Effects of high ammonia concentrations on three cyprinid fish: Acute and whole-ecosystem chronic tests

Article *in* Science of The Total Environment · November 2017

DOI: 10.1016/j.scitotenv.2017.04.070

CITATIONS
2

READS
232

11 authors, including:

Haijun Wang
Chinese Academy of Sciences
35 PUBLICATIONS **380** CITATIONS

SEE PROFILE

Xucheng Xiao
Chinese Academy of Sciences
7 PUBLICATIONS **87** CITATIONS

SEE PROFILE

Qing Yu
Chinese Academy of Sciences
7 PUBLICATIONS **30** CITATIONS

SEE PROFILE

Xiao-Min Liang
Chinese Academy of Sciences
12 PUBLICATIONS **228** CITATIONS

SEE PROFILE

Science of the Total Environment 598 (2017) 900–909

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of high ammonia concentrations on three cyprinid fish: Acute and whole-ecosystem chronic tests

Hai-Jun Wang [a,*], Xu-Cheng Xiao [a,b,1], Hong-Zhu Wang [a], Yan Li [a,b], Qing Yu [a,b], Xiao-Min Liang [a], Wei-Song Feng [a], Jian-Chun Shao [b,c], Marcus Rybicki [d], Dirk Jungmann [d], Erik Jeppesen [e,f]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] National & Local Joint Engineering Laboratory for Ecological Mariculture, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[d] Institute of Hydrobiology, Technische Universität Dresden, 01062 Dresden, Germany
[e] Department of Bioscience, Aarhus University, Aarhus, Denmark
[f] Sino-Danish Centre for Education and Research, Beijing 100049, China

HIGHLIGHTS

- Fish might be more tolerant to high ammonia in natural than under lab conditions.
- Fish adaptation and environmental mediation may reduce the toxicity of ammonia.
- Planktivores and omnivores differ as to growth responses to ammonia exposure.
- Stimulated phytoplankton by ammonia results in increased growth of planktivores.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form 5 April 2017
Accepted 11 April 2017
Available online xxxx

Editor: Henner Hollert

Keywords:
Ammonia
Cyprinid fish
Acute test
Chronic test
Whole-ecosystem experiment

ABSTRACT

A number of studies have revealed ammonia to be toxic to aquatic organisms; however, little is known about its effects under natural conditions. To elucidate the role of ammonia, we conducted 96-h acute toxicity tests as well as a whole-ecosystem chronic toxicity test for one year in ten 600-m^2 ponds. Three common cyprinids, silver carp *Hypophthalmichthys molitrix* Val. (*H.m.*), bighead carp *Aristichthys nobilis* Richardson (*A.n.*), and gibel carp *Carassius auratus gibelio* Bloch (*C.g.*), were used as test organisms. The 96-h LC_{50} values of un-ionized ammonia (NH_3) for *H.m.*, *A.n.*, and *C.g.* were 0.35, 0.33, and 0.73 mg L^{-1}, respectively. In the ponds, annual mean NH_3 ranged between 0.01 and 0.54 mg L^{-1}, with 4 ponds having a NH_3 higher than the LC_{50} of *A.n.* (lowest LC_{50} in this study). No fish were found dead in the high-nitrogen ponds, but marked histological changes were found in livers and gills. Despite these changes, the specific growth rate of *H.m.* and *A.n.* increased significantly with NH_3. Our pond results suggest that fish might be more tolerant to high ammonia concentrations in natural aquatic ecosystems than under laboratory conditions. Our finding from field experiments thus suggests that the existing regulatory limits for reactive nitrogen (NH_3) established from lab toxicity tests might be somewhat too high at the ecosystem conditions. Field-scale chronic toxicity tests covering full life histories of fish and other aquatic

* Corresponding author.
E-mail addresses: wanghj@ihb.ac.cn (H.-J. Wang), 20030901009xiao@163.com (X.-C. Xiao), wanghz@ihb.ac.cn (H.-Z. Wang), liyan_891222@126.com (Y. Li), yuqzoe@163.com (Q. Yu), liangxm@ihb.ac.cn (X.-M. Liang), fengweisong@ihb.ac.cn (W.-S. Feng), jc_shao@webmail.hzau.edu.cn (J.-C. Shao), marcus.rybicki@tu-dresden.de (M. Rybicki), Dirk.Jungmann@tu-dresden.de (D. Jungmann), ej@bios.au.dk (E. Jeppesen).
[1] Co-first author.

http://dx.doi.org/10.1016/j.scitotenv.2017.04.070

organisms are therefore encouraged in order to optimize determination of the effects of ammonia in natural environments.

1. Introduction

The increased availability of reactive nitrogen in many ecosystems as a result of human activities has had negative impacts on human health, biodiversity, and air and water quality (Vitousek et al., 1997; Galloway et al., 2008; Finlay et al., 2013). In freshwater ecosystems, high levels of human-generated ammonia (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) may produce harmful effects on aquatic organisms (Constable et al., 2003; Camargo et al., 2005; Camargo and Álonso, 2006; Yu et al., 2015). Ammonia is believed to be most toxic in its un-ionized form (NH_3) and less so or non-toxic in its ionic form (NH_4^+) (Camargo and Álonso, 2006; USEPA, 2013). For example, NH_3 can reduce the feeding activity, fecundity, and survival of fish through various negative physiological effects by causing asphyxiation, reducing the oxygen-carrying capacity, and affecting the liver, kidneys, or immune system (Environment Canada, 2001; Constable et al., 2003; Camargo and Álonso, 2006).

