



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

Shao Jun Pang^{a,*}, Feng Liu^{a,b}, Ti Feng Shan^a, Na Xu^{a,b}, Zhi Huai Zhang^a,
Su Qin Gao^a, Thierry Chopin^c, Song Sun^a

^a Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China

^b Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

^c University of New Brunswick, P.O. Box 5050, Saint John, NB E2L 4L5, Canada

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ABSTRACT

In 2008, Qingdao (36°06'N, 120°25'E, PR China) experienced the world largest drifting macroalgal bloom composed of the filamentous macroalga *Ulva prolifera*. No convincing biologic 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 algae 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 algae.

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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, Qingdao coasts experienced an attack of the world's largest drifting green tide, evaluated at a level of one million tons of harvestable biomass (FW). The bloom once covered approximately 13,000–30,000 km² of the Yellow Sea (Sun et al., 2008). The dominating

species was identified as being the filamentous, intensively ramified *U. prolifera* (Müller) J. Agardh (Chlorophyta, Ulvophyceae) (Leliaert et al., 2008, 2009; Sun et al., 2008; Ye et al., 2008). Recent phylogenetic analyses showed that this unique strain forms a clade with representatives of the *Ulva linza-procera-prolifera* (LPP) complex and seems to be ubiquitous in several countries (Leliaert et al., 2009). Field-collected algal samples, as well as those maintained in culture, were both characterized with intensive ramification and demonstrated outstanding capacity of vegetative growth under favorable conditions.

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

* Corresponding author. Tel./fax: +86 532 82898831.

E-mail address: sjpang@ms.qdio.ac.cn (S.J. Pang).

URL: <http://www.mbccc.ac.cn> (S.J. Pang).

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-float-

ing 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 center 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–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 ponds as well as full-

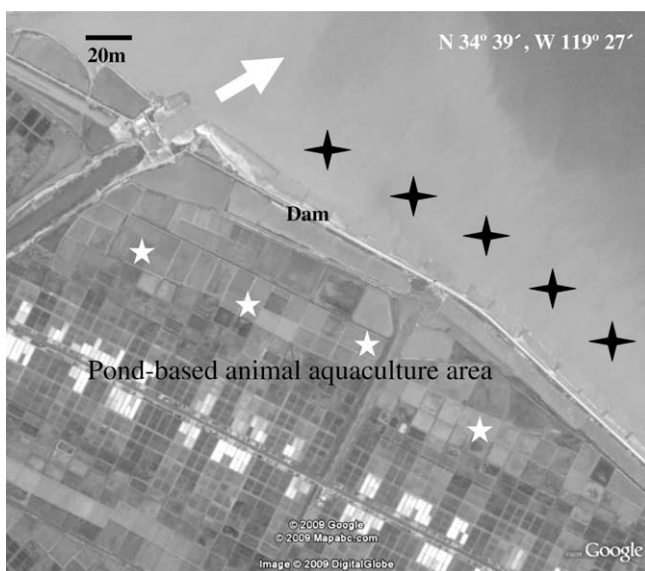


Fig. 1. Satellite image of a typical coastal area in Jiangsu province. The seasonal *Porphyra* farming area (crosses) is located in the intertidal zone. A long dam divides the seaweed farming area from the year-round pond-based animal aquaculture system (stars). Waters from the ponds collectively discharge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

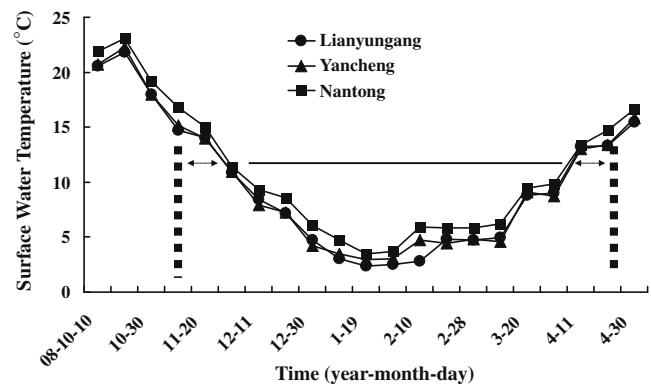


Fig. 2. Changes in surface water temperatures during the *Porphyra* farming period at Lianyungang, Yancheng and Nantong in Jiangsu province (culture period in between vertical dashed lines). The horizontal line represents the period with temperatures below 12 °C during which both growth and sporulation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

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