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Recurrent Toxic Blooms of *Alexandrium* spp. in the East China Sea-Potential Role of Taiwan Warm Current in Bloom Initiation

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Abstract

Large-scale dinoflagellate blooms started to appear in the coastal waters adjacent to the Changjiang River estuary (CRE) in the East China Sea (ECS) at the beginning of the 21st century. The oceanographic and ecological mechanisms, as well as the impacts of these harmful algal blooms (HABs), received much attention over the last decade. During the studies in the coastal waters adjacent to the CRE from 2004 to 2007, recurrent blooms of *Alexandrium* spp. were observed for the first time. The major causative species was identified as *Alexandrium catenella*. Analysis of the samples collected during the blooms of *Alexandrium* spp. revealed the presence of paralytic shellfish toxins (PST) dominated by low-potency N-sulfocarbamoyl toxins C1 and C2. Toxin content in the *Alexandrium* cells ranged from 17.08 to 33.59 fmol. $Cell^{-1}$. Blooms of *Alexandrium* spp. occurred generally in April and May, but the initiation time of the blooms varied from year to year. It's suggested that initiation of the blooms have little connection with nutrients, as other algal blooms co-existed in this area were more significant in terms of scale and intensity. The initiation time of the blooms, however, seems to be more closely related to the seawater temperature at the bottom, which is affected by the intrusion of Taiwan Warm Current (TWC). Patches of the *Alexandrium* blooms were mainly found in the area of 29.0°-31.0°N, 122.0°-123.0°E, with water depth between 20 m and 50 m, but the distribution of the blooms varied from year to year. The distribution pattern, as well as the cell density of *Alexandrium* spp. during the blooms, was related to the initiation time of the blooms. It was proposed that intrusion of TWC in the sea area adjacent to the CRE may trigger the blooms of *Alexandrium* spp. and affect the distribution and intensity of the blooms. This hypothesis, however, requires more detailed studies on the blooms of *Alexandrium* spp. in this region.

Keywords: Algal blooms; Red tides; Neurotoxins; Paralytic shellfish poisoning; *Alexandrium catenella*

Regional Index Terms

China; East China Sea; Changjiang River estuary

Introduction

There is an apparent global increase and expansion of harmful algal blooms (HABs) over the last four decades [1-5]. Recurrent blooms of *Alexandrium* spp., for example, have been found in many coastal regions around the world, such as those in the Gulf of Maine, USA since 1972 [6,7], in the Bay of Fundy, eastern Canada since 1988 [8,9], along the north-west Mediterranean coast since 1998 [10], and in Hiroshima Bay, Japan [11], etc. Some species in genus *Alexandrium* can produce paralytic shellfish toxins (PST), which pose a potential threat to the health of human-beings following consumption of PST-contaminated shellfish products.

Over 300 deaths were reported from intoxication caused by PST worldwide. Therefore, blooms of the toxic species in genus *Alexandrium* are a significant and increasing concern around the world.

In China, cells of *Alexandrium* spp. have been observed in coastal waters near the Nanhuangcheng Island in the Bohai Sea (BS) [11], Jiaozhou Bay in the Yellow Sea (YS), Xiamen coast in the East China Sea (ECS) [12] and Dapeng Bay of Shenzhen in the South China Sea (SCS) [13,14].

In an investigation of phycotoxins in the shellfish samples collected along the coast of China [15], PST were widely detected in shellfish samples collected from the BS in the north to the SCS in the south. Recent investigations revealed that coastal waters adjacent to the Changjiang River estuary (CRE) in the ECS is one of the major areas in China with PST contamination problems in shellfishes [16-18].

Both vegetative cells and cysts of *Alexandrium* spp. have been found in this region [19,20], but there was no report of blooms caused by *Alexandrium* spp. before the year 2000 [21].

Similarly, no information is available so far on the spatial-temporal variation pattern of *Alexandrium* cells in the ECS and the relationship between the bloom formation and environmental factors.

This paper explores information on the distribution pattern of *Alexandrium* blooms and PST profile in the coastal waters adjacent to the CRE in the ECS. In addition, the potential environmental factors that regulate outbreaks of the blooms are also discussed based on data of the 4-year cruises from 2004 to 2007.

Materials and Methods

Cruises

Four cruises were conducted in spring from 2004 to 2007. The study area was located in the coastal waters adjacent to the CRE in the ECS. Transects and stations of the cruises are shown in Figure 1. The dates of the cruises were April 2-May 13 for 2004, March 27-June 4 for 2005, March 30-May 30 for 2006, and April 4-May 21 for 2007, respectively. Data collected within the area surrounded by the dashed lines were used for analyses of relationships between the blooms of Alexandrium spp. and environmental factors.

