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Review

Genetic evidence in tracking the origin of *Ulva prolifera* blooms in the Yellow Sea, China

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ABSTRACT

Recurrent green tides have been recorded in the Yellow Sea for 11 consecutive years. The origin of floating green algae in the Yellow Sea, however, remains a subject of debate. Previous studies suggest that the major bloom-forming green alga *Ulva prolifera* represent a unique ecotype different from other attached populations of *U. prolifera* in China. In this study, 97 green algal samples collected during the 2012 green-tide event and from other locations along the coastline of China were analyzed. Based on the sequences of nuclear ribosomal RNA gene (rDNA) internal transcribed spacer (ITS) region and 5S rDNA spacer region, the green alga *U. prolifera* in the samples were identified. The intraspecific genetic diversity within *U. prolifera* was then examined using sequences of 5S rDNA spacer and a marker of sequence characterized amplified region (SCAR) highly specific for bloom-forming *U. prolifera* in the Yellow Sea. The screening results for SCAR marker demonstrated that *U. prolifera* attached to aquaculture rafts in Subei Shoal belong to the same ecotype of the bloom-forming *U. prolifera* in the Yellow Sea. These findings offer genetic evidence that aquaculture rafts in Subei Shoal are a major source of floating green algae in the Yellow Sea.

1. Introduction

Massive blooms of green algae, generally termed as green tides, started to appear in the Yellow Sea from the summer of 2007, and greatly impacted aquaculture, tourism, and marine ecosystems (Ding et al., 2009; Liu et al., 2010a; Ye et al., 2011; Zhang et al., 2008). The green tide in the Yellow Sea in 2008 was reported to be the world's largest macroalgal bloom ever recorded (Liu et al., 2010a, 2009; Ye et al., 2011). According to the statistics from State Oceanic Administration of China, green tides in the Yellow Sea usually start from the end of April, and last for around 2 months till the middle of July. The sea area affected by green tides is approximately 10–30 thousand square kilometers, and the biomass of green algae could reach millions of tons each year (Liu et al., 2013a, 2015).

The formation of massive green tides in the Yellow Sea are closely related to the appearance and rapid growth of the floating green algae, and to find out the origin of floating green algae is a key process to effectively control green tides. The green tides in the Yellow Sea have been shown not to originate from shorelines of Shandong peninsula (Jiang et al., 2008). Based on the data of satellite remote-sensing and numerical simulations, it can be found that floating patches of green algae always appear in the coastal waters of Subei Shoal prior to the formation of large-scale green tides in the southern Yellow Sea (Hu et al., 2010; Liu et al., 2010a; Liu et al., 2009; Qiao et al., 2009, 2011). Due to the high concentration of nutrients and shallow water depth, Subei Shoal has become the largest cultivation base of red alga *Pyropia yezoensis* (basionym *Porphyra yezoensis*) in China (Li, 2011). Field investigations found large amounts of green algae attached to aquaculture rafts of *Pyropia* in this area. The attached green algae are often removed from the aquaculture rafts and discarded on the mudflat, which is likely to be the major origin of green tides in the Yellow Sea (Fan et al., 2015; Keesing et al., 2011; Liu et al., 2010a, 2009; Wang et al., 2015; Zhang et al., 2014a, b). However, some surveys have proposed rival hypotheses as to whether floating green algae come from animal culture ponds or arise by “floating germination” of microscopic propagules of green algae in the coastal waters (Liu et al., 2012; Pang et al., 2010). As

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Table 1
A list of green algal samples used in the study.

