Cyanobacterial blooms act as sink and source of endocrine disruptors in the third largest freshwater lake in China

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Abstract

Cyanobacterial blooms are of global concern due to the multiple harmful risks they pose towards aquatic ecosystem and human health. However, information on the fate of organic pollutants mediated by cyanobacterial blooms in eutrophic water remains elusive. In the present study, endocrine disruptive potentials of phytoplankton samples were evaluated throughout a year-long surveillance in a large and eutrophic freshwater lake. Estrogenic agonistic, anti-estrogenic, anti-androgenic, and anti-glucocorticogenic effects were observed in the phytoplankton samples using in vitro reporter gene bioassays. 27 endocrine disrupting chemicals (EDCs) of different modes of action were detected in the samples via UPLC-MS/MS system. Results from mass balance analysis indicated that the measured estrogenic activities were greater than the predicted estrogenic potencies from chemical analysis, demonstrating that chemical analysis of targeted EDCs is unable to fully explain the compounds responsible for the observed estrogenicities. Results from Spearman’s correlation analysis concluded that the concentrations of ten EDCs in phytoplankton samples were negatively correlated with cyanobacterial biomass, suggesting the potential occurrence of biomass bio-dilution effects of EDCs due to the huge biomass of cyanobacteria during bloom seasons. The present study provided complementary information about the potential endocrine disruptive risks of cyanobacterial blooms, which is important for understanding and regulating EDCs in eutrophic lakes.

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1. Introduction

Chemical pollutants, such as endocrine disrupting compounds (EDCs), are of great concern due to their industrial and domestic applications and potential adverse effects on metabolism, development, growth and reproduction in exposed freshwater and marine wildlife (Tan et al., 2007). The effects of EDCs include reduced fertility, feminization, reproductive organ anomalies, and changes in sexual behavior of a variety of aquatic organisms (Kidd et al., 2007; Pal et al., 2010). Research has shown the occurrence of EDCs in wastewaters (Yu et al., 2013), natural waters (Yang et al., 2014), and drinking waters (Benotti et al., 2008) all around the world, including synthetic steroid hormones, pharmaceutical drugs, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, alklyphenols, pesticides, but also natural products such as phytoestrogens. Men-made pollutants are released from manufacturing processes and since they are not fully removed by sewage treatment systems, they can enter aquatic systems.

In addition to chemical pollution, aquatic ecosystems are exposed to additional multiple stressors (Ormerod et al., 2010). Eutrophication of freshwaters and coastal marine ecosystems...
resulting from increased anthropogenic nutrient input into receiving waterbodies has become a global problem (Smith, 2003). Harmful algal blooms are frequently concomitant with over-enrichment of nutrients (particularly nitrogen and phosphorus). Eutrophic waterbodies with severe cyanobacterial blooms are observed in Lake Taihu in China (Paerl et al., 2011), Lake Biwa in Japan (Nalewajko & Murphy, 2001), Lake Erie and Michigan in North America (Rinta-Kanto et al., 2005), Lake Winnipesauke in Canada (Schindler et al., 2012), Lake Victoria in Africa (Verschuren et al., 2002), the Baltic Sea in Northern Europe (Suikkanen et al., 2007), and other ecologically and economically important lakes, rivers and estuaries globally (Huisman et al., 2006). Dense cyanobacterial blooms increase the turbidity of eutrophic waters, and subsequently suppress the growth of aquatic plants and thereby negatively affect the underwater habitat for many invertebrates and fish species (Scheffer, 2004). In the last few decades, of great concern is the increase in toxin-producing strains of the blue-green algae Microcystis sp. that produce hepatotoxic microcystin (Michalak et al., 2013). More than 100 structural variants of microcystins have been identified and classified as “possibly carcinogenic to humans” (Crosse et al., 2006). A number of studies have focused on the toxicity of microcystins, such as hepatotoxicity (Takami et al., 2010), genotoxicity (Zegura et al., 2003), oxidative stress (Amado & Monferrat, 2010), and endocrine-disruptive potentials (Rogers et al., 2011). Besides the estrogenicity from microcystins, more recent studies suggest that cyanobacterium Microcystis might be a natural source of environmental estrogen (Sychrowa et al., 2012; Essa & Fathy, 2014; Prochazkova et al., 2018).

