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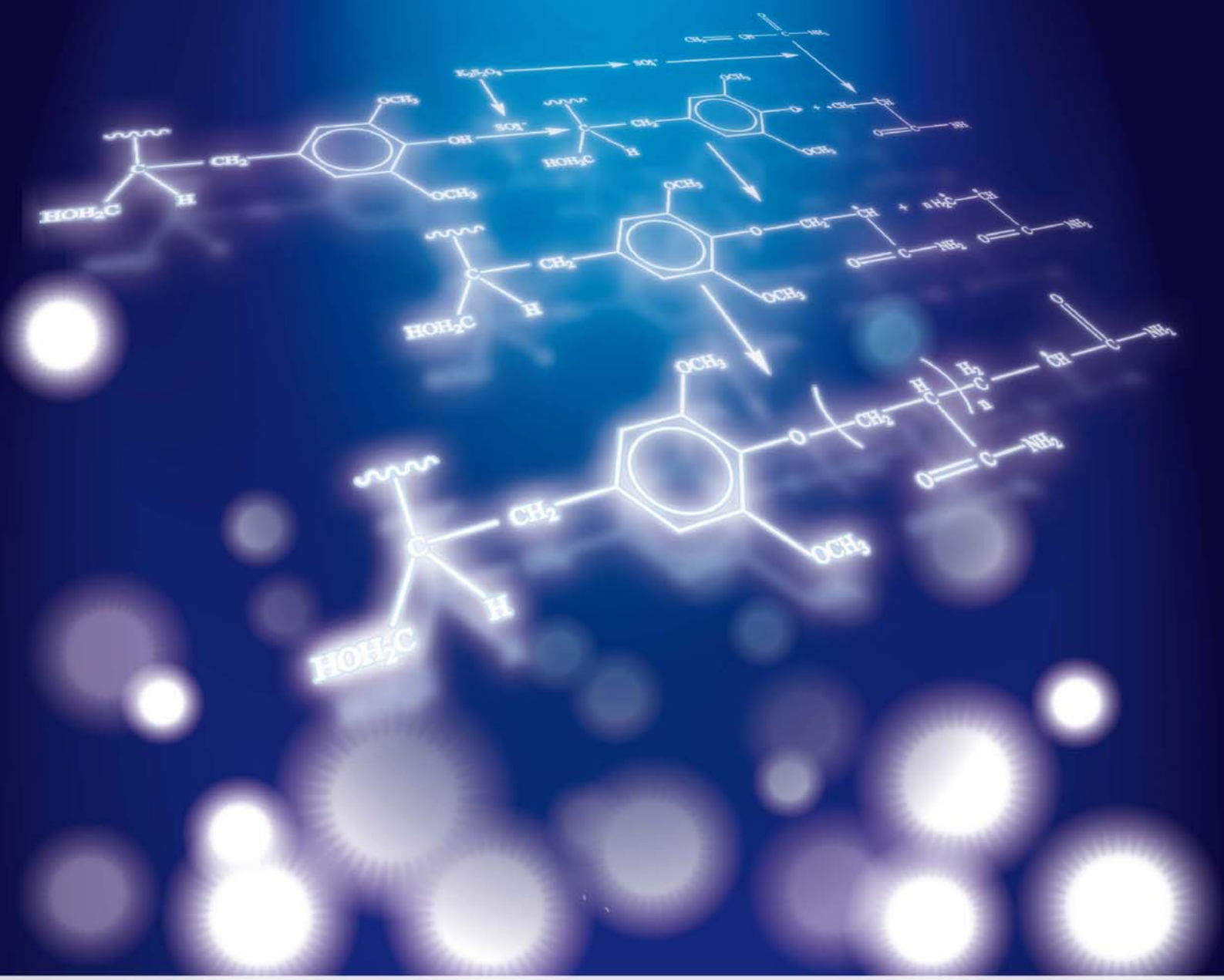

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A fishy odor episode in a north China reservoir: Occurrence, origin, and possible odor causing compounds

