The dominant *Ulva* strain of the 2008 green algal bloom in the Yellow Sea was not detected in the coastal waters of Qingdao in the following winter

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Abstract The region of Qingdao, China, experienced the world's largest green tide from May to July 2008. More than one million tons of fresh algal biomass of the green alga *Ulva prolifera* was harvested, while more was suspected to have sunk to the bottom. The original source of this seaweed was suspected to be from the south as revealed by satellite images. The floating biomass drifted with the water current northward and flourished in nearshore waters around Qingdao. However, direct biological evidence for "seed" source is lacking. It is still unclear whether this alga could survive the Qingdao local coastal environment and pose future danger of potential blooming. Systematic and seasonal sampling of waters in the intertidal zone at six collection sites along the Qingdao coast was conducted from December 2008 to April 2009. Forty-eight water samples were analyzed. From these, nine different morphotypes of *Ulva* were grown in the laboratory under standard temperature and light regimes. Growth of *Ulva* was observed in all water samples. However, molecular phylogenetic analyses revealed that the dominant *U. prolifera* strain of the 2008 bloom was absent in all the water-derived cultures during the sampling period.

These results provide evidence that the dominant bloom-forming alga was unlikely able to survive the coastal waters (the minimal surface water temperature in February is 2°C) in winter conditions in Qingdao, even though all the sampling locations were heavily covered by this alga in June 2008.

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Introduction

Green tides are vast accumulations of unattached green macroalgae associated with eutrophic marine environments (Shimada et al. 2003; Charlier et al. 2007). They have had major ecological and economic impacts (Hiraoka et al. 2003; Sun et al. 2008). In nutrient-rich habitats, *Ulva* species grow rapidly and are often among the dominant species causing green tides and marine fouling (Leskinen et al. 2004). In May to July 2008, before the Olympic sailing competition, the Qingdao coastline experienced what was believed to be the world's largest green tide (Liu et al. 2009), composed of more than one million tons of drifting biomass covering an area of 13,000 to 30,000 km^2 (Sun et al. 2008; Leliaert et al. 2008). The dominant green alga, which was included in the *Ulva linza–procera–prolifera* complex (LPP clade; Shimada et al. 2008; Leliaert et al. 2009), was identified to be *Ulva prolifera* (Müller) J. Agardh (Chlorophyta, Ulvophyceae; formerly known as *Enteromorpha prolifera*) through morphological and molecular analyses (Sun et al. 2008; Ye et al. 2008; Leliaert et al. 2009). The drifting biomass was believed to have been transported across the Yellow Sea offshore from Jiangsu province by seasonal winds and surface currents (Liu et al. 2009). The large-scale

occurrence of this alga in the Yellow Sea was thought to be related to a series of complex coupled processes (Sun et al. 2008). This large-scale inter-province green tide bloom in the Yellow Sea reminded people of coastal management issues and brought intense attention from the local and national governments because of the potentially damaging consequences on the environment and local economy.

Species in the genus *Ulva* are widely distributed in marine and brackish water environments all over the world (Hayden and Waaland 2002; Shimada et al. 2003; McAvoy and Klug 2005). Recently, molecular phylogenetic analyses and culture data provided convincing evidence that *Ulva* and *Enteromorpha* are not distinct evolutionary entities and should be recognized as one genus of the Ulvophyceae, *Ulva* (Tan et al. 1999; Hayden et al. 2003; Shimada et al. 2003). *Ulva* species are highly tolerant of variations in salinity, temperature, irradiance, and water quality (Taylor et al. 2001; Dan et al. 2002; Cohen and Fong 2006; Conley et al. 2009). They show great intraspecific morphological and cytological plasticity in growth phases with season and environmental conditions (Blomster et al. 1998, 1999). This high level of plasticity often leads to difficulties in species identification when only based on morphological and cytological features (Blomster et al. 2002; Leskinen et al. 2004). In view of these facts, molecular methods were introduced on the basis of sequence comparisons of the nuclear encoded internal transcribed spacer DNA (ITS nrDNA) region, including the 5.8S gene, the more conserved plastid encoded large subunit of the ribulose-1,5-bisphosphate carboxylase/oxgenase (*rbc*L) gene, and the 5S spacer region (Marks and Cummings 1996; Leskinen and Pamilo 1997; Blomster et al. 1998; Woolcott and King 1999; Shimada et al. 2003, 2008; Hayden and Waaland 2004).

Localization of the source of the green tide bloom and analyses of the outbreak potential are two prerequisites before any management decisions could be made (Blomster et al. 2002; Ye et al. 2008). Species of *Ulva* release microscopic gametes or spores which rapidly grow into adult thalli. These sporulation events were found to be accelerated at elevated light and temperature levels and can be facilitated by thallus fragmentation (Dan et al. 2002; Lüning et al. 2008). The microscopic stages of the germlings can be free-floating in seawater and grow into adult filamentous thalli when a more stagnant water environment is encountered (Dan et al. 2002; Shimada et al. 2008). These microscopic gametes, zygotes, spores, or germlings constitute the "seed bank" in nature for future blooms when favorable light, temperature, and pulse of nutrient enrichment occur (Hoffmann and Santelices 1991; Santelices et al. 1995; Zhang et al. 2009).

