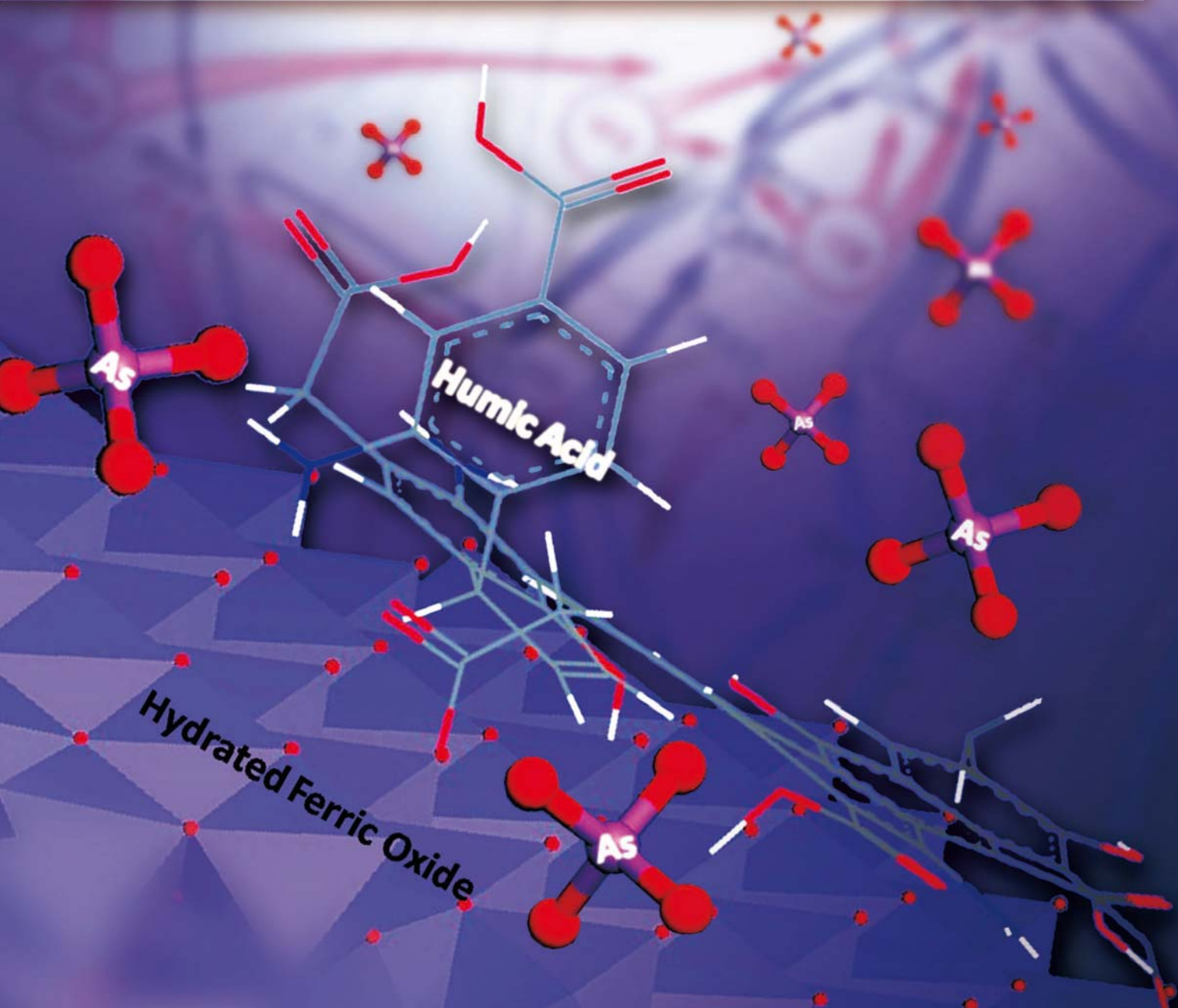


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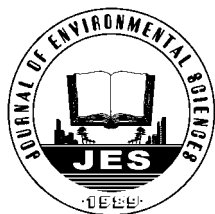
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## Beyond hypoxia: Occurrence and characteristics of black blooms due to the decomposition of the submerged plant *Potamogeton crispus* in a shallow lake