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Abstract

A significant outbreak of fishy odor occurred in a reservoir located in Inner Mongolia, China, in the winter of 2011, and the odor rating, algal density and concentrations of some potential odorous compounds were monitored over a period of two months. The peak odor rating of the fishy odor was 7 according to flavor profile analysis. Among the dominant algal species (two diatom and one chrysophyte species) observed during the survey, the chrysophyte *Dinobryon* sp. was the most abundant species, with the peak density recorded at 88,520 cells/mL. Seven potential algal metabolites including heptanal, 2,4-heptadienal, 2,4-decadienal, nonanal, 2-octenal, 2,6-nonadienal and hexanal were detected. The principal component analysis result showed that *n*-hexanal, *n*-heptanal and 2,4-decadienal, possibly the metabolites of diatoms, and 2,4-heptadienal, possibly the metabolite of *Dinobryon* sp., might have contributed to the fishy odor episode. This study demonstrated that the fishy odor episode in this reservoir might be caused by the abnormal growth of chrysophytes and diatoms under the ice-cover.

Key words: fishy odor; chrysophyte; diatom; reservoir; drinking water; flavor profile analysis

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Introduction

Taste and odor is one of the major problems causing consumer complaints in drinking water (Watson, 2004; Hoefel et al., 2006). Among the taste and odor problems, earthy/musty odors caused by the metabolites of some cyanobacteria (Lanciotti et al., 2003; Fink et al., 2006) and actinomycetes (Lanciotti et al., 2003; Zaitlin and Watson, 2006), such as 2-methylisoborneol and geosmin (Li et al., 2010), have been frequently encountered in water utilities and extensively studied (Watson et al., 2000; Westerhoff et al., 2005; Uwins et al., 2007; Chen et al., 2010). On the other hand, occurrence of fishy odor has also been reported in some regions (Naumenko, 1992; Watson et al., 2001a). Diatoms (Brutemark and Granéli, 2011), chrysophytes, cryptophytes and dinoflagellates (Watson et al., 2001a) have been suspected of being responsible for generating the fishy odor (Gradinger, 1996; Watson et al., 2001a). These algae normally contain large amounts of polyunsaturated fatty acids in the cells, which could be transformed into odorous unsaturated aldehydic derivatives in the environment, including 2,4-decadienal, 2,6-nonadienal, 2,4-heptandienal and 2,4,7-decatrienal with odor thresholds (OTC) of 0.3, 0.08, 25 and 1 μg/L, respectively (Ahlgren et al., 1992; Wee et al., 1994; Haynes et al., 1998; Watson et al., 2001a). In comparison with cyanobacteria and diatoms, knowledge regarding chrysophytes is very limited. It is known that chrysophytes can proliferate even in ice-covered lakes and reservoirs. Chrysophyte episodes accompanied with significant fishy odor occurrence have been recorded in Canadian reservoirs in spring/early summer (Agbeti and Smol, 1995; Watson et al., 2001a), and the unsaturated aldehydes mentioned above were considered to be the main odor causing compounds (Wendel and Juttner, 1996; Watson et al., 2001a, b; Watson and Satchwill, 2003; Venkateshwarlu et al., 2004). Compared with the musty/earthy odors, however, the algal species and compounds responsible for the fishy odors have not been well understood (Watson et al., 2001a; Lin et al., 2002).

Jinhai Reservoir is the main source of drinking water in Inner Mongolia, China. It receives water from the Yellow River and often suffers from fishy odor episodes accompanied with the occurrence of some unknown algae, even in the winter when the water surface is covered with ice. Notably, fishy odor episodes have also been reported to occur in other cities including Jinan and Zhengzhou using

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the Yellow River as source water (Sun, 2012). However, the main reason for the fishy odor has not yet been clarified, which makes it difficult to take effective measures to either prevent the occurrence of the fishy odor in the reservoir or control it in waterworks receiving source water from the reservoir. Aiming at exploring the odor-causing algae as well as the potential metabolites, an environmental survey was conducted to track the changes in odor and algal population from mid-December 2011 to mid-February 2012. The flavor profile analysis (FPA) method, which can provide the odor features (descritors) as well as the intensities of water samples with a minimum of four trained panelists, was employed to characterize the odor profiles of water samples, and 9 potential algal metabolites including *n*-hexanal, 2-octenal, nonanal, 2,6-nonadienal, *n*-heptanal, 2,4-heptadienal, 2,4-decadienal, benzaldehyde and β-cyclocitral in the reservoir were analyzed. Our study will provide useful information for better understanding the fishy odor problems.

