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Plasma biochemical responses of the planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to prolonged toxic cyanobacterial blooms in natural waters

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ABSTRACT

The planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) are the attractive candidates for bio-control of plankton communities to eliminate odorous populations of cyanobacteria. However, few studies focused on the health of such fishes in natural water body with vigorous toxic blooms. Blood parameters are useful and sensitive for diagnosis of diseases and monitoring of the physiological status of fish exposed to toxicants. To evaluate the impact of toxic cyanobacterial blooms on the planktivorous fish, 12 serum chemistry variables were investigated in silver carp and bighead carp for 9 months, in a large net cage in Meiliang Bay, a hypereutrophic region of Lake Taihu. The results confirmed adverse effects of cyanobacterial blooms on two phytoplanktivorous fish, which mainly characterized with potential toxicogenomic effects and metabolism disorders in liver, and kidney dysfunction. In addition, cholestasis was intensively implied by distinct elevation of all four related biomarkers (ALP, GGT, DBIL, TBIL) in bighead carp. The combination of LDH, AST activities and DBIL, URIC contents for silver carp, and the combination of ALT, ALP activities and TBIL, DBIL, URIC concentrations for bighead carps were found to most strongly indicate toxic effects from cyanobacterial blooms in such fishes by a multivariate discriminant analysis.

1. Introduction

Cyanobacterial blooms in fresh surface waters occur worldwide with ever increasing incidence (Duy et al., 2000; Falconer et al., 1994). Several cyanobacteria species, specifically *Microcystis aeruginosa*, are capable of producing a variety of potent toxins, including a group of hepatotoxins called microcystins (MCs) which have strong cytotoxic activity (Codd et al., 1997; De Figereido et al., 2004). Aquatic animals such as zooplankton, fish and mollusks have been reported to bioaccumulate MCs in natural water bodies with toxic cyanobacteria (Williams et al., 1997; Amorin and Vasconcelos, 1999).

Fish mortalities are occasionally reported when toxigenic strains of *M. aeruginosa* dominate the phytoplankton community of freshwater ecosystems (Rodger et al., 1994). Snyder et al. (2002) declared that species native to oligotrophic habitats are more vulnerable than species native to eutrophic ones, where MC exposure is more frequent. The planktivorous fish, which feed more frequently on toxic cyanobacteia, might have developed special mechanisms to counteract MCs in the history of evolution. Actually, the planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) are the attractive candidates for bio-control of plankton communities to eliminate odorous populations of cyanobacteria (Starling, 1993; Tucker, 2006). However, available data have verified that the microcsytins also accumulated in silver and bighead carp in natural waters as other fishes (Chen et al., 2006a,b). But, few studies focused on the adverse effects from the MCs exposure on this fish. Besides, most literature data available are on acute effects or subchronic effects of the toxicants in the lab. Therefore, more studies on prolonged toxic effects from long-term and/or frequent exposure to toxic cyanobacteria in natural conditions are needed.

Marked blood biochemical responses often occur after aquatic organisms have been exposed to microcystins, with injury of organs (Råbergh et al., 1991; Fischer and Dietrich, 2000; Malbrouck et al., 2003; Malbrouck and Kestemont, 2006; Zhang et al., 2007). MCs-induced liver damage has been correlated with the activities of certain plasma enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and λ-glutamyl transferase (GGT) (Falconer et al., 1994; Fischer and Dietrich, 2000; Malbrouck et al., 2003), and with the concentration of bilirubin (Carbis et al., 1996,

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1997), which are usually measured to screen for liver or gall bladder dysfunction. Meanwhile, the secondary toxic effect on basic metabolism of liver was indicated by the alterations of levels of blood glucose (GLU), cholesterol (CHO), triglyceride (TG), and total protein (TP) (Zhang et al., 2007). In fish, elevated concentrations in blood urea nitrogen (BUN) and creatinine (Cr) were recorded in crucian carps with i.p. injection of extracted microcystins, which convinced the MC-induced kidney impairment (Zhang et al., 2007). However, seldom studies focused on the changes of blood parameters in phytoplanktivorous fishes till now. To our knowledge, there is no report on blood biochemical responses of phytoplanktivorous fishes to toxic cyanobacteria blooms in natural water, although they are the main grazers of the cyanobacteria.

