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Molecular phylogenetic analysis and exploration of the sporulation features of *Ulva* spp. in the yellow sea of China provide insight into *Ulva prolifera* being the dominant species in green tides

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Abstract

Annual large-scale green tides in the Yellow Sea initially appeared in the Subei Shoal, Jiangsu Province, with *Ulva prolifera* identified as the dominant species. Molecular identification of *Ulva* species has been a key focus during green tide monitoring. In this study, we used the chloroplast gene *tufA* (encoding elongation factor Tu) as a molecular marker to assess species composition and frequency across 11 *Ulva* populations. These were sampled from three coastal sites in Jiangsu and Shandong Provinces at six time points, totaling 420 biologically identified *Ulva* individuals. Additionally, the sporulation features of *Ulva* spp., which are directly linked to biomass variation, were examined to understand why *U. prolifera* dominates these blooms. (1) *TufA* proved to be a highly accurate and unambiguous molecular marker for *Ulva* species identification. We found that propagules in the coastal waters of Yancheng, Jiangsu, consisted mainly of *Ulva aragoensis* and *U. prolifera*, whereas floating biomass sampled during green tides in Shandong was predominantly *U. prolifera*. (2) A lower sporulation ratio in *U. prolifera* compared to *U. aragoensis* affects their relative proportions in floating biomass. This contributes to *U. prolifera* becoming the dominant floating species during the annual large-scale green tides in the Yellow Sea.

Keywords *Ulva prolifera*, *Ulva aragoensis*, *TufA*, Sporulation, Floating biomass

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Background

Green tides are large-scale blooms of floating green macroalgae, primarily composed of *Ulva* species (Hayden et al., 2003; [1]). In China, massive floating green tides have occurred annually in the Yellow Sea for over 15 years. Phylogenetic analyses of floating samples have identified the dominant species as the filamentous *Ulva prolifera* (Müller) J. Agardh [2, 3]. Satellite data from multiple sources suggest that these green tides first appear on the Subei Shoal off the northern Jiangsu coast and then drift northward, driven by seasonal winds and surface currents [4, 5].

In the scenario of the initial floating *Ulva* biomass outbreak on the Subei Shoal, *Pyropia* aquaculture rafts (comprising nets and ropes) have been identified as a common initial substrate. *Ulva* propagules in the water column attach to these rafts and grow into thalli [6]. Once established, these “epiphytic” green algae rapidly increase in biomass. The thin, tubular filamentous thalli can then detach due to factors such as strong water movement, spontaneous sporulation and the cleaning process during raft recycling. Consequently, the initial floating *Ulva* biomass is formed at this location. Nonetheless, attachment to *Pyropia* rafts is not considered essential for green tide initiation, given that *Ulva* propagules in the water column are also capable of developing directly into floating biomass [6].

The Radial Submarine Sand Ridges (RSSR) feature a unique geographical structure known as the Subei Shoal, where a benthic muddy beach spanning hundreds of square kilometers is exposed during low tide [7]. This distinctive environment provides all essential conditions for green tide outbreaks: seawater enriched with nutrients from terrestrial agriculture and freshwater crab aquaculture via riverine inputs [5], ample solid substrate, extensive offshore *Pyropia* aquaculture nets stretching up to

10 miles, and the perennial presence of the green bloom-forming species *U. prolifera* in both water and sediments [8].

Among the plausible explanations for the spatiotemporal patterns of these outbreaks, a fundamental issue remains unresolved. Why is *U. prolifera* almost exclusively identified in the floating biomass that reaches Shandong coast after drifting over a thousand kilometers? Where do other filamentous *Ulva* species go? Furthermore, *Ulva* thalli lose viable biomass when sporulation occurs [9]. The remaining vegetative bases will regenerate in a semilunar rhythm [10, 11]. Hiraoka et al., (2021) emphasized that inhibiting biomass allocation to sporulation is crucial for *Ulva* to form large-scale blooms. This raises a pivotal question: what are the sporulation features of the major *Ulva* species on the Subei Shoal? Answering this will provide critical new evidence for understanding the mechanisms behind the dominance of *U. prolifera* in the Yellow Sea’s green tides.