A very pertinent question after the large bloom in Qingdao in 2008 is whether this considerable biomass left behind germlings in the seawater along the Qingdao coastline, which could become the direct source of future blooms besides the annual arrival of drifting biomass from the south. The goal of this study was to survey whether the dominating algal strain of the 2008 bloom was still present in the coastal waters of Qingdao in the next winter and, if so, investigate the phylogenetic relationship of the bloom-forming strain with native isolates.

Materials and methods

From December 2008 to April 2009, seawater was randomly sampled eight times at six locations (Ferry, Tuandao, Zhanqiao, Institute of Oceanology Chinese Academy of Sciences, Olympic, and Shilaoren) along the Qingdao coast where the green tide was observed in June 2008 (Fig. 1). Seawater was subsequently aerated after adding NO_3^- (823 μmol L^{-1}) and PO_4^{3-} (73 μmol L^{-1}) to reach the levels of Provasoli-enriched seawater medium (PES; Berges et al. 2001) in 1 L glass beakers (two per sampling location) placed in temperature-controlled rooms at 15°C under 80 μmol photons $m^{-2} s^{-1}$ with a 12-h light per day light-dark regime. Five hundred microliters of saturated GeO_2 aqueous solution was added to the medium to prevent growth of diatoms, and the medium was renewed every 7 days. After 4 weeks, the green algal germlings, attaching to the wall and bottom of the glass beakers, grew
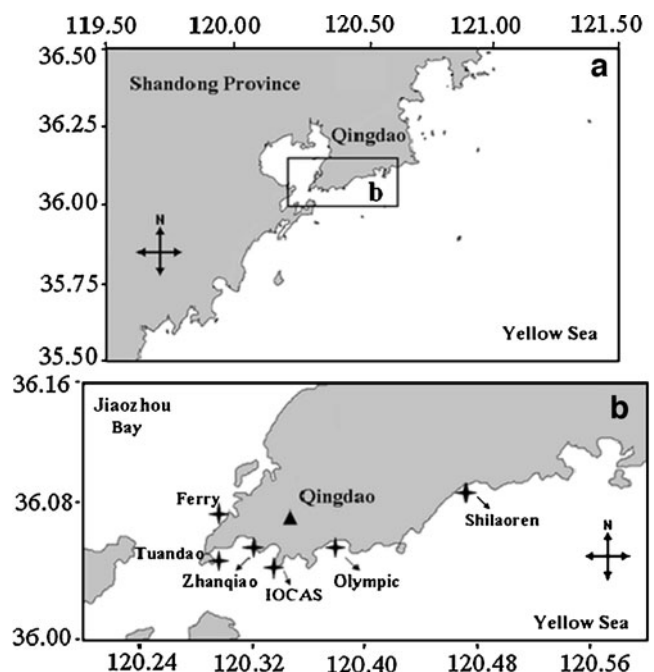
Fig. 1 Maps of Qingdao coast. **a** The *insert* is where water samples were collected. **b** Detailed map of Qingdao and its vicinity showing the six locations where seawater was sampled

up to 1–3 cm. These germlings were derived from microscopic spores, zygotes, gametes or those that were attached to microscopic particles invisible to the naked eye at the time of sampling. After counting, the algae were removed from the beakers and further grown in suspended culture under the same conditions until the morphology of individual plants could be analyzed (6–7 weeks). Twenty-six *Ulva* strains were preliminarily classified into nine groups according to their gross morphological characters including thallus morphology and the degree of branching (Table 1). Eight strains of *Ulva* spp. from the 2008 Qingdao green tide were also analyzed in this study.

DNA extraction, ITS nrDNA, and *rbc*L gene amplification and sequencing

The unialgal samples of 34 *Ulva* strains were washed three times with sterilized seawater and dried with filter paper. Then, 100 mg of each material was ground to fine powder in liquid nitrogen with a mortar and pestle and transferred to a 2-mL tube. DNA was extracted using the CTAB method (Wang et al. 2006). The concentration and the quality of isolated DNA were assessed by electrophoresis on 1.0% agarose gel. DNA concentration of each sample was adjusted to 50 ng μL^{-1}. Primers sequences and polymerase chain reaction (PCR) amplification of ITS nrDNA and *rbc*L gene were described by Leskinen and Pamilo (1997) and Hayden et al. (2003). Total genomic DNA (30–40 ng) was added to 50 μL PCR reactions containing final concentrations of 1×PCR buffer (Takara, Japan), 2 mM $MgCl_2$ (Takara), 0.8 mM dNTPs (Takara), 25 μM of each primer, and 1.6 U Taq polymerase (Takara). Amplification products were separated by 1.0% agarose gel electrophoresis, and fragments of an expected length were cut from the gel and purified by use of a DNA gel extraction kit (Bio Basic Inc, Canada) according to the manufacturer's instructions. ITS nrDNA and *rbc*L gene were sequenced on both strands using ABI 3730 XL automated sequencers (Shanghai Biosune Biotechnology Co., Ltd.). Each sequencing reaction was repeated twice.

