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Concentration and Bioaccumulation of Cyanobacterial Bioactive and Odorous Metabolites Occurred in a Large, Shallow Chinese Lake

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Abstract The distributions of cyanobacterial bioactive and odorous metabolites were investigated in the fifth largest lake in China in the fishing season 2012. The highest microcystins (MCs) concentration in water reached 3.4 μg L^{-1}. A high β-ionone concentration reached 35.6 ng L^{-1} in water. Mean MCs concentration in muscle was highest in omnivorous *Carassius auratus* (20.9 ng g^{-1} dry weight), followed by phytoplanktivorous *Hypophthalmichthys molitrix* (7.4 ng g^{-1} DW) and carnivorous *Coilia ectenes* (3.0 ng g^{-1} DW). The maximum off-flavor concentrations kept 9.5 μg kg^{-1} wet weight for geosmin (*C. auratus*), 5.5 μg kg^{-1} WW for β-cyclocitral (*C. ectenes*) and 25.5 μg kg^{-1} WW for β-ionone (*C. ectenes*). Positive correlation was found between the off-flavor and fat contents in *C. ectenes*. To be different with MCs, β-cyclocitral content was highest in fore-gut contents (87.6 μg kg^{-1} WW) in *H. molitrix*. It should not be reliable to predict odorous compounds level in fish muscle by only measuring the off-flavor in lake water only.

Keywords Off-flavor · Microcystins · Bioaccumulation · Lake Chaohu

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Introduction

The occurrence of heavy cyanobacterial blooms in eutrophic fresh water has been a worldwide problem. Cyanobacteria are well known to produce two secondary metabolites, biotoxins and/or taste and odor (T&O) compounds, which severely impact the supply of drinking water (Watson 2003; Peter et al. 2009). Microcystins (MCs), the main bioactive toxic metabolites, are the most frequently encountered cyanotoxins in fresh water (Chorus and Bartram 1999). MCs are potent hepatotoxins due to their inhibition of several serine/threonine protein phosphatases including PP1 and PP2A, leading to the disruption of normal cell metabolism and functions (Carmichael 1994). Extensive studies have confirmed that MCs can accumulate in the edible part of various aquatic animals and enter into the food chain (Xie et al. 2005; Zhang et al. 2009; Papadimitriou et al. 2012).

The term "off-flavor" is used to describe the accumulation of T&O compounds within water or tissue produced from biological origins. Fish that largely absorb these compounds from water are not acceptable for commercial processing and sale. Geosmin (GEO) and 2-methylisoborneol (MIB) are responsible for the majority of reported off-flavor odors, which are known to be produced by some species of actinomycetes and cyanobacteria (Zimba and Grimm 2003). Previous studies focused on the detection for the odor threshold values for both MIB and GEO in fishes (Young et al. 1996; Robertson et al. 2005). β-cyclocitral and β-ionone, as the oxidation degradation products of carotenoids are the indicators of microbial activities in natural waters (Jüttner 1992). During the period of cyanobacteria blooms, GEO, MIB, β-cyclocitral and β-ionone can be frequently found in the water or tissue of organisms as dominant odorants (Yang et al. 2008). Aquatic organisms are exposed to the odorous and bioactive secondary metabolites through the ingestion of cyanobacteria, consumption of contaminated food items (e.g., prey or detritus), and/or absorption of dissolved compounds from the water column (e.g., after leakage from cells or cell lysis). The contamination of both cyanotoxins and odorous metabolites in aquaculture species will cause a risk for the consumption of aquatic products to humans.

Lake Chaohu is the fifth largest freshwater lake in China. It is an important source of drinking and industry water as well as aquatic products for Hefei City and Chaohu City. Unfortunately, an 11-year observation indicated Lake Chaohu was highly eutrophic and water quality showed no substantial improvement during 2001 through 2011 (Yang et al. 2013). Toxic cyanobacterial blooms (primarily *M. aeruginosa*) occured frequently in the surface water of the bay in warm seasons every year (Jiang et al. 2010). Xie et al. (2005), Peng et al. (2010) and Zhang et al. (2013) ever reported MCs bioaccumulation in some fishes and evaluated the risks associated with consuming fishes contaminated by MCs in Lake Chaohu. However, no references could be found to focus on the off-flavors of water and aquatic products in this lake.