To address these questions, we conducted two field excursions at the Subei Shoal in 2019, collecting water, sediment and algal samples along the drift path. For accurate species identification, the chloroplast gene *tufA* (encoding elongation factor Tu) was used as a molecular marker [12–14]. The *tufA* gene has been demonstrated to be more suitable for differentiating *Ulva* species than other common markers, such as the nuclear-encoded rDNA internal transcribed spacer (ITS) region or the ribulose 1,5-bisphosphate carboxylase gene (*rbcL*), which sometimes provide insufficient resolution within this genus [15, 16]. Concurrent individual culture experiments were conducted to characterize the sporulation features of the isolated algae.

Methods

Sample collection

Sediment (silty sand, 100 g/sample) and green algae samples were collected from *Pyropia* aquaculture nets during low tide in Yancheng, a major cultivation zone (Figs. 1 and 2A). Seawater samples (500 mL/sample) were collected across a 1,000 m² area at the same site during high tide. Rope samples were also cut from the nets during low tide. Floating *Ulva* samples were collected after the *Pyropia* rafts had been retrieved at the end of the production season (Fig. 2B). At the sampling site, seawater temperatures ranged from 12 °C to 21 °C, with a photoperiod of 12.5 to 14 h during the sampling period. Additionally, seawater samples were collected from the Qingdao coast prior to the appearance of floating biomass. Floating *Ulva* samples were obtained from the Qingdao coast (twice) and the Rongcheng coast (once) during the 2019 green tide outbreak in Shandong Province (Fig. 2C, D). All samples were transported to the laboratory in cool boxes

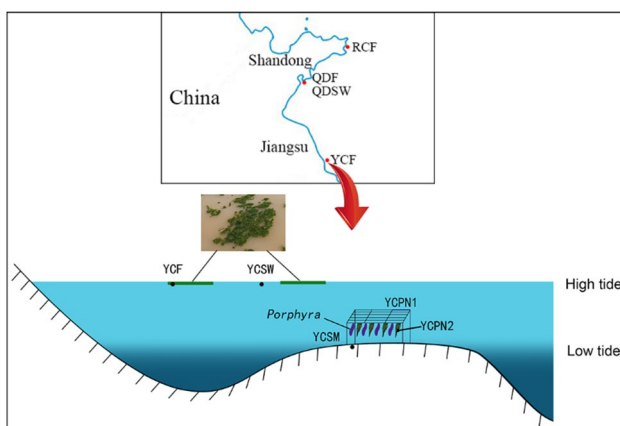


Fig. 1 Coastal sampling sites in the Yellow Sea, China. Sampling locations: Rongcheng (RC), Qingdao (QD), Yancheng (YC). Solid dots denote sampling points on the Yancheng coast

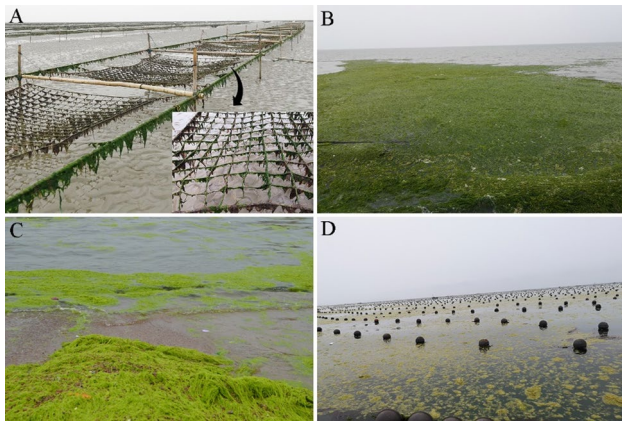


Fig. 2 Green tide development from source to outbreak areas. **(A)** Benthic algal biomass on *Pyropia* rafts (Subei Shoal, Apr 4, 2019). **(B)** Floating *Ulva* mats after *Pyropia* harvest (Subei Shoal, May 31, 2019). **(C)** Floating biomass at the onset of the green tide (Qingdao coast, Jun 28, 2019). **(D)** Floating biomass during the outbreak (Rongcheng coast, Jul 17, 2019)

