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An investigation of the space distribution of *Ulva* microscopic propagules and ship-based experiment of mitigation using modified clay

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ABSTRACT

Previous studies suggested that the removal of *Ulva* microscopic propagules (UMP) from cradle water might restrict the formation and expansion of green tides in the Yellow Sea, China. In this study, the distribution characteristics of UMP in the southern Yellow Sea was investigated, and then a flocculation experiment of UMP using modified clay (MC) was conducted at a selected station of the research cruise. The results indicated that the distribution of green algae thalli is one of the main factors that directly influence UMP distribution. UMP density was strongly negatively correlated with the distance between the sampling station and the centre of the area containing floating *Ulva* ($r = -0.618^{***}$, $n = 83$). >80% of the UMP was removed from the water column after MC application at a concentration of 0.1 g/L, and MC applied at a concentration of 0.5 g/L reduced the germination rate to 0.3%.

1. Introduction

After a decade, green tide continues to overwhelm the southern Yellow Sea. There is still some uncertainty about whether this ecological disaster can be blamed on *Porphyra* aquaculture occurring in Jiangsu province. The *Porphyra* aquaculture industry has operated for approximately 50 years, much longer than the history of green tide blooms. Thus, why did green tide problems start after 2007 and continue from that time? Lin et al. (2011) assumed that the full usage of the Three Gorges Dam beginning in 2006 promoted the formation and development of the green tide event because of large amounts of freshwater and nutrient input when the Dam releases stored water from January to June. Relatively low salinity and enriched nutrient content boosted vegetative growth of *Ulva prolifera* (Lin et al., 2011). Liu et al., (2013a) suggested that from 2006 to 2008 the significant expansion of *Porphyra* aquaculture (approximately 10,000 ha) to the sand ridges between Yancheng and Nantong (Fig. 1) triggered a green tide bloom beginning in 2007, after which green algal fragments were able to be transported offshore.

Several generally accepted conclusions have also been confirmed. Favourable temperature, high nutrient supply, appropriate hydrodynamic transport, high rates of nutrient uptake, growth, and photosynthesis, and diverse reproductive strategies are the mechanisms that turn small patches of macroalgae into the world's largest green tide (Ding et al., 2009; Gao et al., 2010; Gao et al., 2014; Keesing et al., 2011; Lin et al., 2008; Lin et al., 2009; Liu et al., 2013a; Liu et al., 2013b; Pang et al., 2010; Ye et al., 2011).

Propagules of *Ulva* including gametes, zygotes, spores, and microscopic germlings can develop into macroalgal vegetation under favourable environmental conditions (Liu et al., 2012). Propagules can survive winter on their own (Liu et al., 2012) or by developing from somatic cells over winter (Zhang et al., 2010). They play the role of *Ulva* "seeds" that proliferate in spring in suitable niches under elevated nutrient levels, forming the initial biomass. Produced and released from mature thalli or vegetative fragments (Dan et al., 2002; Gao et al., 2010), propagules can then settle and germinate on *Porphyra* aquaculture rafts, suspended particles, or the floating thali, supporting the further development of a green tide.

It has been suggested that removal of *Ulva* microscopic propagules (UMP) from the water column at a suitable opportunity may help alleviate a bloom (Huo et al., 2014; Liu et al., 2013b). Laboratory study has already indicated that modified clay in a concentration of 0.4 g/L could remove >80% of the *Ulva prolifera* microscopic propagules from culture and significantly decrease germination rates (Li et al., 2015). However, the natural composition and distribution of UMP was far more complex than in the laboratory simulation. Propagules found throughout the southern Yellow Sea are composed of a mixture of multiple types of

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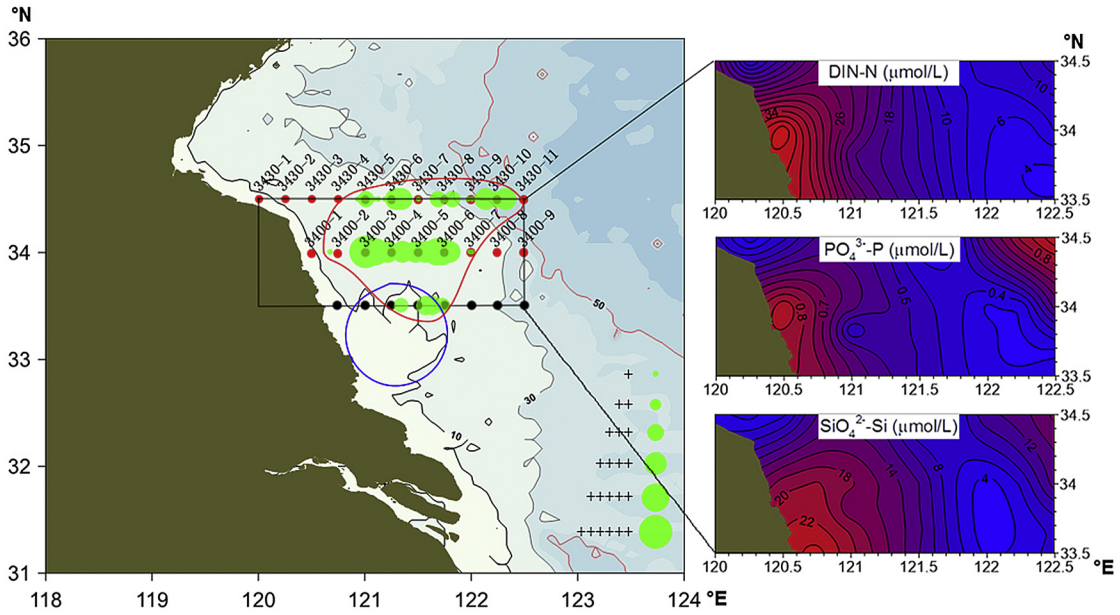

