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Tracking the algal origin of the *Ulva* bloom in the Yellow Sea by a combination of molecular, morphological and physiological analyses

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Abstract

In 2008, Qingdao (36°06' N, 120°25' E, P. R. China) experienced the world largest drifting macroalgal bloom composed of the filamentous macroalga *Ulva prolifera*. No convincing biological evidence regarding the algal source is available so far. A series of field collections of both *Ulva* sp. and waters in various sites along Jiangsu coasts were conducted in March to May of 2009. Density of microscopic *Ulva* germlings in the waters sampled from different sites ranged from 7 to 3140 individuals L⁻¹, indicating the wide-spreading and long-term existence of the algae in the investigated region. Morphological and the nuclear ribosomal internal transcribed spacer ITS

nrDNA and the chloroplast-encoded *rbcL* gene comparisons of 26 algal samples revealed that the algae collected from land-based animal aquaculture ponds mostly resembled the dominating blooming alga in 2008. Mismatch of *Porphyra* farming period with the occurrence of the green tide bloom, as well as the negative identification results of the sampled green algae from the *Porphyra* rafts eliminated *Porphyra* rafts as the principal and original source of the dominating blooming alga.

Key words: *Ulva prolifera*; *Porphyra*; green tide; algal bloom; eutrophication; ITS nrDNA, *rbcL*

1. Introduction

Green tides are massive accumulations of unattached green macroalgae, principally belonging to the genus *Ulva*, and are intimately associated with eutrophicated marine environments (Nelson et al., 2008). One of the green tide algae, the filamentous alga *Ulva prolifera*, formerly known as *Enteromorpha prolifera* (Hayden et al., 2003), is broadly distributed along the nearshore coasts of the north-eastern Asia (Tseng, 1983, Shimada et al., 2008). Conspicuous growth of this alga was usually found in environments with sufficient input of nutrients, such as estuaries, from where land-derived nutrient rich effluents are combined and discharged into coastal waters (Leskinen et al., 2004; Conley et al., 2009). This alga can tolerate a wide range of temperatures, salinities and irradiances (Tan et al., 1999; Dan et al., 2002; Cohen and Fong, 2006). From May to July 2008 before the Olympic sailing competition,

45 Qingdao coasts experienced an attack of the world's largest drifting green tide,
46 evaluated at a level of one million tons of harvestable biomass (FW). The bloom once
47 covered approximately 13,000-30,000 km² of the Yellow Sea (Sun et al., 2008). The
48 dominating species was identified as being the filamentous, intensively ramificated *U.*
49 *prolifera* (Müller) J. Agardh (Chlorophyta, Ulvophyceae) (Leliaert et al., 2008, 2009,
50 Sun et al., 2008; Ye et al., 2008). Recent phylogenetic analyses showed that this
51 unique strain forms a clade with representatives of the *Ulva linza-procera-prolifera*
52 (LPP) complex and seems to be ubiquitous in several countries (Leliaert et al., 2009).
53 Field-collected algal samples, as well as those maintained in culture, were both
54 characterized as intensively ramified and demonstrated outstanding capacity of
55 vegetative growth under favorable conditions.

56 Accurate localization of the origin and persistence of this green algal bloom is the
57 first step in understanding this large-scale green tide and finding solutions to the
58 problems it could potentially bring. According to satellite images the drifted biomass
59 initiated offshore of the coasts of Jiangsu province and was transported across the
60 Yellow Sea to Qingdao coasts by seasonal winds and surface currents (Liu et al.,
61 2009). The original "seed" source of the bloom remained unidentified, although
62 hypothesis was recently proposed. Liu et al. (2009) thought that the rapid expansion
63 of *Porphyra* farming along the Jiangsu coasts was the principal cause. It is therefore
64 necessary to analyze the time series of this red alga farming in relation with the green
65 tide event in terms of reproduction and growth of both algae. Simple morphological
66 identification has proven to be insufficient to distinguish species in the genus *Ulva*

because often unattached thalli demonstrate considerable morphological plasticity (Malta et al., 1999). Use of appropriate molecular markers can both identify the algae and provide important information concerning the origins and dynamics of the blooms (Malta et al., 1999; Largo et al., 2004). Nuclear ribosomal internal transcribed spacer ITS nrDNA and the chloroplast-encoded *rbcL* gene sequences were used to combine the previous *Ulva* and *Enteromorpha* into one genus (Hayden et al., 2003) and were popularly used to discern the taxonomic positions of the strains in *Ulva* (Leliaert et al., 2009, Shimada et al., 2008).