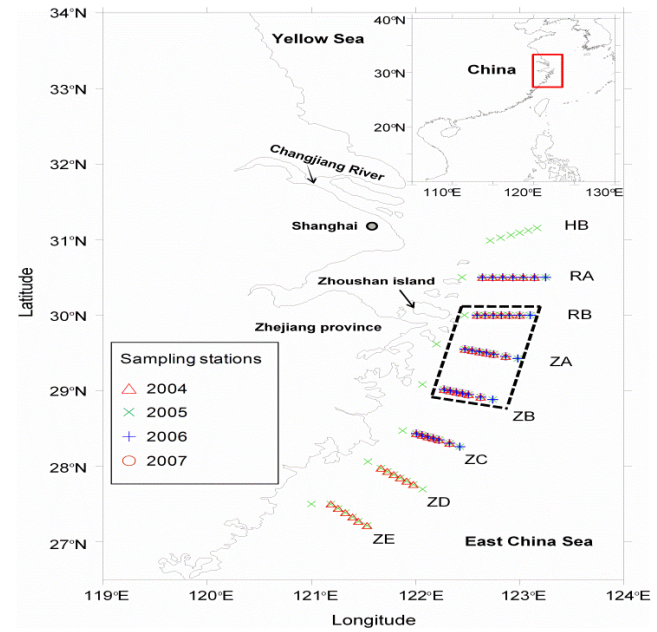

Figure 1: Setup of transects and stations during the cruises from 2004-2007.

Sample collection and preparation

Samples for identification and enumeration of *Alexandrium* cells: Water samples were collected from the surface (0-1 m), the bottom (1 m above the bottom) and the subsurface layer (the layer with the maximum Chl-a level as indicated by a Chl-a sensor on a YSI-6600, usually at the depth of 8-15 m) by a Niskin water sampler at the stations shown in Figure 1. Hydrographic data of the water column were collected with a SBE37-CTD (Seabird Inc., USA) at all stations.

If no maximum Chl-a layer was observed, a mid-water sample was collected at the depth of about 8-15 m depending on the water depth of the station. At some stations, supplementary samples were collected at the depth of 20 m or 30 m when necessary. For identification and enumeration of *Alexandrium* cells, 1 L seawater was collected and sieved onto a 20 μm mesh and backwashed with filtered seawater to a final volume of 20 ml. For stations with low levels of algal cells, 10 L of seawater was sieved. Small portions of the samples were used for live cell observation on board with a light microscope. Other samples were fixed with acid Lugol's solution (3%-5%) for cell counting and for morphological observations in the laboratory with the light microscope (LM) and with scanning electron microscope (SEM).

Samples for analysis of PST: A volume of 100-1000 L of seawater at 5 selected stations containing high densities of *Alexandrium* cells was first concentrated to 100 ml with a 10 μm mesh, 5 ml were fixed with acid Lugol's solution (3%-5%) to enumerate cells of *Alexandrium* spp. The remaining was filtered onto a GF/C glass-fiber filter and kept at -20°C for toxin analysis.

Sample treatment method followed. The filters were cut into pieces with scissors and put into 7 ml centrifuge tubes, 4 ml 0.05 M acetic acid solution was added into the tubes and the mixture was sonicated with a probe sonicator (200 W) for 5 min in an ice-bath until no intact cells were observed.

The mixture was then centrifuged at 10,000 rpm for 5 min, and the supernatant was collected. The residue was extracted again with 1 ml 0.05 M acetic acid solution, and the two supernatants were combined and filtered with a 0.22 μm membrane filter before analysis with HPLC.

A fraction of the extract was hydrolyzed to convert C toxins into the corresponding GTX toxins, using the following procedure: 68 μl of 1 M HCl was added to 300 μl sample solution, the mixture was kept at 90°C for 15 min, and 150 μl 1 M CH_3COONa was added [22]. Both the hydrolyzed sample and the original sample were analyzed with HPLC.

A Waters high performance liquid chromatography system coupled with a fluorescence detector (HPLC-FLD) was used for the analysis of PSP samples. The toxins were separated with a C18 column (Phenomenex, 150 × 4.6 mm, 3 m). The method was used, with slight modifications. Two mobile phases (flow rate 0.8 ml min^{-1}) were used in separation of GTX toxins and STX toxins, respectively.

For analysis of GTX toxins, the mobile phase was 1 mM sodium heptanesulfonic acid in 10 mM ammonium phosphate buffer (pH 7.1). For analysis of STX toxins, the mobile phase was 1.5 mM sodium heptanesulfonic acid in 30 mM ammonium phosphate buffer (pH 7.1), containing 5 percent acetonitrile. The oxidation solution and acid solution were prepared as described in [23]. The temperature of post column derivatization system was 80°C. The excitation and emission wave length used for detection of PSP toxins were 330 nm and 390 nm, respectively.