Table 1 Sampling information of 11 *Ulva* populations analyzed in this study

Sampling sites	Niches	Codes	Coordinates	Dates	No. of individuals
Yancheng, Jiangsu	Sediment	YCSM1	33°12'N, 121°7'E	2019-04-04	50
Yancheng, Jiangsu	Seawater	YCSW1	33°12'N, 121°7'E	2019-04-04	50
Yancheng, Jiangsu	Rope of rafts	YCPN1	33°12'N, 121°7'E	2019-04-04	57
Yancheng, Jiangsu	<i>Pyropia</i> net	YCPN2	33°12'N, 121°7'E	2019-04-04	25
Yancheng, Jiangsu	Sediment	YCSM2	33°12'N, 121°7'E	2019-05-31	60
Yancheng, Jiangsu	Seawater	YCSW2	33°12'N, 121°7'E	2019-05-31	61
Yancheng, Jiangsu	Floating	YCF	33°12'N, 121°7'E	2019-05-31	50
Qingdao, Shandong	Floating	QDF1	36°2'N, 120°21'E	2019-06-28	20
Qingdao, Shandong	Floating	QDF2	36°3'N, 120°20'E	2019-07-12	17
Qingdao, Shandong	Seawater	QDSW	36°2'N, 120°21'E	2019-05-09	15
Rongcheng, Shandong	Floating	RCF	37°10'N, 122°34'E	2019-07-17	15

within 48 h. Detailed sampling information, including dates, is provided in Table 1.

Quantification of *Ulva* propagules in sediment, seawater and on nets

Ropes collected from *Pyropia* aquaculture nets were rinsed with sterilized natural seawater to remove epiphytes and impurities. For quantification, a standard wet weight of 5 g from each sediment sample or a 10-cm segment of net rope was individually placed into a glass

beaker containing 1 L of 1/4 PES (Provasoli's enriched seawater, [17]). The 1/4 PES medium consisted of sterilized seawater supplemented with the following nutrients (final concentrations): NaNO_3 17.5 mg L^{-1} , NaH_2PO_4 2.5 mg L^{-1} , Na_2EDTA 1.1 mg L^{-1} , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 3.0 $\mu\text{g L}^{-1}$, $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 5\text{H}_2\text{O}$ 10 mg L^{-1} , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 2.5 $\mu\text{g L}^{-1}$, MnCl_2 44.5 $\mu\text{g L}^{-1}$, $\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$ 5.8 $\mu\text{g L}^{-1}$. The cultures were constantly aerated. Seawater samples (500 mL in plastic wide-neck bottles) were enriched with 1/4 PES medium. To suppress potential diatom overgrowth, GeO_2 was added to a final concentration of 2 mg L^{-1} . All beakers and bottles were maintained at 20 °C under fluorescent white light at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a 12:12 h (light: dark) photoperiod in a GXZ intelligent light incubator (Jiangnan Co. Limited, Ningbo, China). After three weeks of culture, *Ulva* thalli grown from the samples were carefully removed from the beakers and bottles one by one with tweezers and counted. Selected *Ulva* thalli were transferred to 40-mL glass bottles for sporulation assays. The remaining thalli were transferred to larger beakers and maintained separately by source population.

DNA extraction, PCR and sequencing

Ulva thalli were cultured until a size sufficient for DNA extraction. Individual thalli were then rinsed with sterilized seawater and dried with absorbent paper. Leaf tissue samples were placed in 2-mL tubes, air-dried at room temperature for 48 hours, and stored at -20°C until analysis. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentration and quality were assessed by electrophoresis on a 1% agarose gel. The chloroplast-encoded *tufA* gene (813 bp) was amplified by polymerase chain reaction (PCR) in a 20- μL reaction volume using a T-gradient thermocycler (Bio-metra, Göttingen, Germany). Each reaction contained 10 μL of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, USA), 0.5 μM of primers *tufGF4* (5'-GGNGCNGCNCAAATGGAYGG-3') and *tufAR* (5'-CCTTCNCGAATMGCRAAWCGC-3'), and 5 ng of genomic DNA [16, 18]. The PCR program was as follows: initial denaturation at 94 °C for 4 min, 38 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 7 min [13]. PCR products were separated on 1% agarose gels, purified and then sequenced in both directions on an ABI 3730 XL automated sequencer. Sequences of the PCR products were verified according to overlaps of the forward and reverse sequences using BioEdit 7.1.9 (Hall, [19]).