The main aim of this study was to evaluate the impact of toxic cyanobacterial blooms on health of two planktivorous filter-feeding fishes, silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), in regard of plasma biochemical responses. Plasma enzymes activities of ALT, AST, ALP, GGT and LDH, and the concentrations of blood GLU, CHO, TP, TBIL, DBIL, BUN and URIC (uric acid) were monitored in both fish for 9 months with and without the blooms. Furthermore, a stepwise back ward discriminant analysis was used to elucidate which combination of parameters discriminated best between the stages with and without the blooms.

2. Materials and methods

2.1. Sampling site and fish collection

Lake Taihu (30°5′–32°8′ N and 119°8′–121°55′E) is located in the east part of China. It is the third largest freshwater lakes in China, and has a surface area of 2338 km^2, a mean water depth of 1.9 m and a maximum depth of about 2.6 m (Qin et al., 2004). Meiliang Bay (water surface area 125 km^2) is a hypertrophic region of Lake Taihu (Dokulil et al., 2000), where vigorous and long-lasting cyanobacterial blooms frequently cover the water surface. A large fish pen was built in the Meiliang Bay (Fig. 1) in 2003, with a total area of 1.08 km^2 and a mesh size of 2 cm × 2 cm. Sampling was carried out in the pen during 2005. Water condition in the pen was monitored monthly at ten locations. Each water sample was a mixture of two subsamples—one from 0.5 m below the surface and the other from 0.5 m above the bottom. Water temperature was recorded by a WMY-01 digital thermometer. Dissolved oxygen (DO) and pH were determined with an Orion 810 dissolved oxygen meter and PHB-4PH meter, respectively. One liter of lake water was fixed with 5% formalin, and *Microcystis* cells were separated with ultrasonic before microscopical examination. *Microcystis aeruginosa* biomass (expressed as mg fresh wt/L) was estimated from its geometric cell volume, assuming a mean density of 1 mg/mm. Seston was filtered from 1 l lake water on the glass-fiber filter (GF/C, Waterman, UK) and the toxins in the seston were examined by HPLC according to Park and Lwami (1998).

The phytoplanktivorous silver carp [*Hypophthalmichthys molitrix*] and bighead carp [*Aristichthys nobilis*] are native to Lake Taihu, with mean wet body weight 162 ± 28 g and length of 26.8 ± 2.9 cm for the *H. molitrix* and with mean wet body weight 186 ± 30 g and length of 24.8 ± 1.2 cm for the *A. nobilis*. Fishes were artificially stocked into the pen at the beginning of the year, for the purpose of counteracting cyanobacteria. Every month from April to December in 2005, 10 individuals per species were randomly captured from the pen by gill net, and then measured, weighed, and sacrificed immediately. Blood were firstly taken via tail vein.

2.2. Plasma biochemical analyses

Blood samples were collected and centrifuged at 850 × *g* for 10 min. Serum was stored at −80 °C. An automated analyzer (Backman coulter LX-20, USA) was used for all serum chemistries. The harvested serum was used to determine serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), λ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and serum total alkaline phosphatase (ALP). Concentrations of total bilirubin (TBIL), direct bilirubin (DBIL), total protein (TP), glucose (GLU), and cholesterol (CHOL) were also measured. Serum urea nitrogen (BUN) concentration and uric acid (URIC) concentration were determined. Test kits from Backman were used for all assays.

2.3. Statistics

Values were expressed as means ± standard deviation (S.D.). The data were tested for normality and homogeneity. Based on the appearance of cyanabacteria, the experiment was divided into three stages, the stage before blooms, the stage during blooms and the stage after blooms. Variations of water conditions among stages were tested with One-way analysis of variance. Multifactorial Analysis of Variance (MANOVA) was used to determine statistical differences of the average plasma enzymes activities (ALT, AST, ALP, GGT and LDH) and the average concentration of blood GLU, CHO, TP, TBIL, DBIL, BUN and URIC among different periods. The level of significance was set at 0.05. A stepwise back ward discriminant analysis (significance level of 0.05 for retaining and entry) was used to analyze elucidate which combination of parameters discriminated best between the stages with and without the blooms for two fishes. Statistical analysis was carried out with SPSS 11.0.