Phylogenetic analysis

ITS nrDNA and *rbc*L genes were sequenced from 34 *Ulva* strains representing 11 different morphotypes of *Ulva* spp. (Table 1). Sequences were aligned using Clustal W (Thompson et al. 1994). Evolutionary relationship was inferred with the neighbor-joining (NJ) and maximum likelihood (ML) methods using Mega 4.0 (Saitou and Nei 1987) and PhyML 3.0 (Guindon and Gascuel 2003), respectively. *Ulvaria fusca* and *Umbraulva amamiensis* served as outgroup taxa. For the NJ and ML trees, the bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option; Tamura et al. 2007). Thirty-five closely related ITS nrDNA sequences of *Ulva* spp. in the *U. linza–procera–prolifera* (LPP) clade were used to construct a parsimony network with TCS 1.21 (Clement et al. 2000). Final results are presented using gaps as fifth character states for bases in DNA sequences.

Table 1 Sample number of different *Ulva* strains derived from the seawater of the different collection sites and their gross morphological characters for preliminary classification in groups 1–9 (Fig. 1)

Group	Gross morphological characteristics	Samples	Source
1	Ribbon, monostromatic, no branch, thin base	*Ulva* strains 1 (a, b, c, d, and e)	Derived from seawater
2	Ribbon, monostromatic, no branch, blunt base	*Ulva* strains 2 (a, b, c, and d)	Derived from seawater
3	Ribbon, monostromatic, few branches	*Ulva* strains 3 (a and b)	Derived from seawater
4	Ribbon, monostromatic, dense branches	*Ulva* strain 4	Derived from seawater
5	Filiform, monostromatic, no branch	*Ulva* strain 5	Derived from seawater
6	Filiform, monostromatic, branches	*Ulva* strains 6 (a, b, c, and d)	Derived from seawater
7	Tubular, monostromatic, no branch	*Ulva* strain 7	Derived from seawater
8	Tubular, monostromatic, branches	*Ulva* strains 8 (a and b)	Derived from seawater
9	Foliated, distromatic, no branch	*Ulva* strains 9 (a, b, c, d, e, and f)	Derived from seawater
10	Filiform and tubular, monostromatic, dense branches	*Ulva* strains 2008 (a, b, c, d, e, f, and g)	Free-floating
11	Tubular, monostromatic, branches	*Ulva* strain 2008h	Free-floating

Collection dates of seawater samples were 30 Dec 2008, 10 Jan 2009, 19 Jan 2009, 3 Feb 2009, 20 Feb 2009, 16 Mar 2009, 31 Mar 2009, and 17 Apr 2009, respectively. Algal samples of *Ulva* strains 2008 (a, b, c, d, e, f, g, and h) were collected from Qingdao in May and June 2008 in groups 10 and 11

Relative growth rate and photosynthetic activity of *Ulva linza* and *U. prolifera* at different temperatures

For testing the growth performance of *U. linza* and *U. prolifera*, 6×0.1 g of healthy biomass of each species without epiphytic algae were weighed and cultured in separate 500-mL glass beakers at 5, 10, 15, 20, and 25°C under 100 μmol photons $m^{-2}s^{-1}$ cool fluorescent white light in a 12-h light per day light-dark regime, in a GXZ-260C incubator (Ningbo Jiangnan Instrument, China). Temperature fluctuation was less than 0.5°C. PES medium was used and renewed every 2 days in the 7-day culture experiment. At the end of the experiment, the biomass and the maximal photochemical efficiency of PS II (F_v/F_m) were measured. F_v/F_m was determined using a Portable Chlorophyll Fluorometer (Mini PAM, Walz, Germany) by the method of Fleming et al. (2007). Before measurement, samples were dark adapted for 20 min. Optimal chlorophyll fluorescence quantum yield was calculated according to the following equation: $F_v/F_m = (F_m - F_o)/F_m$. F_o and F_m refer to the minimal fluorescence and the maximal fluorescence from dark adapted samples, respectively. F_v is the difference between F_m and F_o. The culture experiments were repeated four times.

Results

Quantitative and morphological analyses of the culture-derived algal samples

The total number of culturable *Ulva* individuals from 1 L surface seawater collected at the six nearshore locations increased over the study period (Fig. 2). Each time, the most algal individuals were found in the seawater from Zhanqiao, ranging from the lowest at 113±24 strains L^{-1} on December 30, 2008 to the maximum at 1,840±160 strains L^{-1} on April 17, 2009. Seawater from Ferry gave the lowest number of *Ulva* strains each time. According to gross

morphological characters of *Ulva* spp. derived from the water samples, nine groups of *Ulva* were preliminarily classified (Table 1). The occurrence percentage of each group at each location and at each time was determined (Fig. 3). *Ulva* spp. in group 1 was the dominating species along the Qingdao coasts, representing more than 50% of total amount of *Ulva* strains at each location and at each sampling time, with the exception of Shilaoren. Based on morphological characters, *Ulva* spp. in group 6 (*Ulva* strains 6a, b, c, and d) and group 8 (*Ulva* strains 8a and b), both with branches, were the most similar to the dominating alga of the 2008 green tide.