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### ABSTRACT

Organic matter-induced black blooms (hypoxia and an offensive odor) are a serious ecosystem disasters that have occurred in some large eutrophic shallow lakes in China. In this study, we investigated two separate black blooms that were induced by *Potamogeton crispus* in Lake Taihu, China. The main physical and chemical characteristics, including color- and odor-related substances, of the black blooms were analyzed. The black blooms were characterized by low dissolved oxygen concentration (close to 0 mg/L), low oxidation-reduction potential, and relatively low pH of overlying water. Notably higher  $\text{Fe}^{2+}$  and  $\Sigma\text{S}^{2-}$  were found in the black-bloom waters than in waters not affected by black blooms. The black color of the water may be attributable to the high concentration of these elements, as black FeS was considered to be the main substance causing the black color of blooms in freshwater lakes. Volatile organic sulfur compounds, including dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, were very abundant in the black-bloom waters. The massive anoxic degradation of dead *Potamogeton crispus* plants released dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, which were the main odor-causing compounds in the black blooms. The black blooms also induced an increase in ammonium nitrogen and soluble reactive phosphorus levels in the overlying waters. This extreme phenomenon not only heavily influenced the original lake ecosystem but also greatly changed the cycling of Fe, S, and nutrients in the water column.

## Introduction

Massive cyanobacterial and vegetation blooms are a visible ecosystem response to advanced eutrophication (Diaz and Rosenberg, 2008; Paerl et al., 2011). However, the decrease in dissolved oxygen (DO) levels in bottom waters that results from the degradation of large amounts of organic matter is regarded as the most serious threat from these blooms (Rabalais et al., 2002; Diaz and Rosenberg, 2008). Moreover, excessive organic matter in the water column can result in hypoxia, even anoxia, in the water

and surface sediments. Hypoxia and anoxia can induce black water bloom disasters in freshwater lakes (Stahl, 1979; Yang et al., 2008). Because of the degradation of organic matter from cyanobacteria blooms and/or polluted sediments, some of the most important freshwater lakes in China, such as Lake Taihu, Lake Chaohu, and Lake Dianchi, have been suffering from black blooms for many years. These black blooms have drawn the attention of the government and academicians. All the black blooms occurred unpredictably in late spring or early summer, were near the shore, and usually lasted from 24 hr to 2 weeks. Black blooms are identified by the black color of the water and are often accompanied by offensive odors. Black blooms cause mass death of fishes and benthic

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fauna. Furthermore, when black blooms occur near water-source intake areas, they can result in water supply crises or even panic within local communities (Nanjing Institute of Geography and Limnology, Chinese Academy of Science, 2007; Yang et al., 2008; Lu and Ma, 2010).

Lake Taihu is the third largest freshwater lake (2338 km<sup>2</sup>) and the largest water-source lake in China, and it is also well known for its hyper-eutrophication and notorious cyanobacterial blooms (Guo, 2007; Paerl et al., 2011). Over the past several years, algae-induced black blooms have occurred frequently in Lake Taihu and have caused severe ecological and environmental disasters (Lu and Ma, 2010). While black blooms can be induced by algae, they can also be induced by the degradation of submerged plants and other organic matter. In recent years, black blooms induced by submerged plants have occurred in some areas of Lake Taihu and have caused increasingly serious damage in some eutrophic bays. On May 16, 2012, black blooms induced by *Potamogeton crispus* (*P. crispus*) were found in the Gonghu Bay of Lake Taihu, China. These blooms represented a new type of black bloom that was similar to algae-induced black blooms in that the water was also black in color and emitted a strong foul odor.

There have been some studies of the black bloom phenomenon (Yang et al., 2008; Lu and Ma, 2010; Shen et al., 2011, 2012), but there are no published investigations into submerged plant-induced black blooms. Previous publications (Stahl, 1979; Duval and Ludlam, 2001) suggested that ferrous sulfide (FeS) was responsible for the black color and hydrogen sulfide (H<sub>2</sub>S) was responsible for the offensive odor of water during black blooms. However, other studies have implied that more complex organic compounds resulting from cyanobacteria degradation might be the major source of the offensive odor during black blooms. These complex compounds might include geosmin (trans-1,10-dimethyl-trans-9-decalol); 2-methylisoborneol (MIB); and volatile organic sulfur compounds (VOSCs), such as methanethiol (MTL), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) (Yang et al., 2008; Zhang et al., 2010). Lu and Ma (2009) investigated an algae-induced black bloom in Lake Taihu and reported that total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and soluble reactive phosphorus (SRP) were significantly more concentrated in black-bloom water than in normal water. Furthermore, Shen et al. (2011, 2012) studied the formation and recovery processes of black blooms and determined that DO was the key influential factor for both the generation and disappearance of blooms. However, the factors that are closely related to the black color and offensive odors have seldom been studied effectively because of the unpredictability of the time and location of black blooms. Therefore, the mechanism of these blooms is still unknown and almost nothing is known about black blooms that are induced by submerged plants.