1 Materials and methods

1.1 Study site and sampling

Jinhai Reservoir has a maximum capacity of 1.26×10^7 m^3, an average depth of 5 m and an area of 3.14 km^2. The major parameters of water samples in Jinhai Reservoir are as following: turbidity 7.2 NTU, pH 8.1, conductivity 978 S/cm^2, total nitrogen 3.09 mg/L, total phosphorus 0.61 mg/L, and temperature 4.0°C.

Sites 1, 2 and 3 were the main sampling sites, while there was a comprehensive sampling campaign covering 13 sites on 9th February, 2012 (**Fig. 1**). Water flows into the reservoir through site 3, and out of the reservoir from site 1. Site 2 has no perceptible water flow. The reservoir was covered with ice having an average depth of approximately 1 m during the study period, and holes were drilled through the ice-cover to take water samples using an electric drill.

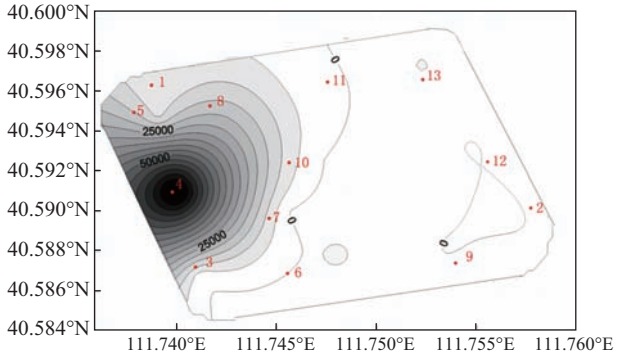

Fig. 1 Sampling sites (1–13) in Jinhai Reservoir, Inner Mongolia, China. Site 3 was near the influent point, site 1 near the effluent point (water intake) and site 2 in the dead zone of water flow. The distribution of *Dinobryon* sp. in Jinhai Reservoir on 9 Feb, 2012 was drawn using Sufer 8.0 with 13 sampling sites.

The water samples were taken from 0–0.5 m under the ice by a sampler. The samples were then divided into three bottles for sensory evaluation, algal analysis and odorant quantification, respectively. The Lugol's solution of 5 mL was added in one bottle for algal fixation, and 10 mL $HgCl_2$ was added in another bottle for the detection of odorants. The water samples were stored in brown glass bottles with airtight stoppers at 4°C and analyzed immediately.

1.2 Chemicals

The 9 potential odorous compounds including *n*-hexanal, 2-octenal, nonanal, 2,6-nonadienal, *n*-heptanal, 2,4-heptadienal, 2,4-decadienal, benzaldehyde and β-cyclocitrial, were purchased from Sigma-Aldrich (USA) as standard reagents. NaCl, KI and $HgCl_2$ of analytical grade were purchased from Beijing Chemicals Ltd., China. NaCl was heated to 450°C for 4 hr before use to remove organic substances. Ultrapure water (18.2 MΩ·cm) was produced with a Milli-Q purification system.

1.3 Odor evaluation

FPA was applied to characterize the odor profile (standard methods 2170; APHA et al., 2005), and seven-point scales of 0–12 were used to evaluate the odor rating of water samples. Each test was performed by at least four qualified panelists. Odor standards with different concentrations were used to remind the panel of the odor group and rating with each batch of samples. The samples were maintained at 45°C in a water bath, at least one of which was a blank that consisted only of reagent water labeled as "odor-free". The descriptor and intensity for each sample was then evaluated and recorded.