Phylogenetic analysis and morphology comparisons

Alignment of ITS nrDNA and *rbc*L gene sequences included about 522 and 1,288 aligned characters, respectively. According to both phylogenetic trees based on ITS nrDNA and *rbc*L gene sequence, all 34 *Ulva* strains were resolved into five distinct clades: the *U. linza–procera–prolifera* (LPP) clade (19 samples), *Ulva* sp. (1–2) clade (six samples), *Ulva rigida* clade (three samples), *Ulva compressa* clade (three samples), and *Ulva pertusa* clade (three samples). The LPP clade included the strains of *U. prolifera* from the Qingdao bloom in 2008 (*Ulva* strains 2008a, b, c, d, e, f, and g), native *Ulva* strains (*Ulva* strains 1a, 1c, 3a, 4, 5, 6a, 6b, 6c, 6d, 7, 8a, and 8b), and Genbank sequences for *U. linza*, *Ulva procera*, and *U. prolifera* (Figs. 4 and 5). The LPP clade was highly supported in both ITS and *rbc*L phylogenies. The other 15 *Ulva* strains were included in the remaining four clades with high bootstrap values (96–100%) in NJ and ML analyses.

The ribotype network of ITS nrDNA from samples in the LPP clade showed that there were at least 14 distinct ITS ribotypes in this clade (Fig. 6). The ITS nrDNA sequences of *Ulva* strains 2008a, b, c, d, e, f, and g from 2008 green algal bloom were identical with *U. linza* (EU888138), *U. prolifera* (FJ426613), *U. prolifera* (AB298314), *Ulva* sp.

Fig. 2 Total culturable individuals of *Ulva* strains derived from 1-L surface water samples at the different collection sites after 4 weeks in culture at 15°C under 80± 5 μmol photons $m^{-2}s^{-1}$ in a 12-h light:12-h dark photo-regime
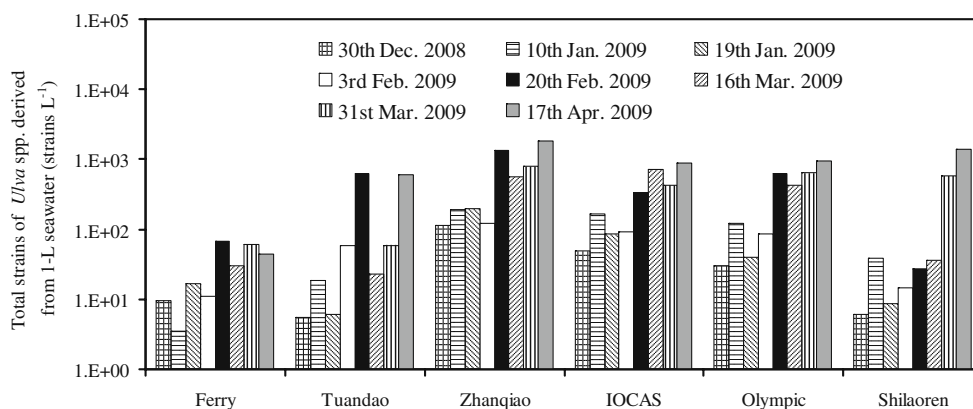
Fig. 3 Proportions of different *Ulva* groups at the designated sampling sites from December 30, 2008 to April 17, 2009
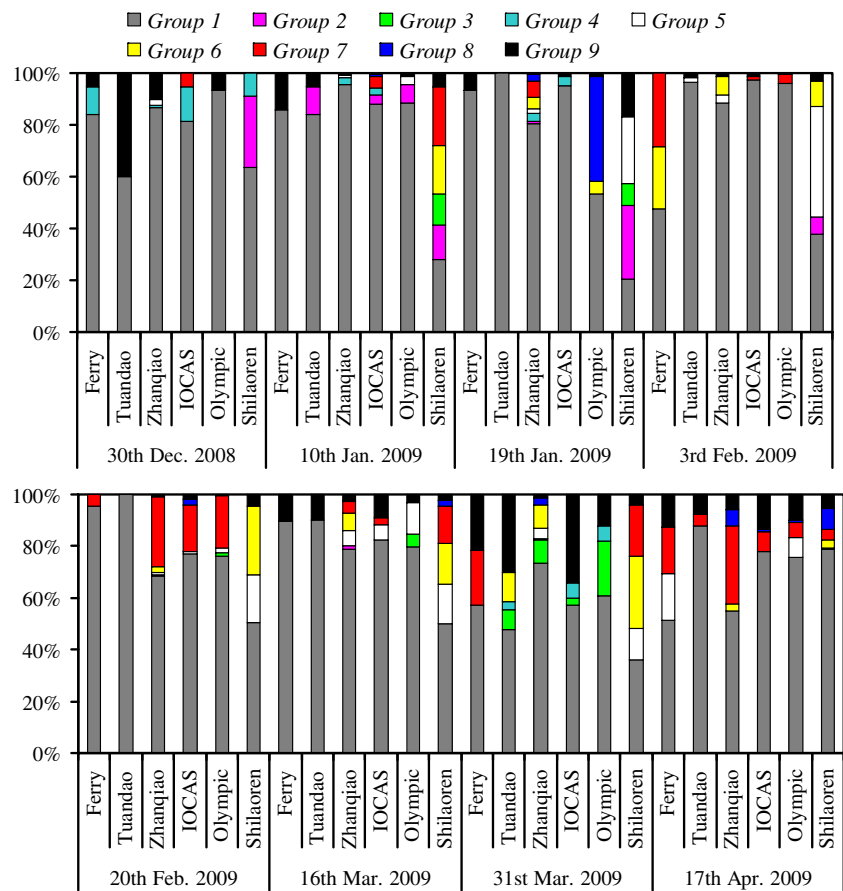
HT7 (FJ194958), and *U. prolifera* (FJ026732). The dominant *Ulva* species forming the Qingdao bloom had at least two mutational steps with the native *Ulva* strains in the LPP clade, which were included in four ribotypes in the network (Fig. 6).

