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The origin of the *Ulva* macroalgal blooms in the Yellow Sea in 2013

Jianheng Zhang [a,b,d], Yuanzi Huo [b], Hailong Wu [b], Kefeng Yu [b], Jang Kyun Kim [c], Charles Yarish [d], Yutao Qin [e], Caicai Liu [e], Ren Xu [e], Peimin He [b,*]

[a] College of Marine Sciences, Shanghai Ocean University, Shanghai 201306, China
[b] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
[c] Department of Marine Sciences, University of Connecticut, 1 University Place, Stamford, CT 06901, USA
[d] Department of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT 06901, USA
[e] East China Sea Environmental Monitoring Center of State Oceanic Administration, Shanghai 200137, China

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ABSTRACT

Green algal blooms have occurred in the Yellow Sea for seven consecutive years from 2007 to 2013. In this study, satellite image analysis and field shipboard observations indicated that the *Ulva* blooms in 2013 originated in the Rudong coast. The spatial distribution of *Ulva* microscopic propagules in the Southern Yellow Sea also supported that the blooms originated in the Rudong coast. In addition, multi-source satellite data were used to evaluate the biomass of green algae on the *Pyropia* aquaculture rafts. The results showed that approximately 2784 tons of *Ulva prolifera* were attached to the rafts and possessed the same internal transcribed spacer and 5S rDNA sequence as the dominant species in the 2013 blooms. We conclude that the significant biomass of *Ulva* species on the *Pyropia* rafts during the harvesting season in radial tidal sand ridges played an important role in the rapid development of blooms in the Yellow Sea.

1. Introduction

Green algal blooms have occurred along the coastal areas of the Yellow Sea for seven consecutive years since 2007. The large-scale blooms of *Ulva prolifera* have resulted in tremendous economic loss due to the destruction of marine ecosystems and damage to ecological service functions. The estimated cost of maintaining algae-free water near Qingdao for the Olympic Games sailing events in 2008 was more than 100 million U.S. dollars (Wang et al., 2009). Since then, green tides have received considerable attention from scientists and governments worldwide and have aroused concerns regarding the marine environments in China. Focusing on the macroalgal blooms at the early developmental stages, by identifying the source, could be a direct way of tackling this issue. Thus, the source of the problem may have been resolved immediately after the macroalgal blooms in 2008.

The excessive growth of green algae which causes the formation of macroalgal blooms has been reported in oceans worldwide (Fletcher, 1996; Blomster et al., 2002; Nelson et al., 2003; Merceron et al., 2007; Ye et al., 2011). However, unlike the bloom events in the Yellow Sea, these were restricted to small coastal areas. The satellite data played a critical role in monitoring the floating route and size of the green tide to further predict its impact in the Yellow Sea for the public and the local administration. To date, the large-scale green tide has been traced by satellite image analysis to the nearshore of the Jiangsu coast, which is about 400 km from Qingdao (Liu et al., 2009; Hu et al., 2010; Zhang et al., 2013).

There are different opinions concerning the origin of the green tide in the Yellow Sea, although the Southern Yellow Sea was confirmed to be the original area of the bloom using satellite data. Pang et al. (2010) first suggested that the *Ulva* blooms originated from land-based animal aquaculture ponds on the Jiangsu coast. Their conclusion was based on the analyses of rbcL and internal transcribed spacer (ITS) molecular markers, as well as morphological analyses. However, the green algae in these aquaculture ponds appeared to be different from the *Ulva* found in the bloom when a different molecular marker, ISSR, was used (Liu et al., 2011). Zhang et al. (2010, 2011) suggested that somatic *Ulva* cells and the settlement of vegetative fragments may serve as a potential propagule bank to support the bloom in subsequent years. Due to the small biomass of *U. prolifera* at the bottom of the Yellow Sea, the settled *Ulva* vegetative fragments could not be more than a supplement for the blooms. Liu et al. (2009, 2010, 2013a), Hu et al. (2010) and Keesing et al. (2011) suggested that the blooms originated

* Corresponding author. Tel./fax: +86 21 61900467.
E-mail address: pmhe@shou.edu.cn (P. He).

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from the cleaning of fouling green algae during *Pyropia* aquaculture along the Jiangsu province coast. However, Pang et al. (2010) reported that *Ulva* from the floating populations and *Pyropia* rafts were not the same species. To date, there is no clear agreement on the source of the blooms in the Yellow Sea.

In this study, we used high resolution HJ-1A/1B data and a systematic shipboard survey to identify a more accurate source of the blooms. In addition, a field investigation which included the species composition and the biomass of *U. prolifera* on the *Pyropia* aquaculture rafts was conducted to determine if *Pyropia* farms in the radial sand ridge area could provide the initial biomass to cause large *Ulva* blooms in the Yellow Sea.