On board oceanographic data collection and nutrient analysis

Vertical profiles of temperature, salinity, and depth were obtained at every station with a SBE37-CTD (Seabird Inc., USA), while Chl-a and turbidity were measured using sensors installed on the YSI-6600 (YSI Inc., USA). 500 ml water samples were collected by a Niskin water sampler and then filtered through a GF/F filter (Whatman) for nutrient analysis on board by the spectrophotometric method described by [24] to obtain temporal and vertical distribution patterns of several important nutrients such as dissolved inorganic nitrogen (DIN), phosphate and silicate.

Results and Discussions

Causative species of the *Alexandrium* blooms

The live cells of *Alexandrium* spp. collected during the cruises were first observed under a light microscope on board. Based on the

morphological features and chain-forming characteristics (chains with 4 or more cells), the major species of *Alexandrium* spp. was primarily identified as *A. catenella* (Figure 2). This result was further confirmed by the observation of fixed cells under scanning electron microscope, which showed that most of the *Alexandrium* cells had no ventral pore in plate 1' (Figure 2). Cells of the *A. tamarense*, identified based on their morphological features (with ventral pore in plate 1), were also found occasionally in the phytoplankton samples.

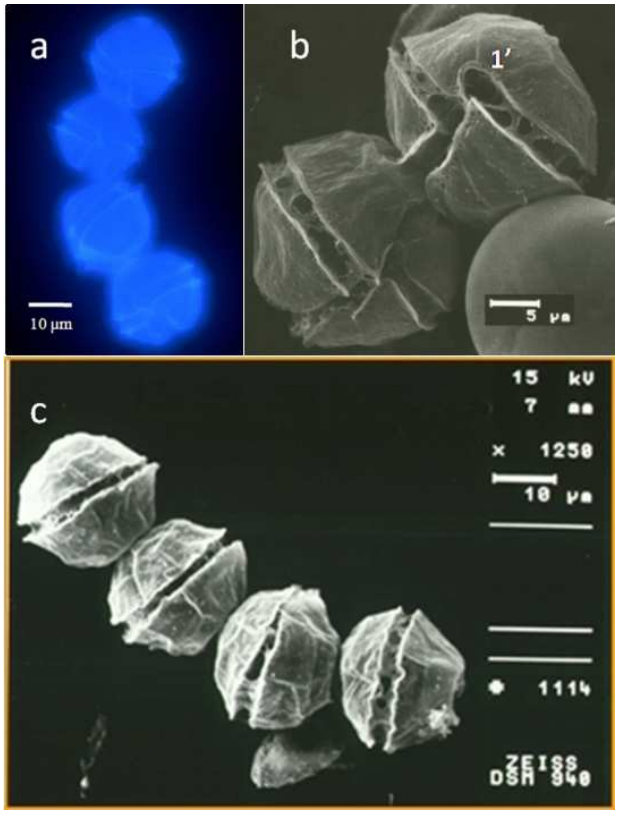

Figure 2: Photos of *Alexandrium* cells collected from the coastal waters adjacent to the Changjiang River estuary in the East China Sea. **2a:** *Alexandrium catenella:* A chain of four cells under light microscope; **2b:** *Alexandrium catenella:* The first apical plate without ventral pore under scan electron microscope; **2c:** *Alexandrium catenella*: A chain of four cells under scanning electron microscope.

A. catenella, A. tamarense and *A. fundyense* were members of the *A. tamarense* species complex, which could be divided into different ribotypes termed as "Temperate Asian", "North America", and "Europe" etc., based on the sequences of small and large-subunit ribosomal RNA genes [25]. These ribotypes were later later renamed as Group I to V by [26]. Both morphotype *A. catenella* and *A. tamarense* were found in several of these ribotypes. In the previous studies, strains of *A. catenella* and *A. tamarense* established from the coastal waters adjacent to the CRE were all assigned to the Group IV of *A. tamarense* species complex, based on the sequences of large subunit ribosomal RNA gene and the ITS regions [27,28]. Recently, [29] revealed the presence of *A. tamarense* species complex (Group I) in the China Seas, but their distribution was confined in the BS and YS, and all the strains of *A. tamarense* and *A. catenella* established from the ECS and SCS up to now belonged to the Group IV of *A. tamarense* species complex. Therefore, the major causative species of the *Alexandrium* blooms in the sea area adjacent to the Changjiang River can be considered as *A. tamarense* species complex (Group IV). The morphological features of A. *catenella* and *A. tamarense* are quite similar, and the major difference is the absence (for *A. catenella*) or presence (for *A. tamarense*) of a ventral pore in the first apical plate. So the two species were not discriminated from each other during most of the investigations.