This study was designed to analyze the diversity and dynamics of the MCs and off-flavors in water and three main economic fish species with different food habits in Lake Chaohu. Furthermore, this study was conducted to identify (1) the possible predictive capability of MCs or off-flavor in water versus levels found in fishes; (2) the variation and production of cyanobacterial odorous and bioactive metabolites among fishes. It should give some new information about the health risks associated with harvesting and consuming aquatic organisms contaminated by MCs and off-flavor in Lake Chaohu during the fishing season.

Materials and Methods

Lake Chaohu [(31°25′–31°43′) N, (117°16′–117°51′) E], located on one of the tributaries of the Yangtze River and was one of the five largest freshwater lakes in China. It was a subtropical lake with a surface area of 760 km^2, a mean depth of 3.06 m. With the increasingly serious pollution occurred in western Lake Chaohu resulting from substantial discharge of urban wastewater and agricultural runoff, fishery catches have been mainly exploited in eastern Lake Chaohu near Chaohu City during the fishing season in recent years. The legal fishing season in Lake Chaohu is from early September to late December every year.

Five sites (Fig. 1) were sampled in the eastern lake at late August and late December 2012. Water temperature, Secchi depth (SD) and dissolved oxygen (DO) were measured in situ. Three duplicate samples were collected at each site from the surface water (0–0.5 m). Water sample was filtered through a filter (GF/C, Whatman, UK) to separate particulate and dissolved toxins and odors. *Hypophthalmichthys molitrix* (*H. molitrix*, phytoplanktivorous fish, body weight: 216 ± 54 g, body length: 23.6 ± 1.5 cm), *Carassius auratus* (*C. auratus*, omnivorous fish, body weight: 167 ± 84 g, body length: 19.9 ± 2.9 cm) and *Coilia ectenes* (*C. ectenes*, carnivorous fish, body weight: 25 ± 15 g, body length: 17.8 ± 5.1 cm) were collected in the eastern lake. These three important economic fish species with different food habits accounted for more than 70 % of the total fishery catch in recent years (Guo et al. 2007). Three groups of *C. ectenes* in different growth stages (based on the fish body lengths) were also collected (group 1: 10.5 ± 0.3 cm, group 2: 18.1 ± 0.6 cm, group 3: 23.4 ± 0.6 cm) in August (Liu et al. 2008). The muscles of fishes were isolated, the fore-gut contents,