Because of the known negative effects of ammonia on aquatic organisms, many acute and chronic laboratory studies have been conducted to determine the effect of ammonia on various aquatic organisms (USEPA, 2013). With the aim of protecting sensitive organisms, the USEPA (1999) recommended a criterion maximum concentration (CMC) (acute, 1-h average) of 24 mg L^{-1} TAN (total ammonia expressed as nitrogen) and a criterion continuous concentration (CCC) (chronic, 30-d rolling average) of 4.5 mg L^{-1} TAN (pH = 7.0, temperature = 20 °C). The CMC and CCC were updated to 17 mg L^{-1} and 1.9 mg L^{-1}, respectively, after including data on the most sensitive mussel glochidia (USEPA, 2013). These guidelines are based on effect studies on the growth, reproduction, and survival of the organisms assessed under laboratory conditions. However, little is known about the effects of long-term chronic exposure in natural aquatic ecosystems. Understanding the effects of ammonia on aquatic organisms in natural ecosystems requires knowledge of the environmental fate and transformation of ammonia in such systems. In water, the percentage of un-ionized ammonia (NH_3) increases with pH and water temperature (Emerson et al., 1975). Thus, the toxicity of total ammonia increases as pH or temperature increases (USEPA, 2013), and pH is increasing with phytoplankton production, which, in turn, may be stimulated by higher loading of ammonia (Moss et al., 2013). However, aquatic ecosystems possess mechanisms reducing accumulation of ammonia: (1) uptake by aquatic algae and macrophytes as their nitrogen source (Mulholland et al., 2000; Peterson et al., 2001; Dodds et al., 2002), (2) transfer to sediments by adsorption on particulates (Rosenfeld, 1979; Mackin and Aller, 1984; Peterson et al., 2001), (3) emission to the atmosphere in the form of N_2 via nitrification-denitrification (Admiraal and Botermans, 1989; Chesterikoff et al., 1992; Mulholland et al., 2000; Peterson et al., 2001), and (4) NH_3 volatilization at the air-water interface (Young and Huryn, 1999; Jha et al., 2001; Hall and Tank, 2003; Passell et al., 2007). Accordingly, the organisms in natural lake ecosystems are often exposed lower ammonia concentrations than would be expected from the loading. Moreover, the ammonia concentration may vary spatially within the ecosystems and animals, including fish, may actively avoid the 'hot' ammonia areas. Therefore, we hypothesize that at given levels of ammonia fish are more tolerant in natural aquatic ecosystems than under laboratory conditions.

To test the toxicity of ammonia under natural conditions, a one-year whole-ecosystem experiment was carried out in 10 ponds with an annual mean total ammonia (TA, including both NH_3 and NH_4^+) gradient ranging between approximately 0.3 and 20.6 mg L^{-1}. Three common cyprinids with different feeding habits were examined: bighead carp (*Aristichthys nobilis* Richardson), silver carp (*Hypophthalmichthys molitrix* Val.), and gibel carp (*Carassius auratus gibelio* Bloch). Bighead carp and silver carp are planktivores and gibel carp is a detritivore. In China, these three cyprinids commonly appear in shallow lakes and ponds along the mid-lower Yangtze Basin. Acute toxicity tests were also carried out for these three cyprinids. The purposes of our study were threefold: (1) to determine the 96-h LC_{50} (concentration lethal to 50% of the test organisms) of ammonia for the three cyprinids under laboratory conditions, (2) to test the chronic effects of ammonia on the growth and health of the three cyprinids when exposed to ammonia under natural conditions, (3) to explore the possible mechanisms mediating the toxicity of ammonia in natural ecosystems.

2. Materials and methods

2.1. 96-h acute toxicity test

Larval silver carp, 5 days post hatching (dph), bighead carp (5 dph), and juvenile gibel carp (1.96 ± 0.1 g) were obtained from a commercial fish farm in Daye, Hubei Province, China. Silver carp and bighead carp were cultured in 1.5-L beakers and gibel carp in 25-L fiberglass tanks. All containers were continuously aerated to ensure oxygen saturation. Twenty fish were placed in each container. In addition to the control, seven concentrations of ammonia were tested in triplicate for each fish. Based on a preliminary test, the concentrations of total ammonia (TA, $NH_3 + NH_4^+$) and un-ionized ammonia (NH_3) (in brackets) for silver carp were 0.70 (0.10), 1.53 (0.21), 2.22 (0.30), 3.18 (0.43), 4.06 (0.55), 4.77 (0.65), and 5.91 (0.80) mg L^{-1}. For bighead carp, the concentrations were 0.31 (0.08), 0.70 (0.18), 1.03 (0.27), 1.50 (0.39), 2.07 (0.54), 2.49 (0.64), and 3.04 (0.79) mg L^{-1}. For gibel carp, the concentrations were 20.94 (0.12), 30.15 (0.18), 40.28 (0.24), 60.7 (0.36), 91.02 (0.54), 169.99 (1.01), and 302.72 (1.81) mg L^{-1}.

The experimental procedure followed that described by the American Public Health Association (APHA, 1980). Solutions of toxicant were prepared from reagent-grade ammonium chloride (AR, Sinopharm Chemical Reagent Co., Ltd., Shanghai), and dilution water was obtained from 24-h aerated tap water. In accordance with the "static renewal methods" for toxicity tests, each test solution with live fish was renewed daily using a 50% volume replacement with freshly prepared test solution. Water temperature was maintained at 19.0 ± 0.1 °C, dissolved oxygen at 7.6 ± 0.2 mg L^{-1}, and pH at 8.63 ± 0.1 for silver carp; 23.0 ± 0.3 °C, 7.55 ± 0.05 mg L^{-1}, and 8.85 ± 0.05 for bighead carp; 19.9 ± 0.2 °C, 7.15 ± 0.15 mg L^{-1}, and 7.18 ± 0.02 for gibel carp. The photoperiod was 12 h light and 12 h dark. Gibel carp were acclimated to the experimental system for 2 weeks before the ammonia exposure. During this period, the fish were fed to satiation twice per day at 8:00 and 16:00. At the beginning of the trial, healthy juvenile gibel carp were collected and randomly allocated to the tanks. Silver carp and bighead carp were carefully counted and then randomly put into the test beakers. Exposure began within 30 min after the test solutions were prepared. No food was supplied to the fish during the exposure to ammonia. Mortality was determined 12, 24, 48, 72, and 96 h after exposure. Dead fish were removed from the containers daily and counted; death was presumed when fish were immobile and showed no response to touch with a glass rod.

