

Genetic and Marine Cyclonic Eddy Analyses on the Largest Macroalgal Bloom in the World

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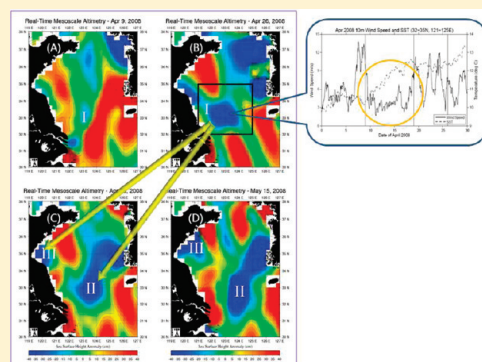
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S Supporting Information

ABSTRACT: In 2008, a massive *Ulva prolifera* bloom, with a 3-million-ton biomass covering an area of $1.29 \times 10^4 \text{ km}^2$ at its largest, suddenly appeared from May to July in South Yellow Sea. The mechanism behind the rapid growth of these seaweeds was investigated. Molecular phylogenetic analysis of free-floating algal samples from the Yellow Sea suggested that *U. prolifera* belong to one population, and that temporary cyclonic eddies in the Yellow Sea in late spring and early summer may help promote the proliferation of this bloom by providing seaweeds with sufficient growth time, abundant nutrition, and favorable habitats. The initial investigation on the relationship between marine cyclonic eddies and the route of free-floating algae extends our knowledge on how the emergence of free-floating macroalgal blooms in coastal areas could yield a large biomass.



INTRODUCTION

Caused by extreme growth and proliferation of free-floating exotic or endemic green algae, green tides are a type of macroalgal bloom occurring in many estuaries and coastal seas in most parts of the world.¹ Many biotic and abiotic factors produce excess nutrients, elevating nitrogen and phosphorus content and thus causing eutrophication.^{2,3} Eutrophication facilitates the growth and blooming of exotic or endemic seaweeds in shallow water, a steady hydrological condition. The blooms usually alter the coastal floral and faunal community structure; when washed ashore, they create repulsive, smelly piles on recreation beaches.^{1,4} The cost of mitigating the impact of green tides is so high that in the French Atlantic coast alone, nearly $1.0 \times 10^8 \text{ kg}$ of seaweed require annual removal from its recreational beaches.⁵ Because green tides typically occur in coastal zones close to fisheries or urbanized areas, many studies focusing on ecological control and economic impact in these regions have been carried out in Europe, America, Australia, and Asia since the 1970s.^{1,6,7} However, the source, route, and life history of free-floating macroalgal blooms in large marine systems have not yet been explicitly studied.

Cyclonic eddies contribute considerably to regional nutrient concentrations. Observations in the middle latitude region-Sargasso Sea⁸ and subtropical region-North Pacific subtropical gyre⁹ indicate that nutrient concentrations, chlorophyll *a* concentrations, photosynthesis rates, and new production were enhanced dramatically in the cyclonic eddies. The cyclonic eddy

in the Luzon Strait in the South China Sea showed similarities to other cyclonic eddies that enhanced productivity by upwelling subsurface nutrients.¹⁰

The Yellow Sea is a marginal sea of the Pacific Ocean, located between mainland China and the Korean Peninsula. The South Yellow Sea is a flood section of the continental shelf, with an area of $3.09 \times 10^5 \text{ km}^2$.¹¹ Its hydrological characteristics are influenced by the branch of the Kuroshio Current, and the seawater exchange with East China Sea is usually obstructed by the Changjiang Diluted Water. The Yellow Sea is one of the world's 64 large marine ecosystems and a crucial region for marine economy development for China, Korea, and Japan.