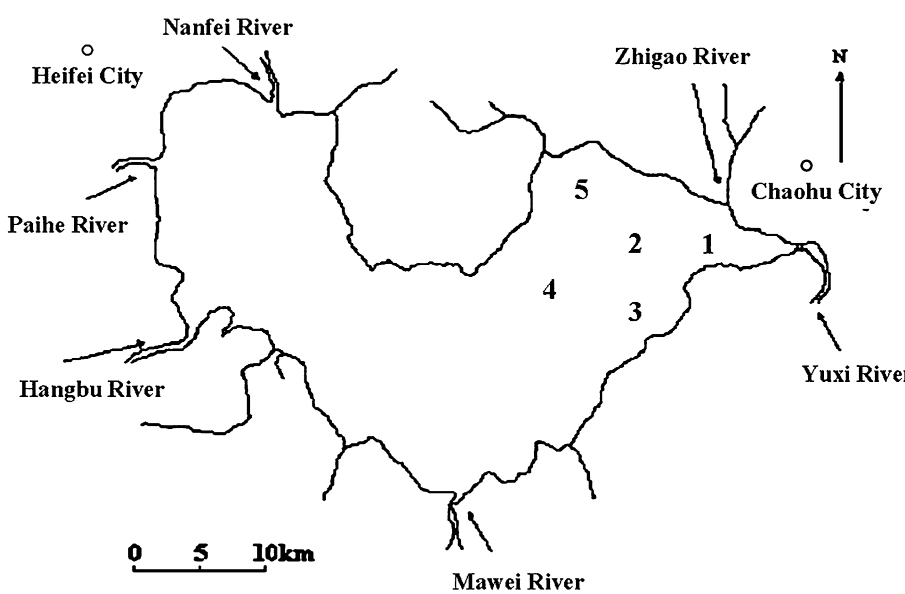

Fig. 1 Map of Lake Chaohu and locations of sampling sites

mid-gut contents and hind-gut contents of *H. molitrix* were collected.

The extraction for MCs in seston, fish muscle and gut contents was followed by the method conducted by Chen and Xie (2005). Recovery experiments were performed in 1,000 mL water or 500 mg freeze-dried fish samples with MC-LR solution at 0.5 μg mL^{-1}. The average recovery of MC-LR in water was 85.4 % (range 71.5 %–98.9 %, n = 10). Average recoveries in fish muscle and gut contents were 78.5 % (ranging from 65.1 % to 95.1 %, n = 10) and 82 % (ranging from 68.3 % to 95.7 %, n = 10), respectively. MCs concentration was measured by enzyme-linked immunosorbent assay (ELISA) method described by Lei et al. (2004) and reported as MC-LR equivalents (detection limit, 0.1 ng mL^{-1}). The assay allows the detection of some types of toxins (including MC-LR, YR, LF, RR, LW) based on the binding of MCs to the antibodies immobilized on well plate (Fischer et al. 2001). The seston on the filter was frozen-thawed, grinded and 10 g fillet sample was homogenized in ice-bath for T&O compounds detection according to the solid phase microextraction (SPME) coupled with GC–MS analysis mentioned by Liang et al. (2005). The procedure to quantify lipid in muscle tissues followed the method conducted by Jiang et al. (2009).

The correlation matrix between different parameters was calculated by SPSS software, version 16.0 for Windows (Chicago, USA). Toxin and off-flavor concentration comparisons were conducted by one-way analysis of variance (ANOVA), followed by Bonferroni tests to identify the sources of detected significance. $p < 0.05$ was considered statistically.

Results and Discussion

In our study, the highest dissolved and particulate MCs concentrations reached 1.0 and 3.4 μg L^{-1} in lake water in August, which were over the safety limit for drinking water (1 μg L^{-1}) provided by the WHO. The mean total MCs values kept 0.6 μg L^{-1} in August and 0.2 μg L^{-1} in December, below the recommended limit. Nevertheless, chronic toxic effects from consumption of MCs-contaminated fish product to human health could not be overlooked. The mean highest MCs content was found in *C. auratus* (20.9, range 16.6–27.9 ng g^{-1} DW), followed by *H. molitrix* (7.4, range 4.3–8.8 ng g^{-1} DW) and *C. ectenes* (3.0, range 0.0–6.7 ng g^{-1} DW) (Fig. 2). *H. molitrix* ingested more toxic *Microcystis* cells, but accumulated less MCs in muscle, probably accounting for the inhibition of the transportation of some MCs across the gutwall (Chen et al. 2006). *C. auratus* might take up MCs through routes other than the gastrointestinal tract (e.g., via gills or skins). The small *C. ectenes* accumulated less MCs, which might be due to the shorter life span and feeding habit (mainly feeding on the zooplankton) (Guo et al. 2007). Previous studies (Xie et al. 2005; Smith and Haney 2006; Zhang et al. 2009) had also found that MCs could be transferred along the food web in lakes and diverse MCs variants and environmental factors could affect different accumulation of MCs variants in fish tissues. Ibelings and Chorus (2007) indicated that toxin content at each trophic level was dependent on biodilution and on the bioaccumulation versus biotransformation capacity of the various organs.