The principal objective of this investigation is to use multiple means to source-track the dominating bloom alga. These means include, (1) standard analyses of ITS nrDNA and the chloroplast-encoded *rbcL* gene sequences of the algal samples collected at different coastal sites of Jiangsu province before the bloom and the samples of 2008 Qingdao's bloom; (2) algal morphological comparisons and sporulation (reproduction) tests under different temperature regimes; (3) quantitative determination of culturable *Ulva* microscopic stages in free seawaters and (4) analyses of time cycle of *Porphyra* farming in relation to the occurrence of the green tide.

2. Materials and methods

2.1. Choices and description of sample collection sites

The entire coast of Jiangsu province (30°44'-35°4'N) is characterized by an extended shallow and muddy intertidal zone, constituting an ideal environment for performing *Porphyra* cultivation by use of floating cultivation methods (Shang et al., 2008). The

world's largest cultivation of *Porphyra yezoensis* has been carried out in this province since 1970s. Until today, *Porphyra* farming occupies 21,000 hectares of intertidal area, producing 126,000 tons (FW) annually (P. Xu, personal communication). Parallel to the *Porphyra* farming area along the coasts are animal aquaculture pond systems on land (AAPs) in which *Eriocheir sinensis* (a fresh water crab with larval stage in the marine environment), and *Penaeus vannamei* (a white prawn species introduced from America) are farmed (Fig. 1). *Porphyra yezoensis* is farmed by use of semi and full-floating rafts composed of bamboo and nets on which the conchospores attach and grow into blades in 2-3 months during the cold season from December to March. Young *E. sinensis* (ca. 1 cm) are produced from February to May in seawater in coastal AAPs, locally called "Natural Ecological Ponds" (NEPs), developed from 2001 onwards. The NEP method, because of its low cost and easiness to manage, became rapidly the dominating one to produce young crabs in Jiangsu province and is characterized with intensive application of organic fertilizers. Jiangsu province is thus becoming the largest young crab production centre in China. *P. vannamei* is principally farmed along the northern coast of Jiangsu province from March to July in shallow coastal ponds with water depth ranging from 1 to 1.5 m. Typical coastal aquaculture areas in this province are characterized by *P. yezoensis* cultivation in the intertidal zone and large numbers of land-based AAPs separated by a dam. Waters from AAPs are collectively discharged from a main sluice channel (Fig. 1). Large volumes of water are exchanged frequently between AAPs and the intertidal water during rainfall seasons. Salinity, temperature and nutrient levels in the water of these

ponds fluctuated, thus making the pond a special niche for species that could tolerate, survive and reproduce, such as species in the genus *Ulva*.

Along the coasts of Jiangsu province, open-sea *Porphyra* cultivation starts with the transfer of the seeded nets to the sea each year in November and ends up with the withdrawal of the nets and bamboos from the sea at the end of April (P. Xu, personal communications). Surface water temperature during this period drops from 15 °C to 3 °C and thereafter increases to 14 °C over the winter with slight variations in different areas (Fig. 2). Blades of *Porphyra* grow most significantly during low temperature periods and cover most of the nets. Filamentous green algae are often found to grow vigorously on the nets where *Porphyra* conchospores sparsely attached. In such cases, the nets are often sun-dried for more than 12 h to kill the epiphytic green algae, while *Porphyra* can tolerate such extreme exposure (Y.D. Yu, personal communications).

Considering the above observations, we collected green algal and corresponding water samples from the rafts of *Porphyra* cultivation system, the intertidal zone, the coastal AAPs adjacent to the algal farming area and from the sluice gates of the AAPs along both southern and northern coasts of Jiangsu province in April to May 2009 (Fig. 3).