2.2. Whole-ecosystem chronic test

The experimental system (N 30°17′17″, E 114°43′45″) is located to the northeast of Lake Bao'an (surface area 48 km^2, mean depth 1.9 m) on the south bank of the middle Yangtze River. The experiment was

conducted in 10 ponds (approximately 600 m^2 each) (Fig. 1), constructed in a lotus pond divided into equal sections by embankments after initial dredging of surface sediments rich in nutrients and organic matter. Lake water and a ca. 10 cm sediment layer were introduced into each pond from the lake in winter 2010 to create a natural lake system. Lake Bao'an is a natural lake with functions of fisheries, flood control, irrigation, drinking water supply, and scenic views. According to lake surveys from 2011 to 2012, annual mean Secchi depth (Z_{SD}) was 0.6 m, pH 8.6, dissolved oxygen (DO) 8.9 mg L^{-1}, conductivity (Cond) 474.8 μS cm^{-1}, total nitrogen (TN) 1.41 mg L^{-1}, total phosphorus (TP) 0.09 mg L^{-1}, and chlorophyll *a* (Chl *a*) 50.36 μg L^{-1}. The macrophyte community was highly dominated by curly-leaf pondweed *Potamogeton crispus*, with a coverage of around 80% in the fast-growing season of May.

Juvenile gibel carp were released into the ponds on May 15, 2011, approximately six months after the system was constructed. Juvenile silver carp and bighead carp were introduced on July 15, the difference owing to their naturally different hatching times. All fish were obtained from the same commercial fish farm as those used in the acute toxicity tests. Fifty silver carp (body weight: 0.6 ± 0.02 g; body length: 0.8 ± 0.06 cm, mean ± S.E.), 20 bighead carp (body weight: 260 ± 5 g; body length: 26.7 ± 1.2 cm), and 100 gibel carp (body weight: 0.8 ± 0.07 g; body length: 1.2 ± 0.07 cm) were randomly selected and transferred to each pond. Regular inspection was performed, at least twice per month throughout the study, to record dead fish floating on the water surface. Recapture of fish was carried out with a trawl net (15 mm in mesh size) on July 7, 2012, after approximately one year of exposure. All captured fish were counted, measured for body length with a measuring board, and weighed with an electronic balance (0.01 g, BL-2200H, Shimadzu Corporation, Japan). Three representative individuals of the median size of each species were selected from each pond and chilled for sampling of hepatopancreas and gills. The histological samples were fixed by immersion in Bouin's fluid for 12 h and dehydrated in a graded ethanol series. The hepatopancreas and gill slices were then embedded in paraffin, cut into 4-μm sections on a rotary microtome, stained with hematoxylin and eosin (H and E), and analyzed using an optical microscope.

Four target concentrations of total nitrogen (TN) were maintained in addition to the control: 2 mg L^{-1} (coded as TN2), 10 mg L^{-1} (TN10), 20 mg L^{-1} (TN20), and 100 mg L^{-1} (TN100). NH_4Cl fertilizer (NH_4Cl, ≥ 99.5%, Sinopharm Chemical Reagent Co., Ltd., Shanghai) was added as a nitrogen source to maintain the target concentrations. Fertilizer was added twice per month after the chemical sampling, and the added amount was calculated relative to the difference between the measured and the target concentrations. The fertilizer was dissolved in pond water in a polyethylene bucket before being poured evenly into the ponds. No phosphate fertilizer was added to the ponds throughout the experiment.

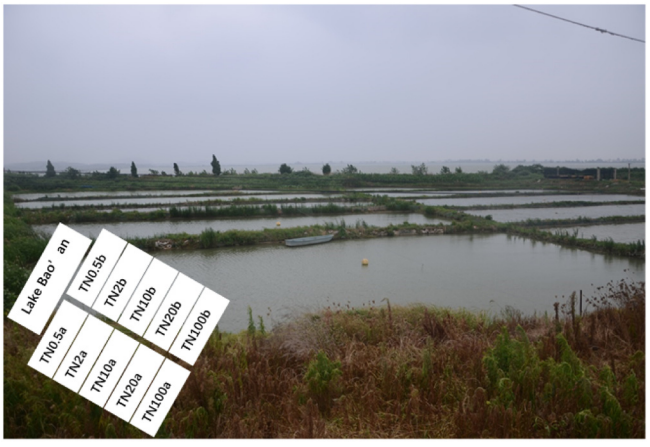

Fig. 1. Photo of the ponds where the whole-ecosystem chronic test was carried out; text indicating treatments and locations. In the code of treatment, the digit after TN indicates the target concentration of TN, 0.5 represents the control treatment, and a and b are discriminate replicates. The photo was taken by Haijun Wang on June 30, 2015.

Environmental investigations began on August 13, 2011. The period between fish release and environmental investigations allowed the fish to acclimate. During the acclimation period, no dead fish were found. Investigations were carried out biweekly, beginning at 09:00 AM. DO, pH, and water temperature (Temp) were measured at the same time using a YSI Pro 1020 multi-parameter meter. Water samples for chemical analysis were collected at 5 randomly chosen locations within each pond by integrating the water column with a tube sampler (1.5 m in height, 10 cm in diameter). TN was determined with an alkaline potassium persulfate digestion-UV spectrophotometric method (PERSEE, TU-1810, Beijing), TA with a Nessler's reagent colorimetric method (ISO 5664, 1984; ISO 7150–1, 1984), NO_3^- with the spectrophotometric method using phenol disulfonic acid (ISO 7890–3, 1988), NO_2^- with the spectrophotometric method (ISO 6777, 1984), and TP with an ammonium molybdate-ultraviolet spectrophotometric method after digestion with $K_2S_2O_8$ solution (Murphy and Riley, 1962; Golterman et al., 1978). Chl *a* was extracted using 90% acetone (at 4 °C for 24 h) after filtration through GF/C filters (Whatman, GE Healthcare UK Limited, Buckinghamshire, UK), and absorbance was then read at 665 nm and 750 nm both before and after acidification with 10% HCl using a spectrophotometer (Lorenzen, 1967).

2.3. Data processing and analysis

The 96-h LC_{50} for NH_3 was calculated using a probit analysis (Finney, 1971). The results are expressed as means ± S. E. Microsoft Excel 2010 and STATISTICA 8.0 were used to process and analyze the data. Specific growth rate (SGR, % day^{-1}) was calculated for each species (Wootton, 1990):

$$SGR_{BW} = 100 \times [\ \ln BW_t - \ln BW_0]/days \quad (1)$$

$$SGR_{BL} = 100 \times [\ \ln BL_t - \ln BL_0]/days \quad (2)$$

where BW_0 and BW_t are the initial and final body weight (fresh weight, g) of fish, and BL_0 and BL_t are the initial and final body length (cm) of fish, respectively.