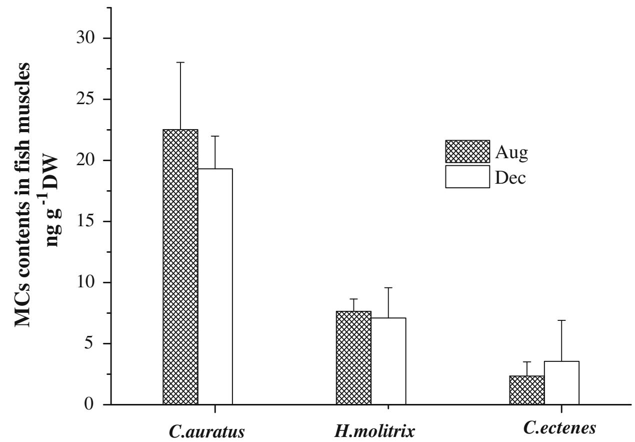

Fig. 2 Microcystins (MCs) content in muscles of each fish species from August to December 2012 (*H. molitrix* = *Hypophthalmichthys molitrix, C. auratus* = *Carassius auratus, C. ectenes* = *Coilia ectenes*)

Figure 3 showed the particulate T&O compounds concentrations in lake water during our study. MIB levels were too low to be detected. GEO also showed low concentrations and were comprised almost 100 % of the particulate fraction. The highest content for particulate fraction of GEO was 1.3 μg L^{-1}. From August to December, mean particulate fraction of β-cyclocitral (>97.2 % of the total) kept from 0.2 to 0.0 μg L^{-1}, below its OTC (0.5–1.0 μg L^{-1}) (Blackburn et al. 1992). Particulate β-cyclocitral correlated significantly with *Microcystis* biomass in lake water (r = 0.793, $p < 0.01$, n = 30), which was consistent with the reports that *Microcystis* could produce β-cyclocitral (Watson 2003). The mean particulate β-ionone (>99.3 % of the total) reached 8.3 μg L^{-1} that exceeded its OTC (7 ng L^{-1}) and a high β-ionone concentration was recorded as 35.6 ng L^{-1} in December. β-cyclocitral and β-ionone were products of oxidative decomposition of β-carotene in *Microcystis* spieces (Watson 2003). The oxidative cleavage of β-carotene during increased oxygen concentrations might yield more norcarotenoid products such as β-ionone (Walsh et al. 1998). β-ionone formed more readily compared to β-cyclocitral when β-carotene was degraded by filamentous fungi (Zorn et al. 2003). It might be concluded that the increased oxygen content (6.2–9.3 mg L^{-1} from August to December) and some filamentous fungi species in water promoted

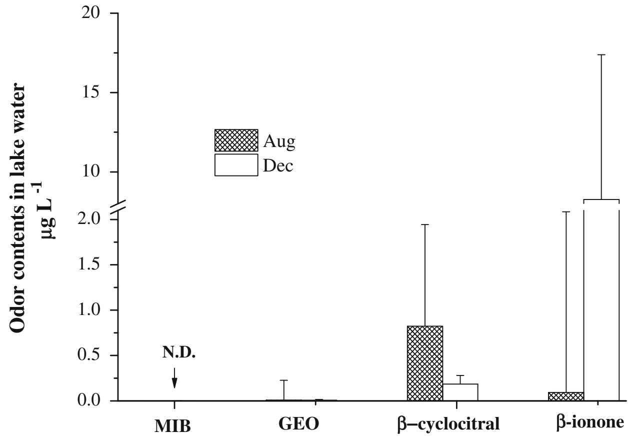

Fig. 3 Particulate T&O compounds concentrations in lake water from August to December 2012 (values are averages at each sampling sites. GEO: geosmin; MIB: 2-methylisoborneol)