2.2. Treatments of water and algal samples

In each of the six locations investigated in Jiangsu province (full-floating rafts of *Porphyra* cultivation area, nearshore water and sluice gates of AAPs at Liuwei, coastal pond as well as full-floating rafts of *Porphyra* cultivation area at Xiangshui), 4 L of seawater were collected and mixed thoroughly. Seawater was subsequently

aerated after adding NO_3^- ($823 \mu\text{mol L}^{-1}$) and PO_4^{3-} ($73 \mu\text{mol L}^{-1}$) to reach the levels of Provasoli Enriched Seawater (PES) medium in 1 L glass beakers (2 per sampling location) placed in temperature-controlled rooms at 15-18 °C under 80-100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in a 12 h light per day light-dark regime. The medium was renewed every 7 d in the following culture period. After 3 weeks, the green algal germlings, attaching to the wall and bottom of the glass beakers, grew up to 1-5 cm and were counted and identified by both morphological and molecular analyses as explained below. These germlings were derived from microscopic spores or gametes or those that were attached to particles invisible to the naked eye at the time of sampling. After counting, the algae were removed from the beakers and further grown in suspension culture under the same conditions.

For the field-collected algal samples, the algae were cleaned *in situ* and brought back to the laboratory in cooled box within 24 h. Dominating *Ulva* species in each sample, judged by morphological observations, were individually sorted and cleaned with sterilized seawater and further grown in GeO_2 -added PES medium for a week to remove epiphytic diatoms for further DNA analyses. In all the analyses we performed, no DNA contamination of epiphytic algae was ever observed.

2.3. Algal DNA extraction

The algal samples were washed three times with sterilized seawater, dried with filter paper. Then 100 mg of unialgal material for each sample was ground to fine powder in liquid nitrogen and transferred to a 2-mL tube. 750 μL cetyl trimethyl ammonium bromide (CTAB) buffer including 3% CTAB (w/v), 1.4 M NaCl, 20 mM EDTA, 100

155 mM Tris-HCl (pH 8.0), 1% polyvinyl pyrrolidone (w/v), 1% β -mercaptoethanol (v/v)
156 and 10 $\mu\text{g mL}^{-1}$ RNase A was added to the tube. The mixture was incubated at 65 °C
157 for 60 min with gentle inversion occasionally (Wang et al. 2006). 250 μL of 5 M KAc
158 (pH 8.0) was added to the mixture and kept on ice for 15 min. Centrifugation was
159 followed at 12,000 rpm for 15 min. The upper phase was transferred to a new tube.
160 One volume of chloroform-isoamyl alcohol (24:1) was added and centrifuged at
161 12,000 rpm for 10 min. This step was repeated once more. The aqueous phase was
162 precipitated with one volume of cold isopropanol at -20 °C for 1 h and collected by
163 centrifugation at 12,000 rpm for 10 min. Finally, the precipitate was washed with 75%
164 ethanol, dried and dissolved in double distilled water. The concentration and the
165 quality of isolated DNA were assessed by electrophoresis on 1.0% agarose gel. DNA
166 concentration of each sample was adjusted to 50 $\text{ng } \mu\text{L}^{-1}$.

167 **2.4. ITS nrDNA and *rbcL* gene amplification and sequencing**

168 Polymerase chain reaction (PCR) amplification of ITS nrDNA and *rbcL* gene was as
169 described by Hayden *et al.* (2003). Primers used to amplify and sequence ITS nrDNA
170 and *rbcL* gene were synthesized by Shanghai Sangon Biological Engineering
171 Technology & Service Co., Ltd., with the following sequence:

172 ITS1 (5'-TCTTTGAAACCGTATCGTGA-3'),

173 ITS2 (5'-GCTTATTGATATGCTTAAGTTCAGCGGGT-3'),

174 *rbcL*1 (5'-ATGTCACCACAAACAGAACTAAAGC-3'),

175 *rbcL*2 (5'-AATTCAAATTTAATTTCTTTCC-3').

