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Decadal characteristics of the floating *Ulva* and *Sargassum* in the Subei Shoal, Yellow Sea

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Abstract

The data of field surveys during 2009 to 2018 was analyzed to understand the seasonality and inter-annual variability of the floating *Ulva* and *Sargassum* in the Subei Shoal, the southwestern Yellow Sea of China on decadal scale. The floating *Ulva* biomass was consistently originated from the central region of the Subei Shoal in middle to late April, increased rapidly, drifted and extended into the offshore water in May and June. The average floating *Ulva* biomass in the shoal generally increased over the years with evident inter-annual fluctuations. In contrast, pelagic *Sargassum* was accumulated in the Subei Shoal and formed the spring bloom only in 2013, 2017 and 2018, and the biomass was higher than the co-occurring *Ulva* during the survey in these three years. Compared to the raft-origin floating *Ulva*, genesis and development of the pelagic *Sargassum* was distinct. Based on the current research, the *Sargassum* biomass was exotic and often initiated in the offshore water in March, and intruded into the shoal in April and May. The analysis on the environmental parameters was inconclusive since multiple anthropogenic and non-indigenous factors could influence the green tides in this region. Further research covering both the East China Sea and the Yellow Sea is needed to trace the origin of the floating *Sargassum* and to understand the interactions between these two co-occurring seaweeds.

Key words: seaweed bloom, green tide, golden tide, *Ulva*, *Sargassum*, Yellow Sea

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1 Introduction

In 2008, astonishing huge floating biomass of the seaweed *Ulva prolifera* accumulated in western Yellow Sea (YS), drifted along the coast of Jiangsu Province and farther north to the southern coasts of Shandong Province (Hu, 2009; Leliaert et al., 2009). Since then, the green tide recurred annually and was considered to be the world's largest green tide in terms of its massive biomass and wide geographic distribution (Liu et al., 2009, 2013; Keesing et al., 2011; Ye et al., 2011). The subsequent remote sensing data traced the floating biomass back to the southwestern coastal water of YS (near the Subei Shoal), and this drifting pathway was confirmed by a number of drifters and modeling experiments (Hu, 2009; Liu et al., 2009; Ciappa et al., 2010; Hu et al., 2010; Keesing et al., 2011; Garcia et al., 2013; Bao et al., 2015; Zhang et al., 2017). The field observations then directly pointed out that the green macroalgal wastes from the *Pyropia* aquaculture rafts on Subei Shoal were the primary source for the initial floating biomass (Liu et al., 2013, 2016; Zhang et al., 2014; Wang et al., 2015; Zhou et al., 2015). Compared to the numerous studies on the large-scale green tides in open water (Keesing et al., 2011; Qi et al., 2016; Hu et al., 2017; Cao et al., 2019; Xiao et al., 2019), little was known about the detailed distribution, development and inter-annual variation of the floating *Ulva* biomass in the source region, Subei Shoal.

Meanwhile, the blooming incidences of the pelagic *Sargassum* (comprised exclusively *S. horneri*) were reported in the YS recently (Xing et al., 2017; Liu et al., 2018), which brought more difficulties on tracking and monitoring macroalgal blooms and aroused more chaos on environmental and ecological issues in this region. *Sargassum horneri* is a cosmopolitan benthic seaweed species distributed widely along the coasts of northwestern Pacific, including the coasts of China (Tseng, 1984; Hu et al., 2011). A recent invasive expansion of the benthic population was observed in the coastal waters of southern California, USA and Baja California, México in the 2000s (Marks et al., 2015). At the same time, the significant blooms of the pelagic *Sargassum* were reported in the East China Sea (ECS) since the early 2000s (Komatsu et al., 2007, 2008). Nevertheless, the pelagic *S. horneri* was

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scarce in the YS in the 2000s and not concerned with the harmful macroalgal bloom until the recent years. In October of 2016 to early 2017, Xing et al. (2017) detected a large-scale *Sargassum* bloom in the YS through the high-resolution satellite remote sensing, which caused significant losses for the local *Pyropia* aquaculture (Liu et al., 2018). In following spring of 2017, Qi et al. (2017) reported another unusual inundation of pelagic *Sargassum* biomass in the ECS, which even invaded into the YS. Although the remote sensing and genetic analyses indicated a few initiation locations along Zhejiang and Shandong coasts (Qi et al., 2017; Xing et al., 2017; Liu et al., 2018), the exact sources and the causes for these unusual *Sargassum* blooms have not been clarified due to lacking of extensive field observations. Even less was known about the detailed seasonality, distribution, biomass and progression in any specific ecosystem.

The Subei Shoal is a large inter-tidal muddy flat in the near-shore water of the southwestern YS, and featured by dozens of elongated sand ridges and grooves distributed alternative and extending radically from the central raft region to the offshore water. The strong tidal forces in this region caused the permanent turbidity maxima. As a traditional aquaculture base for the *Pyropia* crops, the anthropogenic activities and biological process in this region played key roles for the genesis of green tides in the YS (Liu et al., 2010a, 2013; Zhang et al., 2014; Wang et al., 2015). Besides, the geochemical and ecological environment within and around this region interacted (with the strong tidal force) closely to influence or even regulate the whole ecosystem in the YS. As described above, both *Ulva* and *Sargassum* blooms were found co-occurring in this region recently. However, little is known about the detailed dynamics, micro-structure and inter-annual variations of these two blooming species in this region. In this study, the field observational data during 2009–2018 was compared and analyzed to understand the inter-annual and inter-specific variability of the two blooming species (*U. prolifera* and *S. horneri*) in the Subei Shoal on decadal scale. This research would provide an insight into the genesis, seasonality, biomass and any difference of these two occurring seaweeds, which is necessary for developing appropriate management to mitigate their impairments to local ecosystem and marine aquaculture.