At the end of May 2008, a massive macroalgal bloom caused by the free-floating green alga *Ulva prolifera* suddenly appeared from the south to Qingdao in the Yellow Sea. According to the Moderate Resolution Imaging Spectroradiometer Satellite (MODIS) images,¹² the free-floating bloom was first observed on May 15 in the west coastal area of the South Yellow Sea¹² and transported to Qingdao after one month. The macroalgal bloom persisted for approximately two months in the coastal area of Qingdao. Its biomass was an extraordinary $3.0 \times 10^9 \text{ kg}$ fresh weight ($1.0 \times 10^9 \text{ kg}$ removed from beaches and coastal waters

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and an estimate of 2.0×10^9 kg that settled in deep waters). At its largest, it covered an area of approximately 1.29×10^4 km².¹³ A prebloom occurred in the summer of 2007 and it was demonstrated that this free-floating algae was not from Qingdao.¹⁴

The hypothesis of the origin and rapid expansion functions in situ of this free-floating green algal bloom were hotly debated based on the field investigation, as well as morphological and nucleotide sequence analysis.^{12,15–18} The genetic markers internal transcribed spacer (ITS), chloroplast gene encoding ribulose 1,5-bisphosphate carboxylase large subunit (*rbcL*),¹⁵ and gene encoding D1 reaction center protein of the photosystem II complex (*psbA*)¹⁸ were applied on the algal samples collected along coasts of Jiangsu Province and in Yellow Sea to track the source of this bloom event, demonstrating that the ITS, *rbcL*, and *psbA* sequences show high identities among free-floating samples.

The genetic structure analysis within *U. prolifera* species on attached and floating samples collected from the South Yellow Sea and the south of the East China Sea demonstrated that floating *U. prolifera* samples in the Yellow Sea in 2007 and 2008 had a close genetic relationship, and the floating samples were genetically distinct from the attached samples, suggesting this alga may be not an endemic population in Yellow Sea.¹⁹

The sudden emergence of this free-floating macroalgal bloom suggests that this ecological phenomenon has to be studied in the context of large marine ecosystems, and involved in interdisciplinary research considering more marine environmental factors and detailed algal biological characteristics. It is possible that large habitats may be “temporary venues” for the rapid growth and proliferation of *U. prolifera* because of the relatively steady hydrological and biological conditions present in these ecosystems. The purpose of this work, therefore, is to investigate how this green algal bloom could suddenly develop with a large biomass in coastal areas using molecular phylogenetic and marine cyclonic eddy analyses. The effects of cyclonic eddies in the growth of free-floating macroalgal blooms were inferred, and cyclonic eddies were used to forecast the route of the blooms in large marine ecosystems.

EXPERIMENTAL DETAILS

Study Sites and Sampling. Free-floating green algae *U. prolifera* were sampled from the coastal zone and the Yellow Sea (32°33′–36°46′ N, 119°32′–22°00′ E) in the summer of 2007 and 2008 (Table 1 in the Supporting Information). The collection sites are shown in Figure 1. Samples were washed with cold boiled seawater 3–4 times and cultivated in Von Stosch's Enriched Medium (VSE medium).

DNA Extraction and Polymerase Chain Reaction Amplification. The 5S spacer region is a useful nucleotide marker to infer interspecies phylogeny of algae and higher plants.^{20,21} In fact, interspecies phylogeny of Phaeophyceae has been successfully distinguished by this marker.^{22,23} Both the polymerase chain reaction (PCR) profiles and primers for four molecular markers including ITS (the nuclear rDNA ITS 1, 5.8S rRNA gene, and ITS 2 sequence), 5S rDNA spacer region, *rbcL* gene, and *psbA* gene are listed in Table 2 in the Supporting Information. Amplifications were checked and purified on 1% Tris-acetate-EDTA agarose gels.