The potential odorants including *n*-hexanal, 2-octenal, nonanal, 2,6-nonadienal, *n*-heptanal, 2,4-heptadienal, 2,4-decadienal, benzaldehyde and β-cyclocitrial were quantified using headspace solid phase microextraction (Suffet et al., 1999) combined with gas chromatograph/mass spectrometry (GC/MS) (Satchwill et al., 2007). Solid phase microextraction fibers (No.57334-U, Supelco) were inserted into the headspace of the vials containing water samples and maintained at 65°C for 30 min, with constant stirring of the vials by a Teflon bar in a water bath. After this, the fibers were retracted and inserted directly into the injector of the GC/MS where the odorants were thermally desorbed (250°C for 2 min). The GC temperature program was as follows: 40°C initially for 2 min, then programmed at 8°C/min to 240°C and held for 2 min. The GC/MS system included an HP 6980 GC with a 60 m × 0.25 μm × 0.25 mm DB-WAX MS capillary column and an HP 5973 mass spectrometric detector (Agilent, USA). It was operated in selective ion monitoring mode for quantification (Watson et al., 2001a). The linearity of the calibration curves for the aldehyde standards was good with high regression coefficients ($R^2 > 0.99$).

1.4 Algal enumeration

Algal cell number was counted with a 1 mL phytoplankton counter chamber using a microscope under a 20 × objective lens (Zeiss Axioskop 2 Mot Plus, Germany). For water samples with a high density of algae, cell numbers were counted without concentration. For the other samples, cell numbers were counted after preconcentration by a factor of 10 (from 100 to 10 mL). *Dinobryon* sp. was identified according to published pictures (Nicholls, 2000; Hu and Wei, 2006). Cells of *Dinobryon* sp. were surrounded by a vase-like loria shell (Franke and Herth, 1973) as shown in **Fig. S1**, and counted by individual cells. The photo was taken under bright field mode.

1.5 Statistical methods

Principal component analysis (PCA) (Parinet et al., 2010) was used to help find the correlation between different aldehydes detected and algae observed during the fishy odor episode. PCA converted all kinds of odorants and algae into new variables and principal components (PCs), which were orthogonal and non-inter-correlated (Barbieri et al., 1999; Shrestha and Kazama, 2007). PCA and all statistical processing of the data were carried out using STASTICA 8 software.

2 Results and discussion

2.1 Odor and algal characteristics

The fishy odor episode in Jinhai Reservoir lasted for two months, from December 2011 to February 2012. FPA described the odor of the water samples at all of the three sampling sites as predominantly fishy. As shown in **Fig. 2**, the FPA rating changed between 2 and 7. Two diatom species, *Cyclotella* sp. and *Melosira* sp., and one chrysophyte species, *Dinobryon* sp., were detected (**Table S1**) with *Dinobryon* sp. being the most abundant one during the fishy odor episode. There is no report available regarding the relationship between *Cyclotella* sp. and fishy odorants. *Melosira* sp. have been reported to produce polyunsaturated aldehydes such as 2,4,7-decatrienal (Wendel and Juttner, 1996; Lanciotti et al., 2003; Fink, 2007), a compound related with some fishy odor episodes, which was not analyzed in this study due to the lack of standard material. The cell density of *Melosira* sp . was in general one order of magnitude lower than that of *Dinobryon* sp., which has also been reported to cause fishy odors (Watson et al., 1996, 1999, 2001a; Parker and Beaty, 1997; Watson and Satchwill, 2003; Satchwill et al., 2007). Therefore, the relationship between the fishy odor and *Dinobryon* sp. was further focused on in this study. However, the relationship between fishy odor and diatoms also needs more attention in further study.

Figure 2a shows variations of the odor intensity and cell density of *Dinobryon* sp. at site 1, which was located near the effluent of the reservoir. It was found that both the odor intensity and the cell density exhibited similar trends, although a kind of "lag time" was observed between them. The density of *Dinobryon* sp. reached the peak value of 88,520 cells/mL on 11 January, 2012, while the peak fishy odor was detected 3 weeks later. Chrysophytes such as *Dinobryon* sp. could produce severe fishy odors through the production of unsaturated aldehydic derivatives after cell rupture (Watson et al., 2001a). Distribution of *Dinobryon* sp. in the whole reservoir was further investigated on February 9, 2012. The highest density of 87,720 cells/mL was observed at site 4, which was located between the influent and the effluent of the reservoir, suggesting the growth of *Dinobryon* sp. in the reservoir. It is known that *Dinobryon* sp., belonging to the chrysophyte group, can grow at low temperature (4.0–15°C) with low light availability (Barbiero and McNair, 1996; De Hoyos et al., 1998; Clegg et al., 2003).