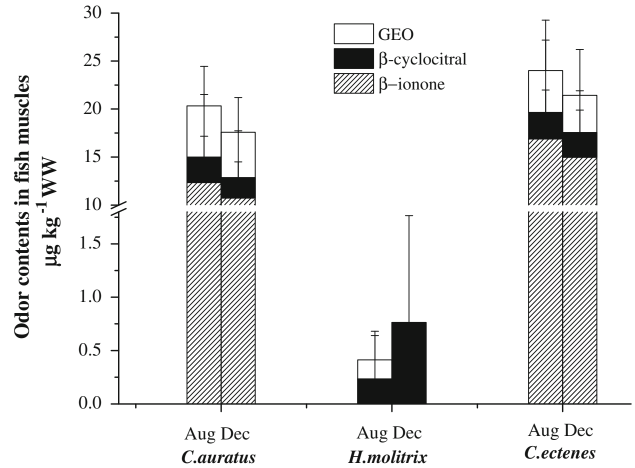

Fig. 4 T&O compounds concentrations in muscles of each fish species from August to December 2012 (*C. auratus* = *Carassius auratus, H. molitrix* = *Hypophthalmichthys molitrix, C. ectenes* = *Coilia ectenes*)

the substantially amounts of β-ionone in lake water in winter.

Our study was the first to report odorous metabolites contents in muscle tissues of wild fishes in Lake Chaohu (Fig. 4). Stable high mean concentrations of β-ionone were found in *C. ectenes* (15.9 µg kg^{-1} WW) and *C. auratus* (11.6 µg kg^{-1} WW), whereas this taint appeared below detectable levels in *H. molitrix*, suggesting there were considerable differences in the accumulation patterns of off-flavors among fish species. Johnsen and Lloyd (1992) found that the bioaccumulation of earthy taints in fish appeared to be depended on the concentration of the compound in the water supply, water temperature, fat content and mass of fish. So in the present study, we investigated not only the variable accumulation of the evident taints but also MCs in the same species *C. ectenes*, and in fish of different sizes collected at the same time. Table 1 showed the lipid content, β-ionone and MCs contents in muscle tissues of three groups of *C. ectenes*. Positive correlations were found between the odorous compounds and fat contents in *C. ectenes* (r = 0.671, $p < 0.01$, n = 15). It was found that the violet-taint problem was greater in the larger *C. ectenes* than in the smaller ones. Percival et al. (2008) ever found there was a significantly greater GEO and MIB taints concentration in large compared to small barramundi in a reservoir in Australia. This observation together with our study indicated that differences in lipid content of the fish might result in the different levels of taint compounds detected within these aquatic species. No significant correlation was found between MCs concentration in *C. ectenes* muscle and the weight or length of fish in our study. Similar results were also shown between MCs concentration in Nile tilapia muscle and the weight of fish in lakes in Uganda (Semyalo et al. 2010). However, some significant correlations were observed between MCs concentrations in fishes and total length (Papadimitriou et al. 2012; Zhang et al. 2013).

In phytoplanktivorous *H. molitrix*, the highest mean content for β-cyclocitral was found in the fore-gut contents (87.6 µg kg^{-1} WW), followed by the hind-gut contents (2.7 µg kg^{-1} WW) and mid-gut contents (1.4 µg kg^{-1} WW). There were significant decreases in odor contents when the ingested food moved from fore- to hind-guts. That might be due to the fact that during the process of digestion, a large amount of intact cyanobacterial cells in the fore-gut were ruptured, releasing relatively much more off-flavor taints that rapidly moved to various tissues or organs through the gut-wall, which were then accumulated in the whole body. Johnsen and Lloyd (1992) ever pointed out the bioaccumulation of odorous compounds in fish tissues was rapid. This might explain why we found little amount of these taints in the mid- and hind-gut contents. The study conducted by Chen et al. (2006) and our study both suggested that there were significant increases in MCs when the ingested food moved from fore- to hind-guts for *H. molitrix*. The progress for MCs transporting across the intestines was slow in comparison with that of off-flavor, as higher MCs contents were detected in hind-gut contents which proved to be due to the great resistance to MCs for *H. molitrix*. Thus, it was good evidence that the absorption and distribution for these two sorts of cyanobacterial secondary metabolites derived from food sources might have significant differences in phytoplanktivorous silver carp, which maybe attributed to their different physical/chemical characters.

