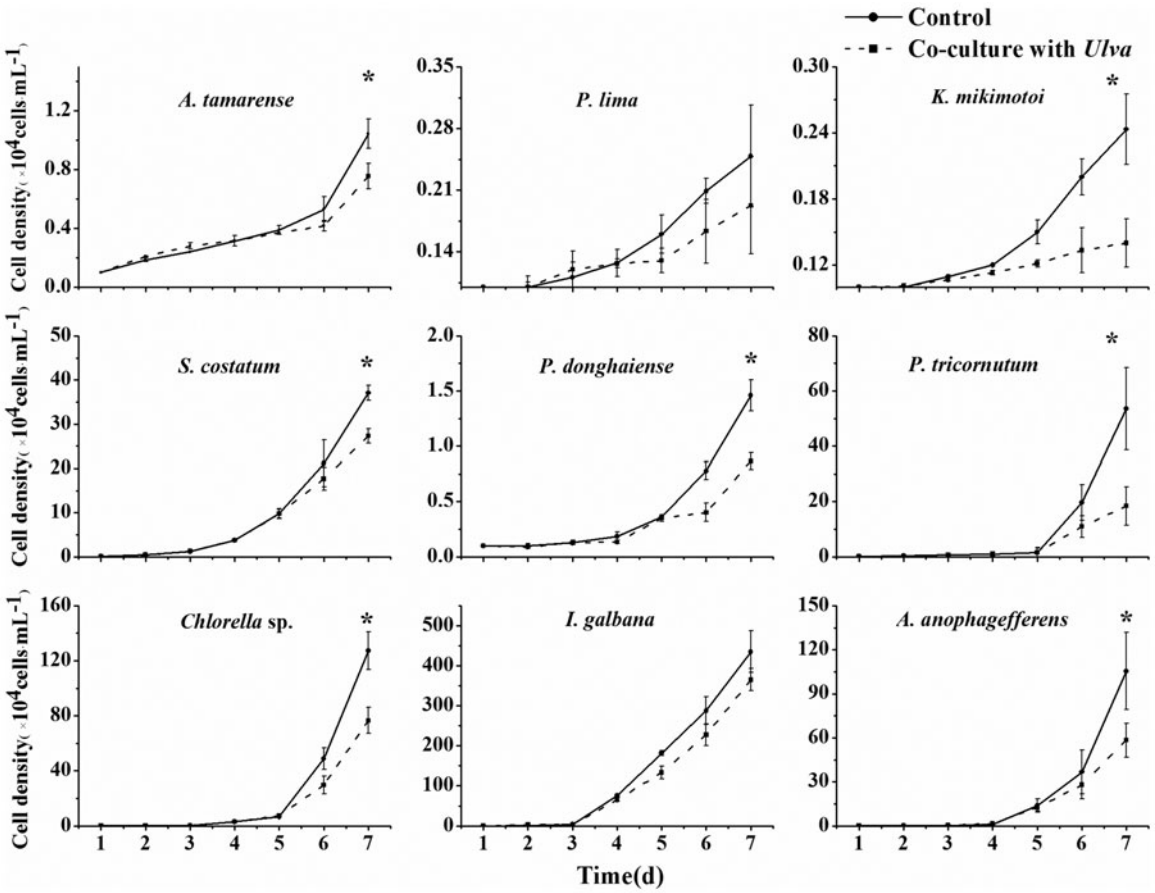


Fig. 5. Effects of germinated gametes on the growth of microalgae. The cell density of microalgae was set at 1.0×10^3 cells ml^{-1}, and the concentration of *Ulva prolifera* gametes was set as 2.0×10^4 cells ml^{-1}. Data points are means ± SD (N = 3). * $P < 0.05$ as compared with the control.

was co-cultured with the gametes, the settled number of gametes was only 2 cells·mm^{-2}, very close to the co-culture with toxic *A. tamarense* (7 cells mm^{-2}) (Liu *et al.*, 2015a). Therefore, it can be implied that the PST produced by *Alexandrium* did not play a major role in affecting gametes. Other bioactive compounds may be involved in the interactions. However, we did not further test the toxins of *P. lima* and *K. mikimotoi* in inhibiting the gametes, for which further elucidation is needed. Recent research has shown that the settlement of *Ulva* propagules was impacted by some bacteria (Joint *et al.*, 2002; Wheeler *et al.*, 2006; Tait *et al.*, 2009). Joint *et al.* (2002) pointed to the bacterial quorum sensing molecules N-acylhomoserine lactone (AHL) as a cue for the site selection of *Ulva* zoospore settlement. So bacteria may be an important factor in the settlement mechanisms of *U. prolifera* propagules; this will need to be carefully evaluated.

Through the germination experiment, we found that the inhibitory effects of *Ulva prolifera* on microalgae started from the early germination stage of gametes. During the 7-day experiment, the germinated gametes significantly reduced the density of the microalgae, even the toxic microalgal species. The impacts of macroalgae are generally not mediated by toxic substances; some metabolites like polyunsaturated fatty acids were thought to cause negative effects on microalgae. Jensen (1977) examined whether propagules could affect their own microenvironment via the release of certain metabolites during settlement and germination stages. Recent studies also showed that mature thalli of *U. prolifera* in YS could produce chemicals that inhibit the growth of microalgae (Nan *et al.*, 2004; Alamsjah *et al.*, 2008; Huo *et al.*, 2010; Wang *et al.*, 2013). However, whether the germlings of *U. prolifera* produce the same compounds as the mature thalli is not clear so far. Some studies have emphasized that certain development stages of macroalgae would associate with their unique characteristics, particularly at the microscopic stage (Amsler *et al.*, 1992).