3. Results

No fish-kill episode was observed throughout the experiments. Temperature, MC content in seston, biomass of cyanobacteria and *Microcystis* all significantly differed with stages. However, pH and oxygen concentration did not change much (Table 1). In cyanobacteria, *Microsystis* was dominant. During the blooms, the average temperature increased to 26.7 °C, and the maximum biomass of cyanobacteria and *Microcystis* in August reached 14.3 and 12.5 mg/l, respectively, when MC content in seston was as high as 1.53 mg/l (Fig. 2). The relatively stable concentration of DO in the lake water in spite of the occurrence of cyanobacterial blooms was probably due to frequent stirring of the lake water by strong wind in this bay (Fig. 1).

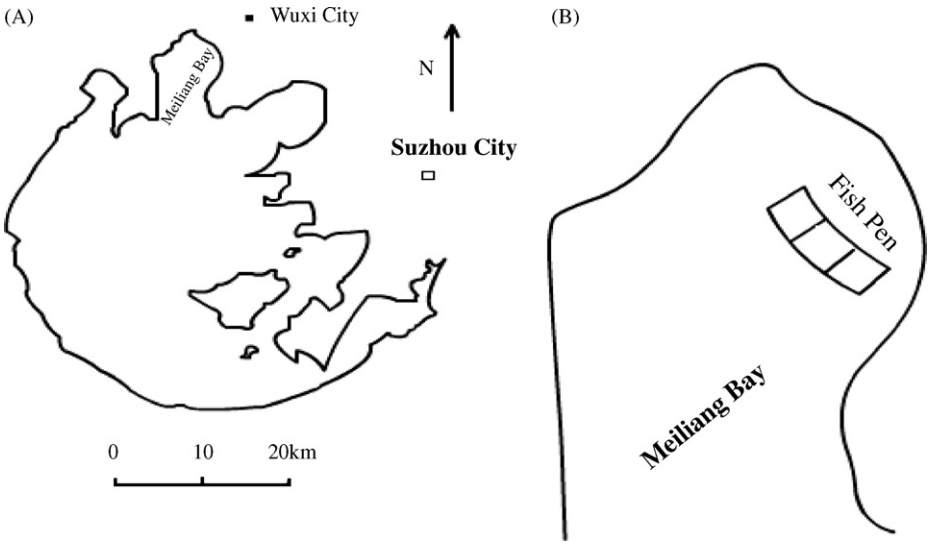

Fig. 1. The sketch of Lake Taihu (A) and the location of the fish pen in Meiliang Bay (B).

Table 1
The mean values of physical and chemical parameters of the lake water in three different periods.

	Before cyanobacterial blooms	During cyanobacterial blooms	After cyanobacterial blooms
Temperature (°C)	16.6 ± 2.7	26.7 ± 4.8*	12.4 ± 7.0
Oxygen concentration (mg/L)	8.4 ± 2.1	7.8 ± 1.2	7.9 ± 0.4
pH	7.5 ± 0.9	8.5 ± 0.4	7.4 ± 0.4
MCs content in seston (μg/L)	0	0.5 ± 0.5*	0.1 ± 0.1
Biomass of cyanobacteria (mg/L)	0	6.31 ± 8.99*	0.27 ± 0.27
Biomass of *Microcystis* (mg/L)	0	5.93 ± 8.51*	0.27 ± 0.11
Time (month)	April, May	June–October	November, December

* $P < 0.05$.

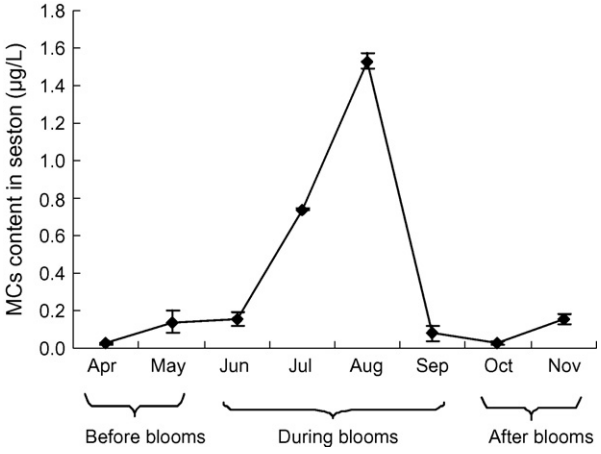

Fig. 2. Seasonal variation of MCs content in seston in the fish pen.