Toxin production and potential impacts of the *Alexandrium* blooms

PST in the net-concentrated phytoplankton samples collected during the *Alexandrium* blooms were analyzed with the HPLC-FLD system. PST components, including C1, C2, GTX1, GTX2, GTX3, GTX4, and neoSTX, were detected. The dominant toxins were C1 and C2, which contributed more than 92% of the total PSP toxins (Figure 3). Toxin levels of the samples collected at the 5 stations in 2005 were summarized in Table 1, with a range of 17.08-33.59 fmol/cell and an average level at 24 fmol/cell. Compared to other toxic species in genus *Alexandrium*, such as *A. fundyense* in the Casco Bay (cellular toxin content 113-155 fmol cell^{-1}) and *A. catenella* in the Argentine Sea (cellular toxin content 45.53-261.4 fmol cell^{-1}) [30,31], *A. catenella* in the ECS produces much lower amounts of PST.

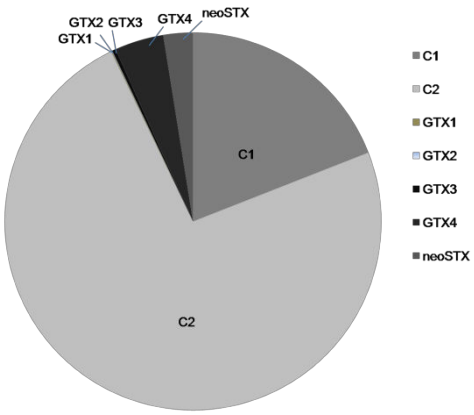

Figure 3: Profile of paralytic shellfish toxins in a net-concentrated phytoplankton sample collected during the *Alexandrium* bloom in 2005.

Toxin profile is a relatively stable characteristic for a specific toxic species when grown under constant conditions. So far two different toxin profiles were found among the strains within the *A. tamarense* species complex (Group IV) isolated in China. Most of the strains from mainland China produce a large proportion of C1 and C2 toxins together with trace amount of other GTX toxins [32,33], while the strains from the coastal waters of Hong Kong produce a relatively high proportion of GTX toxins, particularly GTX1, 4, besides C toxins. Analysis of the strains of *A. tamarense* species complex (Group IV) established from the coastal waters adjacent to the CRE also found high proportion of C toxins (C1 and C2) similar to those of the field samples collected in the current study. This may give another clue that the bloom was caused by the *A. tamarense* species complex (Group IV). Toxin content calculated from the current study is in consistence

with those strains of *A. tamarense* species complex (Group IV) established from the China Seas, with toxin level between 11.9 and 64.0 fmol cell^{-1}.

Some previous studies reported the contamination of shellfish by PST in the coastal waters adjacent to the CRE. Compared to other coastal regions affected by *Alexandrium* blooms in China, however, the status of PST contamination in the CRE region is not that serious, generally being 0-64 μg STX equiv./100 g fresh shellfish soft tissue, and the maximum toxin content in shellfish recorded was 64 μg STX equiv./100 g in Scapharca subcrenata collected from Daishan Island of Zhoushan in 2006. In the northern Yellow Sea, PST content detected in shellfish could be as high as 640 μg STX equiv./100 g in scallops Patinopecten yessuensis. In the SCS, PST content in scallops Chalmys nobilis could reach 100 μg STX equiv./100 g. Both were above 80 μg STX equiv./100 g, the acting limit for shellfish safety in China and many other countries. Toxin content and composition of the *Alexandrium tamarense* species complex (Group IV) in this region may partially account for the relatively lower PST levels in shellfish, as the strains of *A. tamarense* species complex (Group IV) from this region produce not only much lower level of toxins (~ 24 fmol cell^{-1}), but also the low-potency components of PST (C1, C2) (Table 1 and Figure 3).

	C1	C2	GTX1	GTX2	GTX3	GTX4	neoSTX	Total
122.9190°E, 30.7510°N	3.07	28.95	0.01	0.01	0.16	0.49	0.9	33.59
122.7831°E, 30.5618°N	1.42	14.85	0.02	0.01	0.09	0.28	0.41	17.08
122.6110°E, 30.4992°N	7.95	9.07	0.04	0.02	0.05	0.06	0.26	17.45
122.7470°E, 30.5123°N	7.53	8.26	0.01	0	0.03	3.27	0.47	19.57
122.6023°E, 30.0017°N	2.8	27	0	0	0.05	0.93	0.99	31.77
Average	4.55	17.63	0.02	0.01	0.08	1.01	0.61	23.89
Standard deviation	2.98	9.81	0.02	0.01	0.05	1.31	0.32	8.1

Table 1: Toxin content and composition of the net-concentrated phytoplankton samples collected during the *Alexandrium* bloom in 2005

Dynamics of the *Alexandrium* blooms

Alexandrium blooms occurred every spring from 2004 to 2007, and duration of the blooms was usually between 1-2 weeks in the sea area adjacent to the CRE (Figure 4). The blooms appeared from May 2 to 10 in 2004 (Julian days 122-130), May 26-30 in 2005 (Julian days 146-150), April 19-May 4 in 2006 (Julian days 109-124) and April 5-10 in 2007 (Julian days 95-100), respectively.