The goal of this study was to explore the main physical and chemical characteristics of submerged plant-induced black-bloom water and to analyze the major odor compounds. The mechanism of black-bloom formation has been summarized to improve the understanding of this serious ecological disaster.

## 1 Materials and methods

### 1.1 Study site

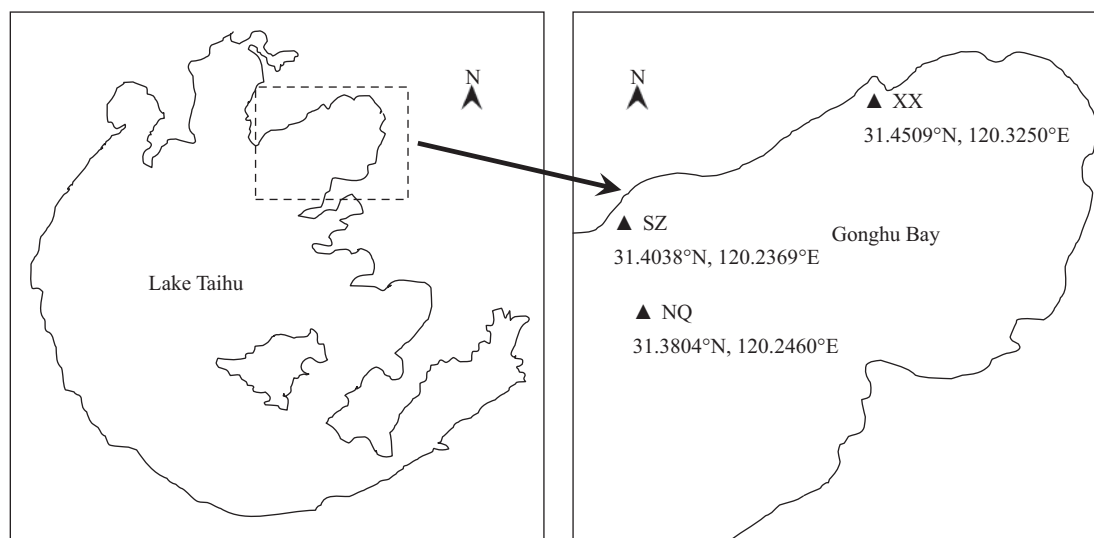
On May 16, 2012, black blooms occurred in two separate nearshore zones in the Gonghu Bay of Lake Taihu. Gonghu Bay is an important water-source intake area for the nearby Wuxi and Suzhou Cities. The bay has an area of about 147 km<sup>2</sup> and an average depth of less than 2 m (Fan et al., 1997). One bloom zone was in Shazhu Port (SZ) and the other was near Xuxian Port (XX) (Fig. 1). Both bloom areas covered less than 1 km<sup>2</sup> and were close to the water-source intake area of Wuxi City. No black blooms occurred within the water-source intake area during the study period. Therefore, the two black bloom areas and the water-source intake area were selected as the study areas. Samples were collected from SZ, XX, and the water-source intake area of the Nanquan Water Plant (NQ). The sampling sites are shown in Fig. 1.

### 1.2 Sampling and pretreatment of water samples

During the black bloom period, triplicate surface and bottom water samples were collected at each sampling site. Aerobic solutions of ferrozine in *N*-2-hydroxyethylpiperazine buffer (Phillips and Lovley, 1987) and basic solutions of zinc acetate (Cline, 1969) were placed into individual 10 mL polypropylene centrifuge tubes. Water samples for the analysis of Fe<sup>2+</sup> were immediately transferred into the bottles containing ferrozine and samples for the analysis of ΣS<sup>2-</sup> (ΣS<sup>2-</sup> = [H<sub>2</sub>S] + [HS<sup>-</sup>] + [S<sup>2-</sup>]) were transferred into bottles containing zinc acetate, in order to avoid oxidation. For the analysis of VOSCs, 50-mL-headspace bottles were completely filled with sample water and allowed to overflow for 5 sec before capping to ensure that no air remained in the bottles. Samples for the analysis of NH<sub>4</sub><sup>+</sup>-N, SRP, and dissolved organic compounds (DOC) were collected in 100-mL polythene bottles and filtered through cellulose acetate filter (Ø47 mm, 0.45-µm pore size) within 2 hr of collection. All bottles were immersed in dilute hydrochloric acid for 12 hr and then washed three times by using deionized water prior to the collection of samples.