Relative growth rate and photosynthetic activity of *U. linza* and *U. prolifera* at different temperatures

At 5°C, both *U. linza* and *U. prolifera* grew slowly (*t* test, $P>0.05$. Fig. 7a). When the temperature was raised to 10, 15, 20, and 25°C, *U. prolifera* grew faster than *U. linza* (two-way analysis of variance, species effect: $P<0.001$, temperature effect: $P<0.001$, species × temperature effect: $P<0.001$). Both species had the highest growth rates between 10°C and 20°C. However, the photosynthetic activity of *U. prolifera* was significantly impacted at 5°C compared to that of *U. linza* (*t* test, $P<0.01$. Fig. 7b). F_v/F_m of *U. prolifera* was reduced to 0.48 ± 0.08, while that of *U. linza* was at 0.61 ± 0.07, indicating that *U. linza* was better adapted to lower temperature than the stain of *U. prolifera* from the 2008 green tide in Qingdao. There was no significant difference in F_v/F_m between *U. linza* and *U. prolifera* at all the other temperatures tested (*t* test, $P>0.05$).

Discussion

This study demonstrates the absence of the dominant *U. prolifera* strain from the 2008 bloom at all six locations near Qingdao from December 2008 to April 2009. This conclusion is based on the phylogenetic analyses, the ribotype network analyses in the LPP clade, and unique features of ramification. These results indicated that *Ulva* strains 2008a, b, c, d, e, f, and g collected from different areas around Qingdao during the bloom represented a unique algal strain with intensive ramifications, different from *Ulva* strains 1a, 1c, 3a, 4, 5, 6a, 6b, 6c, 6d, 7, 8a, and 8b. The latter belong to native *Ulva* species in Qingdao.

Ulva strains 2008 a, b, c, d, e, f, and g with dense branches, as the representative of the dominating *Ulva* strain in the 2008 green tide, were collected from different sites during the summer of 2008. The sequence divergence within the seven samples was between 0% and 0.2% according to the combined ITS nrDNA and *rbc*L sequences (data not given). The low sequence divergence was striking because of the large covering area of the green tide in the Qingdao region. These results confirm a previous study (Jiang et al. 2008) in which the sequence divergences among all free-floating samples collected along the Qingdao