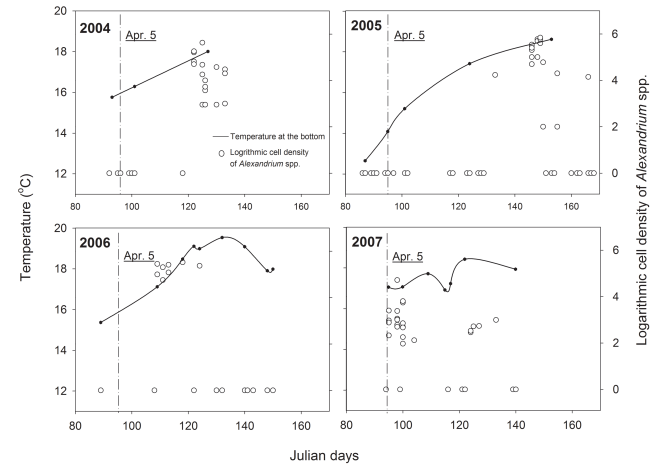

Figure 4: Relationship between bottom water temperature at station ZB11 (122.5°E, 28.95°N) and the initiation of *Alexandrium* blooms in springs of 2004-2007.

The vertical dashed lines indicated the date of April 5, on which the bottom temperature was adopted to analyze its relationship with the initiation of *Alexandrium* blooms in Figure 6.

A. tamanrense spp. was not the only bloom-causative species in the coastal waters adjacent to CRE in spring 2004 to 2007. The major algal blooms in this area were caused by the diatom Skeletonema costatum in early spring (around Julian day 90-100) followed by dinoflagellates like Prorocentrum donghaiense and Karenia mikimotoi (around Julian day 130-150). Generally, two peaks of chlorophyll a would be observed during spring (Figure 5, in the year 2005). The first peak represented the diatom bloom in early spring, and the other peak was a bloom of dinoflagellates.

Interestingly, blooms of *Alexandrium spp.* did not occur synchronously with those of otherdinoflagellates, such as *P. donghaiense* and *K. mikimotoi. Alexandrium* spp. bloomed early with the diatoms in 2007, late during the blooms of other dinoflagellate species in 2004 and 2005, and between the diatom bloom and the dinoflagellate bloom in 2006 (Figure 5).

Nutrients should have little effects on the initiation of the *Alexandrium* blooms, as other algal blooms co-existed in this area, such as those of *S. costatum* and *P. donghaiense*, were more significant in terms of scale and intensity. Cell densities of *S. costatum* and *P. donghaiense* during the blooms could reach 10^7cells/L. The *Alexandrium* cells, however, did not contribute substantially to the total algal biomass due to the relatively low cell density during the blooms (normally less than 10^5 cells l^{-1}). The initiation date of the blooms of *Alexandrium* spp. seems to be more closely related to the seawater temperature at the bottom. Figure 4 illustrates the relationship between water temperature at the bottom in earlier April (~Julian day 95) and the cell density of *Alexandrium* spp. in station ZB11 (122.5°E, 28.95°N), a representative station for the *Alexandrium*

blooms. The investigation results showed that *Alexandrium* spp. bloomed earlier if the bottom water temperature increased earlier and vice versa. For example, *Alexandrium* bloomed in early April in 2007 when the bottom temperature was the highest in early April. In 2005, bloom of *Alexandrium* didn't occur until late May, as the temperature was the lowest observed in early April. This bloom occurred some 50 days later than that in 2007. A strong negative correlation (R2=-0.941) was observed between the outbreak date (Julian days) of *Alexandrium* blooms and the water temperature at the bottom in early April (~ Julian day of 95) as shown in Figure 6.

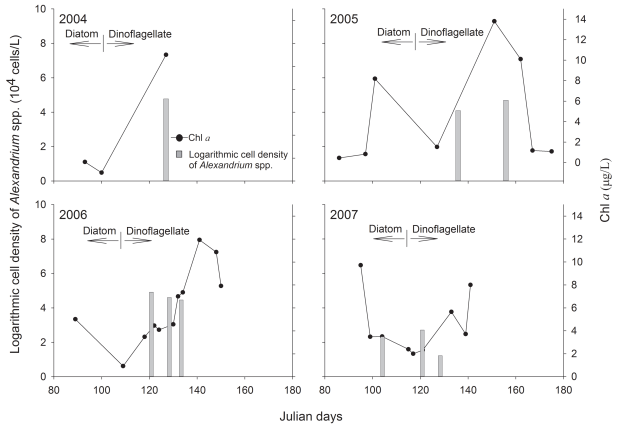

Figure 5: Relationship between *Alexandrium* blooms and other blooms. Arrows roughly indicate the stage of diatom blooms and dinofalgellate blooms in spring.