### 1.3 Analysis of physical and chemical characteristics

The concentration of iron, ΣS<sup>2-</sup>, NH<sub>4</sub><sup>+</sup>-N, and SRP in the water samples was measured using a Shimadzu UV-2550 spectrophotometer. Iron was analyzed using a ferrozine



**Fig. 1** Water sampling sites in Gonghu Bay, Lake Taihu, China. Shazhu Port (SZ) and Xuxian Port (XX) were affected by black blooms, while the water-source intake area of Nanquan Water Plant (NQ) was not.

spectrophotometry method (Stookey, 1970).  $\Sigma S^{2-}$  was analyzed using a methylene blue spectrophotometric method (Cline, 1969).  $NH_4^+-N$  and SRP contents were determined using a Nessler's Reagent method (Jing and Tu, 1990) and a molybdenum blue method (Murphy and Riley, 1962), respectively. A total organic carbon analyzer (LiquiTOCII, Elementar Company, Germany) was used to determine the DOC in the water samples. DO, chlorophyll *a* (Chl-*a*), oxidation-reduction potential (ORP), and pH were simultaneously measured using a multi-parameter water-quality testing instrument (YSI 6820EDS, USA) at the midway between the water surface and lake floor at each sampling site.

#### 1.4 Analysis of odor compounds

Yang et al. (2008) determined that DMDS and other related alkyl sulfide compounds were the main odor-causing compounds in a 2007 black bloom in Lake Taihu. Therefore, we measured VOSCs, including MTL, DMS, DMDS, DMTS, by using a headspace solid-phase micro-extraction method and a gas chromatograph coupled to a flame-photometric detector (Lu et al., 2012).

#### 1.5 Statistical analysis

Differences in the  $Fe^{2+}$ ,  $\Sigma S^{2-}$ ,  $NH_4^+-N$ , and SRP of waters from different sampling sites were evaluated using one-way analysis of variance, followed by Tukey's honestly significant differences test (\* represents  $P < 0.05$ , \*\* represents  $P < 0.05$ ). These statistical analyses were conducted using SPSS 16.0 software. Regressions between DOC and black color-causing ions ( $Fe^{2+}$  and  $\Sigma S^{2-}$ ) were evaluated using linear fit, as implemented in Origin 8.5 software.

## 2 Results

### 2.1 Characteristics of the black bloom

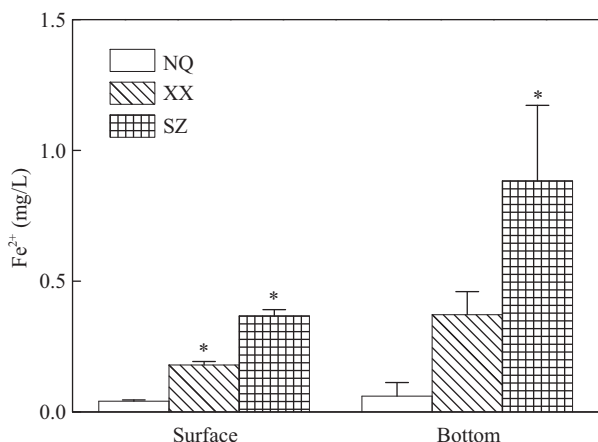
In XX and SZ black bloom areas, the water was black in color and emitted strong offensive odors. In contrast to previous algae-induced black blooms, these two black blooms were not induced by large-scale aggregations of algae, as there were neither algae blooms nor algae accumulations in or near the XX and SZ bloom areas. Dead *P. crispus* was dominant in both black bloom zones during the bloom period. No submerged plants, including *P. crispus*, or cyanobacteria blooms were found at the control site, NQ. Compared to NQ, the waters in XX and SZ were remarkably anoxic, with DO of 0.45 mg/L and 0.83 mg/L, respectively. The ORP and pH of the water at the two black bloom sites were significantly lower than those at NQ, while Chl-*a* levels were dramatically higher at XX and SZ than at NZ (Table 1).