2.2 Typical odorants

As shown in **Table S2**, 7 of the 9 compounds were detected in the water samples, except for β-cyclocitrial, a frequently detected algal metabolite, and benzaldehyde. Of the 7 detected compounds, *n*-heptanal, 2,4-dicadienal, *n*-hexanal and 2,4-heptadienal have been reported to exhibit fishy odors (Watson, 2004). Among these 4 fishy aldehydes, *n*-hexanal exhibited the highest concentration of 12.289

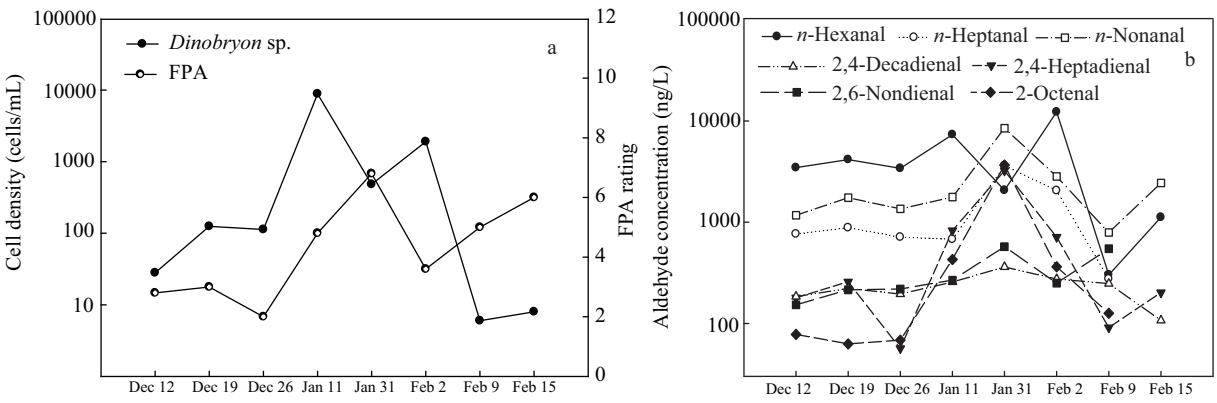

Fig. 2 Variations in *Dinobryon* sp. cell density and FPA rating (a) and variations in aldehyde concentration with time (b) at site 1.

μg/L, about 3 times the OTC value (4.5 μg/L) (**Table S2**). The highest concentration of 2,4-heptadienal was 3.23 μg/L, which was close to its OTC (5.0 μg/L), that of 2,4-dicadienal was 0.36 μg/L a little higher than the OTC (0.3 μg/L), and that of *n*-heptanal was 1.2 times that of its OTC (3.0 μg/L). Although 2,6-nonadienal was present at a relatively high peak concentration, this compound only exhibits a grass and cucumber odor. Thus it is possible that all of the 4 fishy aldehydes might be associated with the fishy odor episode observed in this study.

2.3 Correlation analysis

In order to reveal the relationship between the algal species and the 7 potential algal metabolites, PCA was performed using 30 groups of data obtained over the duration of the investigation (**Fig. 3**). Component 1 had a weight of 36.02% and component 2 had a weight of 19.72%. The *Cyclotella* sp. and *Melosira* sp. were found to be more closely correlated with hexanal, heptanal and 2,4-decadienal, which was in accordance with previous reports showing that these compounds are mainly the metabolites of diatoms (Satchwill et al., 2007; Fink, 2007). 2,4-Heptadienal, 2,4-decadienal and heptanal, which have been reported as fishy odorants (Hargesheimer and Watson, 1996; Watson et al., 2001a; Watson and Satchwill, 2003; Satchwill et al., 2007), were found to be closely related with *Dinobryon* sp. (**Fig. 3**). As shown in **Fig. 4**, the 2,4-heptadienal concentration was significantly correlated with the density of *Dinobryon* sp. (r^2 = 0.431, n = 28, $p < 0.01$), showing that 2,4-heptandienal was possibly produced by the *Dinobryon* sp. However, with an OTC as high as 5 μg/L and a maximum concentration of 3.24 μg/L, the fishy odor episode in this study could not be well explained