2. Material and methods

2.1. Study area and survey methods

The tidal flats along the Southern Yellow Sea are unique in terms of their huge geometric scale, abundant sediment supply associated with large rivers, silt-dominated sediments and an offshore radial sand ridge (He et al., 2012). Due to the complex sediment transport dynamics driven by the wave and current interactions, the tidal flats in this area are well-developed and range in width from several kilometers to tens of kilometers.

The Southern Yellow Sea was monitored daily by HJ-1A/1B satellites which are the new generation of small Chinese civilian earth-observing optical remote sensing satellites with a wide-coverage multispectral charge-coupled device (CCD) camera. The CCD camera has nadir pixel resolution of 30 m and central-pixel matching accuracy of 0.3 pixels (Wang et al., 2010). In the early stage of the blooms, we confirmed when and where the first floating patches were observed.

From January to May, 2013, before the *Ulva* blooms were identified by the satellites, monthly field studies at Rudong, Dafeng, Sheyang (SY) and Binhai were conducted to monitor the distribution characteristics of floating green algae (Fig. 1). Each transect extended offshore for 100 km with five sampling stations, except for the SY transect which extended offshore for 50 km with three stations (Fig. 1). Recording criteria for the status and size of the patches of green algae were described by Huo et al. (2013).

2.2. Quantification of Ulva microscopic propagules

In April 2013, 500 mL water samples from a depth of 0 m, 5 m and the bottom at each station were collected and filtered through a 150 μm mesh size sieve, then transported to the laboratory in dark conditions within 48 h. Although *Ulva* microscopic propagules are invisible to the naked eye, *Ulva* seedlings were observed and quantified after the water samples were cultivated in the laboratory for 3–5 weeks under nutrient enrichment conditions. The cultivation and quantification of *Ulva* propagules have been described by Liu et al. (2013b).

2.3. Biomass measurement of Ulva on Pyropia aquaculture rafts

The water depth is less than 10 m in the radial sand ridge area (Fig. 2A), which is an ideal place for *Pyropia* aquaculture. Semi-floating raft cultivation techniques are widely adopted in these *Pyropia* farms, combining the strong points of the pillar and the floating methods, especially for intertidal cultivation. At high tide the net floats on the water, maximizing the light available to the seaweed; at low tide the net rests on the ground by short legs.

The whole aquaculture area is divided into six regions including Dongsha, Jiangjiasha, Zhugensha, Rudong, Yaosha and Qidong (Fig. 2C). In each region, ten rafts were randomly chosen to collect samples of the attached green algae in early April 2013. Each raft, covering 7.5 m^2, consists of two bamboo poles, two pieces of rope and one nursery net. All the green algae on the selected rafts were removed to estimate the biomass of *Ulva* spp.

To estimate the total aquaculture area, multi-source satellite images including radar satellite ERS-2 PRI, Landsat TM/ETM, CBERS-2B and HJ-1A/1B, were collected by the East China Sea Branch of State Oceanic Administration. The obtained satellite images were examined to identify days which were sufficiently

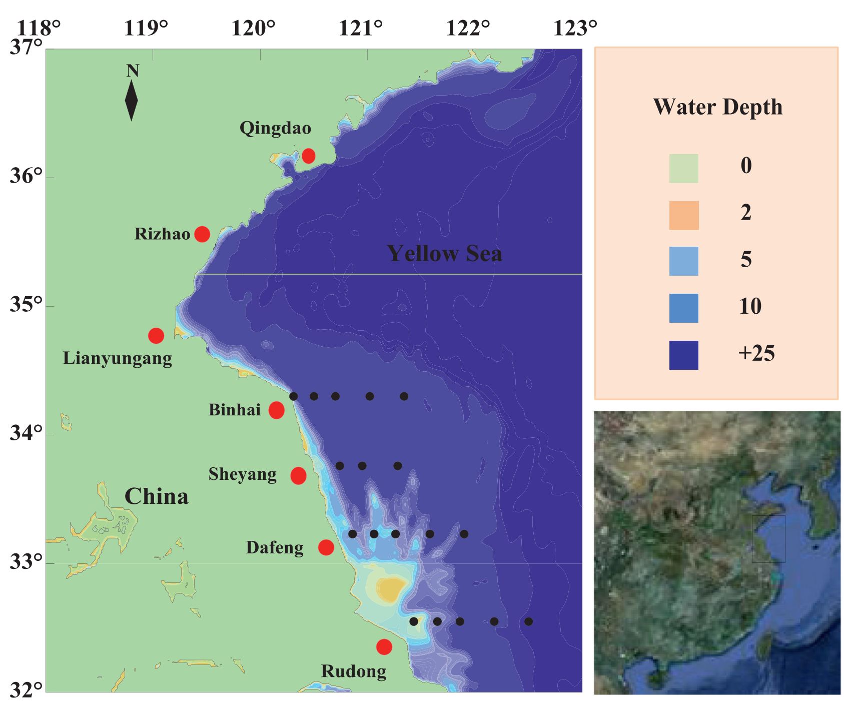


Fig. 1. Study area and monitoring transects in Rudong, Dafeng, Sheyang, and Binhai from January to April, 2013.