### 2.2 $Fe^{2+}$ in waters

As shown in Fig. 2, water samples from different sites had significantly different  $Fe^{2+}$  content. In NQ samples,  $Fe^{2+}$  concentrations were very low in both surface and bottom waters. The  $Fe^{2+}$  concentrations were much higher in black bloom samples from XX and SZ. In XX and SZ

**Table 1** Main physical characteristics of the water sampled from two black-bloom sites and one control site in Lake Taihu, China

Sampling site	DO (mg/L)	Chl- <i>a</i> (mg/L)	ORP (mV)	pH
NQ	8.30	1.6	526.6	8.1
XX	0.45	31.5	358.7	7.74
SZ	0.83	69.2	394.0	7.82



**Fig. 2** Fe<sup>2+</sup> in overlying waters at three sampling sites in Gonghu Bay, Lake Taihu, China. \* Presents concentrations from XX or SZ samples were significantly different ( $P < 0.05$ ) from NQ samples, and also represents the same meaning in the following figures.

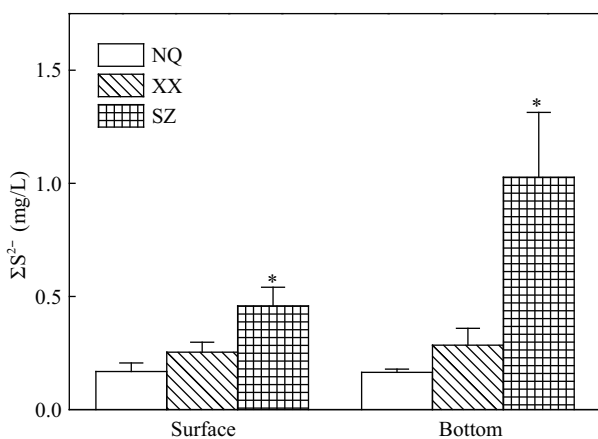
samples, the concentration of Fe<sup>2+</sup> in bottom waters was much higher than that in surface waters. The bottom-water Fe<sup>2+</sup> concentration at SZ was as high as 0.88 mg/L.

### 2.3 $\Sigma S^{2-}$ in waters

The  $\Sigma S^{2-}$  concentrations in overlying waters are shown in **Fig. 3**. Compared to samples from NQ, samples from XX and SZ black-bloom zones contained much higher concentrations of  $\Sigma S^{2-}$ . The  $\Sigma S^{2-}$  concentrations at NQ and SZ were significantly different. Concentrations of  $\Sigma S^{2-}$  were much higher than those in surface waters at XX and SZ. Bottom  $\Sigma S^{2-}$  concentrations reached as high as 1.03 mg/L at SZ, 6.23 times those at NQ.

### 2.4 Nutrients in waters

Dissolved nutrients (NH<sub>4</sub><sup>+</sup>-N, SRP) are important water quality parameters in lakes. Ammonium and SRP are always higher in the black blooms overlying waters than in normal areas (Lu and Ma, 2009). There were no significant