Sequence Divergence and Phylogenetic Analysis. Sequence data were aligned using ClustalX.²⁴ The sequence divergence was calculated using Kimura 2-parameter model by Mega 4.0.²⁵ Phylogenetic tree was performed by neighbor joining

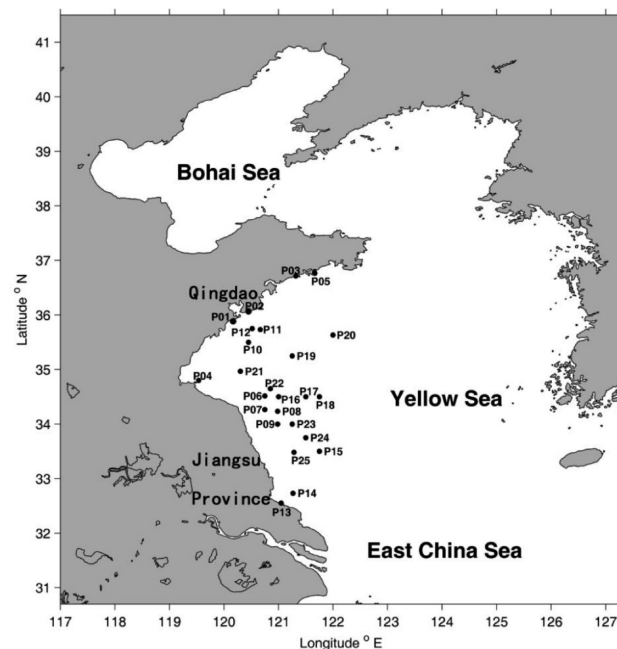


Figure 1. Collection site location of the samples during the summer of 2008 in the Yellow Sea.

(NJ) methods with Mega 4.0. The robustness of the NJ tree was tested by bootstrapping with 1000 replications of the data.

Physical Environment Analysis. We used three meteorological/oceanographical data sets to facilitate the understanding of the physical environment during the spring of 2008. First, maps of SSH anomalies, which indicate marine mesoscale eddies, were produced by the Global Near-Real-Time Altimetry Data Viewer, which is developed by the Colorado Center for Astrodynamics Research (CCAR) at the University of Colorado, Boulder (available at http://argo.colorado.edu/~realtime/gsfsc_global-real-time_ssh/). This data viewer allows the visualization of SSH anomalies field based on multisatellite altimetry data at our regions of interest. To show the atmospheric and oceanic conditions during the period of eddy formation, we further analyzed the hourly time series of 10 m wind speed and sea surface temperature (SST) from the NCEP Climate Forecast System Reanalysis (CFSR) during April 2008.²⁶ Finally, HadSST2 of the Met Office Hadley Centre's sea surface temperature data set was used for the calculation of long-term mean SSTs during the past 46 springs.²⁷

RESULTS

All four nucleotide markers of ITS, *rbcL*, *psbA*, and 5S spacer region showed high sequence identities among samples collected from the Yellow Sea. Through phylogenetic analysis with reference sequences from GenBank, both ITS and *rbcL* placed the free-floating green alga into an *U. prolifera*–*U. linza* group. The 5S spacer region provided a confirmed position for the floating species as *U. prolifera* compared with 38 reference sequences of *U. prolifera* and *U. linza* from the Japan coast.²⁸ This result of 5S spacer marker, the more useful marker to infer interspecies phylogeny, implies that the causative species belonged to one population of *U. prolifera* (Figure 2).

In addition, smaller blooms were observed in Qingdao in the summer of 2007, and the ITS analysis of the floating species and