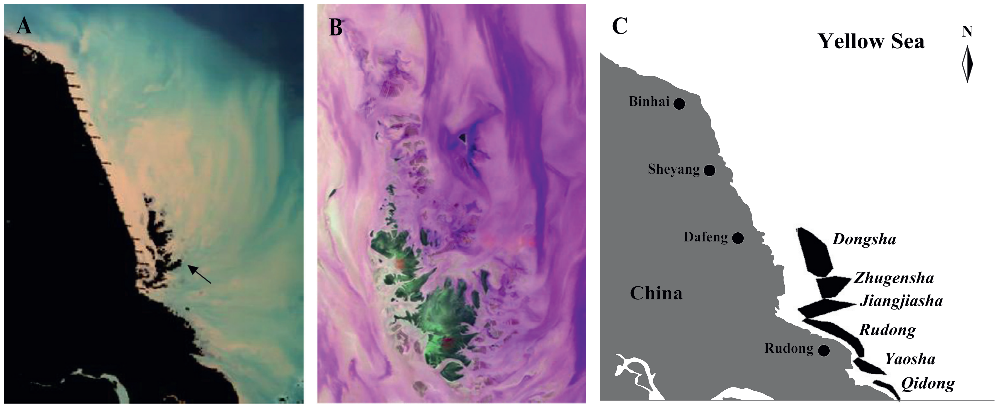


Fig. 2. (A) Satellite images of the radial sand ridge area; (B) satellite images of the *Pyropia* aquaculture area in Dongsha; (C) sketch map of the six regions of *Pyropia* aquaculture in the radial sand ridge area.

cloud-free and to observe the rafts at low tide during *Pyropia yezoensis* cultivation.

2.4. Identification of Ulva species on the Pyropia aquaculture rafts

Morphological and phylogenetic analyses were performed to identify the species of green algae. All samples collected from the rafts were transported to the laboratory in cool dark conditions within 48 h. The algal thalli were quickly cleaned three times with sterile seawater, then observed under a microscopic (Nikon E200, Japan). For morphological identification, the key outlined in the study by Tseng (1983) and Duan et al. (2012) was used.

Phylogenetic analysis was performed for unidentified green algae. Before DNA extraction, the selected single algae were cleaned of debris and epiphytes, and gently rinsed using sterile seawater. The DNA extraction and PCR amplification techniques used were previously described by Han et al. (2013). The nuclear encoded ITS DNA region was amplified using the primers reported by Leskinen and Pamilo (1997). However, *U. prolifera* and *Ulva linza* belonged to one group, called the LPP cluster (*Ulva linza–procera–prolifera*). Therefore, the 5S rDNA spacer region was amplified using the primers to identify the LPP samples according to Shimada et al. (2008). Sequences of the ITS and 5S rDNA regions were aligned using Clustal X (Thompson et al., 1997). Neighbor-joining (NJ) analyses of the aligned sequences were performed using Mega 4.0 (Tamura et al., 2007).

3. Results

3.1. The original area of the Ulva blooms determined by satellite remote sensing

The satellite images showed that the floating green algae patches were first monitored at (32°40′, 121°34′) and (33°21′, 121°36′), approximately 75 km and 60 km east of the coast near Rudong and Dafeng, respectively, on May 12th, 2013 (Fig. 3). The area covered by the bloom at the Rudong coast was about 2.6 km^2, which was much larger than that at the Dafeng coast (<0.6 km^2). On May 20th, the area covered by the blooms was larger. Over the next 10 days, the blooms moved quickly northward and reached 67.3 km^2 spread over 3582 km^2.

3.2. Distribution characteristics of floating green algae by the shipboard survey

Table 1 describes the formation of green algal blooms at the early stage along the coastline of the Southern Yellow Sea in 2013. In January and February, no floating *Ulva* was observed in the study areas. Sporadic individual floating *Ulva* species first appeared at Rudong on March 2nd and sporadic patches of floating algae were found in both Rudong and Dafeng until April. At the same time no floating *Ulva* was observed in Sheyang and Binhai. In May, large-scale floating green algae were observed at all four monitoring sites, and the blooms started to form.

3.3. Quantitative distribution characteristics of Ulva microscopic propagules

Ulva seedlings were obtained from microscopic propagules in the water collected in the field (Fig. 4). The surface water at Rudong had a maximum density of up to 715 ind L^{-1}; whereas less than 20 ind L^{-1} were found in the open sea at Binhai. These results showed that *Ulva* microscopic propagules were widely distributed in the Yellow Sea and the densities decreased gradually northward from Rudong to Binhai and eastward from nearshore to open sea, coinciding with the distribution of *Pyropia* aquaculture.

