

Outbreak of *Skeletonema costatum* Bloom and Its Relations to Environmental Factors in Jiaozhou Bay, China

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Abstract: - The marine diatom *Skeletonema costatum* is commonly found in coastal and estuarine areas from the temperate zones to the tropical zones. Blooms caused by *Skeletonema costatum* have been reported in almost all the eastern coastal regions of China seas. Jiaozhou Bay, located at the middle of Yellow Sea of China, is a shallow semi-closed basin. *Skeletonema costatum* constitutes a major fraction of dominant species among more than forty red tide organisms documented in this bay. This paper presents a dynamic course of *Skeletonema costatum* bloom occurring in July of 1998. Relationship between ambient factors and biomass has been analysed to gain insights into the blooming mechanism.

Key-Words: - *Skeletonema costatum*, Environmental factors, Statistical analysis

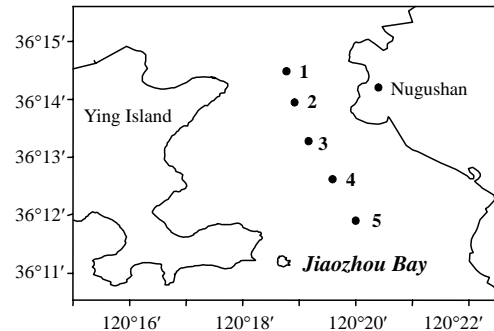
1 Introduction

A *Skeletonema costatum* bloom [1-3] was happening during July 3-8, 1998 in Jiaozhou Bay [4]. The outbreak of the *Skeletonema costatum* bloom was found to be associated with a heavy rain between June 30 and July 1, 1998. Relatively high concentrations of nutrients, iron and manganese were responsible for the growth of *Skeletonema costatum*. After the heavy rain, warm, low-salinity and eutrophic water resulted in rapid proliferation of *Skeletonema costatum*. On nutrient structure, compared with TIN (total inorganic nitrogen), concentrations of silicate and phosphate were relatively deficient. At the developing stage, the concentrations of various nutrients and iron in surface water as well as TIN were declining due to declination of nitrate. Among three forms of TIN, it was possible that *Skeletonema costatum* preferred to absorb nitrate. At the blooming stage, exhaustion of phosphate and silicate led to death of *Skeletonema costatum* and disappearance of the bloom. After the bloom disappeared, the content of TIN and silicate returned rapidly to normal, but the concentration of phosphate still remained low-level and re-normalized after a considerable time. Two statistical methods (correlation analysis and principal component analysis) were used to determine the mechanism of the red tide. Combined results showed that the ecological factors such as temperature, salinity, silicate and phosphate were critical in affecting the occurrence of the *Skeletonema costatum* red tide.

2 Material and Methods

Samples were collected at the five routine stations (Fig. 1) located in the northeast of Jiaozhou Bay. Sampling was performed once a week from May to September 1998 and once a day during blooming period. Because mean water depth was less than 4m, surface water was collected using a plastic barrel.

Fig. 1: Sampling locations in Jiaozhou Bay



The water samples were filtered using 0.45 μ m acetate fiber filters and then separated into two parts. One part for analysing nutrients was preserved (with 0.3% chloroform) in polyethene bottles and frozen immediately. Another part for analysing dissolved iron and manganese was added 1mol/l HCl (1ml of HCl for 500ml filtrates) and also frozen at once. Phytoplankton samples were preserved using acid Lugol solution. Samples for measuring dissolved oxygen were fixed on a board ship.

Water temperature was measured *in situ* by reversed thermometers (precision ± 0.05 °C); salinity

by salinometer (precision $\pm 0.05PSU$); and dissolved oxygen by the Winkler method according to Strickland & Parsons [5] (precision $\pm 0.02ml/l$). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were determined according to Criterion of oceanographic monitoring.