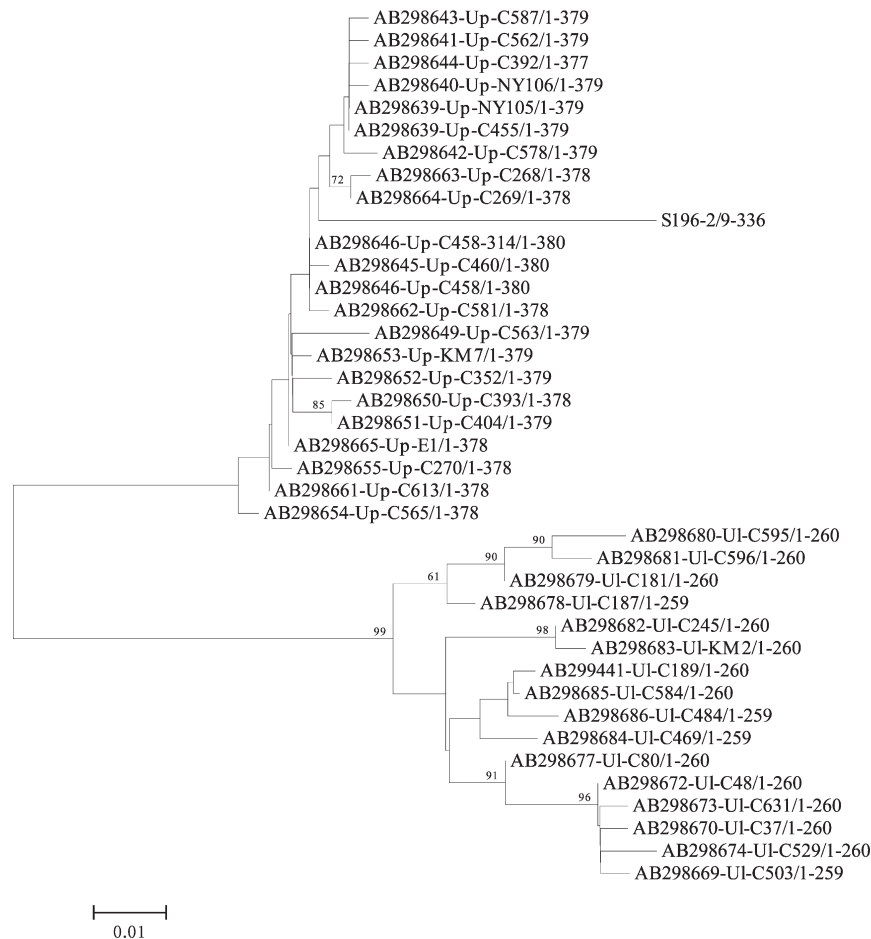


Figure 2. Neighbor-joining phylogenetic tree of 5S spacer region. The S196-2/9-336 is the 5S spacer sequence from samples in the Yellow Sea. Others beginning with “AB” are the *U. prolifera*–*U. linza* reference sequences.

attached local closer species showed that the original blooming species was not from Qingdao. Including the 5S spacer marker, the four genetic markers in this experiment were also tested against the 2007 sample, and high sequence identities with 2008 samples were demonstrated, the same with the inter-simple sequence repeat (ISSR) analysis,¹⁹ indicating that the same population of *U. prolifera* produced the blooms from 2007 to 2008 in the Yellow Sea.

A negative SSH anomaly “I” with a diameter of 100 km appeared in the southern region of the Yellow Sea April 9, 2008 (Figure 3A), and developed gradually in situ until April 19, 2008 (Figure 3B; figures between these two days are not shown here). Before April 9, positive wind stress curl dominated the surface of this region (not shown here), which is usually considered to cause divergence in the Ekman layer and upward Ekman pumping. From April 9 to 19, a comparatively low surface wind speed over the eddy-developing region during this period (Figure 4) implies a very stable weather condition during this period. All the evidence mentioned above implies that this persistent SSH anomaly “I” represented a locally developing cyclonic eddy. Moreover, the steady weather condition resulted in rapid increase of SST by 3° (Figure 4) during this 10-day interval. During April 19 to 26 weather conditions went unstable, but the eddy was strong enough to maintain (not shown here). While the weather took a dramatic change from high to low pressure and the surface wind changed its direction from April 27

to 28, the SSH anomaly “I” split into two parts: one (II in Figure 3C) remained in the original place but the other (III in Figure 3C) was transported toward Qingdao. This negative SSH anomaly remained outside Qingdao in the Yellow Sea for the entire month of May.

