



Changes to the biomass and species composition of *Ulva* sp. on *Porphyra* aquaculture rafts, along the coastal radial sandbank of the Southern Yellow Sea



Yuanzi Huo^{a,b,c}, Hongbin Han^{b,c}, Honghua Shi^a, Hailong Wu^{b,c}, Jianheng Zhang^{b,c}, Kefeng Yu^{b,c}, Ren Xu^d, Caicai Liu^d, Zhenglong Zhang^d, Kefu Liu^d, Peimin He^{b,c,*}, Dewen Ding^a

^a The First Institute of Oceanography, State Oceanic Administration (SOA), Qingdao, Shandong Province 266061, PR China

^b College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, PR China

^c Marine Scientific Research Institute, Shanghai Ocean University, Shanghai 201306, PR China

^d East China Sea Environmental Monitoring Center of State Oceanic Administration, Shanghai 200137, China

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ABSTRACT

Compositions, changes and biomass of attached *Ulva* species on *Porphyra* rafts along the radial sandbank in the Yellow Sea were investigated, and potential contributions to green tides was analyzed. *Ulva prolifera*, *Ulva flexuosa* and *Ulva linza* were all appeared throughout the investigated period. *U. prolifera* and *U. flexuosa* dominated attached *Ulva* population on *Porphyra* rafts. Attached *Ulva* species biomass showed obviously spatial and temporal variations. Temperature, *Ulva* microscopic propagules and human activities were main factors to influence attached *Ulva* species biomass. The total attached *Ulva* species biomass was more than 20,000 fresh weight tons in April, and the green tide causative species *U. prolifera* accounted 51.03% in April 2013 before green tides occurred. The high biomass of attached *Ulva* species would contribute most to green tides in the Yellow Sea. But how attached *Ulva* species on *Porphyra* rafts contributing to green tides in the Yellow Sea should be further studied.

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1. Introduction

Over the past four decades, green macroalgal blooms, of sufficient size to cause green tides, have occurred worldwide, with increasing frequency in coastal areas. Such tides have a significant negative impact on the ecology and economy of coastal areas (Fletcher, 1996; Morand and Merceron, 2005; Hiraoka et al., 2011). The world's largest green tide events, mainly caused by high populations of *Ulva prolifera*, have occurred annually from 2007 to 2014 along the coast of the southern Yellow Sea, China. Green tides are likely to reoccur in the Yellow Sea when exposed to similar oceanographic conditions, which is a matter of great concern to government officials and scientists in China (Liu et al., 2009). The origins, causes, and population dynamics of these green algal blooms have been extensively studied (Liu et al., 2009, 2012; Pang et al., 2010; Zhang et al., 2011, 2013). Although many papers on green tides in the Yellow Sea have been published, the origins,

causes, and mechanisms associated with the growth of these macroalgal blooms remain uncertain.

Numerous hypotheses have been proposed to explain the origin of the green algal blooms that occurred between April and August, from 2007 to 2013, in the southern Yellow Sea. Based on remote sensing data, some researchers postulated that these green algae blooms originated from the *Porphyra* aquaculture regions (Liu et al., 2009; Hu et al., 2010; Keesing et al., 2011). Zhang et al. (2014) also postulated that the *Ulva* spp. biomass attached to *Porphyra* rafts was the main source of green algal blooms in the Yellow Sea. Zhang et al. (2011) suggested that somatic cells, present in marine sediments, provided a propagule bank for the *U. prolifera* blooms, while Pang et al. (2010) postulated that *U. prolifera* thalli and germlings in effluents entering the Yellow Sea originated from aquaculture ponds. Liu et al. (2013) proposed that microscopic propagules of *U. prolifera*, in the sediments and coastal waters of Jiangsu Province, formed overwintering 'seed stocks', which constituted the precursor of green tides in the spring. Based on field investigations of ecological characteristics, such as habitat assessments, Ding et al. (2009) postulated that the green algal blooms probably originated from coastal waters of the southwest Yellow Sea.

* Corresponding author at: College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China. Tel./fax: +86 21 61900467.

E-mail address: pmhe@shou.edu.cn (P. He).

Free-floating green algal blooms were initially recorded in the Rudong area, and the *Porphyra* aquaculture area was believed to be the main resource for green algal blooms in the Yellow Sea (Huo et al., 2013). From 2011 onwards the main causative species, *U. prolifera*, was noted to be widespread on *Porphyra* aquaculture rafts (Xiao et al., 2013; Han et al., 2013). Liu et al. (2010) reported the presence, within six investigated areas, of approximately 5000 tons (fresh weight) of green algae *Ulva* species on *Porphyra* aquaculture rafts, while Zhang et al. (2013) estimated that the fresh weight *Ulva* species on *Porphyra* rafts in the Rudong area was approximately 4.0×10^6 kg fresh weight. These records, together with previous evidence, led to the conclusion that green algal blooms in the Yellow Sea generally originated from the large biomass of *Ulva* spp. attached to *Porphyra* aquaculture rafts.

Although algal blooms in the Yellow Sea have been extensively studied, no large-scale investigations into the attached *Ulva* spp. biomass (on *Porphyra* aquaculture rafts) at the radial sandbank (southern Yellow Sea) have been undertaken. In the present study, six *Porphyra* aquaculture areas—from north to south, along the radial sandbank at the coast of southern Yellow Sea—were investigated on a monthly basis, from December 2012 to April 2013. The primary objective of this study was to identify variations in the species composition of *Ulva* and changes to the biomass of attached algae on *Porphyra* aquaculture rafts. The second objective was to evaluate the potential contribution of attached *Ulva* biomass to green algal blooms in the Yellow Sea.