2 Materials and methods

2.1 *Survey region and cruises conducted since 2009*

To monitor the development process of the floating macroalgae in the Subei Shoal, shipboard field cruises were conducted almost every year since 2009 except 2015 (Table 1). The surveys covered the region in and around the Subei Shoal in the southwestern YS (32.00°–36.00°N, 120.50°–122.50°E, including the intertidal raft region in the central Shoal enclosed by the dashed line in Fig. 1). Before 2017, surveys were performed in spring (March to June), since the primary objective at that time was to monitor the green tides. Then, large amount of pelagic *S. horneri* was unprecedentedly accumulated in the Subei Shoal in the winter of 2016 (Xing et al., 2017; Liu et al., 2018). Subsequently, monthly surveys were performed from March of 2017 to June of 2018 in order to investigate any difference on the seasonality and occurrence of the floating *Ulva* and *Sargassum* in this region. A total of 41 field cruises were conducted in the Subei Shoal since 2009 (Table 1). The survey was not conducted in 2015 due to limited funding. The results from the rest years was graphed and compared to study the inter-annual variability. Partial data has been published previously (Fan et al., 2012; Liu et al., 2015, 2016; Wang et al., 2015), but cited and re-analyzed here.

2.2 *Trawling bioassay on the floating biomass*

In each cruise, the research vessel navigated from the south to the north along the six transects (Fig. 1). Floating biomass was sampled at each station and assessed by a trawling bioassay (Wang et al., 2015). In brief, a plankton net (500 μm mesh size and 80 cm diameter) was used to collect the floating biomass on the sea surface. The vessel was moving at a low speed (2 knots) for 10–15 min to let the net tow through the certain area of the surface water. The biomass (A_i, g/m^2) at the station was then calculated as the total wet weight of the sampled macroalgae divided by the trawling area. When both *Ulva* and *Sargassum* were collected and mixed in the samples, they were separated and weighted individually. The samples were transported back to the laboratory at 4°C for further analysis. The total floating biomass in the Subei Shoal was computed as the average biomass cross all the surveyed stations multiplied by the total area of the Subei Shoal.

2.3 *Species composition of the floating algal mats*

As described above, the green and brown seaweeds were separated manually and weighted individually on board. The biomass proportions of each genus were calculated accordingly. To study the species composition of the floating biomass, six floating clumps were collected from the different survey stations in May of 2018 (Fig. 1). After they were separated into genera, the algal samples were transported back in a cooler for further species identification. In the laboratory, the green macroalgae clumps were re-suspended in DI water, and the individual thallus of green macroalgae was then carefully sorted out based on the gross morphology (Tseng, 1984). Samples of each species

Table 1. Annual field surveys on the floating macroalgae conducted in the Subei Shoal during 2009–2018

Year[1)]	Number of cruises	Month	Number of stations[2)]	Samples[3)]
2009	6	Mar. – Jun.	28	floating *Ulva*, SST, salinity
2010	5	Mar. – Jun.	28	floating *Ulva*
2011	2	Jun.	29	floating *Ulva*
2012	6	Feb. – May	36	floating *Ulva*, nutrients, SST, salinity
2013	3	Mar. – May	28	floating *Ulva* and *Sargassum*
2014	2	Apr. – May	28	floating *Ulva*
2016	1	May	27	floating *Ulva*
2017	9	Mar. – Jun., Oct. – Dec.	36	floating *Ulva* and *Sargassum*, nutrients, SST, salinity
2018	7	Jan. – Jun.	31	floating *Ulva* and *Sargassum*
Total	41			

Note: [1)] No surveys were conducted in 2015; [2)] stations included those in raft region; [3)] only samples relevant to this research are listed.

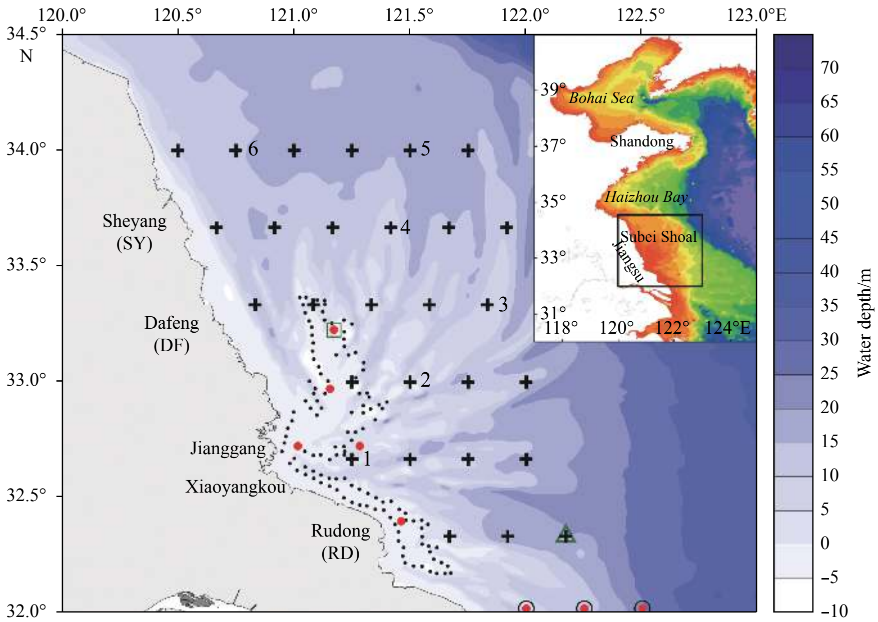

Fig. 1. Survey region and stations in the Subei Shoal. Black + are regular sampling stations for 2009–2010 and 2013–2014, red dots are the extra stations surveyed in 2012 and 2017 beside the regular ones, black circles are the extra stations in 2018, green square and triangle indicate the station added in 2011 and that was not surveyed in 2016, respectively. Nos 1–6 indicated where the six floating green macroalgal samples were collected in 2018 for molecular species identification. A dotted line encloses the major *Pyropia* aquaculture area in the Subei Shoal. Geographic location of the survey region is shown in the insect at the upright corner.

were randomly identified by a molecular assay (Xiao et al., 2013) to justify the reliability of morphological identification. The fresh weight of each species group was measured after they were tap dried on the tissue paper. The percentage of each species in total biomass was estimated accordingly.