DISCUSSION

The causative free-floating green alga was identified as *U. prolifera* based on its morphological and nucleotide marker ITS features.^{13,14,18} Because *U. prolifera* demonstrated a cosmopolitan distribution,²⁹ and the resolving capabilities of ITS, *rbcl*, and *psbA* were unable to provide phylogeny information below the level of the species, the 5S spacer region, along with ITS and chloroplast-encoded genes *rbcl* and *psbA*, was employed to investigate the geographical population genetic characters among samples collected from the coastal zone and the Yellow Sea in the summer of 2008. The phylogeny of 5S spacer region implies that the free-floating *U. prolifera* in 2008 in the Yellow Sea belongs to one genetic population. Evidence by ISSR genetic diversity analysis on attached and floating *U. prolifera* samples in 2008 around Yellow Sea indicates the floating samples were separated from the attached samples.¹⁹ The sudden emergence of the free-floating macroalgal bloom with a spatial and temporal scale of $1.29 \times 10^4 \text{ km}^2$ and 2-month persistence at the end of May led us to believe that a “temporary venue” could possibly

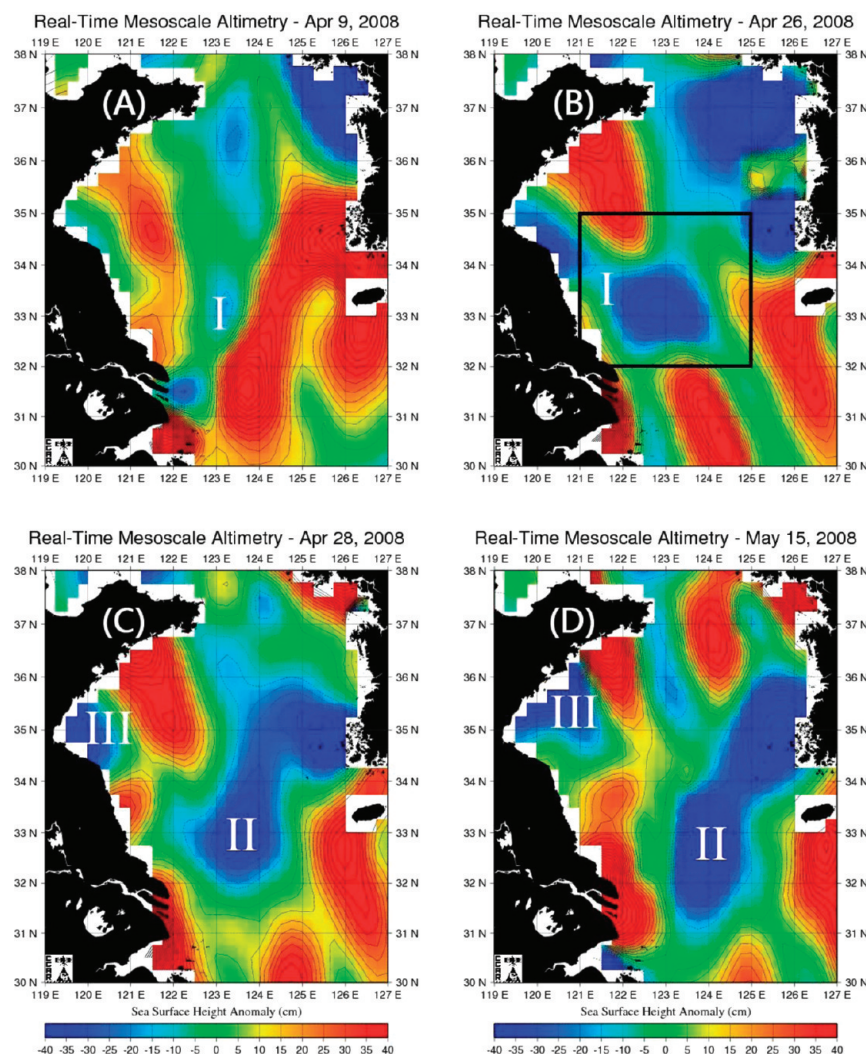


Figure 3. Sea surface height (SSH) anomalies from satellite altimeter show marine cyclonic cold eddies in the Yellow Sea in 2008 spring on four different days. The rectangle in (B) indicates the region where the data are averaged over in Figure 4.

exist in the spring for the free-floating algae to grow and proliferate in the Yellow Sea.