Fig. 4 Phylogenetic tree constructed by neighbor-joining (NJ) method from the analysis of the nuclear encoded internal transcribed spacer DNA (ITS nrDNA) region, including the 5.8S gene, of *Ulva* species. The tree was rooted with *Ulvaria fusca* and *Umbraulva amamiensis*. Bootstrap values (percentage of 1,000 replicates) for neighbor-joining (*above*) and maximum likelihood (*below*) are indicated at nodes. The *numbers under the branches* represent full heuristic bootstrap values (1,000 replicates) greater than 50%. Branch lengths are proportional to the amount of sequence change, which are indicated by the *scale bar below the tree*
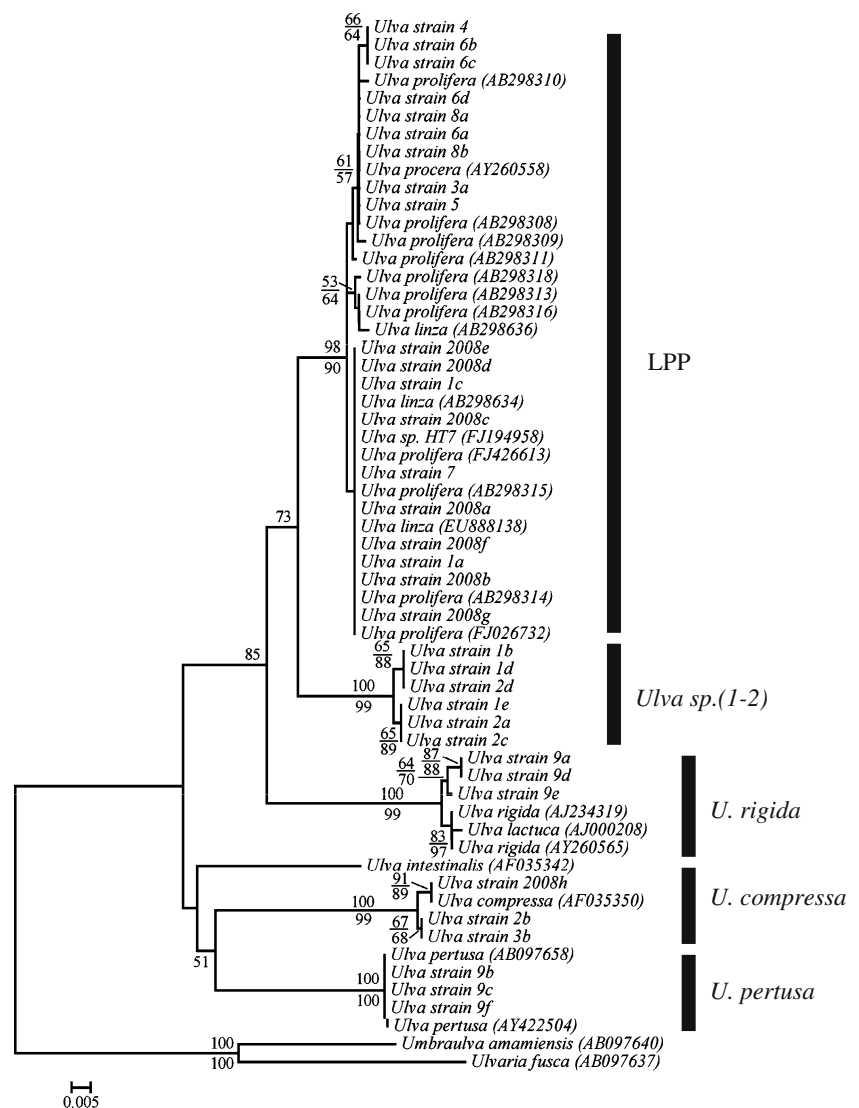

coastline in summer 2007 were between 0% and 2.5% based on the sequencing of the ITS nrDNA including the 5.8S gene. It was reported that data from molecular analyses based on ITS nrDNA showed a clade representing the *U. linza–procera–prolifera* (LPP) complex in the phylogenetic trees (Shimada et al. 2008; Leliaert et al. 2009). We also found that the ITS nrDNA and *rbc*L sequences from the strain of *U. prolifera* in the 2008 green tide were similar to that of *Ulva* strains 1a, 1c, 3a, 4, 5, 6a, 6b, 6c, 6d, 7, 8a, and 8b derived from seawater of Qingdao coasts, with just a few changes of a few bases in the ITS nrDNA sequences. However, a parsimony network analysis of the LPP clade revealed that there were at least two mutational steps between the dominating *Ulva* strain in the 2008 green tide and the native *Ulva* strains 1a, 1c, 3a, 4, 5, 6a, 6b, 6c, 6d, 7, 8a, and 8b. In this analysis, the position of the species forming the Qingdao bloom was fully consistent with that reported by Leliaert et al. (2009). Additionally, it is easy to

distinguish them based on significant differences in the morphological characteristics under identical culture conditions (data not given). The exception was *Ulva* strain 2008h, with few branches and similar to *U. compressa* based on their gross morphology (Tseng et al. 1962). The molecular phylogenetic analysis showed that it was closer to *Ulva* strains 2b, 3b, and *U. compressa* (Figs. 4 and 5).

Species in the genus *Ulva* are famous for their wide tolerance of temperature, salinity, and nutrient levels (Tan et al. 1999; Dan et al. 2002; Cohen and Fong 2006; Conley et al. 2009). Thus, the absence of the dominant 2008 green tide alga in the following winter is surprising, considering that extremely large biomass which was present along the entire coast half a year before the sampling and that large number of gametes or spores were discharged into coastal waters (based on laboratory releasing experiments, data not shown). A logical explanation seems to be that the microscopic stages of the bloom-forming alga were not

