Estuarine, Coastal and Shelf Science 163 (2015) 112–119

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Application of a fluorescence *in situ* hybridization (FISH) method to study green tides in the Yellow Sea

Qing-Chun Zhang [a], Qing Liu [a, b, 1], Ren-Cheng Yu [a, *], Zhen-Jun Kang [a, b], Wei Guo [a, b], Yue-Min Ding [a, b], Jian-Hua Chen [a, b], Yun-Feng Wang [a], Yan Li [c], Tian Yan [a], Ming-Jiang Zhou [a]

[a] Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Key Laboratory of Science and Engineering for Marine Ecology and Environment, State Oceanic Administration, Qingdao 266061, China

ARTICLE INFO

Article history:
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
green tide
Ulva prolifera
fluorescence *in situ* hybridization (FISH)
Yellow Sea

ABSTRACT

Massive green tides of *Ulva prolifera* have been recorded consecutively since 2007 in the Yellow Sea (YS). It has been proposed that the floating green algae in the YS are originally from the culture rafts of *Porphyra yezoensis* in the Subei Shoal. However, there is still much debate about this, mainly due to the difficulty in rapid and accurate identification of *U. prolifera*. In this study, a developed fluorescence *in situ* hybridization (FISH) method was adopted to identify *U. prolifera* and assess its relative abundance in the green algal community. Using this method, several processes related to the formation of green tides were studied, including: (1) variation of the relative abundance of *U. prolifera* in the green algal community attached to *Porphyra* rafts in the Subei Shoal; (2) contribution of the microscopic propagules in seawater to the *U. prolifera* population attached to the rafts; and (3) variation of the proportion of *U. prolifera* in the floating green algae in the YS. *U. prolifera* were detected in the green algae attached to *Porphyra* rafts from March to May 2012, where its relative abundance increased rapidly from 10% at the end of April to 40–60% in mid-May. Microscopic propagules of *U. prolifera*, which could be detected from seawater and sediment, contributed significantly to the dramatic increase of the attached *U. prolifera* on the *Porphyra* rafts. After the attached green algae were removed from the rafts, *U. prolifera* rapidly demonstrated dominance in the floating green algal community, and the proportion of *U. prolifera* increased gradually from south to north. Our conceptual model is that the germination of *U. prolifera* microscopic propagules on *Porphyra* rafts promotes the proliferation of attached *U. prolifera* on the rafts, which release more microscopic propagules into seawater and sediments after they became mature. This positive feedback enhances the dominance of *U. prolifera* in the attached green algal community in late May, which leads to the formation of green tides after the attached plants are removed from the rafts during *Porphyra* harvest. The proportion of *U. prolifera* then increases rapidly in the resuspended and floating green algal community due to its unique structure, making it the most dominant species of floating green algae. The application of the FISH method clearly depicted the early development of green tides in the Subei Shoal and greatly helped to solidify the proposed linkage between *Porphyra* culture rafts and the formation of green tides in the YS.

1. Introduction

The excessive growth of some green algal species, such as those in the genus *Ulva*, *Chaetomorpha* and *Cladophora*, can form macroalgal blooms, which are referred to as “green tides” (Hiraoka et al., 2004b). The number of recorded green-tide events has increased significantly in an extensive geographic range throughout the world (Ye et al., 2011), including North America, South America, Europe, Australia and some Asian countries, such as Japan and the Philippines (Fletcher, 1996; Largo et al., 2004; Hiraoka et al., 2004a; Morand and Merceron, 2005; Merceron et al., 2007; Nelson et al., 2008). In 2007, a large-scale green tide of *Ulva prolifera* appeared in the Yellow Sea (YS) and reached the coastline of Shandong province. In the following years, green tides occurred each summer in the YS and led to dramatic impacts on tourism, mariculture, and the natural ecosystem.

* Corresponding author.
E-mail address: rcyu@qdio.ac.cn (R.-C. Yu).
[1] Equal contribution to this manuscript as the first author.

http://dx.doi.org/10.1016/j.ecss.2014.09.005
0272-7714/

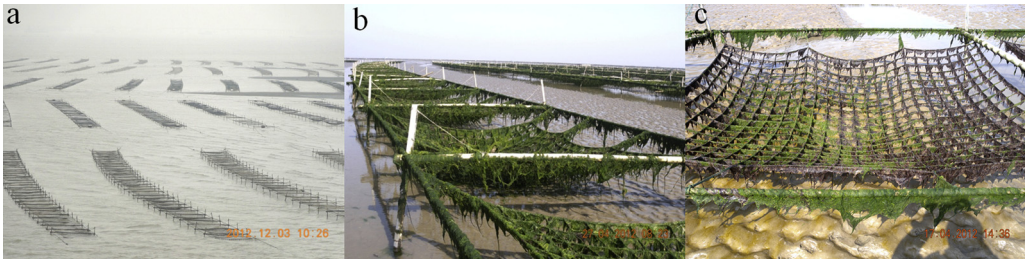

Fig. 1. *Porphyra* aquaculture rafts in the Subei Shoal (a: *Porphyra* aquaculture rafts; b: rafts made of bamboo poles and ropes; c: net curtain used for *Porphyra* culture).

Green tides in the YS have certain unique characteristics. They last for a long period of time (approximately 1–2 months) and generally occur from mid-June to the end of July. The affected area in the YS was massive, covering an area of approximately 10–30 thousand square kilometers. Large-scale green tides have been recorded along the coast of Shandong province each spring-summer. Based on the moderate resolution imaging spectroradiometer (MODIS) data and numerical simulation results, however, it was determined that the floating green algae were transported a great distance from the coastal waters of the Subei Shoal in the Jiangsu province to the coastal area of Shandong province (Hu et al., 2010; Qiao et al., 2009, 2011).