The life history and pathways of reproduction of *U. prolifera* are known to be multifarious including sexual, asexual, and vegetative propagations.³⁰ Studies on life-history of floating *U. prolifera* in Yellow Sea implied this alga could proliferate via floating small vegetative fragments and sporangia attached to these thalli.¹⁶ Somatic cells of this alga are cold-resistant, whose survival rates could reach over 60% from 5 to 15 °C and around 40% at 0 °C, and could begin to germinate from 5 °C and reach the highest germination rate around 15–20 °C.¹⁷ More studies on the life history of *U. prolifera* are necessary in order to clarify their various germination and reproduction pathways at different conditions.

Previous oceanographic survey on South Yellow Sea supported by Marine and Coastal Zone Comprehensive Investigation and Assessment of China in April of 2007 demonstrates the nitrate and phosphate concentrations at the surface layer of rectangle area on Figure 3B were higher than other areas in Yellow Sea, and the ratio of inorganic nitrate to phosphorus is appropriate for marine plants according to the “excess nitrate” calculation³¹ in the most part of South Yellow Sea.³² Meanwhile, a large number of suspended solids in the South Yellow Sea³³

could be the initial small solid substratum for the early germination of spores of *U. prolifera*.³⁴

The cyclonic eddy I and subsequent cyclonic eddies II and III in Figure 3 possibly led to the formation of potential “temporary venues” for the free-floating green algae. The cyclonic eddy I was near the tentative original source at Jiangsu Province coastal zone proposed by Liu et al. and Pang et al.^{12,15} The rotation of the eddies brought the possible somatic cells and fragments remained from 2007 and outside resources, mixed them with the nutrition pumped up from below, enabling a habitat for them to gather, grow, and proliferate in the spring of 2008. Actually, the region where Eddy I was developing (33°N, 124° E) observes frequent eddy occurrence, which is proved by a certain bottom deposition pattern in a mud patch analysis.³⁵ The rapid increase from 10 °C to approximate 13 °C (Figure 4) could supply a favorable temperature condition to facilitate the vegetative growth and germination.¹⁷ The cyclonic eddy III split from cyclonic eddy I (Figure 3C) was transport toward Qingdao continually gathering and feeding the algal community. Then this eddy III remained outside Qingdao in May when the green algae bloom impacted Qingdao. The series of satellite images show this evolving green tide, in accord with our results.¹²

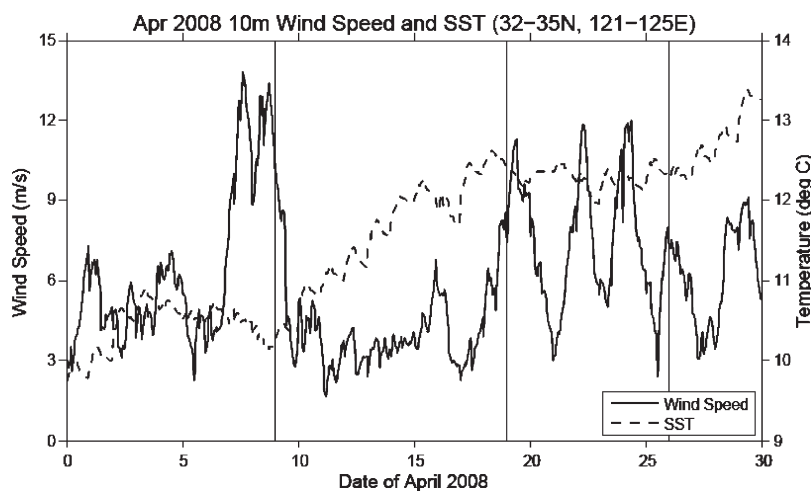


Figure 4. Area averaged hourly time series of 10 m wind speed (solid) and sea surface temperature (SST; dashed) in April 2008. The region used for averaging is shown in Figure 3. Three vertical lines indicate April 9, 19, and 26, respectively.