A total of ten individual thalli of the pelagic *Sargassum* were randomly sampled from the brown seaweeds brought back. They were screened by the ITS markers and sequenced to identify their species affiliation. DNA extraction, PCR amplification and sequencing followed the protocol from Lü et al. (2018). The resulted sequences were aligned with the references of *Sargassum* spp. retrieved from the NCBI database using the MUSCLE algorithm (Edgar, 2004). The phylogenetic analyses were conducted using neighbor-joining and maximum-likelihood methods to identify the genetic affiliation of these samples.

2.4 *Environmental parameters of the survey region*

Surface seawater temperature (SST) and salinity (SSS) were measured at each station by a CTD instrument (SBE25plus). Nutrients of the surface water, including NO_3-N, NO_2-N and PO_4-P, were sampled and measured following the protocol in Shi et al. (2015). The inter-annual variation was described based on the historical data and the references. Particularly, average DIN and DIP were estimated for 2017 and compared with the historical data (Li et al., 2015). Additional field monitoring data of nutrients and SST at the three coastal stations at Sheyang (SY), Dafeng (DF) and Rudong (RD) was provided by the Jiangsu Ocean and Fisheries Bureau (JOFB, 2011–2017).

3 Results

3.1 *The spatio-temporal variation of initial floating Ulva bands*

Initiations of the floating *Ulva* biomass were recorded for five years (2009, 2010, 2012, 2017 and 2018), but not for the others

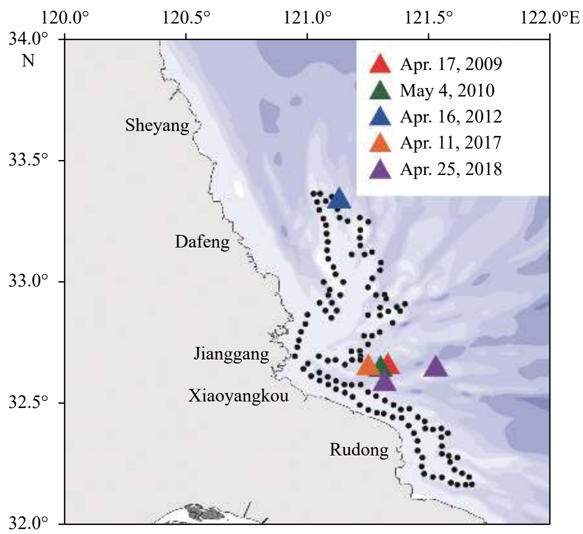

Fig. 2. Initiation locations and dates of the floating *Ulva* bands in the Subei Shoal in 2009 (red), 2010 (green), 2012 (blue), 2017 (orange) and 2018 (purple).

due to limited cruises conducted in those years. The first floating slick detected in 2009 was located at the edge of the raft region close to Xiaoyangkou (Fig. 2). The drifting band was about 20 m in length and 2–3 m in width. Small floating patches could be detected sporadically in the nearshore region, from the coasts of Dafeng to the south of Rudong. Similarly, the floating *Ulva* biomass was initiated from the center region of Subei Shoal in 2010, 2017 and 2018, which was close to the location in 2009. Whereas, the first floating band in 2012 was detected at the northern edge of the raft region (Fig. 2). The dates for these initial floating *Ulva* biomass spanned from mid-April to early May (April 11 to May 4), which were 19–42 d (31 d in average) earlier than first the large-scale floating mats detected by MODIS (Moderate Resolution Imaging Spectroradiometer; MNR, 2009–2018).

3.2 *Inter-annual variations of the floating biomass*

During the survey period (early April to late June), the average biomass of the floating *Ulva* increased rapidly. It started from less than 1.00 g/m^2 in early April, and reached to the maximum in late May (2017) or early June (2009, 2010, 2011, 2012 and 2018, Fig. 3). For example, the average abundance of the floating *Ulva* at the peak was about 100 and 27 times of the initial biomass in April of 2018 and 2012, respectively. Significant inter-annual variation was also evident. Among the five years (2009, 2010, 2012, 2017 and 2018) when relatively complete survey was conducted, the maximum average biomass was about 7.20, 4.60, 7.36, 3.25 and 274.40 g/m^2, respectively (Fig. 3). The average biomass tended to increase in recent years except for 2017, and reached the highest in 2018. Similarly, the total biomass of floating *Ulva* in the Subei Shoal increased even faster, given the combined effects of average biomass increasing and rapid distribution expansion of the floating biomass. During the recent three years (2016–2018), the total *Ulva* biomass in the Subei Shoal at the peak was highest in 2018, while lowest in 2017 (data was not shown).

Unlike the consistent annual occurrence of floating *Ulva* in spring (April–June) in the Subei Shoal, the bloom of floating *Sargassum* was only detected in 2013, 2017 and 2018 during this period (Fig. 3). The floating *Sargassum* in the other years was sporadic and negligible compared to the huge biomass of floating *Ulva*. The *Sargassum* biomass was much higher than the occurring *Ulva* in 2013 and 2017 ((6.49±2.82) g/m^2, (14.15±5.87) g/m^2, respectively). And it was highest in April and declined quickly with a rapid increase of *Ulva* biomass. Although the average biomass of *Sargassum* in 2018 ((20.10±9.33) g/m^2) was higher than that in 2017 ((14.15±5.87) g/m^2), the floating *Sargassum* was restricted in a relatively small area at the peak (April) of 2018 (See description below). So the maximum total biomass of the floating *Sargassum* in 2018 was still lower than that of 2017. The maximum total floating biomass was observed in 2017 when substantial pelagic *Sargassum* biomass widespread in the entire Subei Shoal and even throughout the western YS (Xiao et al., 2020).