The results of the biochemical examinations involved analyses of 12 blood parameters in both fishes (Figs. 3 and 4). Statistical differences of such parameters among different periods were present in Table 2 for silver carp and in Table 3 for bighead carp. In silver carps, ALT, AST and LDH altered during the blooms, while significant increase in LDH activity was up to 119% of that before and distinct decrease in AST accounted for 29% of that before. ALP activity enhanced at the beginning of the blooms and followed by decreases in the last 2 months. GGT activity increased in July and September with a sharp decrease in August. There was little effect on TBIL concentration from toxic cyanobacterial blooms exposure but marked increase in DBIL content was observed during and after blooms. TP and GLU content remained steady throughout the experiments except for a sharply decrease in GLU content after the blooms. Significant decrease in CHOL content was observed during blooms, which was only 70% of that before the blooms. As to indicators of kidney damage, stable level of BUN concentration lasted throughout the blooms, while the URIC content remarkably increased during the blooms and sharply decreased after the blooms.

Table 2
The average values of serum parameters for the silver carp in three stages and the results of multifactor analysis of variance (MANOVA).

	Before cyanobacterial blooms	During cyanobacterial blooms	After cyanobacterial blooms
LDH	666.1 ± 112.4	793.4 ± 211.7**	834.6 ± 129.1**##
ALT	86.5 ± 26.0	93.5 ± 24.9	58.1 ± 13.6**##
AST	258.7 ± 185.7	185.7 ± 71.7*	204.8 ± 37.8*
TP	17.7 ± 3.5	16.8 ± 1.9	18.8 ± 3.6
GLU	3.9 ± 1.1	3.9 ± 1.6	2.4 ± 0.6**##
CHOL	5.0 ± 1.5	3.5 ± 1.1**	4.1 ± 0.8
ALP	44.8 ± 31.0	64.7 ± 16.1	28.9 ± 11.2**##
GGT	3.0 ± 1.6	3.8 ± 2.4	2.5 ± 0.7
TBIL	3.5 ± 1.0	3.8 ± 1.0	3.8 ± 1.1
DBIL	1.6 ± 1.1	2.6 ± 0.8**	2.5 ± 0.8**#
BUN	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.3
URIC	50.1 ± 18.7	83.3 ± 32.7**	14.8 ± 4.4**##

The values are expressed as mean ± S.D. The significance levels observed are *$P < 0.05$, **$P < 0.01$, compared with the values before the blooms, while #$P < 0.05$, ##$P < 0.01$, compared with the value during the blooms.

Table 3
The average values of serum parameters for the bighead carp in three stages and the results of multifactor analysis of variance (MANOVA).

	Before cyanobacterial blooms	During cyanobacterial blooms	After cyanobacterial blooms
LDH	750.2 ± 195.8	1042.8 ± 325.2**	1237.3 ± 269.2**#
ALT	157.1 ± 27.4	93.9 ± 31.5**	121.9 ± 31.1**##
AST	171.0 ± 57.7	150.6 ± 50.2	159.7 ± 22.7
TP	20.9 ± 3.6	17.0 ± 2.4**	17.9 ± 1.9
GLU	6.2 ± 1.5	6.6 ± 2.0	3.8 ± 0.9**##
CHOL	4.1 ± 1.2	2.6 ± 0.5**	3.1 ± 0.3**##
ALP	56.5 ± 39.7	95.2 ± 27.2**	67.1 ± 30.7##
GGT	3.2 ± 1.0	5.2 ± 3.2**	2.9 ± 1.3##
TBIL	2.5 ± 0.6	3.3 ± 0.7**	2.7 ± 0.7##
DBIL	1.6 ± 1.1	2.5 ± 0.9**	1.9 ± 0.7#
BUN	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
URIC	51.7 ± 28.8	77.7 ± 34.9*	24.2 ± 6.6**##

The values are expressed as mean ± S.D. The significance levels observed are *$P < 0.05$, **$P < 0.01$, compared with the values before the blooms, while #$P < 0.05$, ##$P < 0.01$, compared with the value during the blooms.