NH_3 was calculated following the equation from Wood (1993) and Emerson et al. (1975):

$$NH_3 = TA\text{–}TA/\left[1 + 10^{(pH - pKa)}\right] \quad (3)$$

where $pKa = 0.09018 + (2729.92 / (273.2 + Temp))$, TA is the measured concentration of total ammonia (including both NH_3 and NH_4^+), pH is the measured pH of the solution, and Temp is the temperature in °C.

Normalization of the acute value of toxicity test (AV_{TA}, i.e. LC_{50}) to the AV for TAN (total ammonia expressed as nitrogen) at pH 7 ($AV_{t,7}$) followed the method of USEPA (2013) where the AV for TA is firstly converted to the nitrogen equivalents (AV_t) and then normalized to pH 7 ($AV_{t,7}$):

$$AV_t = AV_{TA} \times (14/17) \quad (4)$$

$$AV_{t,7} = AV_t/[0.0114/\left(1 + 10^{(7.204 - pH)}\right) + (1.6181/\left(1 + 10^{(pH - 7.204)}\right)] \quad (5)$$

where AV_{TA} is the acute value of the toxicity test for total ammonia and pH is the test pH.

The same procedure was applied to normalize the TA values of the chronic test to standard conditions (TAN_{pH7}).

3. Results

3.1. Acute toxicity test

No fish were found dead in the control test. The LC_{50} values of NH_3 at 96 h for silver carp larvae, bighead carp larvae, and gibel carp juveniles were calculated to be 0.35 mg L^{-1}, 0.33 mg L^{-1}, and 0.73 mg L^{-1}, respectively (Fig. 2). The corresponding 96-h LC_{50} values of TAN_{pH7} for silver carp, bighead carp, and gibel carp were 30.8 mg L^{-1}, 22.3 mg L^{-1}, and 120.3 mg L^{-1}, respectively.

3.2. Whole-ecosystem toxicity test

3.2.1. Environmental factors

In the ponds, TN exhibited a significant treatment gradient, with means ranging from 0.8 to 41.2 mg L^{-1} (Table 1). The measured TA followed a similar pattern of variation as TN ($R^2 = 0.87$, $p < 0.001$) and constituted a high proportion of the TN (TA%), on average 41.6%, ranging from 34.6% to 48.8%. The calculated NH_3 ranged from 0.01 to 0.54 mg L^{-1}. Relatively low variations among treatments were found for pH (7.84–8.33) and water temperature (17.93–18.14 °C). During the year, the nitrogen variables (TA, NH_3) showed pronounced variations between months in the high N treatment group (TN10, TN20, and TN100) (Fig. 3A, B). Generally, TA began to increase after the start of the experiment, peaked in the cold season, and then decreased to relatively low levels. NH_3 concentrations remained at a high level after the peak because of the increasing temperature (Fig. 3C), a negative factor in calculating the NH_3 fraction of TA. The amounts of fertilizer added during the whole experimental period are shown in Fig. 3D.

3.2.2. Histological examination

Histological examination showed similar changes in livers among the three cyprinids with increasing NH_3 concentrations after one year of exposure. In the control and TN2 treatment groups, hepatocytes had normal shapes with regular gross morphology and clearly located cell nuclei (Fig. 4A, B, F, G, K, L). In the TN10 and TN20 treatment groups, hepatocytes were enlarged, with a swollen appearance and fatty vacuolation (Fig. 4C, D, H, I, M, N). In the TN100 treatment group, liver lesions consisted of irregularly shaped hepatocytes with cloudy swelling and hydropic degeneration (Fig. 4E, J, O). A similar pattern of changes was found in gills. In the control and TN2 treatment groups, the gills had normal shapes with straight filaments and secondary lamellae (Fig. 5A, B, F, G, K). The gibel carp in the TN2 treatment group was an exception (Fig. 5L), with a modest thickening of the basal epithelium of the gills. In the TN10 and TN20 treatment groups, all three species showed modest thickening of the basal epithelium with telangiectasia and chloride cell hyperplasia (Fig. 5C, D, H, I, M, N). In the TN100 treatment group, the secondary lamellae were severely disrupted, with severe edema underlying the lamellar epithelium and modest epithelial thickening (Fig. 5E, J, O).

3.2.3. Survival and growth of fish

During the experiment, only two gibel carp were found dead (TN10b, on November 11, 2011). The recapture rates (RRs) of fish were relatively low as established experimental facilities in the ponds prevented effective trawling. The RR of silver carp ranged between 24% (12/50) and 40% (20/50) (Table 2). The RR of bighead carp ranged between 12% (6/20) and 100% (20/20) and that of gibel carp between 3% (3/100) and 20% (20/100). All three species grew well in all the treatments as judged from the body weight (BW) and body length (BL) measured at the end of the experiment (Table 2). Compared with their initial sizes, the BW of silver carp, bighead carp, and gibel carp increased 29.7–1840, 1.0–9.8, and 40.8–230.5 times, respectively, and the BL increased 15.1–59.6, 1.0–2.3, and 9.8–17 times. The BW-derived SGR of silver carp, bighead carp, and gibel carp ranged between 0.95 and 2.11, 0.01–0.64, and 1.04–1.53, respectively, and their BL-derived SGR ranged between 0.76 and 1.15, 0.01–0.23, and 0.64–0.80, respectively.