Fig. 1. Sampling transects along 33.30′, 34.00′ and 34.30′N off the southern coast of the Yellow Sea and nutrient distribution (color filled counter map) in the investigated area. Red spots represent nutrient and propagule density sampling stations along each transect. Black spots represent nutrient sampling stations. The blue circle represents the location of the sand ridges. The red circle marks the area where we observed floating Ulva patches along the cruise from May 17–19. Green bubbles represent a quantitative description of the floating algal thalli amounts along the cruise on May 17, 18 and 19 (from north to south).

green algae. These varieties are difficult to identify because propagules are nearly identical at early life stages. Although there is evidence that UMP are broadly distributed in the southern coastal region of the Yellow Sea, there is also some uncertainty regarding the spatio-temporal distribution of this complex because of a lack of quantitative data.

The main purpose of this study was to determine the proper dosage of MC when eliminating propagules from natural abundance levels and to assess the influence of MC application on water nutrient levels. Basic distribution patterns of UMP were investigated based on our own observations and were also based on a literature review of previous works in the field.

2. Materials and methods

2.1. Study area

Three transects located at 33.30′N, 34.00′N and 34.30′N (referred to as 3300, 3400, and 3430, respectively) were assessed during green tide events along the southern coast of the Yellow Sea in Jiangsu Province. These areas are assumed to have been affected by floating green algae based on remote sensing images taken from May 17 to 19, 2016. Specific sampling locations are shown in Fig. 1.

2.2. Oceanographic conditions

At each sampling station, vertical temperature (Tem.), salinity (Sal.) and turbidity (Tub.) profiles were recorded using a Sea-Bird (SBE 911) instrument lowered from the sea surface to the bottom. Seawater samples were collected at different water depths using Niskin bottles, to determine the amount of ammonium (NH_4-N), nitrate (NO_2-N), nitrate nitrogen (NO_3-N), soluble reactive phosphorus (PO_4-P), soluble reactive silicate (SiO_4-Si), total nitrogen (TN), and total phosphorus (TP) in each. Samples collected for the assessment of dissolved inorganic nutrients were filtered through GF/F membranes, after which a small amount of $HgCl_2$ was added. A small amount of H_2SO_4 was added to samples collected for TN and TP measurements, to bring the pH down to a level of <2.0. Seawater pH was measured in the field, using a Mettler-Toledo pH metre (model: SevenExcellence S400). All of the water samples were kept chilled during transportation to the laboratory, after which they were stored at temperatures below −20 °C until analysis. In the laboratory NH_4-N, NO_2-N, NO_3-N, PO_4-P, TN, TP and SiO_4-Si concentrations were measured using the SKALAR-1000 nutrient auto analyser (model: SA 3000/5000 chemistry unit).

2.3. Microscopic propagule quantification

Water samples were collected at different depths at each sampling site to assess approximate UMP density. The process for quantification of UMP was based on the method established by Liu et al. (2010) with some slight modifications. Specifically, 800 mL of the water was filtered through a sieve of 150 μm mesh size. It was then enriched with prepared nutrient stock until it reached a nutrient level similar to the *L*1 medium without silica(Guillard and Hargraves, 1993). The enriched water samples were then transported to the laboratory in 1 L glass conical flasks and incubated at 18–20 °C under 80–100 μmol m^{-2} s^{-1} in a 12 h light:12 h dark cycle. During the subsequent culture period, the culture medium was renewed every 7 days. After 2–3 weeks, green algal germlings that had attached to the walls and bottoms of the glass conical flask and had grown to a diameter of >1 cm were counted.

2.4. Modified clay preparation

The MC slurry used in this study was based on polyaluminum chloride (PAC, analytical reagent), which modified Kaolin (1:5) in hyper pure water at 25.0 g/L as the stock solution(Yu and Zou, 1994). The slurry was not mixed until required in the algae flocculation experiments. The PAC was obtained from Guangfu Fine Chemical (Tianjin, China). The Kaolin was obtained from Wuxian, Jiangsu, China.

2.5. Flocculation experiment

Custom acrylic barrels (diameter of 0.4 m, height of 1.0 m) were produced for the study. One hundred litres of surface seawater at station 3400-3 was pumped into each barrel, and 0, 400, 1200, and 2000 mL of the MC slurry (25.0 g/L) was added to the barrels, respectively. This resulted in final concentrations of 0, 0.1, 0.3, and 0.5 g/L MC,

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