176 Total genomic DNA (30-40 ng) was added to 50 μL PCR reactions containing final

177 concentrations of 1×PCR buffer (Takara, Japan), 2 mM MgCl₂ (Takara), 0.8 mM
178 dNTPs (Takara), 25 µM of each primer, and 1.6 U Taq Polymerase (Takara).
179 Amplification products were separated by 1.0% agarose gel electrophoresis and
180 fragments of an expected length were cut from the gel and purified by use of a DNA
181 Gel Extraction Kit (Bio Basic Inc., Canada) according to the manufacturer's
182 instructions. ITS nrDNA and *rbcL* gene were sequenced on both strands using ABI
183 3730 XL automated sequencers (Shanghai Biosune Biotechnology Co., Ltd). Each
184 sequencing reaction was repeated twice.

185 **2.5. Phylogenetic analysis**

186 ITS nrDNA and *rbcL* gene were sequenced from 26 algal samples including 20 from
187 2009's field sampling and 6 from 2008's bloom in Qingdao. Additional 15 ITS
188 nrDNA and 10 *rbcL* sequences were downloaded from the GenBank. The ITS nrDNA
189 and *rbcL* sequences were aligned for phylogenetic analyses using Clustal W
190 (Thompson et al., 1994). For the *rbcL* sequence data set, the alignment was
191 unambiguous. But the ITS nrDNA alignment contained many insertions and deletions.
192 The combined data set contained the ITS nrDNA and *rbcL* sequences (Hayden et al.
193 2003). Prior to analysis of the combined data, the incongruence length difference test
194 was conducted. The phylogenetic trees were constructed by neighbor-joining (NJ)
195 method using the program Mega 4.0 (Jiang et al., 2008). *Blidingia* sp., collected from
196 rafts and sluice gate from Liuwei, served as an outgroup taxon. The bootstrap
197 consensus tree inferred from 1000 replicates is taken to represent the evolutionary
198 history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions

reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

2.6. Sporulation tests

Unialgal culture (individual plant) isolated from Qingdao's bloom was used throughout the test. In addition, a filamentous strain isolated from Jiangsu coast was used in the temperature tests for comparison. The filamentous algal material was cut into short fragments (2-3cm). Ten fragments were selected and put into each well (5-7 ml) of a multi-well culture plates filled with PES. A series of temperature (5, 10, 12, 15, 18 °C) and salinities (30, 24 and 18) were tested. For the salinity test, all cultures were maintained at 18 °C. All cultures were exposed to $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in a 12 h light per day light-dark regime. For each testing condition, sporulation rate was calculated as the averaged percentage of total sporulated fragments as identified by microscopic checking on day 6 (n=6, totally 60 fragments were checked and counted in each condition). The cultures were performed in six GXZ-260C light and temperature-controlled photo-incubators (Ningbo Jiangnan Instrument, China) with temperature deviation less than 0.5 °C.

221 3. Results

222 3.1. Phylogenetic analysis of the algal samples

223 Analyzed together with control sequences downloaded from the Genbank, the more
224 conserved *rbcL* gene sequences (1288 bp in length) revealed that the dominating algal
225 samples collected from 11 AAPs, and one sample derived from coastal water, all
226 tubular and filamentous with ramifications, were in the same clade as all the six
227 unialgal cultures isolated from the Qingdao 2008 bloom (Fig. 4; 2008 a-f). Three
228 dominating algal samples from *Porphyra* rafts and one from intertidal rocks, all
229 ribbon in structure, were closely forming two adjacent clades. One algal sample from
230 *Porphyra* rafts collected from Neisha (filamentous with ramifications) was in the
231 fourth clade together with *U. prolifera* (AF499670) and *U. prolifera* (AY422554).
232 One filamentous algal sample from Waisha was classified in the same clade as *U.*
233 *compressa* (AY255859) and *U. compressa* (AB097615). Two fine filamentous algal
234 samples, one from *Porphyra* rafts and one from the pond sluice gate, respectively,
235 were identified as *Blidingia minima* var. *minima* (according to the ITS nrDNA
236 sequences below) and were treated therefore as an outgroup.

237 The ITS nrDNA sequence analyses showed that 12 algal samples - one from
238 intertidal rocks (filamentous), two from *Porphyra* rafts (one filamentous, one
239 ribbon-like), seven from AAPs, one from coastal water (obtained from microscopic
240 germings after 3 week culture in the lab) at Liuwei and one from AAPs at Xiangshui
241 were closely related to the Qingdao algal bloom samples (filamentous) (Fig. 5; 2008
242 a-e). Samples isolated from *Porphyra* rafts at Neisha, Waisha and some of the AAPs

at Liuwei were in the third clade. Two samples from AAPs at Xiangshui were in the fourth clade together with *U. compressa* (AF035350). Two dominating filamentous algal samples, isolated from *Porphyra* rafts and from the sluice gate, respectively, were identified as belonging to the genus *Blidingia* and were treated as an outgroup, together with *B. minima* (AJ000206).