3.3 *The spatio-temporal development of Ulva and sargassum blooms*

To investigate the occurrence time of the floating *Ulva* and *Sargassum* in the Subei Shoal, year-round surveys were conducted during April 2017 to June 2018 except July, August, September and November 2017, and February 2018. It was found that beside the spring bloom, low biomass of pelagic *Sargassum* was commonly observed even in fall (October 2017), winter (December 2017) and early spring (March 2018) when no floating *Ulva* could be detected (Fig. 4). Then the *Sargassum* biomass increased rapidly and accumulated in the Subei Shoal in spring. As the case in 2018, the pelagic *Sargassum* biomass grew rapidly in early spring and reached the maximum in April, which was earlier than the floating *Ulva* (May and June). The pelagic *Sargassum* in 2017 also occurred earlier (before March, data was not shown) than the floating *Ulva* (April). And it spread to the entire Subei Shoal rapidly, reached and maintained at high biomass in May and even in June of 2017 (Fig. 4).

In comparison, floating *Ulva* biomass was usually detected in spring to early summer in the Subei Shoal. Although some floating *Ulva* biomass was observed in September of 2012 in the shoal (data was not shown), it was not reported or observed in the

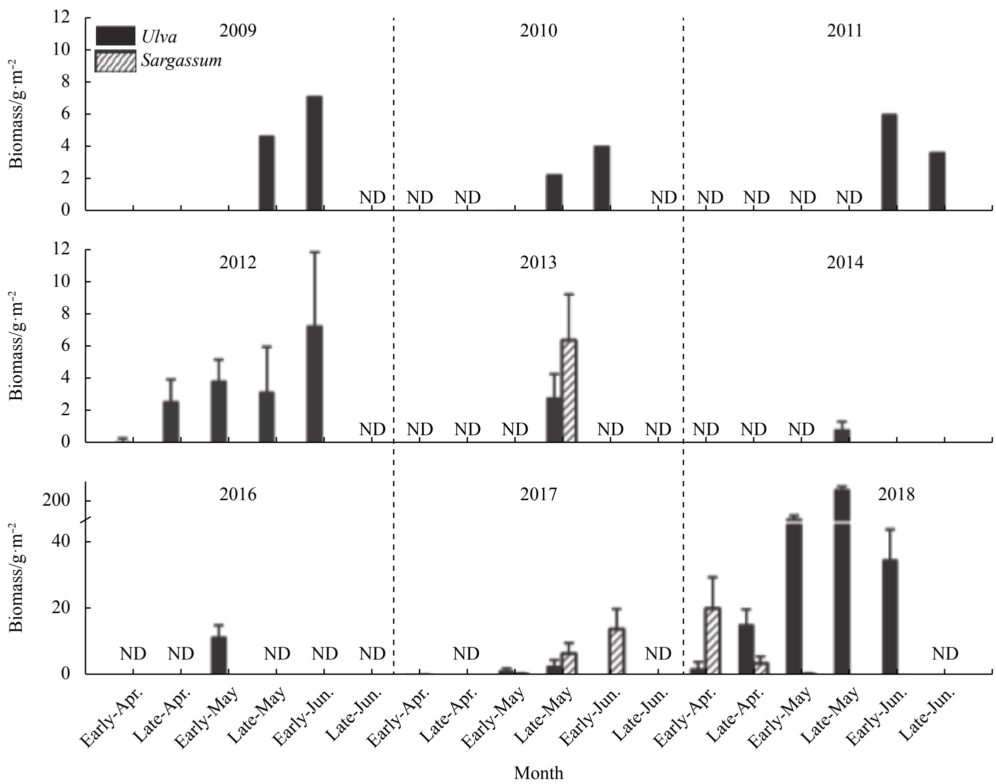

Fig. 3. Inter-annual variation of the average floating biomass, *Ulva* and *Sargassum*, in the Subei Shoal during 2009–2018 except 2015. ND indicates no data. The *Y*-axis was rescaled for 2016–2018 due to the high biomass in 2018.

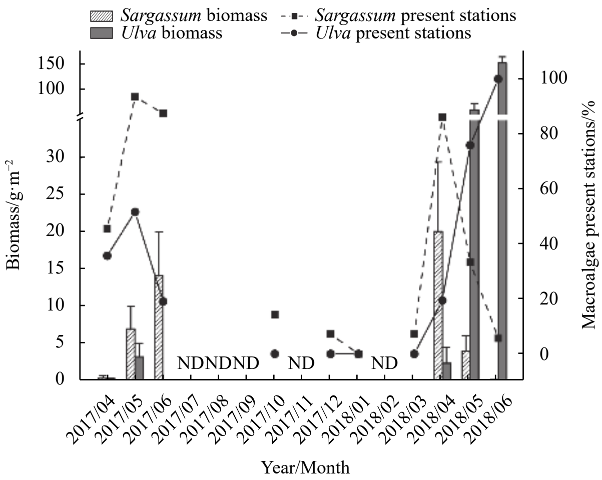

Fig. 4. Seasonal variation of floating *Ulva* and *Sargassum* in the Subei Shoal during April 2017–June 2018. Columns are average biomass of *Ulva* or *Sargassum*. Symbols and lines are percentages of stations with floating macroalgal biomass observed. ND indicates no field surveys were conducted. The average biomass was used and graphed for the months that multiple surveys were performed (May 2018 and June 2018).

months other than the typical *Ulva* blooming season (spring to early summer). As described above, it started in April to early May, grew rapidly in May and June. Unlike in 2018, the floating *Ulva* in 2017 diminished quickly after May and drifted out of the Subei Shoal region in June. Similar fluctuation patterns were observed for the percentage of positive stations for these two floating species (Fig. 4). The difference between the cases in 2017 and 2018 indicated probably variable blooming dynamics of the pelagic *Sargassum* among years in this region, which was further corroborated in the following spatial progression below.