Phytoplankton is the first link in the trophic chain, performing an important function in the transfer of organic compounds between biotic and abiotic elements of aquatic ecosystems. It has been indicated that significant accumulation of contaminants in phytoplanktonic phase can occur for example with organic contaminants such as polychlorinated biphenyls (Lynn et al., 2007), polycyclic aromatic hydrocarbons (Wan et al., 2007), dioxins (Wan et al., 2005), polybrominated diphenyl ethers (Frouin et al., 2013), and other EDCs (Staniszewska et al., 2015). Thus, in the context of cyanobacterial blooms, the loading and transformation of EDCs in phytoplankton matrix warrant concern. Particularly, colonial Microcystis, which form by a heterogeneous high-molecular-weight mucilaginous matrix (Forni et al., 1997), might play a considerable role in regulating the biogeochemical cycles of organic pollutants in waterbodies affected by Microcystis blooms.

Lake Taihu, the third largest freshwater lake in China, serves as an important resource for drinking water, aquaculture, irrigation and industrial waters, in addition to being a popular recreational and tourist attraction (Song et al., 2007). Unfortunately, decades of intensive utilization of water resources has transformed this once meso-oligotrophic lake in the 1950s into its present hypertrophic state (Chen et al., 1997). Every spring, large areas of the lake turn green with dense Microcystis blooms that persist well into the autumn (Otten et al., 2012). In addition, the pollution by synthetic organic chemicals has been reported in abiotic and biotic phases from Lake Taihu (Yan et al., 2014; Xie et al., 2015). However, little information is available about the occurrence of endocrine disruptors from cyanobacterial blooms in eutrophic freshwaters.

In the present study, phytoplankton samples were collected throughout a one-year surveillance of Lake Taihu. Firstly, the situations of cyanobacterial blooms and cyanotoxins were evaluated in the north, west, and south of Lake Taihu. Particularly, the phytoplankton compositions and biomass were monitored. Secondly, a bioanalytical approach was employed to evaluate the endocrine disruptive potentials in phytoplankton matrices. Rapid, highly sensitive in vitro reporter gene bioassays were used to measure endocrine disruption potentials, as these have been previously demonstrated to successfully identify endocrine disruption effects in environmental matrices (Kunz et al., 2017; Gehrmann et al., 2018; Kase et al., 2018). For environmental monitoring, one of the advantages of the use of bioassays over chemical analysis is that bioassays can measure both known and unknown EDCs, whereas chemical analysis can only detect known EDCs. Thus, bioassays evaluate the total endocrine disruption potency, offering a more robust and accurate assessment of the risk of EDCs in the environment than chemical analysis (Wernersson et al., 2015; Könemann et al., 2018). However, important quantitative information about concentrations of EDCs in the environment can still be obtained from chemical analysis. Thus, in the third step of the present study, chemical analysis was performed in parallel to the bioassays to gain complementary results about the presence and risk of EDCs in the environment. Mass balance analysis was performed between the two approaches. Finally, multivariate analysis was conducted to gain more insights into the relationship between EDCs contents and phytoplankton compositions.

2. Methods and materials

2.1. Chemicals and materials

All chemicals used in the present study were reagent grade and purchased from Sigma Aldrich (Schnelldorf, Germany). Reference compounds used for bioassays, including estradiol (E2), dihydrotestosterone (DHT), dexamethasone (Dexa), tamoxifen, flutamide, and mifepristone, were prepared in dimethyl sulfoxide (DMSO). Cell culture medium [Dulbecco’s Modified Eagle Medium (DMEM)] was obtained from Sigma Aldrich (Schnelldorf, Germany) and Invitrogen (Darmstadt, Germany). Twenty-six natural and synthetic hormonal chemicals and four commonly detected industrial contaminants were selected as the target EDCs based on previous studies in Lake Taihu (Lu et al., 2011; Yan et al., 2012). Descriptions of these substances are provided in Table 1.