2. Materials and methods

2.1. Study area and survey methods

The Jiangsu Province coastline ($30^{\circ}44'–35^{\circ}4'N$) is 954 km long and the total shoal area is characterized by an extensive shallow and muddy intertidal zone of about 5100 km². Coastal waters, located within the 20 m isobaths, are usually suitable for *Porphyra* aquaculture using semi-floating cultivation methods (Shang et al., 2008). In such coastal areas aquaculture rafts are set up in the September–October period, for the purpose of cultivating *P. yezoensis*, from October to April. Rafts are usually removed during the following April–May period. During our investigation we collected monthly samples, from December 2012 to April 2013. Ten sampling sites were selected for sampling attached *Ulva* algae, with two sites being located in each of the following areas: the Rudong, Jiangjiasha, Zugensha, and Dongsha sea areas. Additional single sites were located in the Qidong and Yaosha sea areas (Fig. 1). Each raft was comprised of bamboo, a mooring rope, and a net, as described in Han et al. (2013). During cruises, at least three rafts were randomly selected, for the purpose of sampling attached *Ulva* species and the collection of the bamboo and rope to which the *Ulva* was attached. Following collection, the *Ulva* samples were rinsed with filtered sea water and immediately transported (under cool conditions) back to the laboratory where morphological assessments were made, as described below, for the purpose of compiling biomass statistics for phylogenetic analysis. A total of 450 *Ulva* samples were collected during the study period.

2.2. Oceanographic conditions

Transects were undertaken during each cruise, to investigate oceanographic conditions. Samples were taken at 20 designated sampling sites in the investigation area (Fig. 1). Sea surface temperature, salinity and pH values were recorded at each site, using a Sea-Bird (SBE 25) instrument. Seawater samples were collected, using Niskin sampling bottles, for later determination of ammonium (NH₄-N), nitrite (NO₂-N), nitrate nitrogen (NO₃-N), soluble

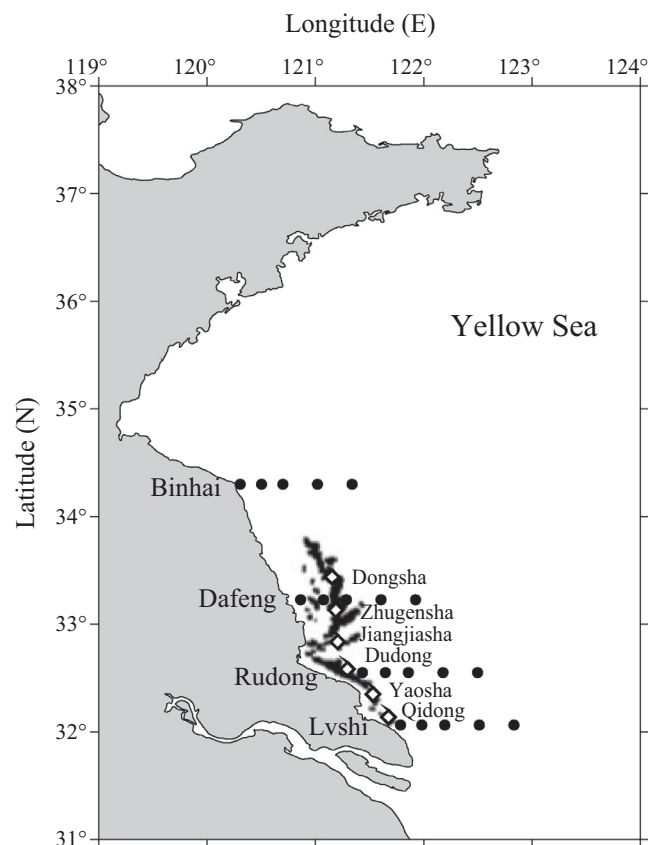


Fig. 1. *Ulva* species samples, collected in turn at the Qidong, Rudong, Yaosha, Jiangjiasha, Zugensha and Dongsha *Porphyra* aquaculture areas, from northern to southern areas, along the radial sandbank, southern coast of the Yellow Sea (◇). Water samples were collected from 20 sites along the Lvshi, Rudong, Dafeng and Binhai transect (●).

reactive phosphorus (PO₄-P) and silicate (SiO₃-Si). Each sample was initially filtered through a cellulose membrane (0.45 μm)—which had been immersed in 10% HCl for at least 10 h and repeatedly rinsed with distilled water—after which a small amount of HgCl₂ was added. Samples were then transported to the laboratory under cold conditions and preserved in a refrigerator, at a temperature of $-30^{\circ}C$, until further analysis. Sampling assessments were based on protocols developed for the Joint Global Ocean Flux Study (JGOFS, 1994), as outlined in Huo et al. (2013).

2.3. Species identification, phylogenetic analysis and biomass statistics

Morphological identification of green algal samples was undertaken by means of microscopic examination (Nikon E200) and photographic records. Internal transcribed spacer (ITS) sequences and 5S ribosomal deoxyribonucleic acid (5S rDNA) spacer sequences were chosen for the purpose of species identification. Phylogenetic analysis of *Ulva* samples was based on the methods outlined in Huo et al. (2013) and Han et al. (2013). The biomass of *Ulva* samples per unit attached on rope (g m⁻¹), bamboo (g m⁻¹) and net (g m⁻²) was obtained according to the wet weight measured using an electronic balance. Based on *in situ* investigations and measurements, the net area, length of rope, and length of bamboo on each raft were estimated as 11.88 m², 8.0 m, and 3.3 m, respectively. Based on the number of rafts containing *P. yezoensis*, as described by Liu et al. (2010), we calculated that the density of rafts was 450 per hectare within the aquaculture area. Based on these assumptions, the total biomass of attached green algae per hectare of *P. yezoensis* aquaculture was estimated by multiplying the mean

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