Spatial development of the floating biomass was quite different between the two seaweed species. As described above ("dates and locations for the initial floating *Ulva* biomass"), the *Ulva* biomass was consistently initiated from the central region of Subei Shoal, close to the raft region, at middle April to early May (Fig. 2). It grew rapidly after its initiation, filled the entire Subei Shoal and even drifted out of this region in about two weeks. However, the initiation of floating *Sargassum* in Subei was not consistent among these years. In 2018, low biomass of *Sargassum* was drifting constantly at the east edge of the Subei Shoal in fall, winter and early spring (Figs 5d–f). And it intruded into the nearshore water in April followed by a rapid growth (Fig. 5g), then spread throughout the Subei Shoal in May and declined in June (Figs 5h, i). In 2017, two initiation locations were observed for the floating

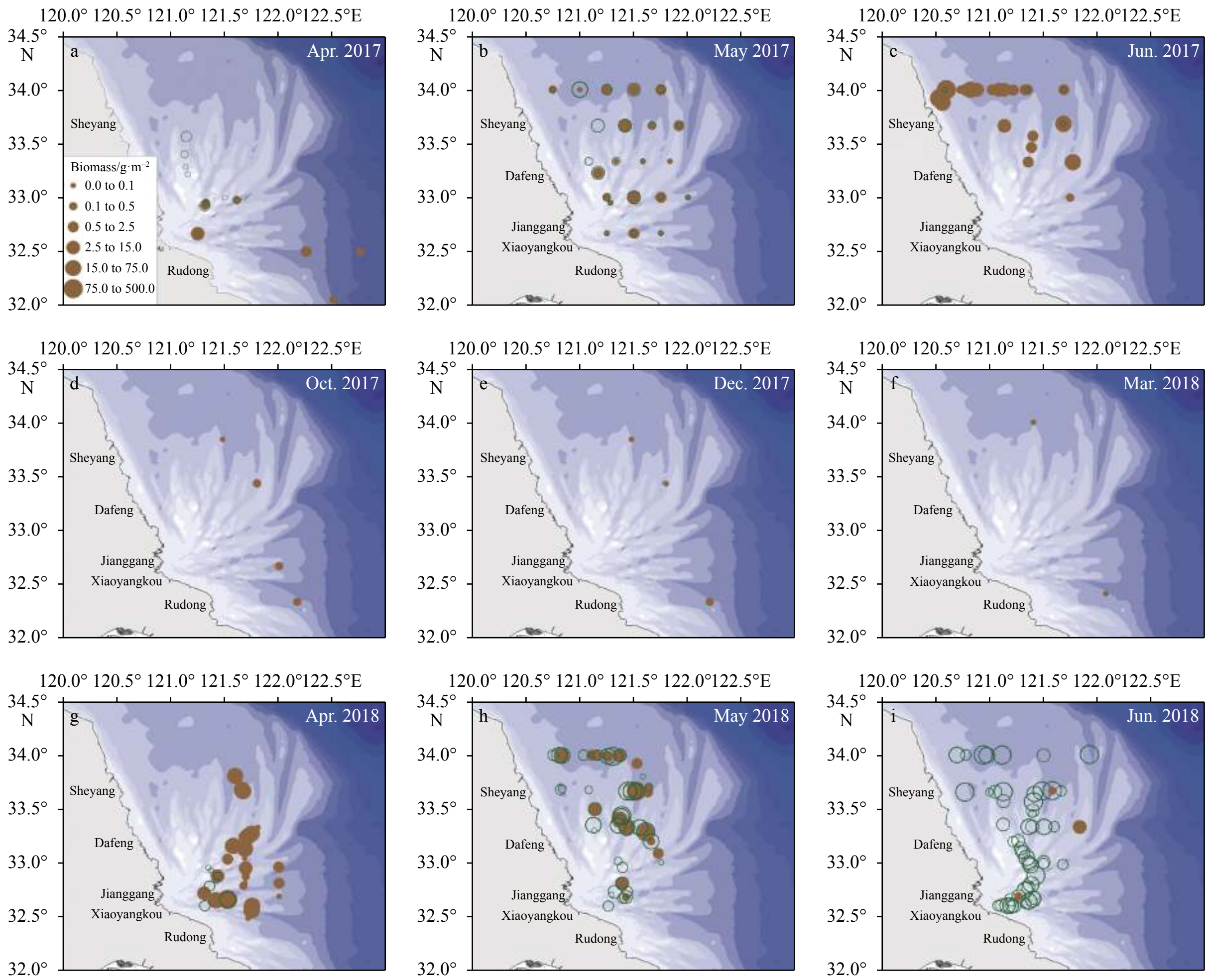

Fig. 5. Spatial progression of the floating *Ulva* (green open circles) and *Sargassum* (brown filled circles) in the Subei Shoal during April 2017–June 2018.

Sargassum, one was at the central Subei Shoal (close to the raft region) where *Ulva* biomass was contemporarily starting, the other was at the southeast edge of the shoal where the floating *Sargassum* biomass was commonly detected in early spring (Fig. 5a). The *Sargassum* spread rapidly in May along with the *Ulva* biomass, drifted further north in June (Fig. 5b), but was mostly restricted in the region between the northern Subei Shoal and southern Haizhou Bay which was south to the major distribution of *Ulva* biomass (Xiao et al., 2020). Based on our field observations, substantial amount of free *Sargassum* was also wrapping on the *Pyropia* aquacutlure rafts in early spring (March–April 2017). The *Sargassum* biomass on the rafts and that drifting around the raft region (central Subei Shoal) was believed deriving from the last winter bloom in this region (Xing et al., 2017), and contributed partially for the *Sargassum* bloom in spring of 2017. Another source of the pelagic *Sargassum* in the Subei Shoal was likely from the offshore water south to the shoal, which could be testified by the satellite remote sensing (Qi et al., 2017).