2.2. Sample collection and preparation

Samples of phytoplankton were collected using 40 μm phytoplankton net from four sites in each of the north, west, and south regions of Taihu Lake over the period from September 2010 to August 2011 (Fig. 1). The sampling sites were not directly adjacent to the inflows of major effluents. Water samples for phytoplankton species composition were collected at each site from 0 to 0.5 m depth using a vertical sampler, and fixated with Lugol’s iodine solution. Fixated phytoplankton samples were identified and enumerated by light microscopy according to the commonly used method by Watanabe and co-workers (Watanabe et al., 1992). In the laboratory, the phytoplankton samples were settled overnight at 4°C to separate the zooplankton and suspended particles (Watanabe et al., 1992). The phytoplankton samples were lyophilized and stored at −80°C for further analysis.

2.3. Sample extraction

Due to the limited biomass obtained during the cold season, sites with abundant algal biomass were selected for cyanotoxin and EDCs measurements. Firstly, one portion of the dried phytoplankton samples (50 mg) were weighed for cyanotoxins analysis, and prepared with 5% acetic acid and 80% aqueous methanol used for cyanotoxins extraction (the ratio of solvent volume per dry weight biomass is 20 mL per 50 mg). The obtained extracts were cleaned up using C18 solid phase extraction cartridge (6 cc, 500 mg, Waters, US) and the eluted solutions were stored at −20°C until high performance liquid chromatography (HPLC) analysis, as
Table 1
The 30 selected EDCs and their measurement parameters for UPLC-MS.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Abbreviation</th>
<th>Internal standards</th>
<th>Mode</th>
<th>Parent Ions</th>
<th>Quantification Ions</th>
<th>Confirmation Ions</th>
<th>Retention time (min)</th>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>Estrone</td>
<td>E1</td>
<td>E1-D2</td>
<td>ESI−</td>
<td>269.2</td>
<td>145.1</td>
<td>159</td>
<td>3.58</td>
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<td>E1-D2</td>
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<td>ESI−</td>
<td>271.3</td>
<td>185.1</td>
<td>171.1</td>
<td>3.53</td>
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<td>E1-D2</td>
<td>ESI−</td>
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<td>182.9</td>
<td>145</td>
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<td>E1-D2</td>
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<td>237</td>
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<td>ESI−</td>
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<td>3.65</td>
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<td>Dino</td>
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<td>119</td>
<td>134</td>
<td>3.79</td>
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<td>Proges-D9</td>
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<td>105</td>
<td>77</td>
<td>5.8</td>
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<td>82.9</td>
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<td>TES-D3</td>
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<td>109</td>
<td>3.35</td>
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<td>Norges-D6</td>
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<td>245.6</td>
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<td>Norgestrel-D6</td>
<td>Norges-D6</td>
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<td>ESI+</td>
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<td>251.7</td>
<td>301.5</td>
<td>3.9</td>
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<td>Proges-D9</td>
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<td>123</td>
<td>97</td>
<td>4.18</td>
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<td>Progesterone</td>
<td>Proges</td>
<td>Proges-D9</td>
<td>ESI+</td>
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<td>109</td>
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<td>113.1</td>
<td>4.67</td>
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<td>Me-ace</td>
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<td>385.3</td>
<td>224.2</td>
<td>267.2</td>
<td>4.62</td>
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<td>Hydrop</td>
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<td>253.5</td>
<td>271.3</td>
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<td>2.17</td>
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<td>90.9</td>
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<td>Prednin</td>
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<td>361.4</td>
<td>147.1</td>
<td>307.2</td>
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<td>Me-prednl</td>
<td>Proges-D9</td>
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<td>375.4</td>
<td>161</td>
<td>120.9</td>
<td>2.52</td>
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<td>Four Industrial compounds</td>
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<td>Bisphenol S</td>
<td>BPS</td>
<td>DES-D8</td>
<td>ESI−</td>
<td>248.4</td>
<td>108</td>
<td>91.9</td>
<td>0.76</td>
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<tr>
<td>4-n-Octyl Phenol</td>
<td>OP</td>
<td>DES-D8</td>
<td>ESI−</td>
<td>205.2</td>
<td>189.1</td>
<td>133.3</td>
<td>5.56</td>
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<td>BPA</td>
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<td>3.06</td>
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<td>Bisphenol F</td>
<td>BPF</td>
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<td>93</td>
<td>197.4</td>
<td>2.46</td>
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</tbody>
</table>