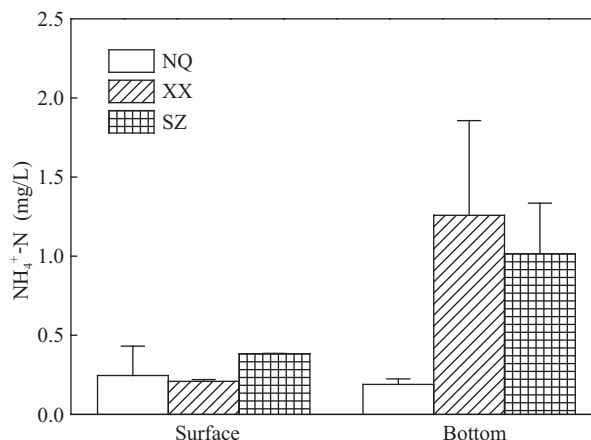


**Fig. 3**  $\Sigma S^{2-}$  in overlying waters at three sampling sites in Gonghu Bay, Lake Taihu, China.

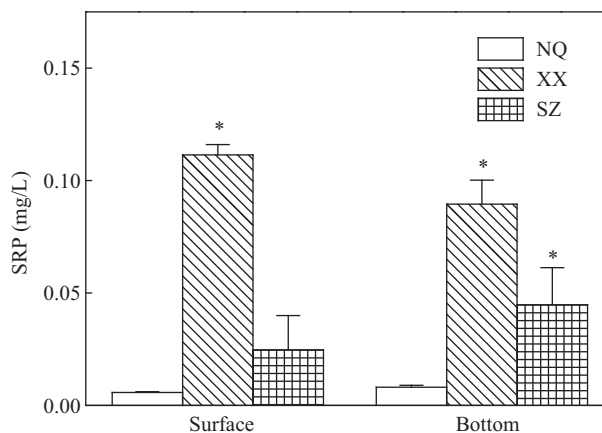
differences in the ammonium concentration between NQ and the two black-bloom zones. However, as shown in **Fig. 4**, ammonium concentrations in the bottom waters at XX and SZ were higher than that at NQ. Moreover, ammonium concentrations in the bottom waters at XX and SZ were higher than those in the surface waters at these sites. SRP concentrations in both surface and bottom samples at NQ were low, but were significantly higher at XX and SZ (**Fig. 5**). The maximum SRP was 0.11 mg/L in XX surface water. This maximum value was 19.39 times that of the NQ surface sample.

### 2.5 VOSCs in waters

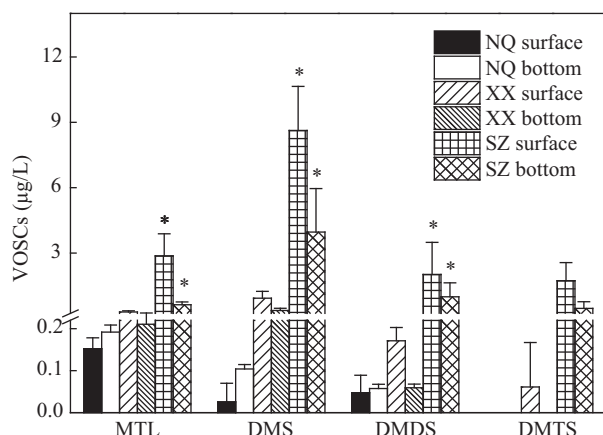
VOSCs were remarkably different in NQ, XX, and SZ samples (**Fig. 6**). Low levels of MTL, DMS, DMDS, and DMTS were detected in samples from NQ. In contrast, with the exception of MTL in XX samples, VOSCs were much more concentrated in XX and SZ black-bloom waters. The highest concentration of VOSCs was found in SZ samples, in which the DMS concentration in surface water reached 8.63  $\mu\text{g/L}$ , 336.9 times that of NQ surface water. Among the VOSCs in black-bloom waters, DMS was the



**Fig. 4** NH<sub>4</sub><sup>+</sup>-N in overlying waters at three sampling sites in Gonghu Bay, Lake Taihu, China.



**Fig. 5** SRP in overlying waters at three sampling sites in Gonghu Bay, Lake Taihu, China.



**Fig. 6** VOSCs in overlying waters at three sampling sites in Gonghu Bay, Lake Taihu, China.

most abundant, and the samples contained significantly more DMS than MTL, DMDS, or DMTS.

### 3 Discussion

#### 3.1 Effects of hypoxia/anoxia on the black bloom

Hypoxia occurs when DO concentrations are less than, or close to 2 mg/L (Turner et al., 2005; Diaz and Rosenberg, 2008; Bianchi et al., 2010; Vaquer-Sunyer and Duarte, 2008). Such low DO concentrations are commonly found in global marine ecosystems (Diaz and Rosenberg, 2008) and some freshwater systems (Conroy et al., 2011), and are always caused by excessive organic matter. In the study area, there were abundant submerged plants (mainly *P. crispus*) and the DO concentration was high in water samples from the control site. However, most of the *P. crispus* died of an unknown cause and sank to the floor of the lake, thus contributing a great deal of organic matter to the top layer of the sediment. The degradation of this organic matter likely caused DO depletion, thereby inducing hypoxia and anoxia in the surface sediment and overlying water.