3.4 *Species composition of floating biomass*

As described above, macroalgae of two genera (*Ulva* and *Sargassum*) were commonly observed floating in the coastal water of Subei Shoal. Floating *Sargassum* was abundant in spring of 2013, 2017 and 2018, and was 56.94%±11.34%, 73.21%±14.10% and 19.42%±2.41% in average, respectively (Table 2). In the other years, *Ulva* dominated the floating biomass and the sporadic *Sargassum* was negligible.

Based on the genetic sequencing, the floating *Sargassum* comprised a single species, *S. horneri*, while *Ulva* comprised multiple species. As an example, samples of floating brown seaweeds collected in May of 2018 from the Subei Shoal were sequenced and their sequences were 100% identical to the references of *S. horneri*, a species widespread in the YS and the ECS (Komatsu et al., 2007, 2008; Liu et al., 2018; Su et al., 2018). The floating green macroalgal patches comprised three common species, *U. prolifera*, *U. linza* and *Blidingia* sp. Among the three species, *U. prolifera* was dominant and accounted for 90.45% of the total biomass in average. The other two species only accounted for 7.88% and 1.67%, respectively. No obvious spatial variation of the species composition was detected (data was not shown), probably due to the few number of samples screened and small geographic range sampled.

3.5 *Nutrients, SST and SSS*

The detailed descriptions of the nutrients, SST and SSS in the Subei Shoal and western YS as well have been published in Shi et al. (2015) and Li et al. (2015). In summary, the shallow water of Subei Shoal was featured by high temperature and nutrients. The salinity ranged from 18 to 34, and was generally increasing from inshore to offshore. The SST increased rapidly during blooming season (<10 to around 20°C, Liu et al., 2010a) and was generally decreasing seawardly. Similarly, the nutrients were significantly higher in the inshore region, especially the Sheyang River Estuary (Shi et al., 2015). Beside the spatial variation, the nutrients (esp. DIN) were found increasing significantly over the past decades (Li et al., 2015). The average DIN and DIP along the coasts of Jiangsu Province (JOFB, 2011–2018) were even higher than the averages of YS (Fig. 6), which was congruent with the overall spatial variation of these nutrients in the YS. Consistent with the increasing trend, the average DIN in the YS and Subei Shoal in 2017 was higher than those in previous years (Fig. 6), indicating the continued eutrophication in this region.

The average SST in spring seasons (March to June) at the coasts of Sheyang (SY), Dafeng (DF) and Rudong (RD) varied during the recent seven years (2012–2018, Fig. 7). The SST was generally higher in the south (e.g., RD) than the north (e.g., SY, p=0.032, Fisher post hoc). No significant different was observed among the years (One-way ANOVA, p=0.181).

4 Discussion

This decadal field data illustrated the detailed biomass distribution, bloom development and occurring time of the floating *Ulva* and *Sargassum* in the shallow coastal water of Subei Shoal. Congruent with the previous studies (Leliaert et al., 2009; Liu et al., 2010b; Xiao et al., 2013), the *Ulva* bloom in this region was dominant by *U. prolifera*, the major causative species for the large-scale green tide in the YS. The *Sargassum* bloom in the shoal was comprised of a single species, *S. horneri* (Liu et al., 2018; this study). The distinctive blooming dynamics of these two species were revealed by this study, which implied different origins and appropriate measures should be adopted for the floating biomass in this region.

In this study, we provided more details about the initiation of the floating *Ulva* biomass in the Subei Shoal, which further corroborated the raft-origin of the floating *Ulva*. Unlike initiation of the large-scale floating mats observed by the satellite remote sensing (MNR, 2009–2018), the field observations narrowed the initiation of the floating *Ulva* biomass into two specific locations, which were both adjacent to the aquaculture raft region in the central shoal (Fig. 2). The initiation dates were also closely related to the cleaning and disposal of the macroalgal wastes from the rafts. Combined the bio-physical and chemical environment of the Shoal, one of the *Ulva* blooming mechanisms was hypothesized that epiphytic green macroalgae disposed from the aquaculture rafts was accumulated and transported offshore by the tidal water, grew and expanded rapidly with the optimal water temperature and sufficient nutrients in this region, and it has been confirmed by a number of studies (Liu et al., 2013; Shi et al.,

Table 2. Proportions (%) of *Sargassum* and *Ulva* biomass in the Subei Shoal during 2009–2018

Year	*Ulva*		*Sargassum*	
	Range/%	Mean±SE/%	Range/%	Mean±SE/%
2009	100.00	100.00	0.00	0.00
2010	100.00	100.00	0.00	0.00
2011	100.00	100.00	0.00	0.00
2012	100.00	100.00	0.00	0.00
2013	0.04–100.00	43.06±10.03	0.00–99.96	56.94±11.34
2014	100.00	100.00	0.00	0.00
2016	100.00	100.00	0.00	0.00
2017	0.00–100.00	26.79±13.90	0.00–100.00	73.21±14.10
2018	0.00–100.00	80.58±2.48	0.00–100.00	19.42±2.41