Fig. 1. Map of the sampling sites in Lake Taihu, China.
Signalsample Signalsample 1 0 10% Signalsample

processed throughout the extraction and clean-up. The performance of sample extraction, procedure controls were performed for solid samples (Pojana et al., 2007). Briefly, 100 mg freeze-dried phytoplankton samples were sonicated with an extraction solvent mixture (hexane/acetone, 70:30, v/v) for 2 h (the ratio of solvent volume per dry weight biomass is 20 mL per 100 mg). The sonication frequency was 20 kHz. The extraction procedure was repeated three times and the obtained three 20 mL extracts were then combined and filtered through a glass fiber membrane (GF/F, Whatman, Maidstone, UK) and concentrated under gentle nitrogen flow to ca. 500 µL under 20 C. The concentrated extracts were further purified with a Florisil clean-up column (60–100 mesh, activated at 150 C overnight) as described elsewhere (Pojana et al., 2007). Meanwhile, in order to evaluate the performance of sample extraction, procedure controls were processed throughout the extraction and clean-up. The final extracts were then dissolved in 300 µL Milli-Q water and stored at 2 C prior to chemical and bioanalytical measurements.

2.4. U2OS cell culture and reporter gene assay

The assay protocols have been extensively described elsewhere (Van Der Linden et al., 2008; Grund et al., 2011; Maletz et al., 2013). The human U2OS Osteosarcoma 1 cells used in the Estrogen receptor α (ERα), androgen receptor (AR), and glucocorticoid receptor (GR) reporter gene assays were provides and licenced by BioDetectionSystems (BDS, Amsterdam, The Netherlands). To avoid false negative results, only the extracts tested as non-cytotoxic (cell viability >80%) within the MTT assay were applied in the CALUX assays according to the protocol developed by BDS. Briefly, U2OS-luc cells were cultured at 37 C under 5% CO2 and high humidity in DF medium (1:1 mixture of Dulbecco’s Modified Eagle’s Medium and Ham’s F12) supplemented with 7.5% fetal calf serum (FCS). Cells were seeded into 96 wells plates with DF medium (without phenol red and supplemented with DCC stripped FCS). After 24 h of incubation, the medium was replaced with medium containing stripped FCS and reference compounds with or without extracts for agonist and antagonistic response testing. After 24 h of exposure, the medium was removed, and the cells were lysed in Triton lysis buffer. The amount of luciferase activity was measured using a luminometer (Berthold, Germany). All measurements were performed in triplicate wells; experiments were conducted at least in triplicate for all samples. For agonistic assays, cells were exposed to reference hormone-agonist agents (17β-estradiol (E2, 10-12, 10-10 M), 5α-dihydrotestosterone (DHT, 10-12-10-7 M), and dexamethasone (DEX, 3 x 10-11-10-7 M) for ER-, AR-, and GR-CALUX, respectively) or extracts. For antagonistic effects, the assay medium was spiked prior to exposure with the EC50 of agonist, i.e., final concentrations of 3 x 10-11 E2, 4.2 x 10-10 DHT, 1.2 x 10-9 DEX for the anti-ER CALUX, anti-AR CALUX, and anti-GR CALUX, respectively. Tamoxifen (10-7-10-3 M), flutamide (10-6-10-4 M), and mifepristone (10-8-10-3 M) were used as the reference compounds for anti-ER, anti-AR, and anti-GR assays, respectively.

For each bioassay, concentrations of samples were expressed as relative enrichment factors (REF), which can be calculated by enrichment factor of the sample extraction or concentration multiplied by the dilution factors of the factor of the factor (Jia et al., 2015).