Ecological implications of the interactions between microalgae and propagules of green algae in the YS

In many eutrophic waters, apparent succession of primary producers has been reported (Schramm, 1999). To study the interactions between opportunistic macroalgae and microalgae is an important approach to understand the succession process and the formation of harmful algal blooms. In this study, we found that some bloom-forming microalgal species could inhibit the settlement of gametes. Based on the studies of green tides in the YS, the Subei Shoal is a key region for the early development of green tides (Keesing *et al.*, 2011; Liu *et al.*, 2013). Microscopic propagules are present in this region throughout the year, which offer important seed stock for the recurrent green tides (Li *et al.*, 2014; Song *et al.*, 2015). Besides, the intensive *Porphyra*

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culture rafts deployed in this region offer important substrates for the settlement of microscopic propagules, in particular the large amounts of gametes produced in spring (Liu *et al.*, 2015b; Song *et al.*, 2015). In Subei Shoal, however, there are few reports of microalgal blooms and the biomass of phytoplankton is generally low (Kang *et al.*, 2013), probably due to the highly turbid seawater and the strong mixing. Besides, the presence of large amounts of green algae and cultured *Porphyra* could also inhibit the growth of microalgae (Huo *et al.*, 2010; Sun *et al.*, 2010; Wang *et al.*, 2013). Therefore, the settlement of microscopic propagules is not likely to be inhibited by microalgae in this region.

The occurrences of harmful algal blooms caused by *Alexandrium tamarense*, *Prorocentrum lima* and *Karenia mikemotoi*, however, have been recorded widely in the coastal waters of China (China Marine Environmental Bulletin, 2001–2012). Due to their production of toxins such as paralytic shellfish toxins, diarrhetic shellfish toxins and haemolytic toxins, these bloom-forming species have led to considerable concern regarding their potential ecological consequence. The outbreak stage of these harmful algal blooms coincides well with the green tides in the YS (from May to August). The occurrence of such algal blooms could impair the settlement of coexisting gametes of *Ulva prolifera*, and the subsequent population dynamics of *U. prolifera*. The green tides of *U. prolifera* occurred consecutively in the YS for at least 8 years (2007–2014), but there is no record of an associated population of this bloom-forming *U. prolifera* in the coastal region of China, except for the Subei Shoal. And the floating population of bloom-forming *U. prolifera* still remains a unique ecotype in the YS (Zhao *et al.*, 2015). Whether this phenomenon is related to the interactions between microalgae and bloom-forming *U. prolifera* is not clear but worth further study.

In this study, it was also found that bloom-forming *Ulva prolifera* exhibited negative effects on the growth of microalgae from the germination stage of gametes. This result, together with some previous studies (Huo *et al.*, 2010; Wang *et al.*, 2012, 2013), reflected the impacts of *U. prolifera* on the phytoplankton community. During the green tides in the YS, there were huge amounts of floating green algae, which required large amount of nutrients to support such a high biomass. Macroalgae generally showed a higher efficiency in absorption of nutrients than microalgae. Hence, macroalgal blooms could reduce the abundance of phytoplankton, and even the occurrence of HABs through competition on nutrients in the sea. Besides, the floating thalli of *U. prolifera* could lead to shading effects on phytoplankton, and the high rate of photosynthesis of *U. prolifera* may draw down CO_2 concentration and lead to the variation of pH level in seawater (Xu *et al.*, 2012). Our results regarding the inhibition of *U. prolifera* on some harmful algae fitted with the situation in YS. Through comparing the records of harmful algal blooms before and after the green tides occurred in YS, we found the HAB events decreased by nearly a half over the last five years, from nine times in 2001–2005 to four times in 2008–2012 (China Marine Environmental Bulletin, 2001–2012). Similar situations have been reported in other sea areas. Investigations by Smith & Horne (1988) showed a dramatic decline of phytoplankton blooms with the introduction of *Ulva* in San Francisco Bay-Estuary, California. Sfrifo & Pavoni (1994) also found that phytoplankton blooms were relatively scarce when macroalgae were found in the central Venice lagoon.

From the current study, it can be seen that *Ulva prolifera* inhibit not only the HAB-forming species of microalgae, but also microalgae that could serve as food for zooplankton. Therefore, the inhibition on phytoplankton will decrease marine primary productivity, and influence the energy transfer through the food chains. Qin *et al.* (2011) proved that the diversity and abundance of dominant species in the phytoplankton community changed significantly during the green tides in 2008, compared with those reported prior to the year 2002. Zhang *et al.* (2013) also pointed out that green tides of *U. prolifera* simplified the structure of phytoplankton community in the YS. Therefore, the potential ecological consequences of green tides are closely related with the interactions between *U. prolifera* and microalgae.

CONCLUSION

In this study, we demonstrated the complex interactions between the propagules of bloom-forming *Ulva prolifera* and microalgae through two co-culture experiments. Some microalgae could inhibit the settlement of gametes, although the mechanism is not yet clearly understood. The bloom-forming *U. prolifera*, however, showed strong and non-selective inhibitory effects on the growth of microalgae early from the germination stage of gametes. A better understanding of the interactions between microalgae and the propagules of bloom-forming *U. prolifera* is necessary in order to understand the mechanisms and ecological consequences of green tides in the Yellow Sea.

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