3.4. Identification of Ulva species

Based on previously published morphological methods, four green algal species were identified, including *U. prolifera*, *Ulva flexuosa*, *U. linza* and *Blidingia* sp., from the *Pyropia* aquaculture rafts. Although the morphological differences were significant between these green algae, the young *Ulva* seedlings looked very similar. All were tubular without branches. Molecular analysis was conducted when *Ulva* species were not identified by morphological analysis.

NJ trees were constructed between the ITS, 5S dataset from *Ulva* samples and additional taxa from GenBank (Figs. 5 and 6). Phylogenetic analyses based on ITS datasets showed that all 36 samples fell into two clades including the *U. linza–procera–prolifera* (LPP) clade (18 samples) and the *U. flexuosa* clade (18 samples). Phylogenetic analyses of the 5S rDNA spacer region dataset revealed that 18 samples in the LPP clade also fell into two clades: the *U. prolifera* clade (12 samples) and the *U. linza* clade (six samples). The ITS and 5S rDNA spacer sequences indicated that *U. prolifera* on the rafts was identical to the free-floating green algae observed from 2008 to 2013.

3.5. Distribution characteristics of the green algae on Pyropia aquaculture rafts

According to the satellite remote sensing images, the *Pyropia* cultivation areas in Dongsha, Zhugensha, Jiangjiasha, Rudong,

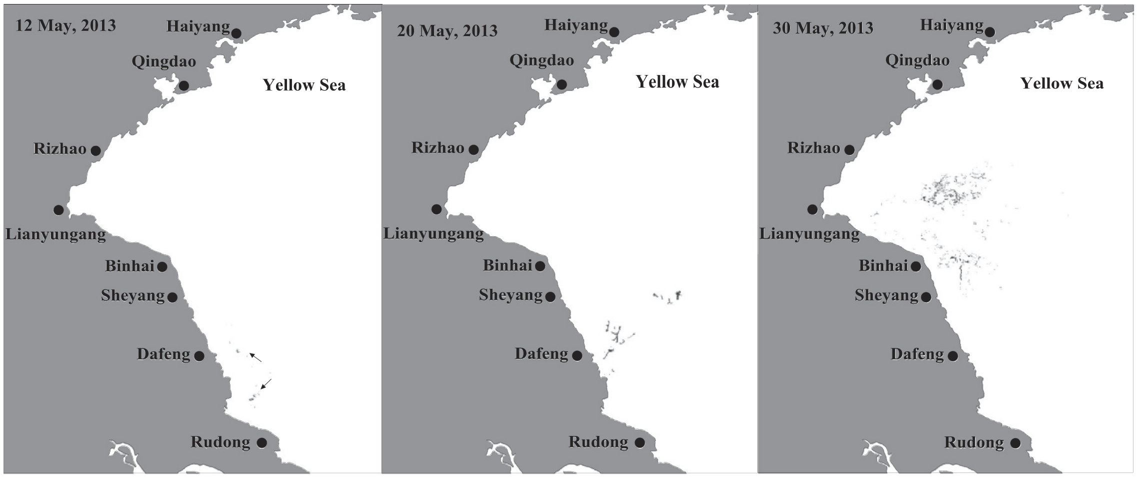
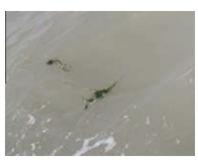
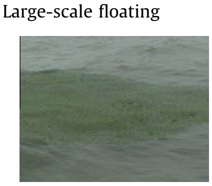
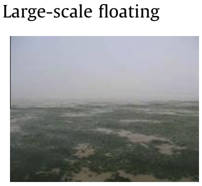


Fig. 3. The satellite remote sensing images of green algae patches at the early stage of the blooms in the Yellow Sea.

Table 1
Floating status of *Ulva* blooms along the coast of the Southern Yellow Sea from January to May in 2013.

Date	Rudong	Dafeng	Sheyang	Binhai
January	No floating *Ulva* found	No floating *Ulva* found	No floating *Ulva* found	No floating *Ulva* found
February	No floating *Ulva* found	No floating *Ulva* found	No floating *Ulva* found	No floating *Ulva* found
March	Sporadic individual floating 	No floating *Ulva* found	No floating *Ulva* found	No floating *Ulva* found
April	Sporadic patches floating 	Sporadic patches floating 	No floating *Ulva* found	No floating *Ulva* found
May	Large-scale floating 	Large-scale floating 	Large-scale floating 	Large-scale floating 