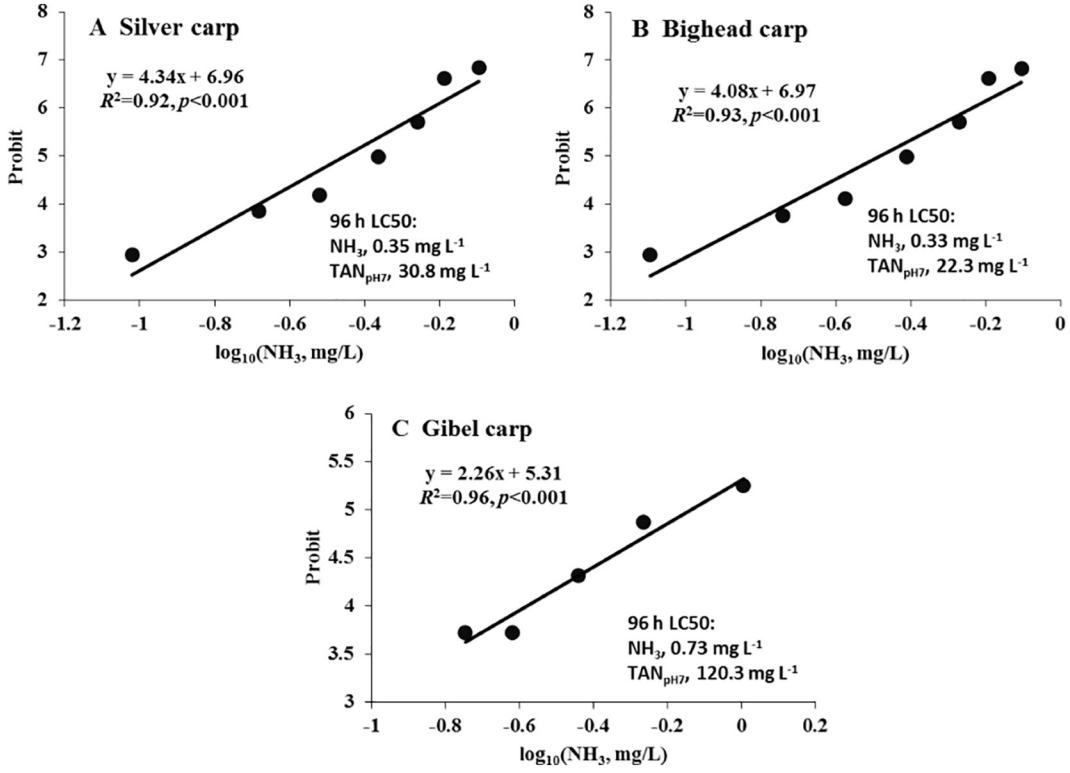

Fig. 2. Probit analysis of ammonia concentrations with mortalities of the three fish for the 96-h acute toxicity test (TAN_{pH7}, total ammonia expressed as nitrogen adjusted at pH 7 following the method of the USEPA (2013); NH_3, un-ionized ammonia).

Table 1
Annual mean ± S.E. (ranges) environmental values in the 10 experimental ponds during August 2011–July 2012.

Treatments	TN (mg L^{-1})	TA	TAN_{pH7}	NH_3	NO_3^-	NO_2^-	TP	pH	Temp. (°C)	Chl *a* (μg L^{-1})
TN 0.5a	0.80 ± 0.12 (0.25–2.26)	0.28 ± 0.06 (0.04–1.39)	2.61 ± 0.23 (0.06–10.74)	0.010 ± 0.000 (0.000–0.001)	0.14 ± 0.05 (0.01–0.92)	0.014 ± 0.007 (0.005–0.145)	0.054 ± 0.004 (0.016–0.095)	8.17 ± 0.03 (7.92–8.33)	18.11 ± 1.98 (4.9–34.3)	9.71 ± 0.92 (2.27–15.92)
TN 0.5b	1.03 ± 0.23 (0.27–2.33)	0.40 ± 0.13 (0.01–3.42)	2.68 ± 0.35 (0.06–11.05)	0.012 ± 0.000 (0.006–0.015)	0.10 ± 0.03 (0.01–0.47)	0.013 ± 0.006 (0.005–0.121)	0.043 ± 0.006 (0.008–0.162)	8.10 ± 0.03 (7.86–8.32)	18.07 ± 1.90 (5.3–34.3)	7.81 ± 0.95 (1.82–15.92)
TN 2a	1.14 ± 0.16 (0.28–3.55)	0.45 ± 0.12 (0.01–2.35)	6.55 ± 0.67 (0.36–68.33)	0.023 ± 0.003 (0.008–0.029)	0.30 ± 0.08 (0.01–1.36)	0.016 ± 0.003 (0.005–0.067)	0.052 ± 0.009 (0.005–0.232)	8.33 ± 0.05 (8.14–8.73)	18.05 ± 1.93 (5.0–34.3)	11.52 ± 1.84 (0.91–30.9)
TN 2b	1.31 ± 0.18 (0.32–3.56)	0.51 ± 0.12 (0.02–2.45)	3.86 ± 0.58 (0.19–19.60)	0.022 ± 0.008 (0.008–0.032)	0.39 ± 0.08 (0.01–1.31)	0.033 ± 0.017 (0.33–0.005)	0.043 ± 0.006 (0.008–0.122)	8.20 ± 0.04 (8.02–8.45)	18.14 ± 1.94 (5.1–34.3)	13.66 ± 2.08 (2.73–46.86)
TN 10a	3.93 ± 0.50 (0.31–8.85)	1.90 ± 0.45 (0.01–9.11)	19.66 ± 3.68 (1.12–146.44)	0.176 ± 0.070 (0.067–0.299)	0.82 ± 0.19 (0.01–2.69)	0.043 ± 0.007 (0.005–0.160)	0.067 ± 0.011 (0.008–0.122)	8.31 ± 0.08 (8.00–8.84)	18.03 ± 1.94 (4.7–34.3)	30.90 ± 6.51 (2.27–101.91)
TN 10b	7.80 ± 1.86 (0.26–23.76)	2.70 ± 0.68 (0.08–12.77)	16.92 ± 4.02 (0.59–85.81)	0.166 ± 0.076 (0.099–0.201)	0.95 ± 0.16 (0.03–2.74)	0.087 ± 0.055 (0.015–1.081)	0.037 ± 0.005 (0.008–0.274)	8.17 ± 0.03 (8.07–8.37)	18.12 ± 1.92 (5.2–34.3)	15.69 ± 2.54 (2.73–53.91)
TN 20a	12.08 ± 1.86 (0.36–36.44)	5.62 ± 1.44 (0.12–32.71)	51.32 ± 7.55 (0.64–564.69)	0.374 ± 0.064 (0.125–0.413)	0.89 ± 0.18 (0.01–2.54)	0.173 ± 0.080 (0.012–1.491)	0.071 ± 0.009 (0.004–0.112)	8.21 ± 0.08 (7.72–8.73)	18.00 ± 1.94 (4.9–34.3)	32.42 ± 4.78 (2.73–90.08)
TN 20b	17.51 ± 2.95 (0.41–49.13)	7.30 ± 1.44 (0.11–29.16)	38.55 ± 6.87 (0.51–157.33)	0.379 ± 0.038 (0.166–0.479)	1.36 ± 0.28 (0.01–3.59)	0.164 ± 0.077 (0.019–1.467)	0.052 ± 0.008 (0.008–0.178)	8.14 ± 0.02 (8.02–8.25)	17.99 ± 1.93 (5.0–34.3)	20.10 ± 3.26 (2.27–70.29)
TN 100a	41.16 ± 7.04 (0.53–135.44)	20.08 ± 3.95 (0.13–63.86)	113.76 ± 14.61 (0.63–570.15)	0.541 ± 0.056 (0.383–0.627)	2.12 ± 0.51 (0.01–8.57)	0.262 ± 0.049 (0.070–0.833)	0.104 ± 0.013 (0.008–0.286)	7.84 ± 0.07 (7.35–8.19)	17.93 ± 1.92 (4.8–34.3)	37.72 ± 8.33 (6.37–154.4)
TN 100b	35.47 ± 6.87 (0.49–139.51)	15.71 ± 3.27 (0.18–54.35)	55.87 ± 11.25 (0.81–241.58)	0.528 ± 0.055 (0.466–0.679)	1.98 ± 0.46 (0.01–7.60)	0.306 ± 0.070 (0.075–1.289)	0.124 ± 0.016 (0.020–0.338)	7.90 ± 0.08 (7.59–8.58)	17.96 ± 1.91 (5.0–34.3)	36.63 ± 5.03 (7.28–91.45)