In the agonistic assays, the response as % effect was obtained by dividing the sample response by the maximum response at the same experiment after subtracting control signal. The control signal refers to solvent control (equation (1)).

\[
\text{Agonistic effect} = \frac{\text{Signal}_{\text{sample}} - \text{Signal}_{\text{control}}}{\text{Signal}_{\text{max}} - \text{Signal}_{\text{control}}} \times 100\% \quad (1)
\]

\[
\text{EC}_{10} = 10\% \text{ effect} \quad (2)
\]

\[
\text{Antagonistic effect} = 1 - \frac{\text{Signal}_{\text{sample}} - \text{Signal}_{\text{control}}}{\text{Signal}_{\text{agonist}} - \text{Signal}_{\text{control}}} \quad (3)
\]

\[
\text{EC}_{20} = \frac{0.2}{\text{slope}} \quad (5)
\]

2.5. Chemical analysis

The target analytes were analyzed according to a previously described method (Zhang et al., 2011). Briefly, the analysis was performed using Waters ACQUITY UPLC system coupled with Waters Xevo TQ MS (Waters, Milford, MA). The tandem MS system was operated in both positive-ion and negative-ion multiple-reaction-monitoring (MRM) mode. UPLC-MS data were acquired using Masslynx 4.1 software package (Waters, Milford, USA). Recoveries of EDCs in phytoplankton samples were determined by use of external standards. Limit of quantitation and recoveries are shown in Table S1. The detailed detection parameters for UPLC-MS and quality assurance and quality control (QA/QC) are presented in the supplemental file. Estrogenic equivalents predicted from chemical analysis were calculated from concentrations of the target estrogenic compounds determined by UPLC-MS analyses and relative potencies (estradiol equivalency factor, EEF) obtained from the in vitro assays of previous studies (Table S4). The compounds that were below the limit of quantification (LOQ) were not included in the calculations of predicted estrogenic equivalents. Calculation of the predicted estrogenic equivalents (EEQ) was according to the following equations:

\[
\text{Predicted EEF (total)} = \sum_{i=1}^{n} \text{compound } i \times \text{EEF } i
\]

2.6. Data processing

Statistical analyses were performed using R Studio (Version 1.0.143, R Studio, Inc.) and GraphPad Prism 6 (GraphPad Software Inc.) for Windows. Prior to data analysis, data were checked for assumptions of normality and homogeneity of variances using the
Shapiro-Wilk test and Bartlett test. The concentration-response curve was generated using a four-parameter logistic model (drc package, R). The final data are shown as mean ± standard deviation. The non-parametric two variables Spearman’s correlation of the phytoplankton and EDCs contents was analyzed using ltm package in “R” (Rizopoulos, 2006). In comparison to Pearson’s correlation analysis which assesses linear relationships, Spearman’s correlation assesses monotonic relationships. The results of all tests were accepted as significant at p < 0.05.

3. Results and discussion

3.1. Status of cyanobacterial blooms and cyanotoxins in Lake Taihu

Eutrophication has long been acknowledged as a driver of the production of phytoplankton biomass in freshwater (Conley et al., 2009). In Lake Taihu, total nitrogen (TN) and total phosphorus (TP), which are largely associated with eutrophication, were present at relatively high concentrations throughout the duration of the sampling period (Fig. 2A and B). The average concentrations of TN and TP were 3.7 mg/L and 0.2 mg/L, respectively. Sampling sites in the western part of the lake contained greater concentrations of both TN and TP loading in comparison with the northern and southern regions (p < 0.05). Overall, the concentrations of nutrients from the present sampling campaign are similar to China environmental quality report for Lake Taihu in 2011, indicating the predominant occurrence of eutrophication in the lake.

Given the abundance of nutrients in the lake, severe cyanobacterial blooms are one of the major problems threatening water quality and function in Lake Taihu. As presented in Fig. 2C, high percentages of cyanobacteria in total phytoplankton biomass were observed during the year surveillance. Cyanobacteria were observed to predominate more than 90% of the total phytoplankton cell density in 65%, 44% and 58% of the 48 phytoplankton samples collected in each of the west, north and south areas of the lake, respectively. The phytoplankton cell densities during the different time points of sampling were shown in Figure S1. Cyanobacterial blooms in Taihu Lake exhibited an increase in coverage in all open lake sections over the sampling year, suggesting that situations in these lake regions continue to worsen.