The determinative methods of nutrients were phosphomolybdenum blue method for $PO_4\text{-P}$, silicomolybdenum blue method for $SiO_3\text{-Si}$, reduction method by copper-cadmium for $NO_3\text{-N}$, Griess-Ilosvay method for nitrite, and oxidation method by sodium hypobromite for ammonia. All water samples were determined by colorimetry.

Chlorophyll *a* was measured according to the recommended methods by UNESCO [6] and calibrated using the equation presented by Jeffrey & Humphrey [7].

3 Results

The abnormal watercolor was found in the morning of July 3 in the region of $36^{\circ}14'N$, $120^{\circ}19'E$. The examination of phytoplankton samples indicated that the cell density of *Skeletonema costatum* reached to $4.5 \times 10^6 \text{ cells ml}^{-1}$ and accounted 95% of the phytoplankton population. On July 4, a large-scale survey was conducted in the whole bay and the results showed that brown water covered the areas

Table 1: Concentrations of nutrients before occurrence of red tide in Nugushan of Jiaozhou Bay ($\mu\text{mol l}^{-1}$)

Region	$NH_4\text{-N}$	$NO_3\text{-N}$	$NO_2\text{-N}$	TIN	$PO_4\text{-P}$	$SiO_3\text{-Si}$	$\Sigma\text{N-N/P}$	$Si/\Sigma\text{N-N}$
Nugushan	10.42 (41.8%)	12.16 (47.8%)	2.74 (10.4%)	25.32	0.51	5.64	49.6	0.22
Jiaozhou Bay*	7.5 (79.0%)	1.3 (13.7%)	0.69 (7.3%)	9.49	0.36	3.90	26.4	0.41

* mean concentration of nutrients in whole Jiaozhou Bay in summer [12]

There was no river input in the studied area and the water dynamics was mainly controlled by periodic tide. Results showed that the tidal velocity was relatively small and the water mixing was also weak [9,10]. Organic matters resulted from municipal sewage were remained for a relatively long time and a large portion was accumulated in sediment. Therefore, the capacity of water self-purification reached its maximum [11]. In last decades, aquiculture had rapidly increased in this area. The increasing load of nitrogenous and phosphorous compounds from shrimp farming had led to the severe eutrophication that was extremely available to the growth and proliferation of phytoplankton [12].

Variation of environmental parameters during

from $36^{\circ}14'N$, $120^{\circ}19'E$ to $36^{\circ}18'N$, $120^{\circ}23'E$. The spatial patterns presented dense stripe parallel to the shore or clot distribution. The blooming area was estimated about 10 Km^2 . It was the afternoon of July 5 that the bloom began to disappear gradually. The color of seawater returned to that of normal *aqua*. The concentration of chlorophyll *a* also decreased to a low level.

Variation of environmental factors before blooming

In Jiaozhou Bay, the concentration of chlorophyll *a* ranged from 1.84 to 5.44 mg m^{-3} and the average was 3.46 mg m^{-3} . Compared with other regions in China, it belonged to normal range [8]. The mean water temperature, salinity and pH were 22.7°C ($21.8\text{-}23.4^{\circ}\text{C}$), 31.24 ($31.20\text{-}31.26$) and 7.85 ($7.83\text{-}7.89$) respectively.

The concentration of total inorganic nitrogen (TIN) in the studied area, Nugushan, was obviously higher than that in Jiaozhou Bay (see Table 1). The proportions of the three forms of inorganic N in TIN were 41.8% for $NH_4\text{-N}$, 47.8% for $NO_3\text{-N}$ and 10.4% for $NO_2\text{-N}$. $NH_4\text{-N}$ accounted 79.0% of the total inorganic N in whole Jiaozhou Bay. The concentrations of $PO_4\text{-P}$ and $SiO_3\text{-Si}$ were slightly higher than that of whole bay average. The atomic ratio between TIN and $PO_4\text{-P}$ in the studied area was 49.6 and higher than its normal value of 16 in ocean water.