Phylogenetic analysis for species identification

A total of 436 *tufA* sequences were used for phylogenetic analysis, comprising 420 newly obtained in this study and 16 reference sequences downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/>). All sequences were aligned using the Clustal W multiple sequence alignment program [20], implemented in BioEdit 7.1.9 (Hall, [19]). A maximum likelihood (ML) phylogenetic tree (Tamura et al., 1993) was constructed using Mega X [21]. The analysis employed the General Time Reversible (GTR) model with a Gamma distribution with invariant sites (G + I) to model rate variation among sites. Three *Blidingia* spp. sequences were set as the outgroup. Missing data were handled by complete deletion, and branch support was assessed with 1000 bootstrap replicates [22, 23].

Sporulation assay

Individual *Ulva* thalli from sediment (YCSM1) and seawater samples (YCSW1 and YCSW2) were selected post-quantification for sporulation assays. Each thallus was placed into a separate 40-mL glass bottle. Individuals from a floating population (QDF1, Qingdao) were included as a control. All thalli were rinsed with sterilized natural seawater before cultivation. Cultures were maintained in sterilized PES at 20 °C under fluorescent white light at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a 12:12 h (light:dark) photoperiod in the GXZ intelligent light incubator. We selected a cultivation temperature of 20 °C, which represents a critical sporulation threshold for *U. prolifera*

(Hiraoka et al., 2021), rather than replicating the in situ temperature range observed during sampling (12–21 °C from April to May). Although field temperatures increase through the green-tide season, the 20 °C threshold is biologically more relevant for assessing sporulation potential. Similarly, while the natural photoperiod lengthened from 12.5 to 14 h during sampling, a fixed 12:12 h (light:dark) cycle was used to represent a stable daily light phase and to account for weather-related reductions in actual field illumination. A constant light intensity of 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was maintained as simulating natural fluctuations under controlled conditions is impractical. The sporulation rate (SR) was calculated using the equation $SR (\%) = (N_s/N) \times 100$, where N_s and N are the numbers of sporulated individuals and total individuals, respectively. Following the incubation period, all *Ulva* thalli were identified by *tufA* sequence analysis. The *U. aragoensis* and *U. prolifera* derived from sediment or seawater samples displayed highly uniform growth stages and physiological states, thus reducing individual variation and enhancing result reliability.

A positive sporulation response was confirmed by two primary observations: (A) partial bleaching of the *Ulva* thallus, and (B) the appearance of new seedlings on the inner wall of the culture bottle after approximately two weeks (Fig. 3A, B). A third, supplementary criterion (C) involved the microscopic visualization of swarmers within cells (Fig. 3C). This microscopic check was employed when bleaching alone was ambiguous and could not distinguish between sporulation and mechanical damage.

Statistical analysis

Experiments were performed in triplicate. Results are expressed as the mean $\pm$ standard deviation (SD). Statistical analysis of sporulation data was performed using one-way analysis of variance (ANOVA) using SPSS ver. 22.

Results

Quantification of *Ulva* propagules

After two weeks of incubation, *Ulva* seedlings were observed in all the sediment, seawater and *Pyropia* net samples collected along the Yancheng coast. Counts were conducted after an additional week of growth (Table 2). The maximum propagule density in sediments was observed on May 31, 2019, with 215 ± 103 individuals per 5 g wet weight. In contrast, seawater samples reached their peak density earlier, on April 4, 2019, with 850 ± 230 individuals per 500 mL. Large numbers of *Ulva* propagules were also detected on the nets. Notably, seawater samples collected from Qingdao before the green tide outbreak already contained 714 ± 134 individuals per 500 mL.