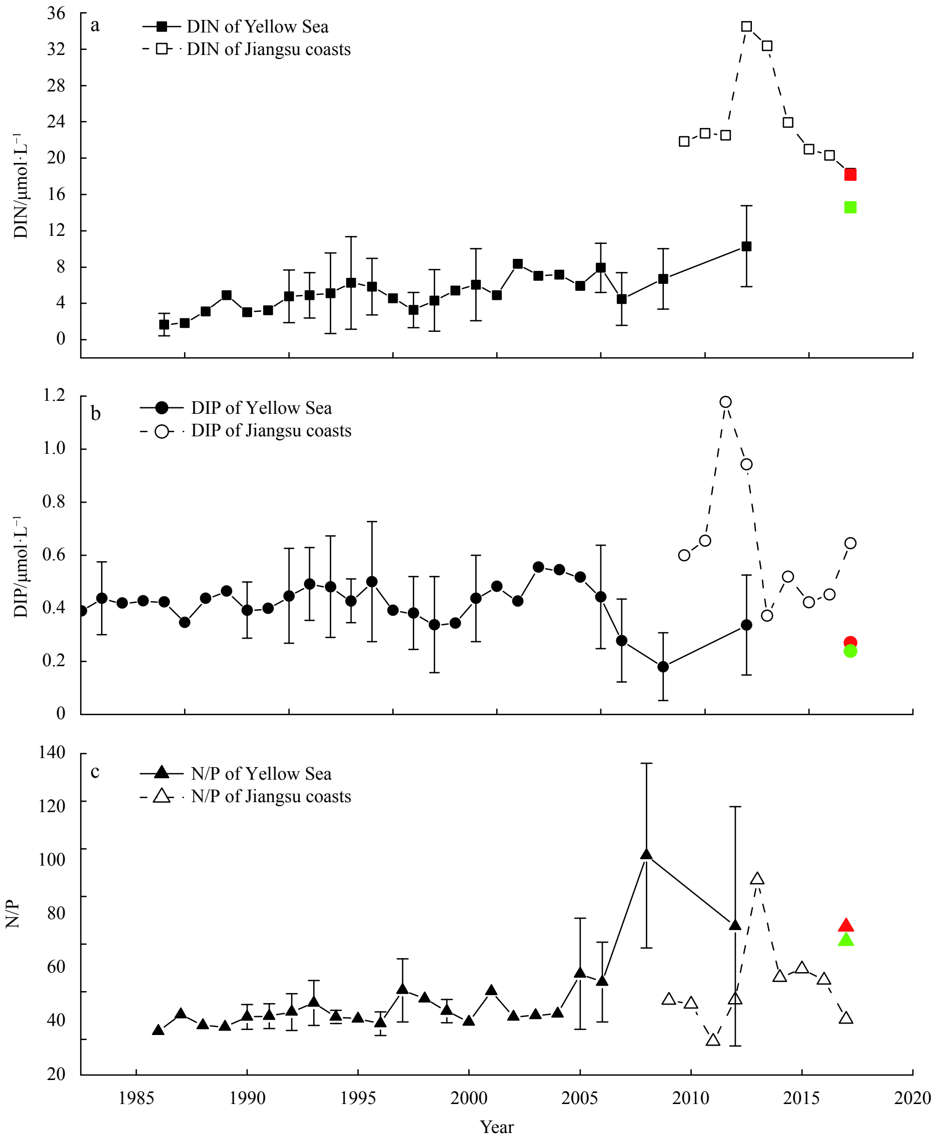

Fig. 6. The average DIN (a), DIP (b) and N/P (c) values in the Yellow Sea (solid line and filled symbols, redrawn from Li et al. (2015)) and along the coasts of Jiangsu (dashed line and open symbols, from JOFB 2011–2017) from the 1980s to 2017. The colored symbols are average values of the Yellow Sea (green) and Subei Shoal (red) in 2017 (from this research).

2015; Wang et al., 2015; Zhou et al., 2015; Zhang et al., 2017). It is worth noting that this initiation and development process was the only one that has been illustrated clearly and confirmed repeatedly by the intensive field observations and experiments so far. Some other hypothesized sources, such as the coastal mariculture pond, developing directly from the extensive environmental micro-propagules (Liu et al., 2010b, 2012; Pang et al., 2010; Zhang et al., 2010, 2011), have not been fully proved due to lacking the key development process or stages linking the hypothesized source and the large-scale green tides. Recently, Cao et al. (2019) summarized the distribution and drifting pathways of the green tides in recent three years (2016–2018) based on the satellite remote sensing data, and suggested an additional source for the floating *Ulva* biomass from the offshore water southeast to the Subei Shoal, especially in 2017. This study, however, did not take into account the substantial floating *Sargassum* biomass in 2017. Based on the field observation and the remote sensing data (Qi et al., 2017; Liu et al., 2018), the significant pelagic *Sargassum* was drifting in 2017 off the coasts of the ECS, the Changjiang River Estuary and the southern YS, which covered this additional source region indicated in Cao et al. (2019). Our field data further suggested presence of the floating *S. horneri* in offshore water of Subei Shoal in non-blooming seasons (e.g., winter and fall) and illustrated the significant bi-macroalgal bloom with both *U. prolifera* and *S. horneri* in and around the shoal in spring season. Therefore, the additional source suggested by Cao et al. (2019) was probably for the floating *Sargassum* instead of *Ulva*.

Unlike the obvious local origin of the floating *Ulva* in the Subei Shoal, the pelagic *Sargassum* (notably *S. horneri*) in this region was exotic and invasive from the offshore water. The spatial progression of pelagic *S. horneri* was distinctive to that of *Ulva*. As the case of 2018, low biomass of the drifting *S. horneri* often persisted in the offshore water east to the shoal region in fall and winter seasons. The biomass then invaded into the Subei Shoal by the strong tides, grew and expanded rapidly, forming the bloom in spring (Fig. 5). Benthic *S. horneri* was believed to be ab-

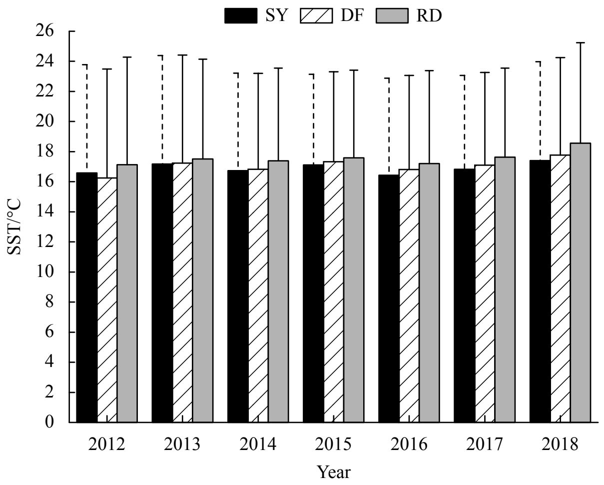

Fig. 7. The average sea surface temperature in spring (March to June) at Sheyang (SY), Dafeng (DF) and Rudong (RD) during 2012 to 2018. Error bars are standard deviations of the means.