DO deficiency in overlying water initiates a redox state change and causes a cascade of alternative terminal electron acceptor use by anaerobic organisms (Middelburg and Levin, 2009). Subsequently, sulfates and iron (hydr)oxides are reduced as terminal electron acceptors in biochemical reactions (Middelburg and Levin, 2009; Nielsen et al., 2010); as the terminal products of these reduction processes,  $H_2S$  and  $Fe^{2+}$  begin to accumulate. Thus, hypoxia and anoxia accelerate  $H_2S$  release (Roden and Tuttle, 1992; Diaz and Rosenberg, 2008) and ferric iron reduction (Gerhardt and Schink, 2005) in surface sediments and overlying water. Moreover, black blooms or black water in freshwater lakes is closely related to  $FeS$  content.  $FeS$  is considered to be the main substance causing the black

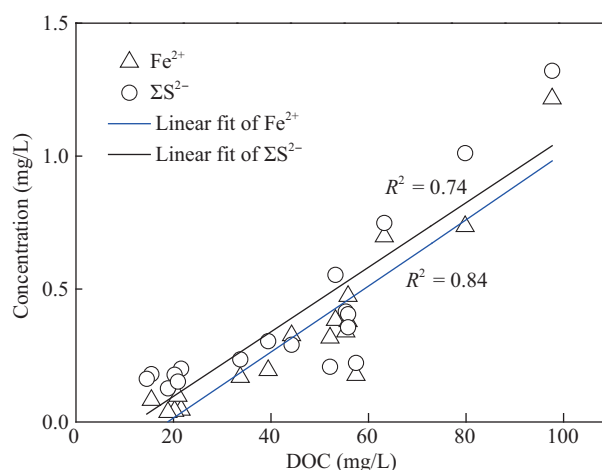
color of black blooms and is generated from ample  $Fe^{2+}$  and  $\Sigma S^{2-}$  (Stahl, 1979; Duval and Ludlam, 2001).

In the present study, black-bloom waters contained notably higher concentrations of  $Fe^{2+}$  and  $\Sigma S^{2-}$  than were found in NQ water. Correlations between DOC and  $Fe^{2+}$  and between DOC and  $\Sigma S^{2-}$  (Fig. 7) indicate that organic matter degradation consumed a large amount of DO, causing first hypoxia and then anoxia. The low DO concentration induced changes in electron acceptor use by anaerobic organisms that ultimately caused the production of large amounts of  $FeS$  in low pH, low ORP, anoxic waters, which finally caused the black color of the water that marked the formation of black bloom. Therefore, the increase in  $Fe^{2+}$  and  $\Sigma S^{2-}$  in anoxic overlying waters may be an important material preparation to the bulk synthesis of  $FeS$ , which finally causes the formation of black blooms.

#### 3.2 Sources of the odor-causing substances

Offensive odors in natural waters are mainly caused by MIB, geosmin, VOSCs, and other complex organic compounds released because of the degradation of various algae (Ikawa et al., 2001; Bentley and Chasteen, 2004; Kiene et al., 2007; Li et al., 2007). In algae-induced black blooms, VOSCs are regarded as more important odor-causing substance than MIB, geosmin, or other organic compounds (Yang et al., 2008; Zhang et al., 2010). Previous studies indicated that VOSCs, including MTL, DMS, DMDS, and DMTS, are mainly found in marine (Bentley and Chasteen, 2004) and anoxic or hypolimnion lake waters (Hu et al., 2007), whereas the concentrations of these offensive odor-causing substances are very low in well-oxidized freshwater lakes (Hu et al., 2007; Peter et al., 2009).

In the black bloom phenomena studied, DMS, DMDS, and DMTS concentrations are significantly higher than those in normal water (Fig. 6). The concentration range of DMS, DMDS, and DMTS in XX and SZ surface black-



**Fig. 7** Regression between black-causing ions ( $Fe^{2+}$  and  $\Sigma S^{2-}$ ) and DOC in overlying water at three sampling sites in Gonghu Bay, Lake Taihu, China.



bloom waters were 0.93–8.63  $\mu\text{g/L}$ , 0.17–2.02  $\mu\text{g/L}$ , and 0.09–1.73  $\mu\text{g/L}$ , respectively, and were significantly higher than those in NQ surface water. VOSC concentrations in SZ and XX black-bloom waters were much lower than those reported by Zhang et al. (2010) for algae-induced black blooms (DMS 93.9  $\mu\text{g/L}$ , DMDS 2.51  $\mu\text{g/L}$  and 46.1  $\mu\text{g/L}$ , DMTS 17.17  $\mu\text{g/L}$ ), although DMTS concentrations were similar to those reported by Yang et al. (2008) (1.77 and 11.40  $\mu\text{g/L}$ ). In water, the odor threshold concentrations for DMS, DMDS, and DMTS are 0.3–1  $\mu\text{g/L}$ , 0.2–5  $\mu\text{g/L}$ , and 0.01  $\mu\text{g/L}$ , respectively (Chen et al., 2010; Zhang et al., 2010). Trace concentrations of these VOSCs could generate a strong putrid odor and taste. Therefore, the concentrations of VOSCs in SZ and XX black-bloom waters were high enough to account for the offensive odors. DMS, DMDS, and DMTS clearly caused the offensive odors emitted by SZ and XX black blooms.