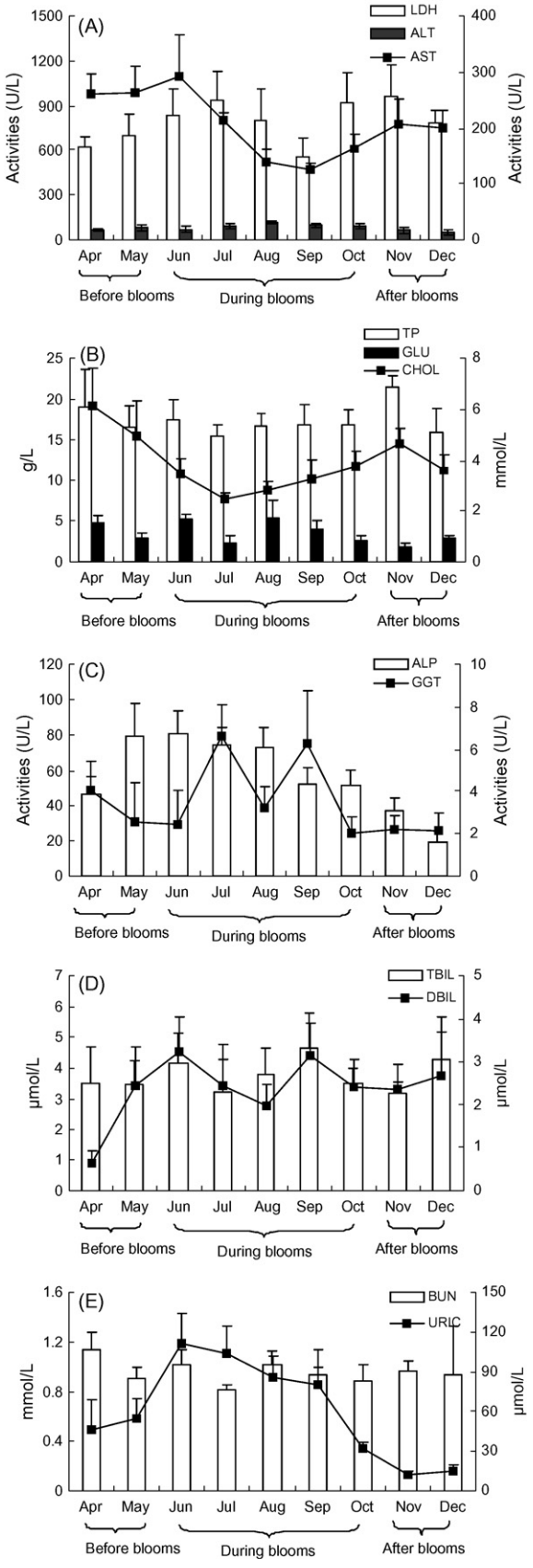

Fig. 3. Plasma biochemical changes of silver carp during the experiment. Data are expressed as mean values ± standard deviation. Vertical bars showing standard deviation of each data set.