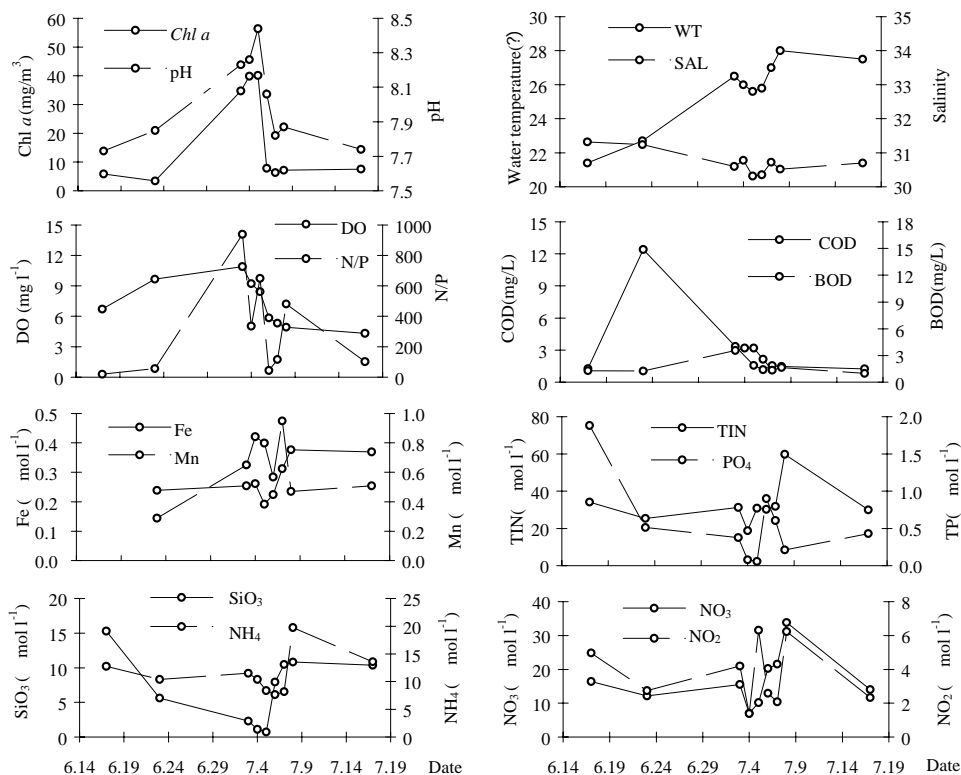
blooming

The dynamic variations of environmental factors were indicated in Fig. 2.

Nutrient concentration: Before this bloom, there was a relatively heavy rain between June 30 and July 1 which carried large terrigenous pollutants into this region. The rapid increase in TIN concentration stimulated the growth of *Skeletonema costatum*. Along with the bloom developing, $NO_3\text{-N}$ and $NH_4\text{-N}$ declined gradually. In the blooming climax, the concentrations of $NO_3\text{-N}$ and $NH_4\text{-N}$ decreased from $11.51 \mu\text{mol l}^{-1}$ and $15.54 \mu\text{mol l}^{-1}$ to $10.39 \mu\text{mol l}^{-1}$ and $7.06 \mu\text{mol l}^{-1}$ respectively. During the dying period, the concentrations of all forms of nitrogen rapidly backed to the level of the initial period. It might be ascribed to low uptake capacity in the late blooming

stage and the nutrient released from decomposing dead algal cells.

Fig. 2: Variations of environmental factors during the red tide



The concentrations of $\text{PO}_4\text{-P}$ and $\text{SiO}_3\text{-Si}$ in the surface layer decreased all the time in the overall blooming stage which fell from $0.51\ \mu\text{mol l}^{-1}$ and $5.64\ \mu\text{mol l}^{-1}$ in the initial period to $0.06\ \mu\text{mol l}^{-1}$ and $0.72\ \mu\text{mol l}^{-1}$ respectively. In the blooming climax, $\text{PO}_4\text{-P}$ and $\text{SiO}_3\text{-Si}$ were almost depleted. Moreover, there were three stations where $\text{PO}_4\text{-P}$ and $\text{SiO}_3\text{-Si}$ could not be detected (lower than the detection limit). During the dying period, $\text{PO}_4\text{-P}$ recovered a little and remained in a low level, but for $\text{SiO}_3\text{-Si}$, its concentration recovered rapidly and returned to the comparable level of the initial period ($>10\ \mu\text{mol l}^{-1}$).