Fig. 5 Phylogenetic tree constructed by neighbor-joining (NJ) method from the analysis of the *rbc*L gene sequences of *Ulva* species. The tree was rooted with *Ulvaria fusca* and *Umbraulva amamiensis*. neighbor-joining (*above*) and maximum likelihood (*below*) are indicated at nodes. The *numbers under the branches* represent full heuristic bootstrap values (1,000 replicates) greater than 50%. Branch lengths are proportional to the amount of sequence change, which are indicated by the *scale bar below the tree*
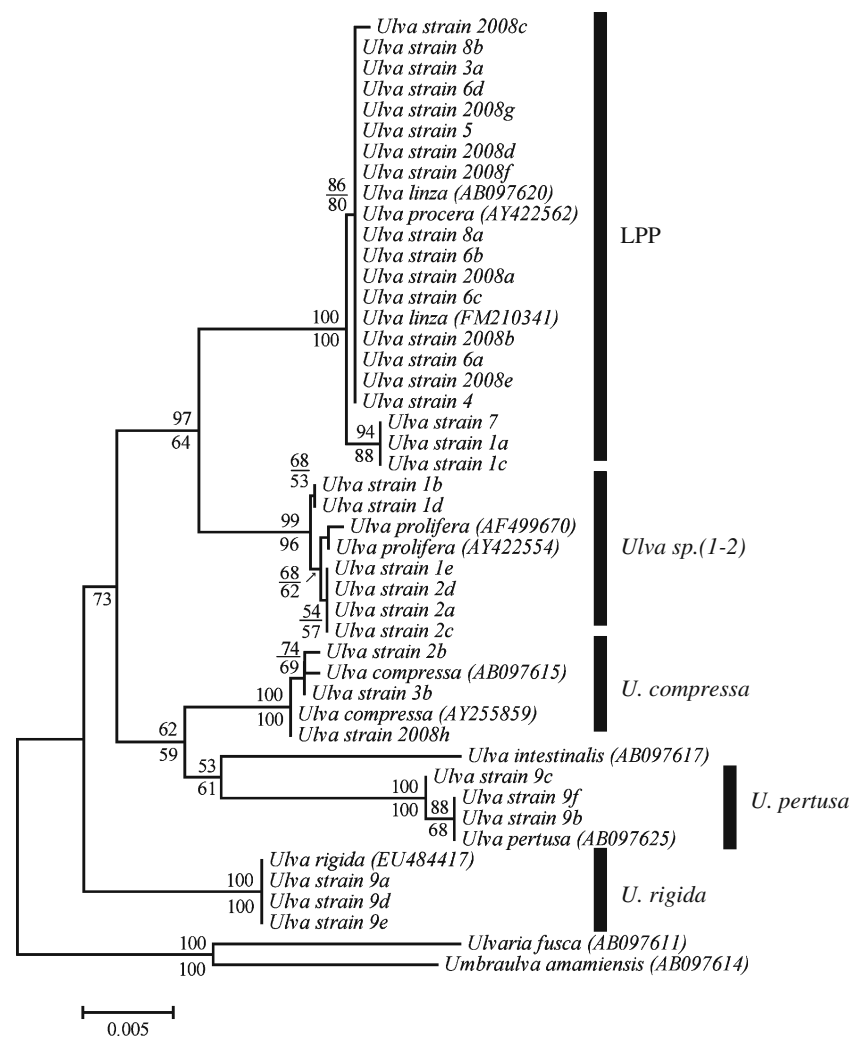

able to survive the winter conditions around Qingdao. Another research team found that the microscopic stages of the dominating 2008 green tide alga (thalli or fragments) were also not detected, as investigated by trawling, along

the Qingdao coasts from November 2008 onwards (Z.L. Wang, personal communication). This conclusion is further strengthened by the fact that significantly reduced photosynthetic activity was found at 5°C in the bloom-forming

Fig. 6 Ribotype network of internal transcribed spacer nrDNA from samples in the *Ulva linza–procera–prolifera* (LPP) clade constructed using TCS 1.21. A *line between ribotypes* and the *small circles* represent one mutational step and ribotypes absent in the network, respectively
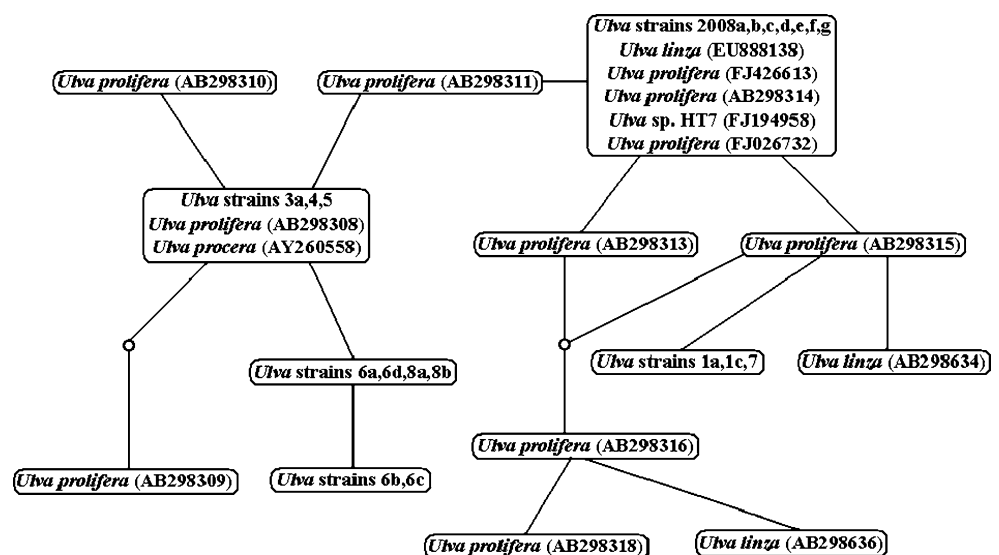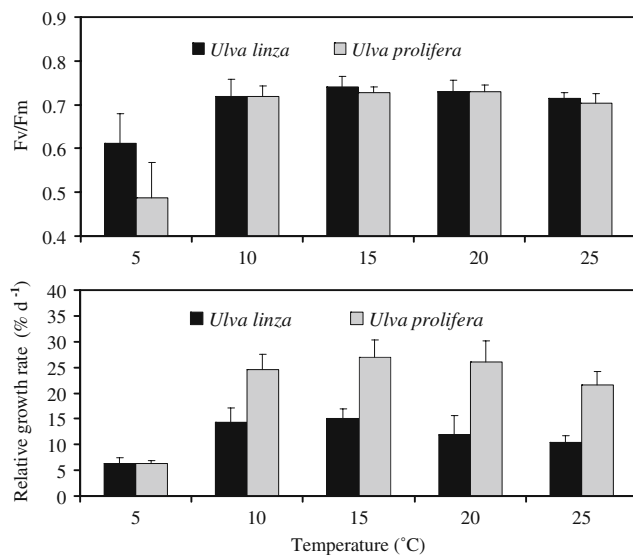
Fig. 7 Comparisons of the relative growth rates and photosynthetic activities between *Ulva linza* and *Ulva prolifera* at different temperatures (5°C, 10°C, 15°C, 20°C, and 25°C)