Meanwhile, cyanobacterial blooms are also notorious for the production of cyanotoxins, such as the heptatoxins, microcystins (MCs). We measured three variants of MCs, i.e., MC-RR, MC-YR, and MC-LR in phytoplankton samples from Lake Taihu (Fig. 2D and Figure S2). The three microcystin variants were widely detected in the samples with the highest concentrations of 1128, 527 and 354 ng/L, respectively (Fig. 3B, C, and D). Jian-liang Zhao et al. (2011) reported that the levels of anti-estrogenic activity were up to 1296 µg tamoxifen/L in surface water and 89.5 µg tamoxifen/g in sediment of Pearl River in China. In vitro reporter gene assays offer a highly sensitive and cost-effective way to evaluate a series of hormone receptors agonistic effects. However, particularly in environmental monitoring, the co-existing antagonist compounds might suppress the agonists binding to receptors. In this case, the in vitro bioassays represent net endocrine-disruption activity in the balance between agonistic and antagonistic effects (Ihara et al., 2014). A previous study has reported that phthalate esters including dibutyl phthalate and dibutyl phthalate were identified as major contributors to an AR antagonistic potency in source waters in China (Hu et al., 2013). Our recent study identified 4-methyl-7-dieethylaminocomarin as a highly potent environmental pollutant that acts as AR antagonist (Muschket et al., 2017). Additionally, it has been reported that several commercially available humins elicited significant anti-estrogenic effects, likely through the sorption of E2 on humic substances, changes in membrane permeability for E2, or another specific mechanism (Janosek et al., 2007). However, the number of pollutants that are responsible for antagonistic effects is far from being fully determined.

3.3. Chemical analysis and mass balance

A wide range of EDCs in phytoplankton samples were detected analytically during all sampling periods (Fig. 4). As shown in Fig. 4, 27 of 30 analytes were detected in at least one of the phytoplankton samples. Four estrogens (i.e., estriol, diethylstilbestrol, dienoestrol, and hexestrol), two androgens (i.e., 19-nortestosterone and boldenone), two progesterones (i.e., norethisterone and hydroxyprogesterone), an adrenocortical hormone (i.e., Cortisone), and three industrial pollutants (i.e., bisphenol A, bisphenol S, and bisphenol F) were detected in all samples. The highest concentrations of estrogens, androgens, progesterons, and adrenocortical hormones in phytoplankton samples were 6.1 (estriol), 54 (boldenone), 22.7 (hydroxyprogesterone), and 151.7 (Cortisone) ng/g d.w., respectively. The concentrations of five adrenocortical hormones were consistently greater than other classes of EDCs, suggesting further studies are required to examine potential effects from GR CALUX assays in comparison to the relatively high content of adrenocortical hormones determined by chemical analysis. Among the four industrial pollutants, bisphenol
A was detected with the highest concentration of 3954 ng/g, followed by bisphenol S (547 ng/g), bisphenol F (324 ng/g), and 4-n-octyl phenol (58.1 ng/g). The demand and production capacity of bisphenol A in China has grown rapidly due to its importance in the manufacturing of many products such as engineered plastics, food cans, and dental composites/sealants (Huang et al., 2012). This trend will lead to much more bisphenol A contamination in various environmental media. It has been extensively reported that the presence of bisphenol A is ubiquitous in aquatic environments with concentrations of up to 5030 ng/L (Quirós et al., 2005; Voutsa et al., 2006; Huang et al., 2012). More recently, the substitutes of bisphenol A, such as bisphenol S and bisphenol F, are also frequently detected in aquatic environment (Yamazaki et al., 2015). Unfortunately, research has demonstrated that these structural analogues of bisphenol A cause similar effects on ER and AR activities (Rosenmai et al., 2014).