$\Sigma\text{N-N/P}$: According to this survey, $\Sigma\text{N-N/P}$ was 25 before blooming which is adjacent to the value ($\Sigma\text{N-N/P}=29$) monitored by Jiaozhou Bay Ecological web from May 1991 to February 1993 [12,13]. It was higher than normal value of 16 in ocean water and organism. In the initial blooming stage, the average of $\Sigma\text{N-N/P}$ in surface water rose promptly from 25 to 56.7 and even reached to 938 in its climax until the phosphate was depleted. This increase was primarily caused by the decrease of phosphate concentration.

Dissolved iron and manganese: During blooming,

mean concentration of dissolved iron decreased from $0.24\ \mu\text{mol l}^{-1}$ to $0.19\ \mu\text{mol l}^{-1}$ and returned to a normal level after blooming, whereas the concentration of dissolved manganese varied a little.

Variation of other environmental parameters: Due to lasting two-day rainfall before the bloom, salinity decreased from 31.24 to 30.59. Water temperature rose from 22.7°C to 26.5°C (increase about 4°C). During this bloom, water temperature and salinity ranged $25.6^\circ\text{C}\sim 28.0^\circ\text{C}$ and $30.31\sim 30.78$ respectively. The variations of dissolved oxygen (DO) and pH had a good positive correlation with biomass. When algae grew and propagated quickly, the increases of DO and pH were attributable to algal cells absorbing a large amount of carbon dioxide and releasing oxygen due to photosynthesis. When the bloom began to collapse, algae died away and were decomposed by microorganism such as bacteria. DO and pH decreased simultaneously owing to the consumption of dissolved oxygen.

4 Statistics of Environmental Parameters during Blooming

Correlation Analysis (CA): Coefficients between chlorophyll *a* and other environmental parameters were listed in Table 2. It shows that DO and pH had a strong positive correlation with chlorophyll *a*, and

salinity, PO₄-P, SiO₃-Si and NO₃-N had a negative correlation with chlorophyll *a*. However, there was no relationship between chlorophyll *a* and water temperature, COD, BOD, Fe, NH₄-N and NO₂-N.

Table 2: Spearman rank coefficient of chlorophyll *a* and environmental factors

Chl <i>a</i>	pH*	T	S*	DO**	COD	BOD	PO ₄ *	SiO ₃ **	Fe	Mn	NH ₄	NO ₃	NO ₂
<i>R</i>	0.52	0.20	-0.49	0.29	0.19	0.18	-0.42	-0.32	0.00	0.22	0.06	-0.21	0.07
<i>t_s</i>	4.07	1.34	-3.81	2.02	1.31	1.23	-2.92	-2.15	-0.02	1.52	0.39	-1.47	0.44
<i>α</i>	<0.01	0.19	<0.01	<0.05	0.20	>0.20	<0.01	<0.05	>0.20	0.13	>0.20	0.15	>0.20

Note: *R* is the Spearman rank correlation; *t_s* is the *t*-test critical value; *α* is the significant level

*: strong correlation under 0.01 level (*t*_{0.01,45}=2.58); **: strong correlation under 0.05 level (*t*_{0.05,45}=1.96)

Principal component analysis (PCA): Forty-seven samples from June 17 to July 17 were used in the PCA. In order to get sufficient information, all

parameters were included in computation and lastly summarized into four principal components with only 20.5% loss of information (see Table 3).