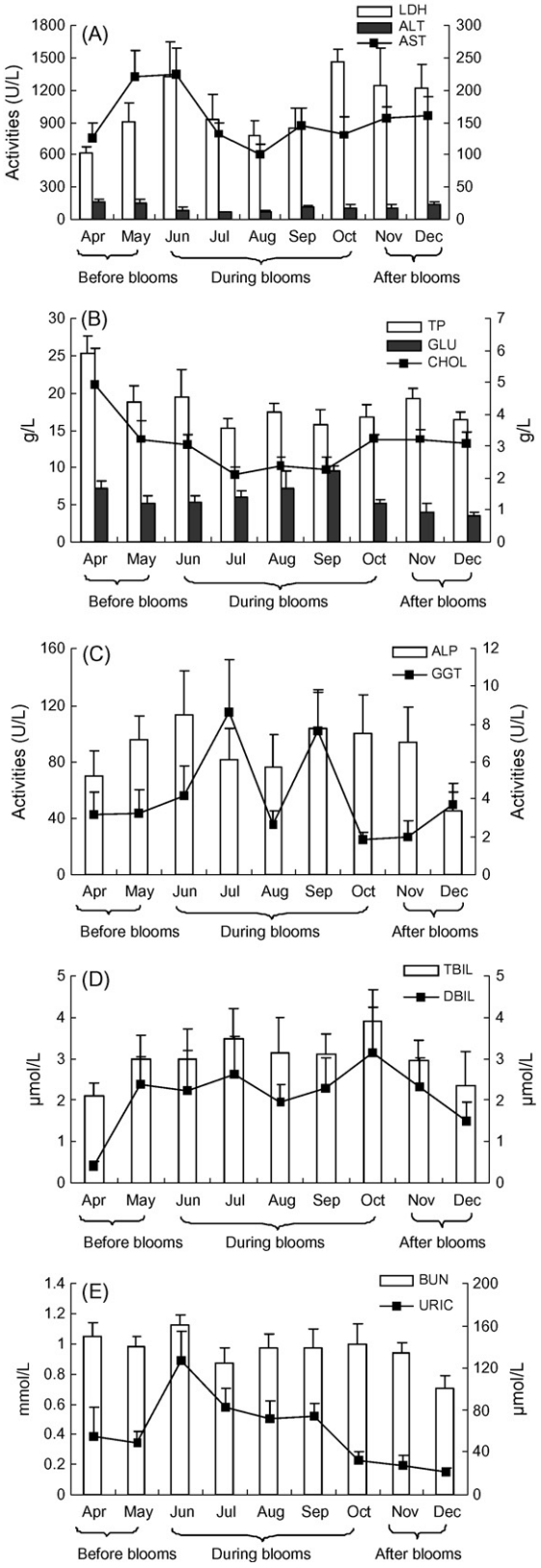

Fig. 4. Plasma biochemical changes of bighead carp during the experiment. Data are expressed as mean values ± standard deviation. Vertical bars showing standard deviation of each data set.

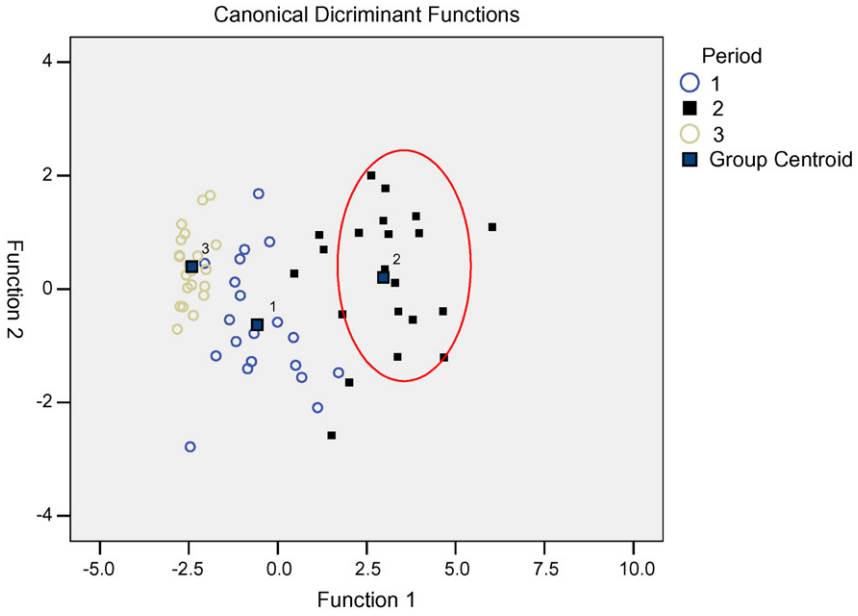

Fig. 5. Multivariate discriminate analysis showing differences serum parameters between the stages with and without the blooms for silver carp (1 ○ represent stage before the bloosm; 2 ■ represent stage during the blooms; 3 ○ represent stage after the blooms). The ellipses represent the 75% confidence level of the sample means of such stage. The serum chemistry parameters subjected to this plot were LDH, ALT, DBIC and URIC.