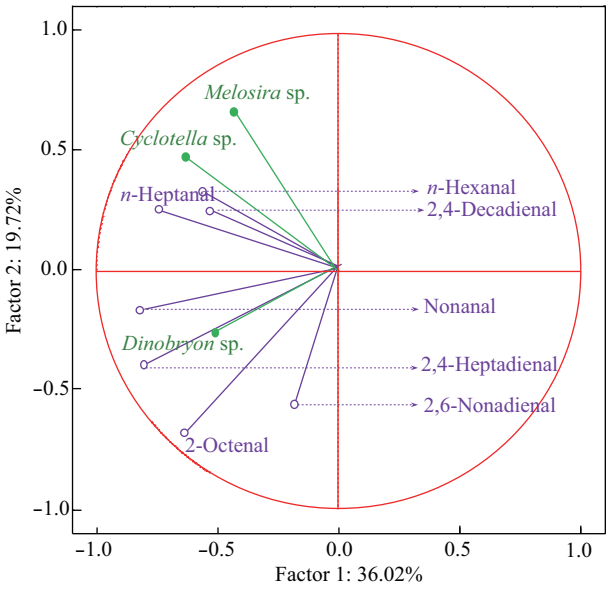

Fig. 3 Principal component analysis of the 7 aldehydes and 3 algal species. Factor 1 explained 36.02% of the observed variation, and factor 2 explained 19.72% of the variation.

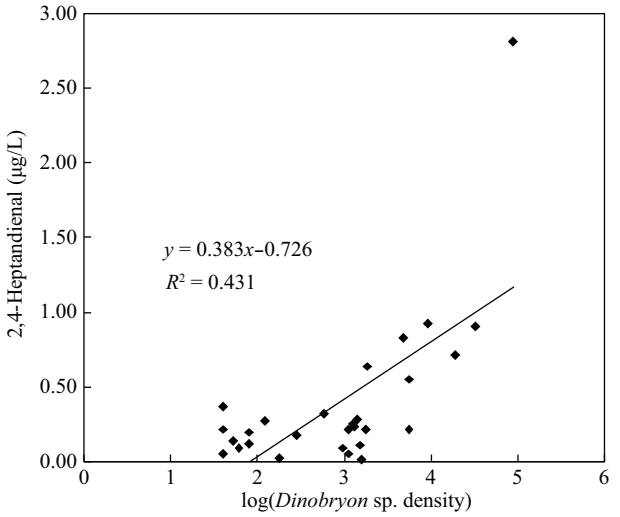

Fig. 4 Correlation of 2,4-heptandienal concentration and *Dinobryon* sp. density. *Dinobryon* sp. density (10^3 cells/mL).

by the mere presence of 2,4-heptadienal. So it was possible that *n*-hexanal, *n*-heptanal and 2,4-decadienal, possibly the metabolites of diatoms, and 2,4-heptadienal, possibly the metabolite of *Dinobryon* sp., might have contributed to the fishy odor episode in this study. It should be noted that the contribution of diatoms to the fishy odor episodes may not be able to be neglected, although the cell density of diatoms was significantly lower than that of *Dinobryon* sp.

3 Conclusions

Through intensive investigation over a two-month fishy odor episode in a reservoir in north China, the following conclusions could be drawn: (1) Among the dominant algal species (two diatom and one chrysophyte species) observed during the survey, *Dinobryon* sp. belonging to the chrysophyte group was the most abundant species, with a peak value of 88,520 cells/mL. (2) The PCA result showed that, of the 7 aldehydes detected, *n*-hexanal, *n*-heptanal and 2,4-decadienal, possibly the metabolites of diatoms, and 2,4-heptadienal, possibly the metabolite of *Dinobryon* sp., might have contributed to the fishy odor episode in this study. It is possible that some unknown compounds produced by *Dinobryon* sp. may also be responsible for the fishy odor episodes.