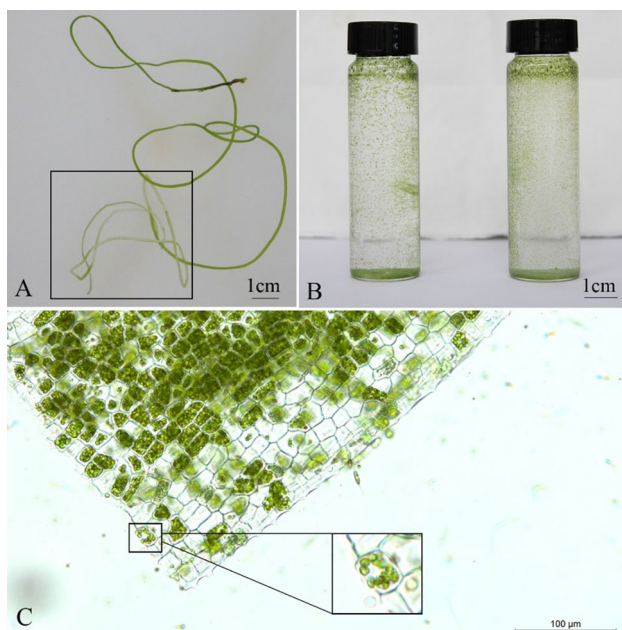


Fig. 3 Criteria for sporulation detection in *Ulva*. (A) Thallus bleaching; (B) Seedling emergence on bottle walls after 14 days; (C) Microscopic swarmer visualization. Sporulation confirmed if both A and B occur. C provides ancillary verification when bleaching is ambiguous

Table 2 *Ulva* individuals cultivated from three sample types

Populations	Sampling dates	Niches	Number of <i>Ulva</i> individuals					Average
			Sample 1 [#]	Sample 2 [#]	Sample 3 [#]	Sample 4 [#]	Sample 5 [#]	
YCSM1	2019-04-04	Sediment	159	157	257	267	83	185 ± 77
YCSW1	2019-04-04	Seawater	678	899	574	943	1157	850 ± 230
YCPN1	2019-04-04	Rope of rafts	319	312	65	1123	-	455 ± 460
YCSM2	2019-05-31	Sediment	242	324	56	271	183	215 ± 103
YCSW2	2019-05-31	Seawater	275	207	271	162	121	207 ± 67
QDSW	2019-05-09	Seawater	922	737	586	605	722	714 ± 134

Source materials: 5 g sediment, 500 mL seawater, 10 cm *Pyropia* net rope

Species composition and relative frequencies based on phylogenetic analyses

Phylogenetic analysis of the *tufA* dataset (~813 bp) assigned all 420 samples into four clades with high bootstrap values (98–100%): *U. prolifera* ($N=166$), *U. aragoensis* ($N=239$), *U. compressa* ($N=14$), and *U. linza* (*U. procera*) ($N=1$) (Fig. 4; Table 3). *U. aragoensis* and *U. prolifera* were the dominant species. *U. compressa* was primarily detected in seawater samples from the Qingdao coast (12 of 15 individuals). A single sediment individual (YCSM2-17) was identified as *U. linza* (*U. procera*). The multiple sequence alignment confirmed that *tufA* provided high resolution for distinguishing these *Ulva* species (Table S1).

Phylogenetic analysis of the *tufA* dataset identified four *Ulva* species among the 420 samples (Table 3). *U. prolifera* was dominant (93.3–100%) in the floating populations collected during the algal bloom (YCF, QDF1, QDF2, and RCF). Among the populations derived from sediments, seawater and *Pyropia* nets on the Yancheng coast, *U. aragoensis* was the most abundant species. Nevertheless, *U. prolifera* still constituted a substantial proportion (34.0% and 54.1%) in Yancheng seawater samples. The individuals derived from the Qingdao seawater of the coast were predominantly *U. compressa*, with *U. prolifera* representing only 20%, a notably lower frequency than that of Yancheng seawater.

Evaluation of sporulation in sampled individuals

Sporulation experiments were conducted on *U. aragoensis* and *U. prolifera*, as they were the most abundant species in seawater and sediment samples. *U. aragoensis* exhibited a significantly higher sporulation percentage than *U. prolifera* in all populations tested (Table 4). The sporulation percentage for the floating *U. prolifera* population was particularly low at 10%. Overall, the average sporulation rate was significantly lower in *U. prolifera* (40.9%) than in *U. aragoensis* (88.3%).

Discussion

Species composition and frequency in the study samples

Our quantitative and molecular analysis demonstrated the abundance and species composition of *Ulva*

propagules in sediments, coastal seawater, and *Pyropia* nets at the Subei Shoal, Jiangsu Province. Phylogenetic analysis based on *tufA* identified *U. aragoensis* and *U. prolifera* as the principal constituents, with *U. aragoensis* being dominant. In April, the algal community attached to the *Pyropia* rafts consisted mainly of *Blidingia* spp., with a minor presence of *U. aragoensis*.