No significant correlations between off-flavor or MCs concentrations in fish muscle tissues and that in lake water were found. Zimba and Grimm (2003) had shown poor predictive capability of measuring off-flavor in water

Table 1 Means (±SD) (n = 5) and ranges of body length, body weight, lipid content, off-flavor (β-ionone) content, microcystins (MCs) content in muscle tissues of three groups of *Coilia ectenes* in August 2012

Group	Body length (mm)	Body weight (g)	Lipid content (%)	Off-flavor content ($\mu g\ kg^{-1}$ WW)	MCs content ($\mu g\ g^{-1}$ DW)
1	105.0 ± 3.4	6.0 ± 0.8	2.2 ± 0.1	7.7 ± 4.0	2.9 ± 2.5
2	180.8 ± 6.1	23.3 ± 2.0	3.5 ± 0.1	11.6 ± 4.7	1.5 ± 1.5
3	233.6 ± 5.7	42.4 ± 2.1	5.2 ± 0.2	17.7 ± 7.3	2.4 ± 2.1

versus levels found in fish muscle tissue. No significant correlation was found between MCs in water or seston and fish tissues by Magalhães et al. (2001). Nevertheless, Magalhães et al. (2003) and Zhang et al. (2013) reported negative or positive correlations between MCs content in seston and *Tilapia rendalli* or silver carp muscle. Volatilization, photolysis, sorption and biodegradation could easily result in the loss of the metabolites, especially semi-volatile odors in water, while the accumulations of MCs and odorous compounds in fishes were chronic and complicated, which reflected a long period of changes of these chemicals in the lake water.

A tolerable daily intake (TDI) of 0.04 μg MC-LR per kilogram body weight per day has been proposed as a provisional guideline (Chorus and Bartram 1999). In the worst situation found in Lake Chaohu (MCs content in muscle = 0.028 $\mu g\ g^{-1}$ DW, the average ratio of dry weight to wet weight was 0.2, consumption in a day of 300 g of fish muscle and a 60 kg person), the TDI proposed by the WHO could not be reached. For off-flavors, the thresholds for GEO in fish flesh ranged 0.3–10.0 $\mu g\ kg^{-1}$ (Robertson et al. 2005). In the present study, the mean contents for GEO ranged from 5.4 to 4.7 $\mu g\ kg^{-1}$ WW in *C. auratus* and 4.4 to 3.9 $\mu g\ kg^{-1}$ WW in *C. ectenes* from August to December, respectively. The presence of GEO in *C. ectenes* and *C. auratus* in the fishing season generated unpleasant smell to people and resulted in a resistance to purchase the fish. The odor thresholds of β-cyclocitral and β-ionone in fishes have not been well determined. The mean concentrations of β-cyclocitral and β-ionone kept from 2.6 to 2.1 and 16.9 to 15.0 $\mu g\ kg^{-1}$ WW in *C. auratus*, and 2.8 to 2.6 and 12.4 to 10.8 $\mu g\ kg^{-1}$ WW in *C. ectenes*, respectively. The highest β-ionone levels reached 22.3 $\mu g\ kg^{-1}$ WW in *C. auratus* and 25.5 $\mu g\ kg^{-1}$ WW in *C. ectene*, respectively. Thus, the distinct β-carotene by-products produced mainly by *Microcystis* species in fish should not be ignored in Lake Chaohu. In general, the presences of cyanobacterial odorous metabolites were concerning in the legal fishing season in Lake Chaohu. The long-term impact of MCs and off-flavors to the aquatic ecosystem and public health should not be overlooked in this lake and some necessary measures of monitoring and control of growth of cyanobacteria are urgently needed in Lake Chaohu.

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