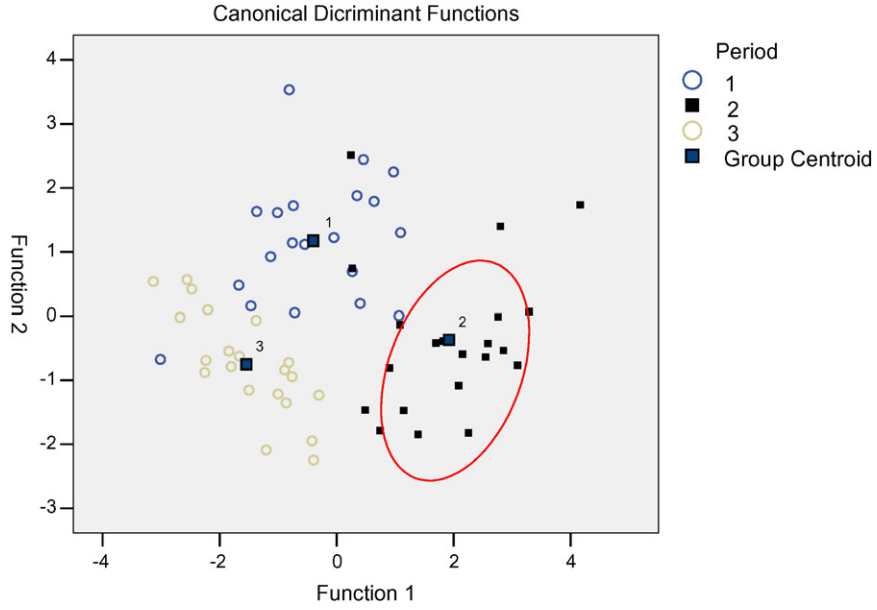

Fig. 6. Multivariate discriminate analysis showing differences serum parameters between the stages with and without the blooms for bighead carp (1 ○ represent stage before the bloosm; 2 ■ represent stage during the blooms; 3 ○ represent stage after the blooms). The ellipses represent the 75% confidence level of the sample means of such stage. The serum chemistry parameters subjected to this plot were ALT, ALP, TBIL, DBIL, URIC.

In bighead carps, prominent increased activities were observed in LDH (139%), ALP (168%), GGT (162%) during the blooms, while the activities of ALT was evidently decreased to 60% of that before the blooms. Contents of both TBIL and DBIL observably elevated during the blooms. The content of TP and CHOL were notably reduced during the blooms, while the GLU level remained stable. Similar to silver carps, stable level of BUN concentration lasted throughout the blooms, while the URIC content remarkably increased during the blooms and sharply decreased after the blooms. Activities of AST and LDH, concentrations of DBIL and URIC for silver carps; and activities of ALT and ALP, concentrations of TBIL, DBIL, and URIC for bighead carps were applied for discriminates analysis (Figs. 5 and 6). The results indicated that multivariate discriminate analysis with selected parameters represented a useful tool to affiliated fish to the different natural environments of with or without the toxic cyanobacterial blooms.

4. Discussion

In previous studies, we revealed the biochemical and ultrastructural changes of the liver and kidney of the phytoplanktivorous silver carp and bighead carp feeding naturally on toxic *microcystis* blooms in Taihu Lake, China (Qiu et al., 2007, the kidney study were submit to Aquaculture). Present results provided plasma biochemical indications of liver and kidney injuries in these two phytoplanktivorous carps and put forward selected parameters which suggested environmental stress from toxic cyanobacterial blooms in natural waters on carps.