A correlation between the presence of targeted EDCs in cyanobacterial bloom samples and the endocrine effects observed in
reporter gene assays could not be established in the present study. For example, cyanobacterial bloom samples in this study contained androgenic and glucocorticoid compounds, such as testosterone, testosterone propionate, cortisone, and dexamethasone, yet results from reporter gene assays suggested that these EDCs were either not present in concentrations sufficient to induce androgenic and glucocorticoid effects in the bioassays or were not bioavailable. There are at least three explanations. Firstly, these EDCs were either not present in concentrations sufficient to induce androgenic and glucocorticoid effects in the bioassays or were not bioavailable. Secondly, certain endocrine-disruptive responses may not have been observed due to the coexistence of other compounds with different modes of actions, such that estrogenic effects might be masked due to the presence of testosterone (Sellin Jeffries et al., 2011; Sychrová et al., 2012). Secondly, the occurrence of antagonistic endocrine disrupting effects suppresses the detectability of agonistic endocrine disrupting effects (Ihara et al., 2014) leading to the undetectable of androgenic and glucocorticoid effects in the present in vitro bioassays.

Mass balance analysis of measured and predicted estrogenic activities are shown in Fig. 5. 17 of 20 cyanobacterial samples were calculated with the ratio between measured and predicted estrogenic equivalents greater than one (with a highest value up to 37.62), suggesting that the measured estrogenic potencies could not be explained by the analyzed estrogens in the present study. Both anthropogenic and natural substances contribute to overall estrogenic activity in cyanobacterial blooms samples. The evidence of endocrine-disruptive potentials of naturally produced compounds from cyanobacterial blooms is now growing (Oziol & Bouaïcha, 2010). One recent study suggested that maximum phytoestrogens accounted for only 1.6 pg/L E2 equivalents, responding to maximal 8.5% of the total estrogenicity of the water samples (Procházková et al., 2017b).
3.4. Multivariate analysis

Statistical analyses were performed to examine possible relationships between the concentrations of EDCs in phytoplankton and phytoplankton biomass. Spearman correlation tests were performed and the results are shown in the datasheet in supplementary file and in Fig. 6. Ten EDCs (i.e., hydroxyprogesterone, methyl testosterone, and dexamethasone, \( p < 0.01 \); bisphenol S, 19-nortestosterone, testosterone, progesterone, estradiol benzoate, megestrol acetate, and epitestosterone, \( p < 0.05 \)) were negatively correlated with Cyanophyta biomass. Eight EDCs (i.e., 19-nortestosterone and testosterone, \( p < 0.01 \); bisphenol F, bisphenol S, progesterone, dexamethasone, methyl testosterone, and hexestrol, \( p < 0.05 \)) were positively correlated with Chlorophyta biomass. Hydroxyprogesterone, progesterone, and methyl testosterone were positively correlated with diatom biomass (\( p < 0.05 \).) Dienoestrol, 4-n-octyl phenol, and estradiol benzoate were positively correlated with Chrysophyta biomass (\( p < 0.05 \).) 4-n-octyl phenol, bisphenol A, and estradiol benzoate were positively correlated with Cryptophyta biomass (\( p < 0.05 \).) Similarly, results from the in vitro bioassays were incorporated into Spearman correlation analysis (Fig. 6), demonstrating that the estrogenicities derived from ER-CALUX were negatively correlated with Cyanophyta biomass but positively correlated with the biomass of diatoms and Chrysophyta. The anti-androgenicities derived from anti-AR CALUX were also positively correlated with the biomass of diatoms and chrysophyta.

Physicochemical sorption of organic pollutants to live algae was influenced by several factors including the properties of the sorbing matrix (e.g., surface area, binding sites, and lipids contents) and the hydrophobicity of the agents themselves. Firstly, in comparison to colonial cyanobacteria, unicellular algae (e.g., chlorophyta and diatoms) provide larger surface area for the uptake of organic compounds from water column onto the surface of algal cells (Chen et al., 2017). Secondly, under certain conditions, diatoms and green algae produce more lipids than cyanobacteria (Hu et al., 2008), which can result in a greater tendency to accumulate hydrophobic organic pollutants in algae. Another plausible explanation is that average concentrations of EDCs in phytoplankton declined as the cyanobacteria biomass increased during the bloom events (i.e., bio-dilution).