U. prolifera strain, in comparison to a Qingdao native species, *U. linza*.

Ulva spp. in the coastal waters of Qingdao in the winter after the 2008 bloom

It has been reported that *Ulva* species are morphologically simple but exhibit considerable plasticity in response to environmental factors (Malta et al. 1999; Blomster et al. 1999; Hiraoka et al. 2004; Leskinen et al. 2004). Environmental conditions such as fluctuating salinity, light intensity, and temperature could cause the wide morphological variations and the differences in branching in some *Ulva* species (Blomster et al. 1998). Based on the above, in our experiments, the *Ulva* strains were cultured under the same condition to minimize the morphological changes caused by the environmental factors.

Under the described culture condition in this investigation, *Ulva* strains in groups 1 and 2 were unbranched, ribbon-like, and often had a frilled margin. However, based on molecular data, *Ulva* strains 1a, 1b, 1c, 1d, 1e, 2a, 2c, and 2d were included in two clades: strains 1a and 1c in the LPP clade and strains 1a, 1b, 1c, 1d, 1e, 2a, 2c, and 2d in the *Ulva* sp. (1–2) clade, which indicated that they might belong to two different *Ulva* species. However, further identification is needed. Meanwhile, *Ulva* strains 1a and 1c were identified as *U. linza* (data not given), a littoral and sublittoral species commonly found on rocks or in tide pools around Qingdao (Tseng et al. 1962). *Ulva linza* was found in surprisingly higher numbers in the seawater and rocks along the Qingdao coasts (Figs. 2 and 3), indicating that it could have the potential to form a local small-scale

green tide. In a field survey in April and May 2009, *U. linza* was found to be the most common *Ulva* species along the Qingdao coasts and was often washed ashore and piling up at a small scale (personal observation). According to the phylogenetic trees, *Ulva* strains in group 9 could be separated into two species, *Ulva* strains 9a, 9d, 9e close to *U. rigida* and *Ulva* strains 9b, 9c, 9f close to *U. pertusa*. Further investigations are required on morphological features including cell arrangement, shape, pyrenoid number, etc.

Quantification of the microscopic stages of Ulva spp. in seawater

Field observation showed that the large-scale drifting *Ulva* biomass in the Yellow Sea in 2008 was gradually accumulating from small patches of drifting algae under the influence of winds and surface currents (Sun et al. 2008; Liu et al. 2009). How the sessile plants started to float and where these small patches of drifting biomass came from are two key issues to be addressed. One hypothesis is that the microscopic gametes or zygotes or spores attached to small particles in the water (high turbidity along the coast of Jiangsu province) and developed into a few cells stage. Adult thalli were derived from these microscopic stages in the water under favorable condition in early spring. However, quantification of culturable *Ulva* individuals at the microscopic stages (gametes, zygotes, spores, or microscopic germlings) has been difficult. For biomass forecasting estimation in green tide investigations, quantification of conspicuous adult plants only will underestimate the real biomass that could potentially be the entire bloom, since the microscopic plants could rapidly grow into adult plants when light, nutrients, and temperature levels reach the optimal combination (Hoffmann and Santelices 1991; Santelices et al. 1995; Worm et al. 2001; Zhang et al. 2009). In practice, quantifying the microscopic stages in the water through filtration at 0.45 or 0.22 μm is not reliable because *Ulva* gametes, zygotes, and spores often are confused with microalgal cells. It is also not possible to obtain enough live material for DNA analyses. In this investigation, large numbers of individuals were detected and determined from different sampling locations, indicating the ubiquitous existence of *Ulva* in seawater along the Qingdao coastline. This culture method will help to accurately delineate species structure of *Ulva* populations in the water and thus help understanding, and possibly forecasting, potential blooms in the investigated region.

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