Toxicogenomic effects from toxic blooms in liver were indicated in both carps. Plasma enzymes, LDH, ALT and AST, are frequently used to determine the toxic effects of varied pollutants (Hilmy et al., 1985; Özcan Oruζ and Üner, 1998). Statistically significant increases in activities of these liver enzymes were found in silver carp, common carp, goldfish, *Heteropneustes fossilis* (Bloch), brown trout and rainbow trout exposed to MCs or pure MC in various routes in experimental conditions (Råbergh et al., 1991; Bury et al., 1997; Tencalla and Dietrich, 1997; Vajcová et al., 1998; Kopp and Hetesa, 2000; Malbrouck et al., 2003; Li et al., 2004, 2007b; Gupta and Guha, 2006; Zhang et al., 2007). On the contrary to most previous results, the reduced activity of ALT was observed in both fishes, while reduction in AST activity was recorded in silver carp. Increased release of ALT into the blood is indicative of damage to the integrity of hepatocyte membranes (Mitchell et al., 1980), and the elevated AST activities are due to mitochondrial disruption as a consequence of heavy hepatitis (Schmidt and Schmidt, 1974). However, we have revealed that the subcellular organs in hepatocytes of *H. molitrix* and *A. nobilis* were in good condition during the blooms (Qiu et al., 2007). A dose-dependent-decrease in ALT activity was confirmed on rats after a prolonged sublethal microcystin-LR exposure (Guzman and Solter, 1999), which resulted from the reduction in both ALT protein concentration and ALT mRNA level (Solter et al., 2000). Therefore, the decreasing trends of aminotransferase activities might be due to the alteration of basal gene expression and protein synthesis in phytoplanktivorous fish. In spite of slight ultrastructure lesion in carps, the alteration in these plasma enzyme activities indicated toxicogenomic effects on fish from the toxic blooms.

Cholestasis was probably involved in the adverse effects of cyanobacterial blooms on bighead carp. ALP and GGT are considered cholestatic-induction enzymes, while serum bilirubin is usually employed to detect cholestasis in addition to such cholestatic enzymes. Elevated ALP activity was recorded in tilapia fish subchronically exposed to microcystins (Molina et al., 2005) and in goldfish treated with crude cyanobacterial extracts (Zhang et al., 2007). In carps exposed to microcystins in lab and to *Microcystis* blooms in natural water body, increased bilirubin concentrations were prominent (Carbis et al., 1996, 1997). In present study, enhanced both enzymes activities and elevated bilirubin concentrations suggested that cholestasis occurred on bighead carp during the blooms. However, such parameters in silver carp remained stable over time, except for the lasting increase of direct bilirubin concentration.

Disorders on hepatic metabolism function were implied by decreases in blood concentrations of certain energy-related biomolecules. The liver in fish plays an important role in several vital functions of basic metabolism, and levels of blood GLU, CHOL, and TP are generally affected by the metabolism of carbohydrate, lipid, and protein, because of hepatic damage (Andenen et al., 1992). In present study, we recorded a decrease in CHOL concentration in silver carp and decreases in both TP and CHOL concentrations in bighead carp during the blooms, indicating a possible nutritional imbalance induced by the toxic blooms. These results are to some extent in accordance with earlier studies (Vajcová et al., 1998; Beasley et al., 2000), in which it is concluded that decreased TP implies a failure of protein synthesis because of liver injury caused by microcystins.

MC-induced kidney impairment was convinced by the increase of URIC content in both carps. Li et al. (2007a, 2008) revealed partial inosculation of foot processes in both silver carp and bighead carp, while in present study similar ultrastucture alterations were observed in these fishes. The inosculated foot processes probably postdisorders on the filtration of glomeruli. Correspondingly, plasma biochemical analyses revealed significantly elevated URIC content during the blooms. URIC is freely filtered by glomeruli. In case of kidney dysfunction, URIC precipitates and causes kidney stones or gout. Generalized urolith-like basophilic precipitate in the tubules in kidney was observed of tilapia exposed to MCs for 21 days (Molina et al., 2005). However, considerable recovery in both fishes presented after the blooms with significant decrease in the URIC content.

In conclusion, through monitoring changes of blood plasma indices, this study confirmed adverse effects of cyanobacterial blooms on two phytoplanktivorous fish, which mainly characterized with potential toxicogenomic effects and metabolism disorders in liver, and kidney dysfunction. In addition, cholestasis was intensively implied by distinct elevation of all four related biomarkers in bighead carp. The combination of LDH, AST activities and DBIL, URIC contents in silver carp, and the combination of ALT, ALP activities and TBIL, DBIL, URIC concentrations for bighead carps were found to most strongly indicate toxic effects from cyanobacterial blooms in such fishes. To some extent, recovery in both fishes was suggested by the decreased value of certain serum parameters. However, to get a better understanding of the mechanism on alterations of serum chemistry parameters, extensively studies are needed to verify the relationship between biochemical changes and histological lesions.

Conflict of interest statement

The authors declared that there were no conflicts of interest.

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