TN, total nitrogen; TA, total ammonia; TAN_{pH7}, total ammonia expressed as nitrogen adjusted at pH 7 following USEPA (2013); NH_3, un-ionized ammonia; NO_3^-, nitrate; NO_2^-, nitrite; TP, total phosphorus; Temp, temperature; Chl *a*, phytoplankton chlorophyll *a*. In the code of treatment, the digit after TN indicates the target concentration of TN, 0.5 represents the control treatment, a and b discriminate between the replicates for each treatment.

3.2.4. Relations of survival and growth with the environments

In the ponds, the correlations between NH_3 and RR of the three species were all weak (silver carp, $r = -0.50$, $p = 0.14$; bighead carp, $r = -0.17$, $p = 0.62$; gibel carp, $r = 0.09$, $p = 0.81$). The correlations between NH_3 and SGR_{BW} for silver carp ($R^2 = 0.66$, $p = 0.004$) and bighead carp ($R^2 = 0.81$, $p = 0.0004$) were positive and highly significant (Fig. 6A). This was not the case for gibel carp, however ($R^2 = 0.00$, $p = 0.88$). The results for SGR_{BL} were similar (Fig. 6B). The correlations of NH_3 with SGR_{BL} were highly positive for silver carp ($R^2 = 0.59$, $p = 0.009$) and bighead carp ($R^2 = 0.84$, $p = 0.0002$) but weak for gibel carp ($R^2 = 0.01$, $p = 0.76$).

Similar results were found when analyzing the regressions between phytoplankton chlorophyll *a* (Chl *a*) and the growth variables of the three fish. Both SGR_{BW} and SGR_{BL} for silver carp ($R^2 = 0.55$, $p = 0.01$;

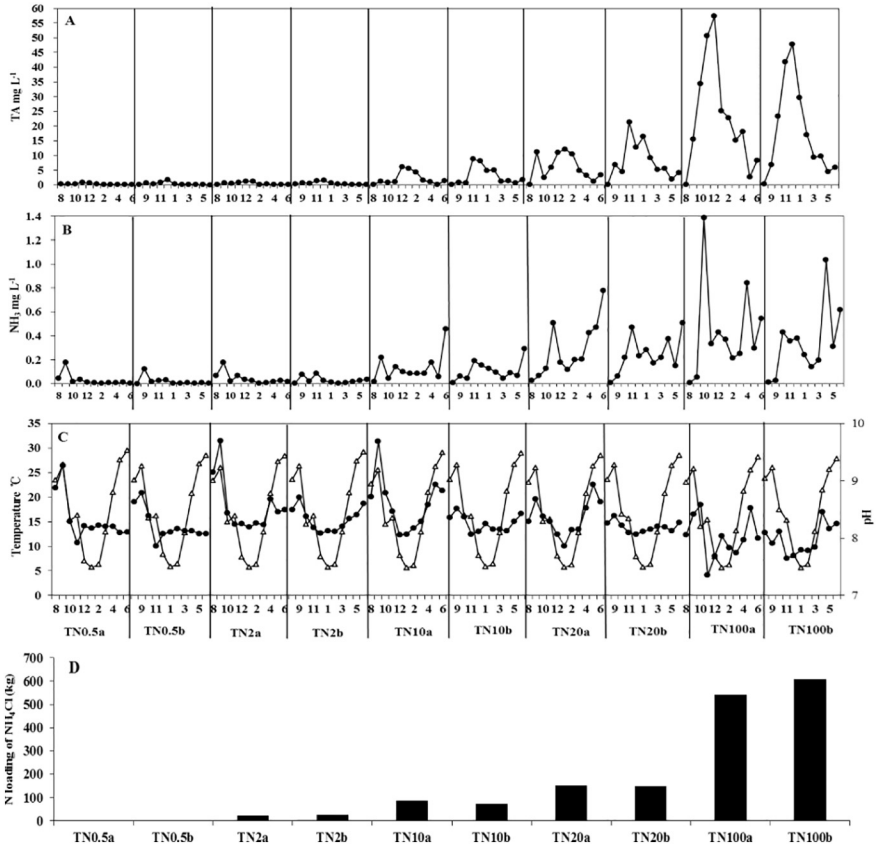

Fig. 3. Monthly dynamics of the measured concentrations of total ammonia (TA) (A), NH_3 (B), temperature and pH (C), and total N loading for the entire experimental period (D) from August 2011 to June 2012 (In the code of treatment, the digit after TN indicates the target concentration of TN, 0.5 represents the treatment of control, and a and b discriminate replicates).