Phylogenetic analysis confirmed that the floating populations from Qingdao (two sites) and Rongcheng in Shandong Province were almost exclusively composed of *U. prolifera*. Similarly, the floating population from the Subei coast (Jiangsu) was also dominated by *U. prolifera* at a comparable proportion. This finding consistently supports the established conclusion that *U. prolifera* is the dominant floating species in Yellow Sea green tides [24].

The species composition of *Ulva* propagules differed markedly between Qingdao and Yancheng coastal waters. In Qingdao, the propagules were dominated by *U. compressa*, whereas *U. prolifera* constituted a significantly lower proportion compared to Yancheng. Although a temperature difference existed (Qingdao: 14 °C, Yancheng: 12–21 °C), this variation alone appears insufficient to explain compositional disparity. We propose that the Qingdao sampling site may represent a historical habitat for *U. compressa*, leading to the persistent presence of its propagules. Importantly, this regional difference does not affect our study's rationale as propagules of the key bloom-forming species *U. prolifera* were present at both locations. Whether and how these propagules contribute to floating biomass formation during green tides warrants further investigation.

Seed source and initial biomass of green tides in the yellow sea

Species of the genus *Ulva*, usually dominating the upper intertidal zone, are classic opportunistic algae. Their biomass proliferates rapidly when seasonal temperature and light conditions become optimal, coinciding with peak nutrient availability in the surrounding waters [25]. These species exhibit a homomorphic alternation of generations, with both gametophytes and sporophytes releasing gametes or spores in a semilunar rhythm, as first