Volatile organic sulfur compounds in freshwater lakes are produced primarily by phytoplankton and algae metabolism or microbial degradation of organic matter (Song et al., 2004; Hu et al., 2007). Because neither cyanobacterial accumulation nor an algae bloom was observed in the SZ and XX black-bloom areas, the offensive odor must have been closely related to the abundant dead *P. crispus* plants. The production and accumulation of VOSCs in black-bloom waters may have resulted from the decomposition of sulfur-containing organic compounds in dead *P. crispus* plants, as this process generates a variety of methylated sulfides (Lomans et al., 1997; Bentley and Chasteen, 2004; Hu et al., 2007; Lu et al., 2012).

### 3.3 Effects on nutrients

High ammonium and SRP concentrations are two notable chemical characteristics of black-bloom waters (Shen et al., 2012). Ammonium concentrations in algae-induced black blooms can reach as high as 4.00–9.06  $\text{mg/L}$  (Yang et al., 2008; Lu and Ma, 2009). Although the ammonium concentrations of SZ and XX black-bloom waters were lower than those reported in the literature, the concentrations in the bottom water samples from these sites were still remarkably higher than the concentrations in the bottom water samples from NQ. The decomposition of dead *P. crispus* plants may have released ammonium into the water column and contributed to the high ammonium concentration at the black-bloom sites. In addition, the anoxic environment of black-bloom sites promotes the growth of denitrifying bacteria and ammonifiers, which would greatly increase denitrification and ammonification and contribute further to the increase in ammonium in the water column (Fan et al., 2000). Resuspended sediment particles and surface sediments can also release ammonium into the overlying water in anoxic environments (Søndergaard et al., 1992). This process may have increased the ammonium concentration in the black-bloom waters.

Degradation of massive numbers of dead *P. crispus*

plants and other organic matter was an important source of SRP in the water column. At the same time, because of the anoxic environment, a large amount of ferric hydroxides was reduced to soluble ferrous ions, which can cause iron-bound phosphorus in the sediments to transform into labile phosphorus (Jensen and Thamdrup, 1993; Hupfer et al., 1995; Rydin, 2000; Kaiserli et al., 2002). Excessive labile phosphorus dissolves into the water and increases the SRP concentration. Consequently, the SRP concentration in the water column increased (Fan et al., 2000). Thus, release of SRP from surface resuspended sediments may be another important source of the elevated SRP found in black-bloom waters.

Obviously, high SRP and ammonium concentrations are the results of black blooms rather than the causes. However, this kind of high-nutrient load aggravates eutrophication and provides sufficient N and P to cause subsequent algae blooms (Dodds, 2006), which might affect long-term N and P cycles and eutrophication problems.

## 4 Conclusions

As an extreme phenomenon of hyper-eutrophication in shallow lakes, black blooms have become a serious threat to the safety of drinking water sources and lake ecosystems. It is clear that the massive degradation of dead submerged plants (*P. crispus*) can induce hypoxia and anoxia and trigger black blooms in SZ and XX. In areas affected by black blooms, black color and offensive odors were directly observable. Low DO, ORP, and pH levels were typical physical characteristics of black-bloom waters. High  $\text{Fe}^{2+}$  and  $\sum\text{S}^{2-}$  concentrations were important chemical characteristics of black-bloom waters and were also important sources of the black substance, FeS, in the water column. Furthermore, VOSCs, including DMS, DMDS, and DMTS, were remarkably high in SZ and XX black-bloom waters, and these VOSCs released during the decomposition of dead *P. crispus* plants under anoxic conditions were the main odor-causing compounds in black-bloom waters. High nutrient loads in the water column were also important characteristics of black blooms. Black blooms may have long-term effects on the eutrophication of lakes.

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