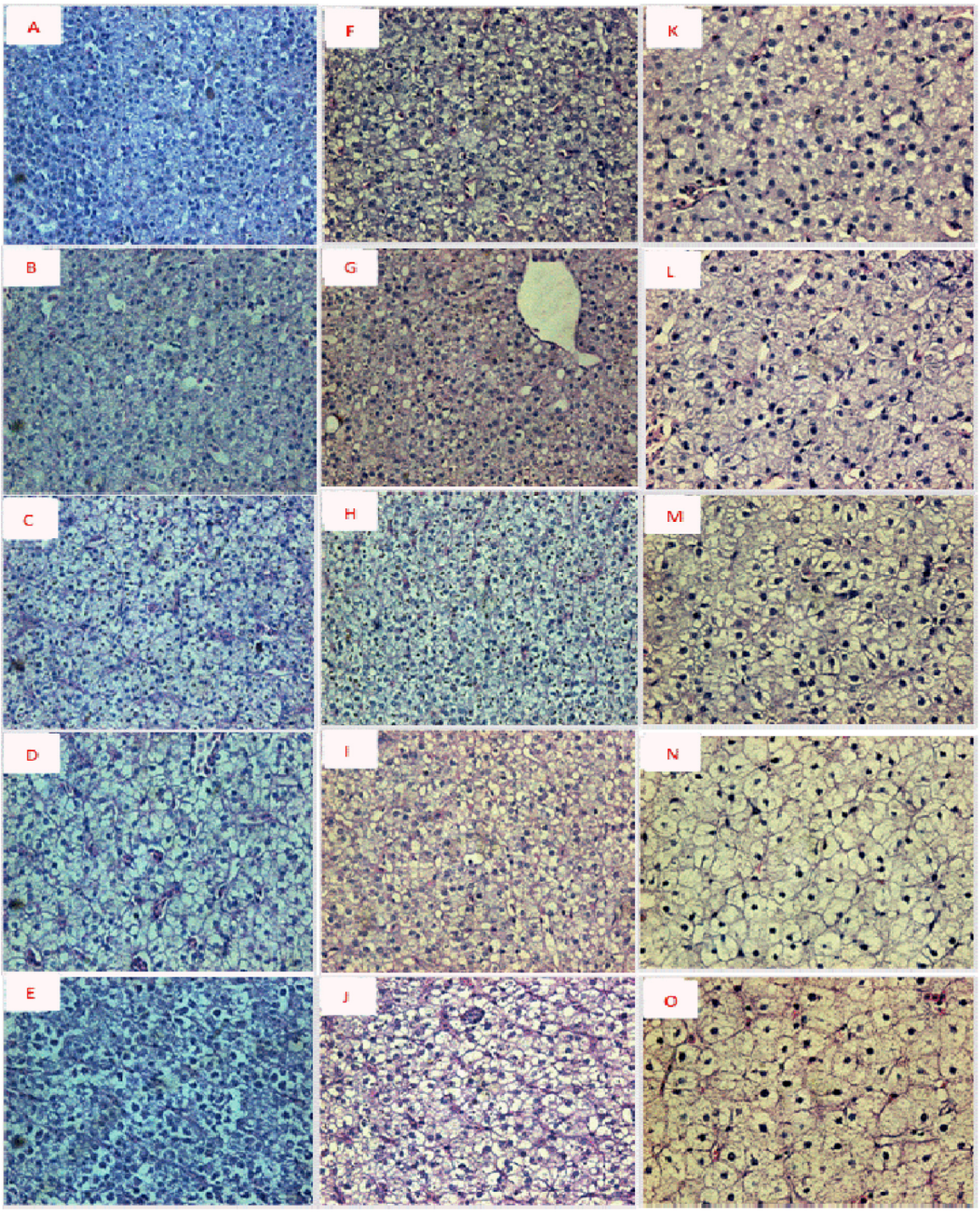

Fig. 4. Livers (400×) of silver carp (A–E), bighead carp (F–J), and gibel carp (K–O) exposed to target concentrations of control (A, F, K), 2 mg L^{-1} (B, G, L), 10 mg L^{-1} (C, H, M), 20 mg L^{-1} (D, I, N), and 100 mg L^{-1} (E, J, O).

$R^2 = 0.50, p = 0.02$) and bighead carp ($R^2 = 0.55, p = 0.01$; $R^2 = 0.63, p = 0.006$) were highly positively correlated with Chl *a*, while those for gibel carp ($R^2 = 0.00, p = 0.97$; $R^2 = 0.02, p = 0.67$) were weakly related to Chl *a* (Fig. 6C and D).

4. Discussion

4.1. Toxicity of ammonia to fish in natural environments

Despite the observed marked histological changes in livers and gills of the fish studied, the low observed dead rate and high growth rate at the highest NH_3 concentrations tend to support our hypothesis that at given levels of ammonia fish are more tolerant in natural aquatic ecosystems than under laboratory conditions. Our findings differ from previous findings revealing that the effect value of a specific toxicant is typically much higher in acute tests than in chronic tests. For example, in a compilation by the USEPA (2013) of species mean acute values (SMAVs) and species mean chronic values (SMCVs) for ammonia criteria, SMAV values are 6.4–31.9 times higher than those of SMCV for the 7 fish for which data are available for both types of tests. It should be noted, though, that due to the large size of our experimental ponds unnoticed fish deaths may have occurred. However, toxic nitrogen