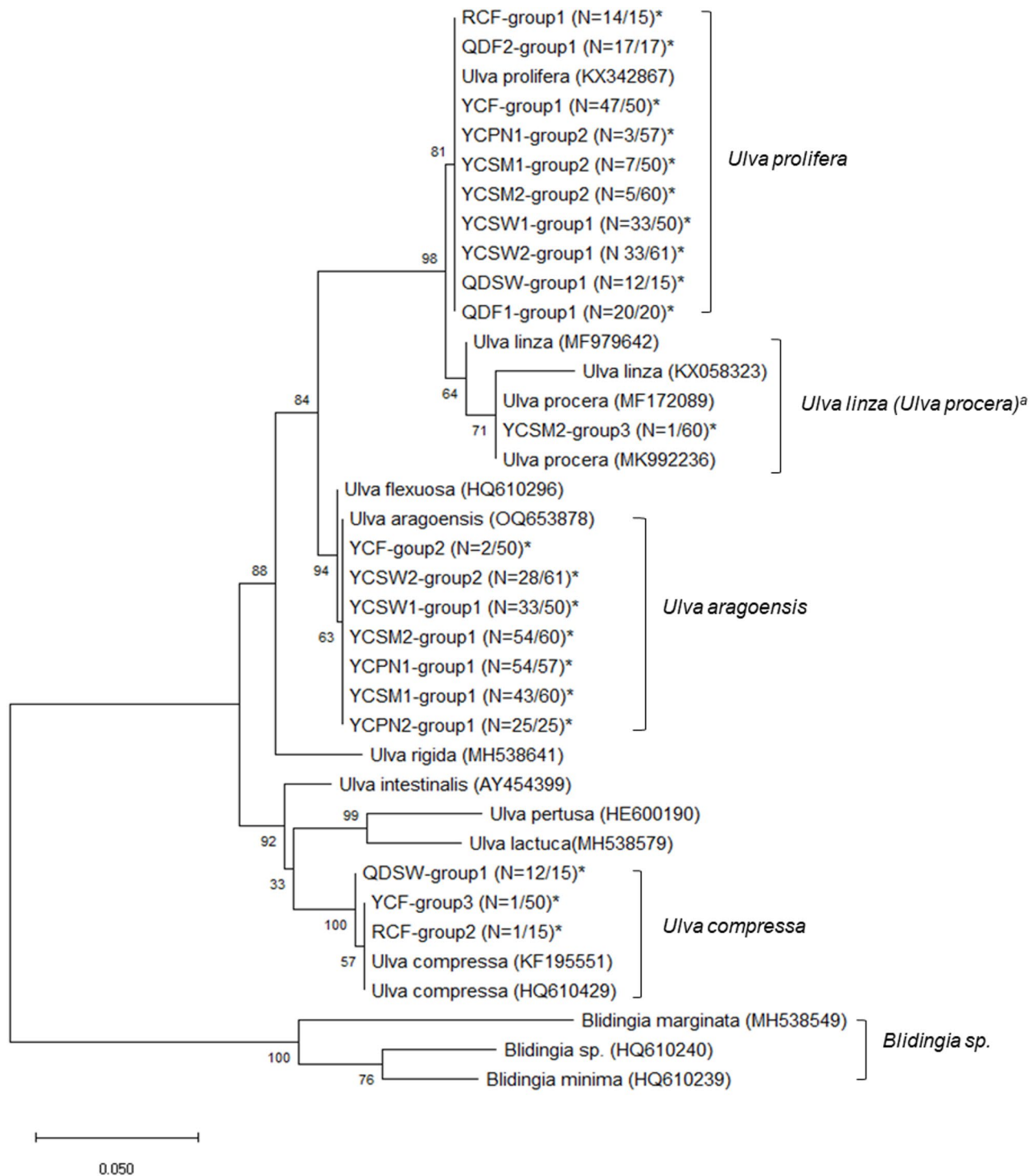


Fig. 4 Phylogenetic tree of *Ulva* and *Blidingia* species based on *tufA* sequences. Population specific clades labeled as “groups” (e.g., YCF-group 1 (N=47/50)). Reference sequences include GenBank accession numbers. Numerals at internal nodes indicate bootstrap support (>50%) from 1000 replicates in maximum likelihood (ML) analysis. The sequences from this study are marked with asterisks (*). ^a Species complexes

Table 3 Species composition and diversity across 11 *Ulva* populations. Data derived from 3 sampling sites via *tufA* phylogenetic analysis. Metrics include species composition, relative frequency, and diversity indices

Population codes	Sampling dates	Number of individuals detected	Number of <i>Ulva prolifera</i>	Number of <i>Ulva aragoensis</i>	Number of <i>Ulva compressa</i>	Number of <i>Ulva linza</i> (<i>Ulva procera</i>) ^a	Ratio of <i>Ulva prolifera</i> (%)
YCSM1	2019-04-04	50	7	43	0	0	14.0
YCSW1	2019-04-04	50	17	33	0	0	34.0
YCPN1	2019-04-04	57	3	54	0	0	5.3
YCPN2	2019-04-04	25	0	25	0	0	0
YCSM2	2019-05-31	60	5	54	0	1	8.3
YCSW2	2019-05-31	61	33	28	0	0	54.1
YCF	2019-05-31	50	47	2	1	0	94.0
QDF1	2019-06-28	20	20	0	0	0	100.0
QDF2	2019-07-12	17	17	0	0	0	100.0
QDSW	2019-05-09	15	3	0	12	0	20.0
RCF	2019-07-17	15	14	0	1	0	93.3
Total		420	166	239	14	1	39.5

^a Species complexes**Table 4** Sporulation rates in *U. aragoensis* and *U. prolifera* populations. Data are derived from one-month observations of populations YCSM1, YCSW1, YCSW2, and QDF1, presenting the total number of individuals and the proportion of sporulating specimens

Populations	Number of <i>U. aragoensis</i>	Number of <i>U. aragoensis</i> releasing spores	Rate of <i>U. aragoensis</i> releasing spores (%)	Number of <i>U. prolifera</i>	Number of <i>U. prolifera</i> releasing spores	Rate of <i>U. prolifera</i> releasing spores (%)
YCSM1	43	37	86.0	7	3	42.9
YCSW1	33	26	78.8	17	8	47.1
YCSW2	28	28	100.0	33	21	63.6
QDF1	0	0	-	20	2	10.0
Average			88.3 ± 10.8^a			40.9 ± 22.5^b

Different superscript lowercase letters indicate statistically significant differences ($p < 0.05$)

documented by Smith [9]. Sporulation frequency is generally higher in warmer summer months than in colder winters [11]. Once sporulated, up to 50% of the cells in vegetative thalli release spores or gametes, resulting in a direct loss of viable biomass. This efficient reproductive strategy has facilitated the expansion and near-global distribution of *Ulva* populations in coastal waters.