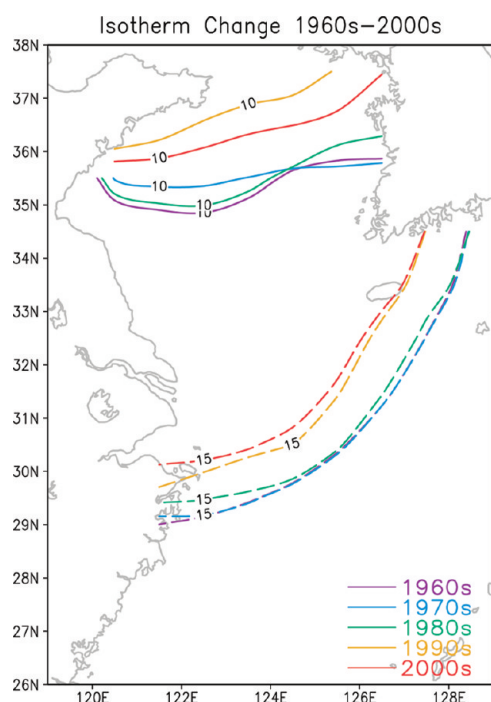


Figure 5. Change of 10 °C (solid) and 15 °C (dashed) sea surface isotherms during the last 40 springs. Colors indicate decades. Monthly mean temperatures of March, April, and May are averaged as 10 year climatology for each decade. Notice that the 2000s only covered six years, from 2000–2005. HadSST2 of the Met Office Hadley Centre's sea surface temperature data set were used for calculation.

Moreover, in the past 50 years, the northward moving trend of the 10–15 °C SST band in the Yellow Sea during spring season (Figure 5) due to global warming indicates that the cyclonic eddies tend to be warmer and temperature becomes less of a limiting factor. It strengthens the role of eddies in promoting the rapid expansion of *U. prolifera* in spring possibly by breaking down the thermocline to ease the upward transport of nutrient, and stimulating algal metabolism and reproduction in an upper warmer ocean.³⁶ Eddy I was somehow far from human habitats and the initial assembly and growth of *U. prolifera* was too dispersive to be

detected timely by satellite imagery, research vessel cruise, and remote sensing airplane. When first detected by satellite imagery on May 15 at the location of Eddy III, the free-floating algal biomass was already overwhelming. In summary, the frequent eddy occurrence in the middle of the Yellow Sea, the trend of eddy temperature increase in spring, and the difficulty of early detection all make our eddy-focusing approach even more valuable.

In order to understand and control the free-floating macroalgal blooms, many studies have focused on the mechanism of these macroalgal blooms by looking for their source. However, it is usually difficult to demonstrate the source accurately since *U. prolifera* is a cosmopolitan species, which means that they are found anywhere around the world,²⁹ with its global interspecies exchange occurring via ocean currents or sea transportations. Considering it is not only a biological phenomenon but a dynamical problem which involves physical and chemical oceanographic factors, focusing on the mechanism seems to be a direct and productive way to tackle this bothersome issue. More attention could be given to the mechanism of rapid expansion of the free-floating green algal blooming in Yellow Sea and other large marine ecosystems, not only to the source itself. The approach of monitoring the real-time SSH, wind, and SST fields shown in this study implies the possibility of an early prediction of free-floating macroalgal bloom by focusing on regions in large marine ecosystems of the Northern Hemisphere where stable marine cyclonic eddies occur in spring and early summer.

To this end, macroalgal blooms, both caused by endemic and free-floating exotic algae, are no longer only related to the problems of some specific estuaries or shallow coastal seas. It is necessary to investigate further detailed genetic population structure analysis among more samples collected in Yellow Sea and obtain more comprehensive physical environment information. Furthermore, the macroalgal bloom's effects on large marine ecosystems must also be considered in light of the ongoing global climate change situation.

■ ASSOCIATED CONTENT

S Supporting Information. Tables 1 and 2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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