In this study, cyanobacteria dominated the phytoplankton community during most of the sampling campaigns. Due to the high biomass of cyanobacterial blooms, bio-dilution effects could be used to explain the negative correlation between several EDCs and Cyanophyta biomass. The biomass bio-dilution of the EDCs might subsequently reduce the uptake of these contaminants in freshwater food webs in Taihu Lake. The rapid proliferation of algal biomass usually results in so-called “bio-dilution” of pollutants in the phytoplankton itself (Söderström et al., 2000; Tiano et al., 2014), and might reduce pollution exposure to other organisms in the environment. Pickhardt et al., (2002) reported that bio-dilution of algal blooms may be responsible for lower methylmercury accumulation by zooplankton and fish in alga-rich waters in comparison to alga-deficient water bodies. Meanwhile, greater occurrence of algal blooms might cause the sedimentation of algae-associated contaminants into the sediment phase after decay of the algal blooms. Concentrations of organochlorine pesticides and PAHs have been observed to be 2.4 to 3.4 times greater in sediments in regions where algal blooms occurred than concentration in regions without the occurrence of algal blooms (Shi et al., 2017). The contaminants thus loaded in sediments might be subsequently redistributed to benthic organisms with events of sediment disturbances and remobilization.

The role of plankton (e.g., as a biological pump) in influencing intercompartment exchange and regional and global distribution of persistent organic pollutants (POPs) has been the focus of recent research (Galbán-Malagon et al., 2012; Nizzetto et al., 2012). In a pelagic lake ecosystem, the primary producers play a key role in alleviating exposure of the trophic web by a) reducing dissolved-phase concentrations of EDCs by uptake or adsorption to phytoplankton; b) enhancing transfer of POPs during the algal bloom and post-bloom seasons; and c) reducing concentrations of EDCs in biota in terms of rapid growth dilution (Nizzetto et al., 2012). Unfortunately, water samples were not collected during our sampling period, thus the determination of bioconcentration factors of EDCs from the water phase to the phytoplanktonic phase were not possible in the present study.

3.5. Future risk assessment of cyanobacterial blooms in eutrophic lakes

Aquatic pollution worldwide is an undesirable by-product of the growing use of industrial chemicals in modern civilization. Sources and fates of these pollutants, such as EDCs, deserve increasing attention. While previous studies have shown that sediments act as a source/sink of bioavailable EDCs, few studies provide information regarding the role of cyanobacterial bloom in the biogeochemistry processes of EDCs. Further studies should focus on the bioavailability of algal bloom-associated EDCs to other aquatic organisms.

Lake Taihu is one of the most extensively studied freshwater lakes in the world. Hundreds of publications have focused on the hyper-trophic situation and frequent occurrence of cyanobacterial blooms in this lake. There is no doubt that the two problematic issues of eutrophication and cyanobacterial blooms contribute to the deterioration of the water quality of Lake Taihu. To this end, monitoring and controlling of cyanobacterial blooms, which are often coupled with the production of cyanotoxins, is of great importance for the environmental and human health risk assessment and water resource utilizations in Lake Taihu. However, the risks of harmful cyanobacterial blooms might be a greater hazard than previously suggested, due to the high amounts of EDCs detected in cyanobacterial bloom samples in the present study. The larger amount of...
cyanobacterial blooms might be a sink or a source of endocrine disruptors, which is a risk of endocrine disruptive effects on aquatic organisms in the water column and humans using cyanobacterial blooms-containing water as drinking source water. The present investigation of endocrine disruptors in cyanobacterial bloom provides insight into the potential risks of cyanobacterial blooms and a comprehensive assessment of cyanobacterial bloom-occurring waterbodies.

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Appendix A. Supplementary data

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Fig. 6. Spearman correlation network between EDC potentials (instrumental and bioanalytical analysis) in phytoplankton and their biomass (mg/L). Phytoplankton was presented in green nodes. The estrogens, androgens, progestogens, and adrenocortical hormones were presented in light yellow, light red, red, and light blue nodes, respectively. The bioanalytical results were presented in grey nodes. BPA, BPS, BPF, and OP were also presented. Solid lines indicated positive relationship; dotted lines indicated negative relationship. Thin lines indicated the relation with p < 0.05 while thick lines indicated strong relation with p < 0.01. The abbreviations of different phytoplankton were: Cyano-cyanophyta; Chloro-chlorophyta; Eugleno-euglenophyta; Chryso-chrysophyta; and Crypto-cryptophyta. The abbreviation of EDCs was presented in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
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