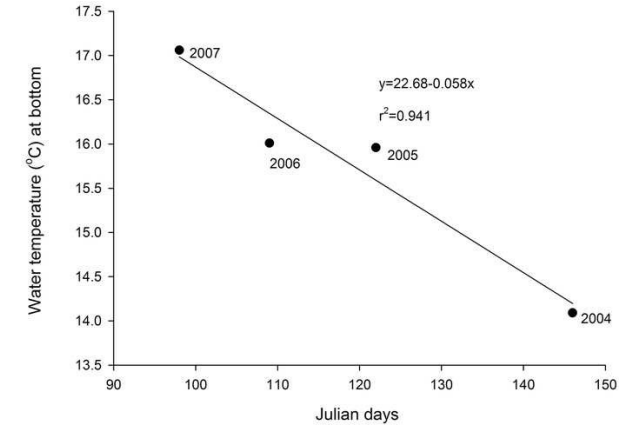

Figure 6: Relationship between water temperature at bottom on April 5 at station ZB11 (122.5°E, 28.95°N) and initiation of *Alexandrium* blooms.

Previous studies on the *Alexandrium* blooms in the Gulf of Maine suggested that cysts were the important seed source and key to the initiation of the blooms [34,35] also found the same phenomenon along the Catalan coast in the Mediterranean Sea. In our study area in the ECS, *Alexandrium* cysts have been widely found in the surface sediment [36], particularly in the region southeast to the Zhoushan Island [37]. The distribution pattern of *Alexandrium* cysts matched well with the distribution pattern of the *Alexandrium* blooms documented in the current study. These cysts could serve as a seed source for the recurrent *Alexandrium* blooms in this area found that the density of *Alexandrium* cysts in surface sediment of ECS had a clear seasonal variation pattern, with the highest values in summer and autumn, intermediate in winter, and lowest in spring. This may reflect cyst germination (for the bloom) in spring and encystment in summer after the blooms of *Alexandrium* [38] studied the germination rate of cysts isolated from surface sediments in the ECS and the survival rate of the germinated cells. Cysts of *Alexandrium* spp. could germinate between 9°C and 21°C, but germination was the fastest when temperature was above 15°C. Therefore, warmer temperature at the sea bottom in spring would lead to large and synchronized cell germination at the time of bloom initiation.

In the coastal waters adjacent to the CRE, intrusion of the Taiwan Warm Current (TWC) at the bottom layer from early spring to late summer is a unique physical oceanographic process in this region. Many studies observed a clear invasion of warm water at the bottom of the bloom area in early spring, a typical upwelling phenomenon driven by TWC. Our investigation also confirmed that the warm water intrusion (Figure 7) into the bottom in the study area was evident in spring but the degree of temperature increase was different from year to year [39]. Therefore, we deduced that outbreak of the *Alexandrium* blooms in this region might be a process driven by the intrusion of TWC and its associated temperature increase at the bottom, which promoted the germination of the cysts in the surface sediment. Besides, the intrusion of TWC (seawater with much higher salinity compared to the Changjiang diluted water) at the bottom also contributes to the formation of stable stratification, which is beneficial for the development *Alexandrium* blooms.

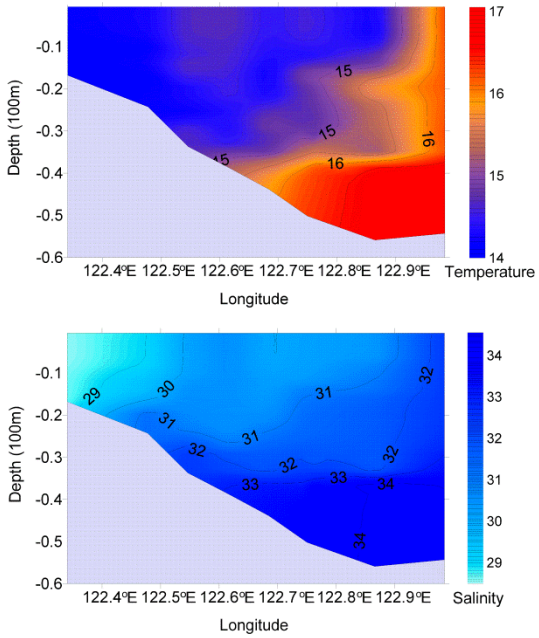

Figure 7: Vertical profiles of temperature and salinity at transect ZA (29.5°N) on April 21, 2006.

Distribution of the *Alexandrium* blooms

During the four-year investigations, patches of dense *Alexandrium* cells were mainly found in the area of 29.0-31.0 ° N, 122.0-123.0 ° E, where the water depth was between 20 m and 50 m (Figure 8). Density of the *Alexandrium* cells in the patches was generally higher than 10^5 cells/L. The area of one patch sometimes covered an area of about 400 km^2.