Combined ITS nrDNA and *rbcL* sequence analyses revealed identical results, in which six filamentous algal samples from AAPs, and one coastal water-derived filamentous sample, were closely related to the dominating Qingdao algal bloom in 2008 (Fig. 6). Other filamentous algal samples joined together in other clades.

3.2. Morphological observations

Green algae including *Cladophora* sp., *Blidingia* sp. and *Ulva* sp. (ribbon and filamentous in structures) were found in the dominating algal samples collected from the full and semi-floating *Porphyra* rafts at six cultivation locations including Xishu (full-floating), Liuwei (semi-floating), Xiangshui (full-floating), Waisha (full-floating) and Neisha (semi-floating). *Ulva* sp. could be easily distinguished under the microscope, while *Blidingia* sp. needs molecular data to be identified. While in most of the investigated AAPs, usually only one dominating *Ulva* species existed as can be judged by the morphology (Fig. 7), probably determined by the unique water environment in those ponds in comparison with the apparent versatile environment on the rafts in the surface water in intertidal zone.

3.3. Microscopic germlings in the water

Filamentous *Ulva* were obtained from microscopic germlings in the waters from all six investigated locations. Density varied from less than 7 to 3140 individuals L⁻¹ (Fig. 8). Higher densities were detected at low tide in Liuwei in nearshore waters. In the relatively clear water sampled from a representative AAPs, the density of the germlings was 500 individuals L⁻¹. A surprisingly low number was recorded at the sluice gates where the effluents from the AAPs were collectively discharged into coastal waters. These results showed the universal existence of the microscopic stages of *Ulva* sp. in all the sampling sites investigated.

3.4. Sporulation tests in the isolated fragments of the dominating *Ulva* sp. of the bloom

In culture conditions, low salinities and higher temperature facilitated sporulation in isolated filamentous *Ulva* fragments (Fig. 9 A, B). At temperatures of 12°C, sporulations were sparsely spotted in the tested fragments, indicating that low temperature greatly hampered the production of the gametes. No significant difference was found in the two dominating filamentous *Ulva* species in the temperature test.

4. Discussion

In elucidating the “seed” source of the largest drifting macroalgal bloom so far observed, data obtained in this investigation points to three important aspects: (1) both morphological and molecular evidences showed that the filamentous *Ulva* sp. ubiquitously living year-round in the AAPs along the Jiangsu coasts mostly resemble the dominating algal species isolated in the 2008 Qingdao green tide event, in

comparison to those isolated from *Porphyra* rafts. AAPs, at the scale of thousands of hectares along the coasts of Jiangsu province, are characterized by higher light penetration, nutrient levels after being artificially fertilized to enrich the pond water and higher temperature in early summer. Thus they constitute the most efficient year-round *Ulva* nurturing niche to sustain this tolerant green alga; (2) the green algal species attached on *Porphyra* rafts were diverse and came originally from the microscopic stages of the algae that were free-living in the water along the entire coast of Jiangsu province. Therefore *Porphyra* raft itself could not become the principal and original source of the blooming alga; and (3) high levels of nutrients derived from AAPs and land-based effluents discharged in the coastal waters of Jiangsu province, in combination with the year-round existence of the green tide forming algae in thousands of hectares of AAPs are potentially constituting two most important triggering factors of the recurrent spring green tides in the Yellow Sea.