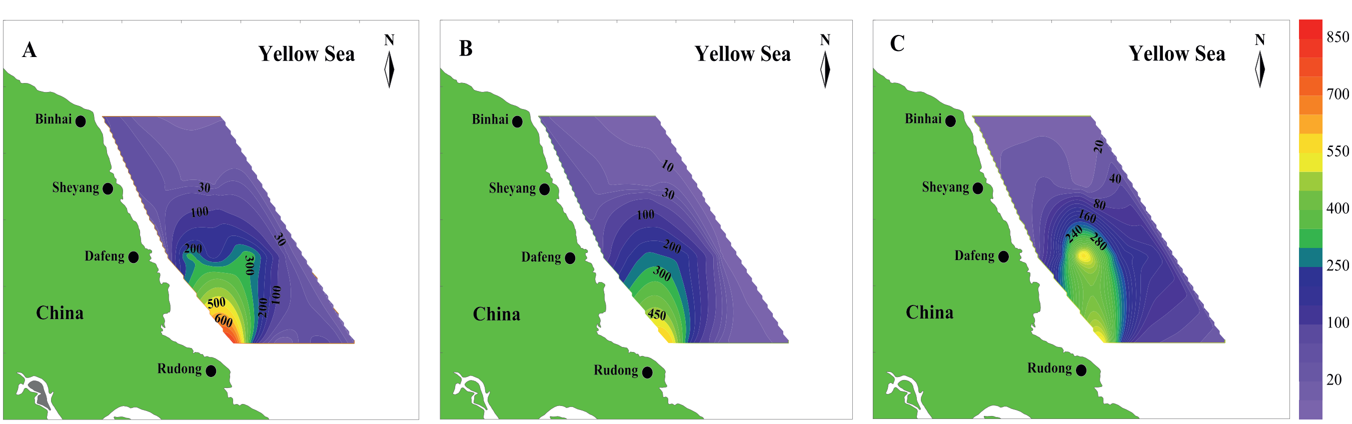


Fig. 4. Distribution characteristics of *Ulva* microscopic propagules with different water depth (A, surface water; B, 5 m water; C, bottom water) in the Southern Yellow Sea.

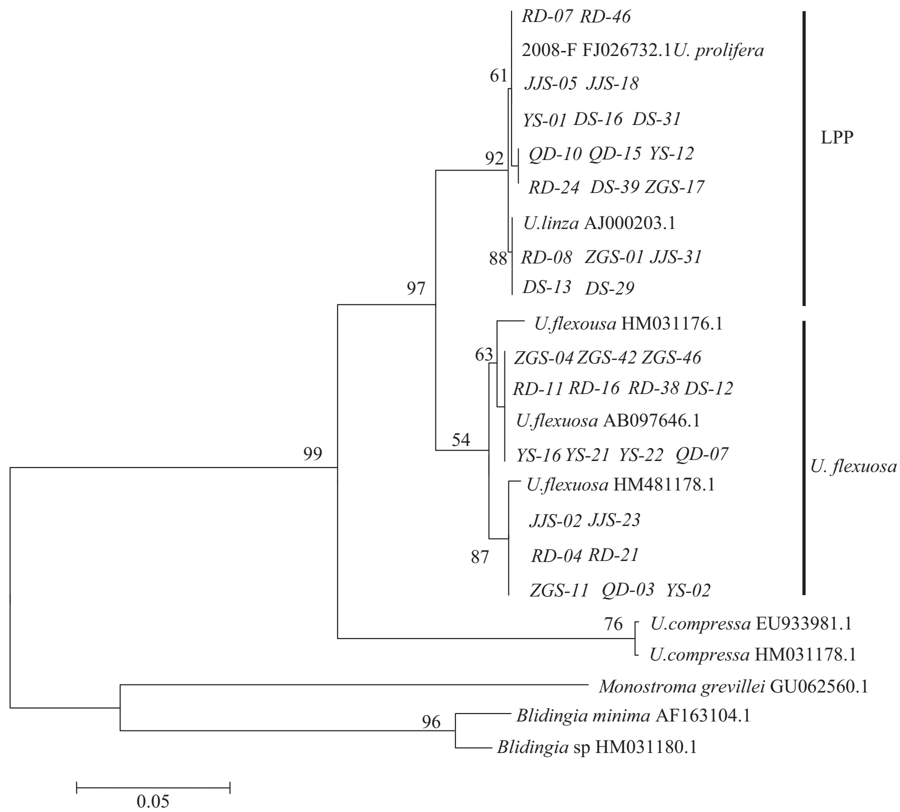


Fig. 5. Neighbor-joining (NJ) tree constructed from the analysis based on the ITS sequences of *Ulva* samples collected from the *Pyropia* aquaculture rafts and those downloaded from GenBank. Bootstrap values (1000 replicates).