The distribution pattern of blooms varied from year to year. The bloom occurred mainly in the sea area southeast to the Zhoushan Island when *Alexandrium* bloomed early in 2007, east to the Island when *Alexandrium* bloomed in mid-season in 2004 and 2006, and northeast to the Island when *Alexandrium* bloomed late in 2005 (Figure 8). It seems that the distribution pattern is closely related to the initiation time of the blooms. When the bloom started earlier, the distribution of the blooms was more southwards. And the blooms appeared more northwards when the bloom started later. Besides, cell density of *Alexandrium* spp. also varied from year to year (Figure 8), with a potential linkage to the initiation of the blooms. Cell density recorded was relatively higher when *Alexandrium* blooms occurred later, such as those in 2004, 2005 and 2006 (10^5-10^6 cells/L), and cell density was much lower when *Alexandrium* bloomed earlier in 2007. The maximum cell density recorded in 2007 was less than 10^4 cells/L.

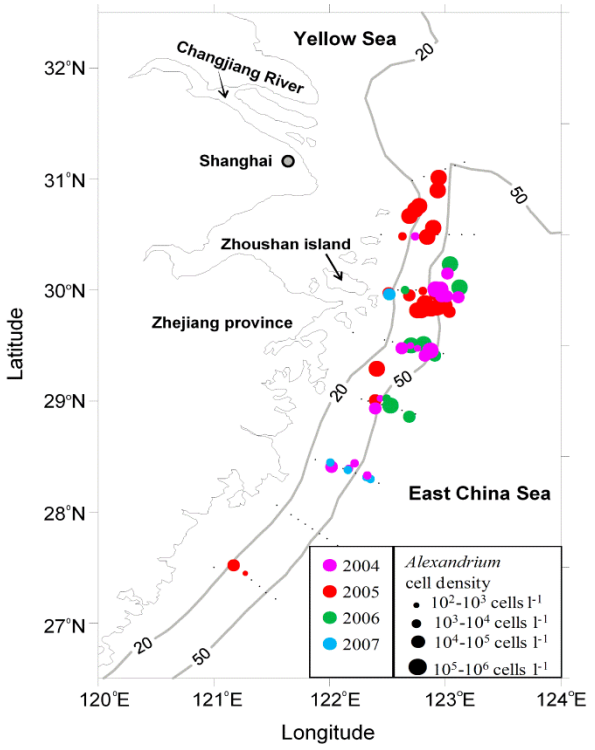

Figure 8: Distribution pattern of *Alexandrium* blooms at surface in the coastal waters adjacent to the Changjiang River estuary in the East China Sea from 2004-2007.

Water temperature and salinity in the bloom area of *Alexandrium* spp. were analyzed to reveal the relationship between cell density of *Alexandrium* spp. and their distribution. It was suggested that the blooms occurred in different water masses in the four years, depending upon the timing of the blooms (Figure 9). If *Alexandrium* spp. bloomed earlier, for example, in 2007, the cells of *Alexandrium* spp. were mainly distributed in the water mass from the open sea, with relatively lower temperature (~13ºC-15ºC) and higher salinity (~30-33). If *Alexandrium* spp. bloomed later, like that in 2005, the cells were mainly distributed in the coastal waters with relatively higher temperature (~19ºC-21ºC) and lower salinity (~28.5-30). This might explain the obscured difference in cell density between the blooms occurred early in 2007 versus that occurred later in 2005.

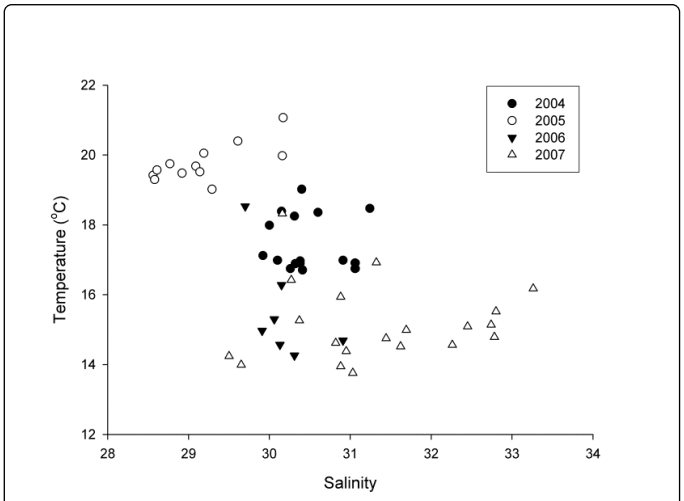

Figure 9: Relationship between surface water mass (classified by temperature and salinity characteristics) and *Alexandrium* blooms.

Coastal water rich in nutrients would support a much higher cell density of *Alexandrium* spp. than the water mass from the open sea (Figure 10). Besides, coastal water is rich in growth stimulants like humic substances, which will also promote the growth of *Alexandrium* spp. [40]. Meanwhile, the relatively high temperature at the surface would also favor the growth of *Alexandrium* spp. if they bloomed later. Therefore, the distribution pattern and cell density of *Alexandrium* blooms was related to the initiation time of the blooms.