In tracking the original source of the 2008 Qingdao bloom, Liu et al. (2009) hypothesized that thousand hectares of *Porphyra* rafts in the intertidal zone along Jiangsu province were the original source of the algae. This was supported by the recent rapid expansion of *Porphyra* farming along the coast of blooming area. However, the authors did not provide biological identification data. Bamboos with attached green algae were found several times in the algal biomass accumulated in Qingdao in 2008, bringing people's attention to the *Porphyra* raft systems. Jiang et al. (2008) pointed out that the dominating alga found in the drifting biomass in Qingdao was not locally derived based on the ITS phylogenetic analysis. In the present

investigation it was shown that *Porphyra* rafts did support the growth of diverse green algae, but with few identical to the dominating alga of the Qingdao's bloom. Free-living microscopic stages of *Ulva* sp. isolated from the costal waters of Liuwei, which were later identified to be very close to the dominating alga in the 2008 Qingdao bloom, were found to be in the waters when the temperature was only 5 °C and there were small amounts of green algae to be spotted at that time on the *Porphyra* rafts (personal observations). High density of culture-derived seedlings of *Ulva* sp. in Jiangsu coastal waters strongly supported the year-round existence of the algae in the water, even when the *Porphyra* cultivation raft systems are not in place. The key issue is where the large amount of the algae in their microscopic stages originated. *Porphyra* cultivation has been carried out for more than 30 years, while large-scale blooms of *Ulva* in the Yellow Sea are only a recent phenomenon in the past three years.

The data of this investigation support the view that AAPs along the Jiangsu province coast are the principal source of the green tide algae. Both morphological identifications and gene sequence data confirmed this point. Accurate identification of *Ulva* species has been puzzling scientists for a long time. Independent analyses of either morphology or gene sequence comparison alone had been shown to be insufficient. A combination of both approaches can lead to reliable identification of the morphotypes in nearly all the cases (Hayden et al., 2003). The dominating alga in the 2008 Qingdao's bloom is filamentous, tubular in structure and characterized with intensive ramifications (Sun et al., 2008). The ITS nrDNA and *rbcL* sequences were

highly uniform in all the samples collected from the Yellow Sea (Ye et al., 2008, Jiang et al., 2008). In this investigation, similar filamentous *Ulva*, with few or intensive ramifications were found in nearly all observed AAPs and were closely related with the 2008 Qingdao bloom in ITS nrDNA and *rbcL* gene sequences. Furthermore, AAPs are operating year-round and, thus constantly contributing to coastal waters the microscopic stages of *Ulva* as well as the thallus fragments. While fragmented thalli tend to undergo sporulation rapidly, releasing even more spores in coastal waters, especially under elevated light and temperature regimes (Lüning et al., 2008; this observation). Spore contribution could be accelerated during early spring when temperature and light levels start to increase. Frequent and efficient sporulation secures a rapid and wide spreading of gametes or spores in the water. The longevity of the gametes or spores that were attached to the muddy particles remains to be resolved in future work. The propagule-attached particles might accumulate in the coastal waters of Jiangsu province over time, thus forming the potential base for future blooming biomass when light, temperature, nutrient and current conditions coincide.

It is worth noting is that filamentous *Ulva* were ubiquitous in nearly all observed AAPs we visited with the only exceptions being those treated with so called “*Ulva*-killer” (Prometryn,; 2,4-Bio(isopropylamino)-6-methylthio-s-triazine). This chemical was used in combination with sand and applied in ponds to block the sessile *Ulva* on the pond bottom. In pond farming, *Ulva* has to be removed periodically to prevent the competition with microalgae for nutrients. An appropriate amount of the latter in the water (locally called “colored water”) is thought to be beneficial for the

353 healthy growth of shrimps or crabs. How the wide application of this chemical affects
354 the balance between the floating *Ulva* biomass and the microalgae populations in the
355 coastal waters (both are nutrient scrubbers) in Jiangsu province remains to be
356 resolved.

357 In summary, this investigation has shown that the *Ulva* biomass in year-round
358 operated AAPs along the Jiangsu province coasts constitutes a potential, principal
359 source of the bloom-forming alga in the adjacent Yellow Sea. How the seasonal
360 climatic parameters, surface water currents, intensive nutrient applications are all
361 converging to prepare the conditions triggering the development of large floating
362 green tide biomass along the coast needs to be comprehensively investigated both in
363 the field and in the laboratory. Results of such investigation will be applicable within
364 the wider perspective of integrated coastal zone management to hopefully find
365 biomitigative solutions to what appears to be a significant and recurrent ecological
366 problem, not only in the Qingdao region but also in other places throughout the world.