The unique geography of the RSSRs at the Subei Shoal provides an ideal habitat for cultivating the commercially valuable red seaweed *Pyropia yezoensis*. Epiphytic green algae, which grow on the *Pyropia* aquaculture rafts (ropes and nets), are routinely removed during farming operations [26]. This discarded biomass has been identified as the primary initial inoculum for green tides in the region, ultimately developing into the large-scale blooms in the Yellow Sea [4]. Our detection of *U. prolifera* propagules in all sediment and seawater samples from the Subei Shoal corroborates earlier findings from multiple studies ([27]; Pang et al., 2010; [28, 29]), further supporting the hypothesis that this area is a key “seed source” for *U. prolifera* outbreaks.

Reasons for the dominance of *U. prolifera*

If the floating *U. prolifera* biomass in the Yellow Sea sporulated at rates comparable to its benthic populations, the rapid accumulation of up to one million tons of biomass over thousands of square kilometers within months would be unlikely. Our finding of a significantly low sporulation rate in *U. prolifera* derived from sediments and water supports this hypothesis. Morphological traits (dense branching) and sporulation characteristics of the studied *U. prolifera* align with those described for subsp. *qingdaoensis* [30]. Although we cannot rule out the presence of subsp. *prolifera* in some samples without molecular confirmation, this does not affect our core conclusion that *U. prolifera* exhibits a lower sporulation rate than *U. aragoensis*. Notably, the sporulation percentage was lowest in the drifted population, probably because thalli with reduced or absent sporulation capacity are retained in the floating biomass more easily.

Although *U. aragoensis* is morphologically highly similar to *U. prolifera* [31], the two species can be clearly distinguished by eight-base-pair difference in the *tufA* gene, which provides better resolution than ITS or *rbcL* (Dafforn et al., 2019; [13]). Surprisingly, despite being a

dominant species in Subei Shoal sediments and water samples, *U. aragoensis* was rarely detected in the local floating biomass. This is notable because *U. aragoensis* possesses key traits for a floating habit: a filamentous branched morphology, rapid growth under suitable conditions, and a tubular thallus that can retain photosynthetic oxygen for buoyancy. Its limited presence in the floating biomass may be explained by its higher sporulation rate compared to *U. prolifera*.

In summary, the low sporulation trait of *U. prolifera* is key to its dominance in the drifting green tides of the Yellow Sea. However, the mechanism sustaining its large propagule population in Subei Shoal waters remains unclear. Alternative sources, such as inputs from adjacent animal aquaculture ponds, may also contribute [26]. These unresolved issues highlight directions for future research.

Conclusions

Chloroplast *tufA* marker analysis confirmed *U. prolifera* as the dominant floating species in green tides. The occurrence of *U. prolifera* propagules in the sediments and coastal waters of the Subei Shoal further supports this area's role as a seed source for the blooms. A key finding is the significantly lower sporulation rate in *U. prolifera* ($40.9 \pm 22.5\%$) compared to *U. aragoensis* ($88.3 \pm 10.8\%$). This suppressed sporulation minimizes biomass loss during dispersal, facilitating the rapid accumulation of floating biomass. Thus, our results not only validate the *tufA* marker for *Ulva* identification, but also reveal that sporulation suppression is a critical adaptive trait underpinning the dominance of *U. prolifera* in the Yellow Sea green tides.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s44377-026-00008-y>.

Supplementary Material 1: Table S1 TufA sequence divergence metrics for *Ulva* and *Blidingia*. Data for five representative *Ulva* individuals and one *Blidingia* spp. outgroup. Above diagonal: pairwise percentage identities. Below diagonal: sequence divergence.

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Authors' contributions

XW designed and executed the experiments, analyzed the data, and drafted the manuscript. TS, SS, and HW critically revised the manuscript. YS conducted sample collection. QL performed **Ulva** culture experiments. All authors read and approved the final manuscript.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Tifeng Shan is an Associate Editor of *Phycology Journal*.

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