Table 3: Factorial loading of each principal component

	Chl <i>a</i>	pH	DO	PO ₄ -P	SiO ₃ -Si	SAL	WT	NO ₃ -N	NO ₂ -N	Fe	Mn	CR*(%)
Z1	-0.71	-0.88	-0.73	0.68	0.92	0.23	0.30	0.08	-0.03	0.20	-0.12	31.47
Z2	0.42	0.35	-0.44	0.13	0.04	-0.90	0.72	0.12	0.15	-0.04	0.24	53.76
Z3	-0.11	0.04	0.03	0.44	-0.12	0.08	-0.40	-0.90	-0.85	0.15	0.20	70.70
Z4	0.09	0.04	0.15	0.28	-0.04	0.01	-0.26	0.22	0.15	-0.88	-0.81	79.49

Principal component: Z₁ = - 0.71Chl*a** - 0.88pH* - 0.73DO* + 0.68PO₄-P* + 0.92SiO₃ - Si*,
Z₂ = - 0.90SAL* + 0.72WT*, Z₃ = - 0.90NO₃ - N* - 0.85NO₂ - N*, Z₄ = - 0.88Fe* - 0.81Mn*

* Standard normal variate; CR is the cumulated proportion

The first, second, third, and fourth component accounted 31.17%, 22.29%, 16.94% and 8.79% for the variation of whole information. The first component mainly included chlorophyll *a*, pH, DO, dissolved phosphate and silicate. Therein, concentration of chlorophyll *a* mostly reflected the biomass of phytoplankton; pH and DO were closely related to the biomass. Their variations were influenced by the photosynthesis of phytoplankton, but they themselves did not exert any influence on the phytoplankton. Dissolved phosphate and silicate also occurred in the first component. According to the CA results, they were inversely related to chlorophyll *a*. The results showed that dissolved phosphate and silicate were possibly the limited nutrients. The second component was composed of salinity and water temperature, which principally implied the hydrological situation of seawater. In terms of CA, salinity had a negative correlation with chlorophyll *a*. Thus, the second component reflected the effects of rainfall in the last two days of June on the blooming formation. The third and fourth components reflected mostly the trophic situation of seawater.

Effects of water temperature and salinity on blooming: Skeletonema costatum could be found in regions with extreme water temperature and salinity. But the optimum temperature and salinity were 14~28 °C and 20~30 respectively. In Jiaozhou Bay, the peak of *Skeletonema costatum* always occurred in June and July during the period when water temperature and salinity had the ranges of 23.0~28.0 °C and 31.5~32.3 respectively [4]. During *Skeletonema costatum* bloom occurred in June of 1990 in East Sea, water temperature and salinity had ranges of 21.8~25.1 °C and 18.7~23.9 [14]. However, in the period of *Skeletonema costatum* bloom occurred in August of 1991 in Changjiang Estuary, water temperature and salinity were 24.0~26.4 °C and 21.5~28.0 respectively [15]. During this bloom, mean water temperature was 26.5 °C, which just laid upon the optimum range. Meanwhile, salinity decreased from 31.24 to 30.59 owing to the rainfall before blooming. Unsteady continuous culture of *Skeletonema costatum* undertaken by Yamamoto & Tsuchiya [16] showed that Si-limited *Skeletonema costatum* presented higher growth rates with an accompanying salinity decrease than that without salinity decrease. Thus, rainfall on June 30 and July 1 made water temperature rising and salinity descending which

5 Discussion

provided a suitable hydrological ambience and directly led to the bloom.