Red alga *Porphyra yezoensis* has been cultured as a type of sea vegetable for a long time in some Asian countries, such as China and Japan. The Subei Shoal, which covers a mudflat zone approximately 22,000 km^2 from the Sheyang River estuary to the Changjiang River estuary along the coast of the southern YS (Li et al., 2011), is an ideal place for the cultivation of *P. yezoensis*. The extensive aquaculture rafts on the Subei Shoal are made of ropes and bamboo poles (Fig. 1A and B), and the net curtains for *Porphyra* cultivation were tied to the rafts (Fig. 1C). Based on studies from the last several years, it has been proposed that extensive aquaculture rafts of *P. yezoensis* in the Subei Shoal may play an important role in the early formation of green tides in the YS (Liu et al., 2009, 2010a, 2013a; Keesing et al., 2011). However, not all studies agree with this hypothesis. Some have cast doubt on *Porphyra* rafts as the major source of floating green algae or even the presence of *Ulva prolifera* on *Porphyra* rafts (Pang et al., 2010; Liu et al., 2011, 2012, 2013b; Shen et al., 2012). To clarify these arguments, the following questions need to be resolved. Is the bloom-forming *U. prolifera* really present on the culture rafts? If so, what is its relative abundance in the attached green algal community? Where did the attached population of *U. prolifera* come from? What is the relationship between *U. prolifera* attached to the *Porphyra* rafts and the floating *U. prolifera* in the YS?

To answer these questions, a rapid and accurate method to identify bloom-forming *Ulva prolifera* is a prerequisite. Presently, identification of *U. prolifera* mainly relies on the traditional morphological approach (Ding et al., 2009) combined with PCR-based sequencing methods (Shimada et al., 2008). However, the morphological features of *U. prolifera* are quite diverse under different environments and often lead to difficulties in the accurate identification of the targeted species. PCR-based sequencing methods can offer accurate and objective data that assist in the identification of *U. prolifera*, but these methods are generally expensive and time-consuming. To fulfill the purpose of rapid and accurate identification of *U. prolifera*, a fluorescence *in situ* hybridization (FISH) method with a probe targeting the 5S rDNA spacer regions was recently developed. The specificity of the FISH method was tested with six species of green algae that are commonly present in the Subei Shoal, including *U. prolifera*, *Ulva linza*, *Ulva flexuosa*, *Ulva compressa*, *Ulva pertusa* and *Blidingia* sp., and only *U. prolifera* had a high labeling efficiency (approximately 96%). In addition, this method offered a quantitative assessment of the relative abundance of *U. prolifera* in the green algal community (Zhang et al., 2015).

In this study, the newly developed FISH method was adopted to study several processes related to the formation of green tides in the YS, including: (1) variation of the relative abundance of *Ulva prolifera* in the green algal community attached to *Porphyra* rafts in the Subei Shoal; (2) contribution of the microscopic propagules in seawater to the *U. prolifera* population attached to rafts; and (3) variation of the proportion of *U. prolifera* in the floating green algal community in the YS. The results of this study will answer the above-mentioned questions and provide insight into the early development of green tides and the role of the *Porphyra* culture in their formation.

2. Materials and methods

2.1. Sample collection

2.1.1. Attached green algal samples

2.1.1.1. Attached green algae on Porphyra rafts.

Green algae attached to *Porphyra* rafts in three representative sites in the Subei Shoal (Fig. 2), i.e., Xiaoyangkou (XYK), Gaoni (GN)

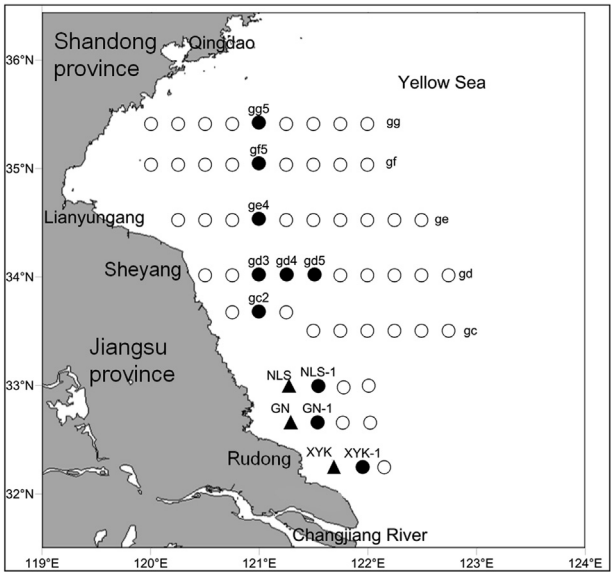

Fig. 2. Sampling sites (XYK, GN, NLS) in the Subei Shoal for attached and floating green algae during 2012 cruises (Subei Shoal is defined as the mudflat zone expanding over 200 km from the Sheyang River estuary to the Changjiang River estuary. Filled triangles depict sampling sites for the attached green algae in Subei Shoal; filled circles show sampling sites for floating green algae. XYK: Xiaoyangkou; GN: Gaoni; NLS: Niluosha; XYK-1: sea area near Xiaoyangkou; GN-1: sea area near Gaoni; NLS-1: sea area near Niluosha; gc-gg: sampling transects in the cruises organized by the "CEOHAB" project in the Yellow Sea).