367

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448

449 Legends of the figures

450

451 **Fig.1.** Satellite image of a typical coastal area in Jiangsu province. The seasonal
452 *Porphyra* farming area (crosses) is located in the intertidal zone. A long dam divides
453 the seaweed farming area from the year-round pond-based animal aquaculture system
454 (stars). Waters from the ponds collectively discharge into the coastal water through a
455 principal sluice gate (arrow).

456 **Fig.2.** Changes in surface water temperatures during the *Porphyra* farming period at
457 Lianyungang, Yancheng and Nantong in Jiangsu province (culture period in between
458 vertical dashed lines). The horizontal line represents the period with temperatures
459 below 12 °C during which both growth and sporulation of *Ulva* spp. slows down
460 dramatically.

461 **Fig.3.** Sampling locations (Xishu, Liuwei, Xiangshui, Waisha and Neisha) along the
462 coasts of Jiangsu province. The accumulation of large amount of drifted *Ulva* biomass

463 in Qingdao in 2008 is indicated by the area circled with a dashed line in which we had
464 another sampling location (Shilaoren).

465 **Fig.4.** Neighbor-joining (NJ) tree constructed from the analysis of *rbcL* gene
466 sequences of collected algal samples along Jiangsu coasts and those downloaded from
467 Genebank for comparison. The tree was rooted with *Blidingia* sp. 1 collected from
468 *Porphyra* rafts and *Blidingia* sp. 2 collected from a sluice gate at Liuwei, respectively.
469 The numbers under the branches represent full heuristic bootstrap values (1000
470 replicates) greater than 50%. Branch lengths are proportional to the amount of
471 sequence change, which are indicated by the scale bar below the tree (in the
472 parentheses, * refer to the samples that were thin filamentous with ramifications; R
473 refers to ribbon in morphology).

474 **Fig. 5.** Neighbor-joining (NJ) tree constructed from the analysis of the nuclear
475 encoded internal transcribed spacer DNA (ITS nrDNA) region, including the 5.8S
476 gene, of collected algal samples along Jiangsu coasts and those downloaded from
477 Genebank for comparison. The tree was rooted with *Blidingia* sp. 1 collected from
478 *Porphyra* rafts and *Blidingia* sp. 2 collected from a sluice gate at Liuwei, respectively.
479 The numbers under the branches represent full heuristic bootstrap values (1000
480 replicates) greater than 50%. Branch lengths are proportional to the amount of
481 sequence change, which are indicated by the scale bar below the tree (in the
482 parentheses, * refer to the samples that were thin filamentous with ramifications; R
483 refers to ribbon in morphology).

484 **Fig.6.** Neighbor-joining (NJ) tree constructed from the analysis of combined ITS

nrDNA and *rbcL* sequences. The tree was rooted with *Blidingia* sp. 1 collected from *Porphyra* rafts and *Blidingia* sp. 2 collected from a sluice gate at Liuwei, respectively. The numbers under the branches represent full heuristic bootstrap values (1000 replicates) greater than 50%. Branch lengths are proportional to the amount of sequence change, which are indicated by the scale bar below the tree (in the parentheses, * refer to the samples that were thin filamentous with ramifications; R refers to ribbon in morphology).

Fig. 7. Photographs of the filamentous algal samples collected from the Qingdao 2008's bloom (A, B), Liuwei (C, D), Waisha (E, F) and Xiangshui (G, H), Jiangsu province. They show the typical tubular and filamentous structure with lateral ramifications.

Fig. 8. Density of microscopic stages of *Ulva* spp. in seawater collected from six locations, including *Porphyra* full-floating rafts in Xishu, *Porphyra* semi-floating rafts in Liuwei, neashore waters at Liuwei, a sluice gate at Liuwei, a coastal pond in Xiangshui and *Porphyra* full-floating rafts in Xiangshui.

Fig. 9. Sporulation (percentages of reproduction) of fragments of the dominating alga in the 2008 Qingdao's bloom under different salinities (A) and temperatures (B). In B, JSFU refers to Jiangsu filamentous *Ulva* for comparison; QDFU refers to Qingdao filamentous *Ulva*.

Fig.1

Fig. 3

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