Effects of nutrients on blooming: An increasing body of evidence suggested that red tide may often be induced in coastal water by escalating eutrophication. Nutrients in the studied area during blooming were higher than that of mean concentration in Jiaozhou Bay. Average dissolved inorganic nitrogen, phosphate and silicate in the studied area were $25.32\mu\text{mol l}^{-1}$, $0.51\mu\text{mol l}^{-1}$ and $3.90\mu\text{mol l}^{-1}$ respectively. According to Justic's criteria [17-19] for stoichiometry and probable nutrient limitation, and the atomic Si:N:P ratios of marine diatoms, dissolved inorganic phosphate and silicate were deficient as compared with dissolved inorganic nitrogen. Shen [12] reported that dissolved inorganic phosphate limited the growth of phytoplankton in Jiaozhou Bay. However, dissolved inorganic nitrogen and phosphate were obviously increased whereas dissolved inorganic silicate was observably decreased since the mid-term of 1980s. Zhang & Shen [13] reported that dissolved inorganic silicate also limited the growth of phytoplankton in Jiaozhou Bay. During this bloom, silicate was depleted by the phytoplankton to lead the bloom to be collapsed. This was certainly true that concentration of dissolved silicate was one of the most important factors influencing formation of diatom bloom in Jiaozhou Bay.

Different forms of dissolved inorganic nitrogen showed the high-low-high tendency in this bloom. But as a whole, variation of ammonia and nitrite had no correlation with chlorophyll *a* and only nitrate showed inversely weak relationship with chlorophyll *a*. It implied that *Skeletonema costatum* preferred to

Table 4: Concentrations of dissolved Fe, Mn before occurrence of blooms in Jiaozhou Bay ($\mu\text{mol l}^{-1}$)

Date	May 18	May 24	June 8	June 17	June 23
Fe	0.36	0.57	0.82	0.31	0.24
Mn	0.87	0.44	0.75	1.05	0.29

6 Conclusion

The Jiaozhou Bay is characterized by the presence of vast aquaculture zones in last two decades. Shrimp and shellfish aquaculture produce a great scale effluents including faeces, feeding wastes and sediments. Heavy loadings of dissolved inorganic nitrogen, phosphate and silicate originated from aquaculture effluents and sewage inputs provide essential trophic matter for the growth of phytoplankton. Furthermore, weak exchange

capacity and limited self-purification are also available for the retention of nutrients in this area. Rainfall in summer is particularly favorable to the silicate complement. Meanwhile, warm, low-salinity seawater after rain is extremely advantageous for the rapid proliferation of diatoms and bloom initiation. The stable synoptic condition is responsible for blooming lasting for a relatively long time. At the blooming stage, phosphate and silicate decline greatly. However, all forms of inorganic nitrogen, ammonia and nitrite change a little. The decreases of nitrate show that

assimilate nitrate rather than ammonia and nitrite and the rate of ammonia supplement was faster than the rate of ammonia consumption by *Skeletonema costatum*. During a *Skeletonema costatum* red tide occurred in Shengshan of Changjiang Estuary in August of 1991, similar phenomenon had also been observed [15]. It was inconsistent with the general results of culture experiments, *i.e.*, that phytoplankton preferred ammonia to nitrite or nitrate. Therefore, the mechanism controlling the dynamics of dissolved inorganic nitrogen in natural condition was rather complicated. Atmospheric deposition, freshwater input, sewage discharge, benthic release and phytoplankton uptake were all-important factors influencing the transformation and variation of different forms of dissolved inorganic nitrogen.

Effects of trace elements on blooming: Iron was an auxiliary component of many enzymes and essential to cellular pigment, ferredoxin and ferri-sulphydyl protein. Meanwhile, algal photosynthesis depended on iron, and, it was important enzymatic components of nitrate reductase and nitrite reductase. Iron contributed to increase the reduction rate and transformation rate of nitrite and nitrite [20,21]. Concentrations of dissolved iron and manganese were relative high in seawater of Jiaozhou Bay (Table 4) which was available for *Skeletonema costatum* bloom initiation [22]. However, dissolved iron decreased slightly at the blooming stage. Manganese showed no obvious relationship with biomass. So their variation was likely regulated by both biotic and non-biotic factors.

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Skeletonema costatum has preferential uptake of nitrate against ammonia or nitrite. At the collapsing stage of bloom, the exhaustion of phosphate and silicate directly lead to the death of *Skeletonema costatum* and disappearance of red tide. Statistical results show that temperature, salinity, silicate and phosphate are the critical ecological factors affecting the occurrence of the *Skeletonema costatum* bloom.

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