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Supporting materials

Supplementary data associated with this article can be found in the online version.

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Supporting materials

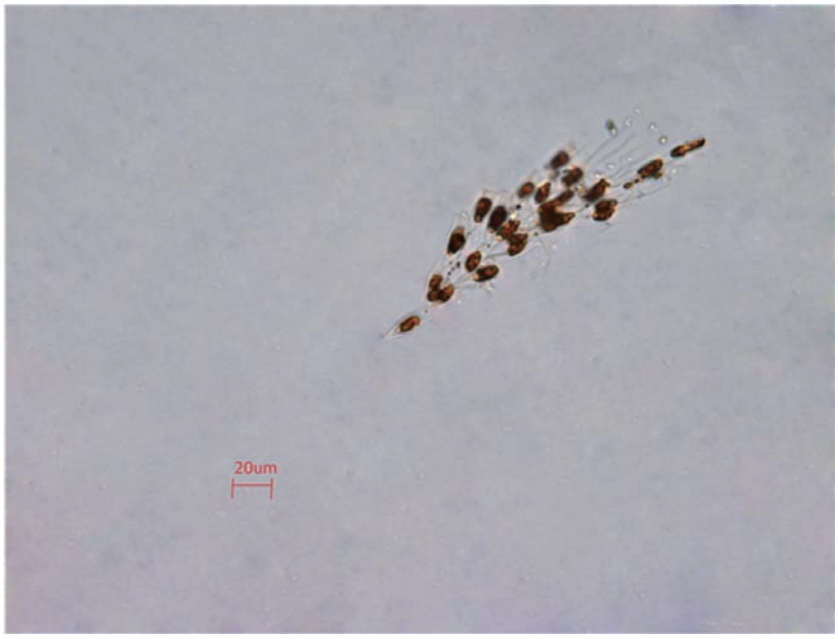

Fig. S1 Photomicrographs of the dominant chrysophyte in Jinhai Reservoir.

Table S1 Algae density in Jinhai Reservoir including one species of chrysophyte: *Dinobyron* sp. and two species of diatom: *Cyclotella* sp. and *Melosira* sp.

Site		*Dinobryon* sp.	*Cyclotella* sp.	*Melosira* sp.
1	Mean (cells/mL)	7155	940	353
	Min (cells/mL)	0	482	0
	Max (cells/mL)	32320	1520	1640
2	Mean (cells/mL)	16434	1188	819
	Min (cells/mL)	60	0	0
	Max (cells/mL)	88520	4000	4280
3	Mean (cells/mL)	1746	857	283
	Min (cells/mL)	80	0	0
	Max (cells/mL)	5520	1800	1440

Table S2 Concentration of several aldehydes

Site		*n*-Hexanal	*n*-Heptanal	2,4-Heptadienal	2,4-Decadienal	2,6-Nonadienal	Nonanal	2-Octenal
1	Mean (μg/L)	4.070	0.929	0.442	0.225	0.355	2.273	0.589
	Min (μg/L)	0.575	0.000	0.000	0.000	0.101	0.505	0.000
	Max (μg/L)	9.251	1.855	0.904	0.279	1.071	3.616	2.377
2	Mean (μg/L)	4.717	1.283	0.765	0.248	0.418	2.589	0.685
	Min (μg/L)	0.302	3.614	0.000	0.184	0.153	1.352	0.000
	Max (μg/L)	12.289	0.275	3.234	0.360	0.573	2.828	2.368
3	Mean (μg/L)	2.520	0.739	0.110	0.203	0.304	2.296	0.429
	Min (μg/L)	0.182	0.000	0.000	0.151	0.000	0.000	0.000
	Max (μg/L)	6.952	1.671	0.216	0.258	0.599	5.764	2.422
OAV		0.0401–2.731	0–1.214	0–0.649	0–1.203	0–7.488	–	0–0.346

OAV: Odorant concentration/OTC; '–' means no OVA available because no OTC of nonanal was obtained.

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