Sample type	Sample description	Total number of samples	Sample ID	Sampling location	Sampling date
1	Green algae attached to culture rafts of *Porphyra yezoensis*	24	1X-1, -2	Xiaoyangkou, Subei Shoal	31st March, 2012
			1X-3, -4	Xiaoyangkou, Subei Shoal	12th April, 2012
			1X-5, -6	Xiaoyangkou, Subei Shoal	27th April, 2012
			1X-7, -8	Xiaoyangkou, Subei Shoal	11th May, 2012
			1X-9	Xiaoyangkou, Subei Shoal	29th May, 2012
			1G-1, -2	Gaoni, Subei Shoal	29th March, 2012
			1G-3, -4	Gaoni, Subei Shoal	18th April, 2012
			1G-5, -6	Gaoni, Subei Shoal	27th April, 2012
			1G-7, -8	Gaoni, Subei Shoal	20th May, 2012
			1N-1, -2	Niluosha, Subei Shoal	28th March, 2012
			1N-3, -4	Niluosha, Subei Shoal	17th April, 2012
			1N-5	Niluosha, Subei Shoal	2nd May, 2012
			1N-6, -7	Niluosha, Subei Shoal	20th May, 2012
2	Green algae attached to new culture rafts of *Porphyra yezoensis* in a new culture season	6	2X-1, -2	Xiaoyangkou, Subei Shoal	25th October, 2012
			2G-1, -2	Gaoni, Subei Shoal	24th October, 2012
			2N-1, -2	Niluosha, Subei Shoal	23rd October, 2012
3	Discarded green algae on mudflats	13	3X-1, -2	Xiaoyangkou, Subei Shoal	16th May, 2012
			3X-3, -4	Xiaoyangkou, Subei Shoal	29th May, 2012
			3G-1, -2, -3	Gaoni, Subei Shoal	28th May, 3rd, 4th June, 2012
			3N-1, -2, -3	Niluosha, Subei Shoal	17th, 20th May, 4th June, 2012
			3E-1, -2, -3	Northern part of Subei Shoal	16th April, 3rd May, 5th June, 2012
4	Green algae grown from microscopic propagules in seawater from Subei Shoal	7	4X-1, -2	Xiaoyangkou, Subei Shoal	21st July, 2nd September, 2012
			4G-1	Gaoni, Subei Shoal	3rd April, 2012
			4N-1, -2, -3, -4	Niluosha, Subei Shoal	3rd April, 21stJuly, 21stJuly, 2nd September, 2012
5	Floating green algae in the Yellow Sea	22	5gg2, 5gg5, 5gg7	Floating in the Yellow Sea	25th May, 2012
			5gf7,5gf5, 5gf3,		26th May, 2012
			5ge1, 5ge4, 5ge10		27th May, 2012
			5gd7, 5gd5, 5gd4, 5gd3, 5gd2, 5gd1		28th May, 2012
			5gc1, 5gc2		29th May, 2012
			5gg9		31st May, 2012
			5gd10		3rd June, 2012
			5gc3, 5gc4		4th June, 2012
			5gc5		5th June, 2012
6	Green algae from different locations along the coast of China	25	6YWG-1, -2, -3	Yanwei Harbor, Jiangsu province	December, 2012
			6ZS-1, -2, -3	Zhoushan Island, Zhejiang province	December, 2012
			6ZS-4, -5, -6	Zhoushan Island, Zhejiang province	November, 2013
			6XM-1, -2, -3	Xiamen, Fujian province	August, 2013
			6QZ-1, -2, -3	Qinzhou, Guangxi province	May, 2013
			6XSG-1, -2, -3	Xiangshan Bay, Zhejiang province	November, 2013
			6XSG-4, -5, -6, -7	Xiangshan Bay, Zhejiang province	May, 2014
			6ST-1, -2, -3	Shantou, Guangdong province	January, 2014

a consequence, the origin of floating green algae in Subei Shoal is still an issue under debate, making it hard to take effective mitigation measures.

The major bloom-forming species in the Yellow Sea has been identified as *Ulva* (*Enteromorpha*) *prolifera* based on the morphological features and sequences of ribosomal rRNA gene spacers (Ding et al., 2009; Jiang et al., 2008; Leliaert et al., 2009; Liu et al., 2009; Zhang et al., 2008). Species within genus *Ulva* often exhibit intraspecific variation of morphological features, and some cryptic diversity and cryptic species have been identified (Flagella et al., 2010; Guidone and Thornber, 2013; Hofmann et al., 2010; Kraft et al., 2010). Recently, studies on different populations of green alga *U. prolifera* in China suggested that intraspecific genetic diversity exist within the species (Han et al., 2013; Huo et al., 2013; Liu et al., 2013c; Zhang et al., 2014b; Zhao et al., 2011). According to the results of Zhao et al. (2011) and Zhang et al. (2014b) who used molecular biological methods like inter-simple sequence repeat (ISSR) fingerprints and expressed sequence tag-simple sequence repeat (EST-SSR), the bloom-forming *U. prolifera* in the Yellow Sea is genetically homogeneous, but different from other attached *U. prolifera* populations collected along the coastline of China. A PCR-based marker of sequence characterized amplified region (SCAR) was then designed to discriminate the bloom-forming *U. prolifera* from other populations (Zhao et al., 2015). Nearly all the specimens of *U. prolifera* collected during green tides from 2007 to 2013 in the Yellow Sea were positively identified with the SCAR marker, while the attached specimens of *U. prolifera* collected along the coastline of China exhibited negative results (Zhao et al., 2015). Those results collectively indicated that the bloom-forming *U. prolifera* in the Yellow Sea may represent a unique ecotype different from other attached populations in China. In those studies, however, no attached samples of *U. prolifera* collected from Subei Shoal were involved. Although huge amounts of *U. prolifera* were found attached to aquaculture rafts of *Pyropia* in spring prior to the massive occurrence of green tides, it's not clear whether they belong to the same ecotype as the bloom-forming *U. prolifera* or not. In another study based on the diversity of 5S ribosomal rRNA gene (5S rDNA) spacer region, Huo et al. (2013)