Another finding regarding the distribution of *Alexandrium* blooms is that *Alexandrium spp.* bloomed only in seawater where turbidity was less than 13 NTU (Figure 11). The CRE and Zhoushan archipelago are surrounded by turbid seawater in winter and spring as a result of huge amounts of sediment carried by the Changjiang River and re-suspended by strong winds in those seasons. The turbid water could partially explain why *Alexandrium* blooms showed such a distribution pattern as seen in Figure 8. Due to the high turbidity, the blooms were always insulated from the inshore area west to the Zhoushan archipelago, where the main shellfish mariculture operations are located. This may protect the mariculture zone from being affected by the blooms. Recently, however, there is a decreasing trend of sediments carried by the Changjiang River following the construction of the Three Gorge Dam [41]. The *Alexandrium* blooms could therefore extend closer to the shellfish farms if the sediment carried by the Changjiang River continues to decrease. Someday this trend may result in conditions that no longer prevent the *Alexandrium* blooms from reaching the inshore area [42-47]. The persistence of recurrent *Alexandrium* blooms may lead to more serious problem of PST contamination in shellfish in the future.

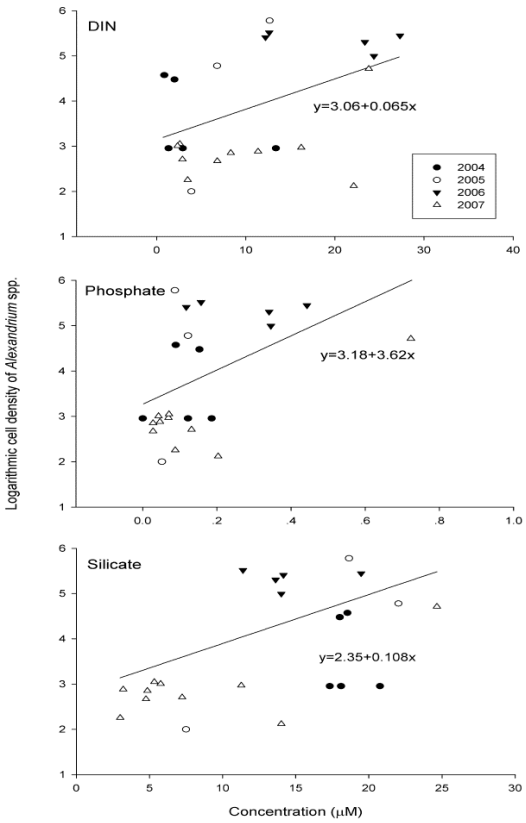

Figure 10: Relationship between nutrient concentrations (DIN, PO_4-P, SiO_2-Si) and cell density of *Alexandrium* spp.

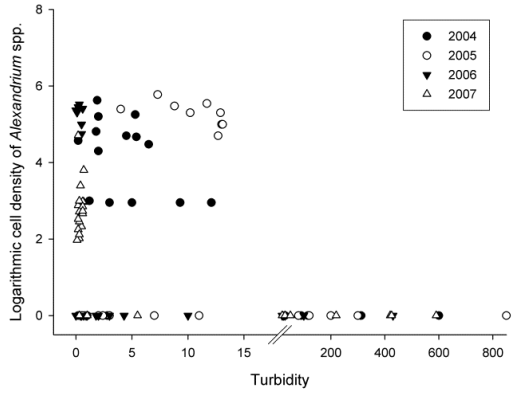

Figure 11: Relationship between surface turbidity and cell density of *Alexandrium* spp.

Conclusion

In the current study, we found recurrent *Alexandrium* blooms from 2004-2007 in the coastal waters adjacent to CRE. The dominant species was identified as *Alexandrium catenella*. During the blooms, PST dominated by the low-potency N-sulfocarbamoyl toxins C1 and C2 were detected. Patches of dense *Alexandrium* cells were mainly distributed in the surface waters near Zhoushan Island with water depth between 20m and 50m. The distribution pattern and intensity of the blooms were closely related to the initiation of the blooms, which had a significant relationship with water temperature at the bottom. It was proposed that intrusion of TWC and its associated temperature increase at the bottom may trigger the initiation of the *Alexandrium* blooms in the ECS, and subsequently affect the distribution and intensity of the blooms. The persistence of the *Alexandrium* blooms, with the long-term change of the turbidity in the coastal waters adjacent to CRE, may lead to increased risk of PSP poisoning in this region.

Limitations

After the four cruises which were conducted in spring from 2004 to 2007, there were no cruises related to the toxic blooms of *Alexandrium* spp. in the East China Sea. Hence, the recent data cannot be provided, and then it's impossible to explain the influence on the current situations.

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