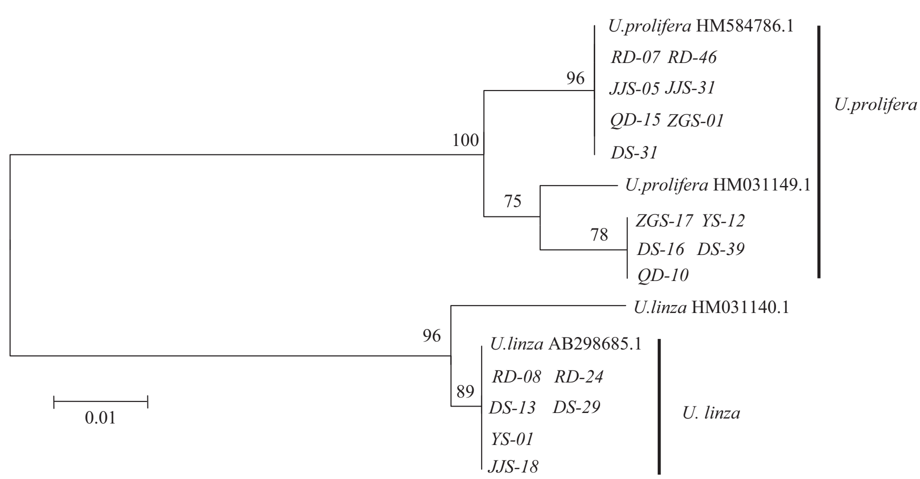


Fig. 6. Neighbor-joining (NJ) tree constructed from the analysis of the 5S rDNA spacer sequences of the *Ulva* LPP samples collected from the *Pyropia* aquaculture rafts and those downloaded from GenBank. Bootstrap values (1000 replicates).

Yaosha and Qidong were 128, 89, 52, 50, 14 and 8 km^2, respectively (Fig. 7). In April 2013, at the early stage of the *Pyropia* harvesting season, the *Ulva* spp. were widely distributed on the rafts, especially the species on the ropes. Fig. 8 shows that a significant amount of green algae grew on the rafts before and after *Pyropia* was harvested. In Dongsha, Zhugensha, Jiangjiasha and Rudong, *U. prolifera* showed the highest biomass followed by *U. flexuosa* and *U. linza*. In Yaosha and Qidong, *U. flexuosa* showed a wider distribution and greater biomass, with *U. linza* showing the least biomass. When the biomass from all six regions was included, the *Pyropia* farms produced 2784 tons of *U. prolifera*, 1314 tons of *U. flexuosa* and 746 tons of *U. linza*.

4. Discussion

In this study, we found that *U. prolifera* on the *Pyropia* rafts showed the same ITS and 5s rDNA sequence as the free-floating *Ulva* species, and a significant amount of the *U. prolifera* biomass dropped into the sea and floated on the sea surface, which supported the rapid development of macroalgal blooms in the Yellow Sea.

4.1. The original area of Ulva blooms in the Yellow Sea

The satellite images provided the most direct evidence of where the floating green algae first emerged each year. Based on the

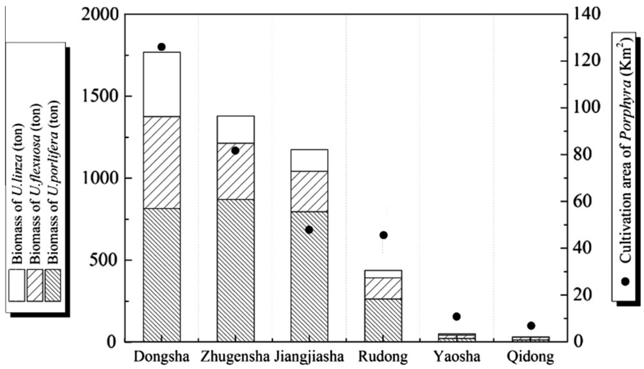


Fig. 7. The total *Pyropia* aquaculture area and the production of *U. prolifera*, *U. flexuosa* and *U. linza* on the rafts in the six regions of the radial sand ridge area in April 2013.

Fig. 9. A significant amount of green algae on the ropes was dragged down by the tractor during *Pyropia* harvesting.

HJ-1A/1B data, the initial bloom patches were observed in the Southern Yellow Sea. However, the satellites were not accurate enough to locate the smaller green algae patches at the early stage of the macroalgal blooms. In addition, floating brown algae patches may have reduced the accuracy of the satellite sensing system. For example, He et al. (2011) and Hu et al. (2010) misidentified *Sargassum* patches as *Ulva* blooms offshore in the Yangtz River even though they used high-resolution Landsat satellite data. In order to overcome this problem, a systematic shipboard survey was carried out at the same time in our study.