sent along the coasts of Jiangsu Province probably due to the unfavorable habitat (Tseng and Chang, 1959). The benthic populations of *S. horneri* was often found growing on the low tidal to sublittoral reefs with relatively low sediment load (Bi et al., 2014; Bi and Wang, 2016), while the large area of muddy seafloor and high suspended sediments along the coasts of southern Jiangsu could restrict the settlement of *S. horneri*, especially in the Subei Shoal, the turbidity maxima. Our decadal field monitoring did not identify any benthic *S. horneri* populations in the Subei Shoal either. Combined all the information above, the pelagic *S. horneri* in the Subei Shoal was not indigenous. The exact origin of the pelagic *S. horneri* biomass is unclear so far and needs further investigation. The satellite remote sensing detected an unusual expansion of the pelagic *Sargassum* in the ECS in 2017, which extended to the northwest, crossed the Changjiang River Estuary and invaded into the coastal water of southern YS (Qi et al., 2017). Consistently, our field data also observed the initial floating *Sargassum* biomass drifting in the offshore water southeast to the Subei Shoal in early spring, and then intruded into the nearshore water laterly (Fig. 5), suggesting a close relationship between the floating *Sargassum* in the Subei Shoal and the ECS. Although a few studies through the computational simulation and buoy-tracking indicated that the floating *Sargassum* in the ECS could be originated from the coasts of Zhejiang Province (Komatsu et al., 2007; Filippi et al., 2010; Qi et al., 2017), the number, scale and seaweed abundance of the natural *S. horneri* beds have not been fully investigated. Without the field observations on initiation and development of the floating biomass, it could not be fully proved about the association between the benthic and blooming biomass in the ECS. Besides, an unprecedented *Sargassum* bloom burst out from the north in October of 2016, drifted southward and accumulated in the southwestern YS in early 2017 (Xing et al., 2017). The *Sargassum* biomass decreased after February 2017, but few seaweeds were still left in the shallow water around rafts area or wrapping on the rafts, as shown by the current field data (Fig. 5). This overwintering residual biomass propagated and amplified rapidly in spring, contributing to the spring bloom of *S. horneri* in the Subei Shoal (Xiao et al., 2020). Therefore, multiple sources of the floating *S. horneri* in the Subei Shoal existed, especially for the spring bloom of 2017.

The inter-annual variation was evident for both floating *Ulva* and *Sargassum* in the Subei Shoal region. The *Ulva* bloom occurred annually, the biomass was relatively low in 2009–2014 and 2017 (<10 g/m^2), significantly increased in 2016 (>10 g/m^2 at the peak), and reached the maximum in 2018 (>250 g/m^2 at the peak, Fig. 3). In comparison, the spring bloom of floating *S. horneri* in the Subei Shoal did not occur annually, but only observed in 2013, 2017 and 2018. Taken into account the both blooming species, the total floating macroalgal biomass (including *Ulva* and *Sargassum*) in the Subei Shoal during spring seasons generally increased over these years (Fig. 3). Little was known about the causes for the regional fluctuations of green tides in the Subei Shoal. The nutrients in the Subei Shoal were obviously higher than that around this region and prominent among the coastal waters in China and even around the world (Li et al., 2015; Valiela et al., 2018). Apparently, the sufficient nutrients in this region favored blooming of these opportunistic seaweeds, and the rising concentrations in these years (Fig. 6) could be ascribed at least partially for the increasing total floating biomass in this region. Coupled with the model prediction, the phosphate concentration likely has a more marked impact on the *Ulva* bloom in this region (Wang et al., 2019), which probably needs to be tested by the field data. Beside the coastal eutrophication, a few of meteorological, hydrological environments (such as global warming, wind trajectory, sea surface temperature anomaly) were postulated to associate with the inter-annual variation of the large-scale green tides in the YS (Keesing et al., 2011; Qi et al., 2016; Valiela et al., 2018; Xiao et al., 2019; Zhang et al., 2019). However, the sea surface temperature in this study did not show obvious correlation with the floating biomass. Other than these, the anthropogenic factors (e.g., the total aquaculture rafts and the associated green macroalgal wastes produced) in the shoal could also influence the *Ulva* bloom (Zhang et al., 2017; Xing et al., 2018, 2019). Additionally, the substantial floating *Sargassum* biomass co-occurring with the floating *Ulva* in spring of these years may also affect the biomass growth and accumulation of the floating *Ulva* in this region, which apparently needs further evaluations. Therefore, more comprehensive analyses were needed to reveal any impacts of the environmental parameters, including those anthropogenic and non-indigenous factors, on the interannual variation of the green tides in the Subei Shoal.

Above all, the floating *Ulva* biomass consistently occurred and originated from the Subei Shoal region. The significant inter-annual variation of the *Ulva* biomass was associated with various anthropogenic and environmental factors in this region. The increasing pelagic *Sargassum* biomass (comprised exclusively *S. horneri*) bloomed in the spring of 2013, 2017 and 2018, and formed unusual bi-macroalgal blooms in these years. Distinct spatial development and seasonality were observed for the floating *Ulva* and *Sargassum* in the Subei Shoal. Although the affiliation between the pelagic *S. horneri* in the YS and the benthic populations along the coasts of China was still unclear so far, the floating biomass of *S. horneri* in the Subei Shoal was not originated locally, but probably derived from multiple sources. Given the difficulties to distinguish *Ulva* and *Sargassum* in such turbidity coastal water (Subei Shoal), field observation is necessarily needed to track the long-term variation of the floating *Ulva* and *Sargassum* and to investigate the ensuing interactions between these two co-occurring species.

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