The results obtained from the monitoring cruises revealed that the first appearance of floating green algae occurred in the Rudong sea area. During the same period, no floating *Ulva* was found in the other areas. The distribution of microscopic propagules showed a similar trend, which also supported the notion that the floating green algal biomass at Rudong was the highest in the Southern Yellow Sea in April, 2013.

Our previous study outlined the reasons why the initial blooms emerged in the Rudong sea area (Huo et al., 2013). Qiao et al. (2011) demonstrated that drifting of macroalgae was mainly controlled by the surface current, which was primarily driven by wind. Keesing et al. (2011) confirmed that the movement of the floating algae was highly consistent with the winds during that period. In addition, under the influence of a freshwater plume from the Yangtze River, there was a weak northward surface current in the Southern Yellow Sea (Rong and Li, 2012). Before May 2013, therefore, the floating green algae drifted southward driven by the southern winds, which was then blocked and accumulated in the Rudong area due to the freshwater surface current. In the following period, suitable environmental conditions supported the fast growth and reproduction of *Ulva*. The winds shifted back towards the north to facilitate the transport of the large accumulated biomass of algae to the Northern Yellow Sea.

4.2. Correlation analysis between the Ulva blooms in the Yellow Sea and the green algae on the Pyropia rafts

Many studies have shown that the rough clean-up of *Ulva* attached to the rafts *in situ* after *P. yezoensis* harvesting caused the green algae to drift into the sea where it continued to grow and formed floating patches (Liu et al., 2009, 2010). The green algae on the ropes were dragged down by the tractor (Fig. 9) as the ropes with significant green algal biomass were too heavy to be transported to the shore. *In situ* cleaning of the intertidal aquaculture infrastructure resulted in the majority of the green algae being deposited into the sea where they floated and bloomed. The satellite remote sensing data indicated the rapid expansion of aquaculture farms in the "Sansha area" including Dongsha, Jiangjiasha and Zhugensha (Fig. 10), whereas the areas of coastal cultivation gradually decreased due to serious seawater pollution (SOA, 2010). In addition, the radial sand ridges of the Jiangsu coast were natural geographical barriers, which prevented the floating *Ulva* in the Rudong area drifting into the Northern Yellow Sea. However, the "Sansha area" is located to the north of the radial sand ridges, and without these barriers, the attached *Ulva* which were dragged down into the sea quickly drifted into the Northern Yellow Sea. In conclusion, the expansion of *Pyropia* aquaculture in the open sea made it much easier for the attached green algae to overcome the varied topography and float into the sea.

Additionally, although *U. linza* and *U. flexuosa* were also important fouling species on the *Pyropia* rafts, they have not been recorded as bloom species in the Yellow Sea. It is generally known that the Yellow Sea green tides were dominated by one species, *U. prolifera*, from 2007 to 2012 (Zhao et al., 2013); however, species succession was found at the early stage of the green algal blooms from 2009 to 2012 (Tian et al., 2011; Han et al., 2013; Huo et al., 2013). Thus, other *Ulva* species dominated the floating macroalgal population in the Rudong and Dafeng areas in April and May,

Fig. 8. A significant biomass of green algae grew on the rafts before (A) and after (B) *Pyropia* harvesting.

whereas *U. prolifera* biomass gradually accumulated while drifting and became the major contributor to the massive green tide. All *Ulva* species have a similar life cycle (Ye et al., 2008; Ma et al., 2009), however, differences in morphology and ecological niche determine their adaptive strategies. The tubular *U. prolifera* thalli showed a higher surface area/volume ratio than other flat *Ulva* species, and exhibited high rates of nutrient uptake (Xu et al., 2012). In addition, distinct physiological characteristics of *U. prolifera*, such as high tolerance to temperature and irradiance (unpublished data) may give this alga an advantage in terms of growth over other *Ulva* species. Therefore, *U. prolifera* survived in the early stage and bloomed quickly when conditions were suitable.

Our previous studies showed that at the early bloom stage, the composition of the attached *Ulva* spp. on *Pyropia* rafts and free-floating *Ulva* population were not only the same, but the sequence similarity rates between these two populations were also 100% (Han et al., 2013). However, we only detected a small quantity of *U. prolifera* on the rafts in March 2011, but not at other times. In 2013, we found that both populations had the same sequence between the two habitats, which was critical evidence in

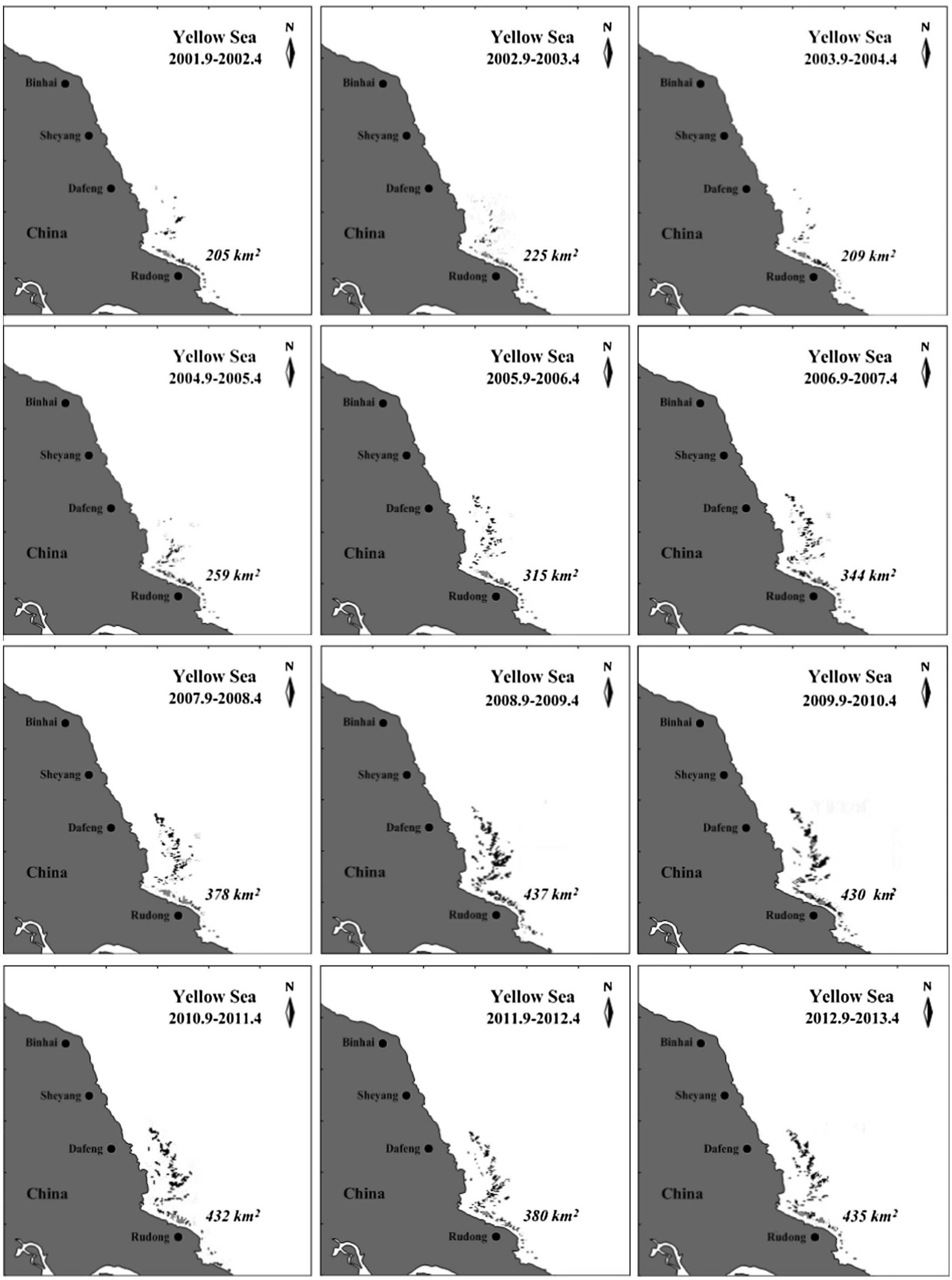


Fig. 10. Yearly variation of *P. yezoensis* in the aquaculture area by satellite remote sensing.

supporting the view that the source of the *Ulva* blooms in the Yellow Sea in 2013 was the *Pyropia* aquaculture area. Estimates of *U. prolifera* biomass accumulation (2784 tons) on the rafts also demonstrated that this alga was the source of the large green-tide in the Yellow Sea. Consistency in the time scale from the harvesting of *Pyropia* to the formation of the bloom patches was also demonstrated by the evidence from field and satellite images. In 2013, the *U. prolifera* biomass on the rafts supported the bloom. Researchers had not found the same sequence for *U. prolifera* on the rafts before, even though a number of papers have been published on this topic. Conversely, the hypothesis that the 2008 blooms in Qingdao resulted in invasion and dominance of the species on the *Pyropia* aquaculture farms cannot be ruled out, as the first satellite remote sensing images in 2008 were taken in the Northern Yellow Sea (Keesing et al., 2011) and the microscopic propagules could have spread quickly throughout the entire Yellow Sea.

This is a controversial issue in the local environment and whether and how *Pyropia* cultivation will impact the evolution of green algal blooms in the aquaculture area is now receiving more attention. However, we cannot ignore the fact that the *Pyropia* aquaculture industry in Jiangsu province has resulted in significant economic benefits to local residents (Shang et al., 2008). A change in the harvesting mode or resource utilization of green algae may be a better way of dealing with these problems.

Acknowledgments

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