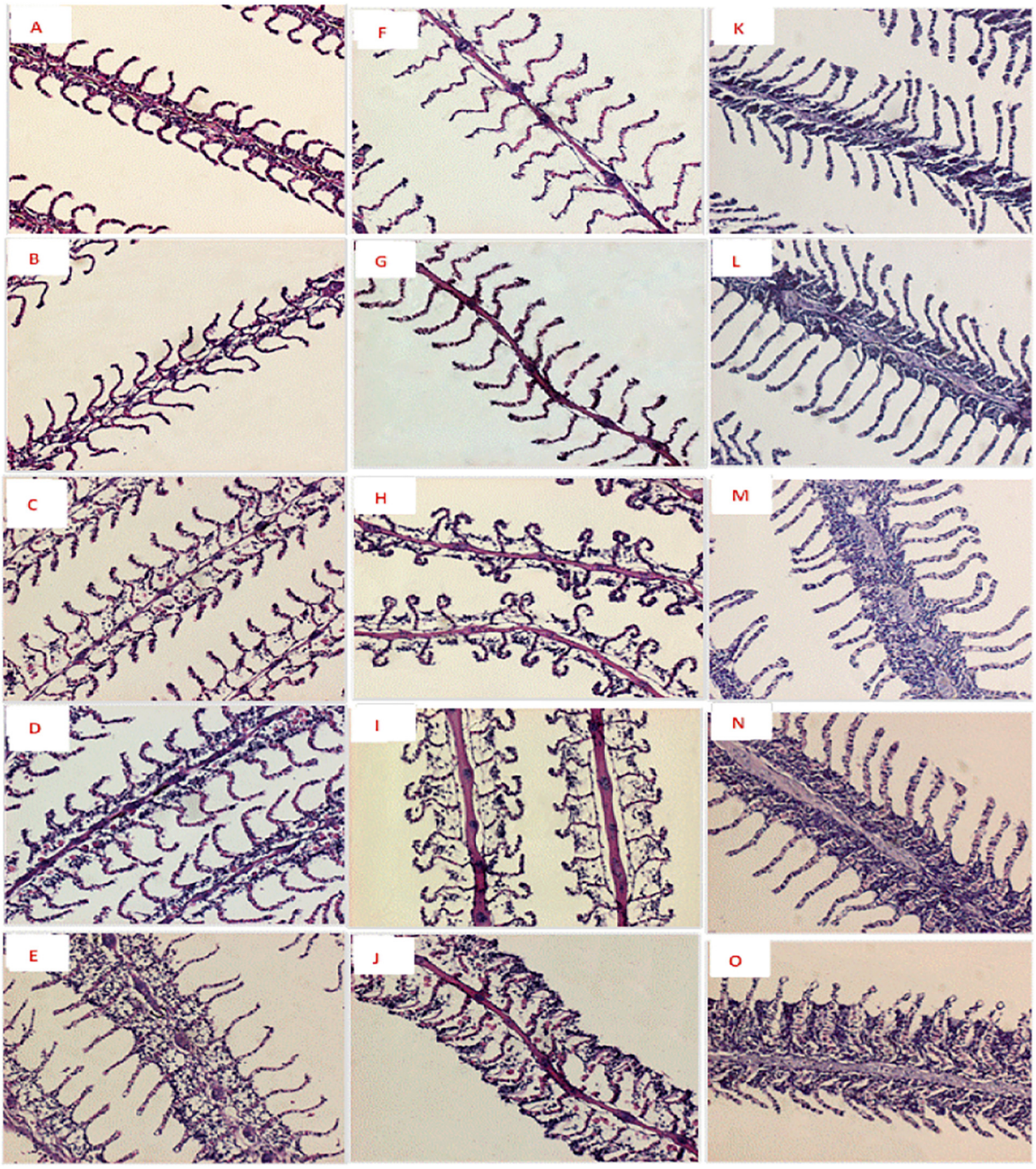

Fig. 5. Gills (400×) of silver carp (A–E), bighead carp (F–J), and gibel carp (K–O) exposed to target concentrations of control (A, F, K), 2 mg L^{-1} (B, G, L), 10 mg L^{-1} (C, H, M), 20 mg L^{-1} (D, I, N), and 100 mg L^{-1} (E, J, O).

stress should not be the main cause for the unnoticed fish deaths (if occurred), as suggested by the weak correlation between NH_3 and recapture rates.

The different findings than in other studies may be attributed to different rearing conditions. In previous studies, chronic tests have primarily been conducted in pure water, while the present study was conducted in ponds where the toxicity of the added ammonium may be modulated by various ecosystem processes. The added ammonia may in part be lost by N_2 emission through nitrification-denitrification (Admiraal and Botermans, 1989; Mulholland et al., 2000; Peterson et al., 2001) and NH_3 volatilization (Young and Huryn, 1999; Jha et al., 2001; Passell et al., 2007). Ammonia may also be taken up by phytoplankton and later, in part, transferred to the sediment when phytoplankton dies or sinks. Considering the observed changes in phytoplankton Chl *a* in the ponds, this uptake did increase with increasing treatment doses as revealed by the highly significant correlation between Chl *a* and the amount of NH_4Cl fertilizer added ($R^2 = 0.69$, $p = 0.003$). However, even when considering these loss factors the observed ammonia concentrations in the ponds were so high that they should have led to negative effects on fish growth and survival when compared with results from the various lab tests. Food availability may also partly explain the differences between ponds recorded in the present study. In lab experiments, fish are often exposed to ammonia without feeding. However, feeding has been proved to be important in the protection of fish against ammonia

Table 2
Body length (BL, cm), body weight (BW, g) and their derived specific growth rates (SGR) for the three fish captured from the ponds at the end of the experiment (mean ± S.E.).

		Silver carp			Bighead carp			Gibel carp		
		BL (0.8 ± 0.06)[a]	BW (0.6 ± 0.02)	*n*[b]	BL (26.7 ± 1.2)	BW (260 ± 5)	*n*	BL (1.2 ± 0.07)	BW (0.8 ± 0.07)	*n*
TN 0.5a	absolute	18.2 ± 0.8	56.2 ± 10.8	20	27.6 ± 1.4	268.9 ± 37.0	20	13.9 ± 0.4	44.1 ± 4.2	19
	SGR	0.88 ± 0.04	1.28 ± 0.07		0.01	0.01		0.69 ± 0.04	1.13 ± 0.02	
TN 0.5b	absolute	14.4 ± 0.4	26.8 ± 1.4	20	26.8 ± 3.7	299.6 ± 136.3	7	17.4 ± 0.5	93.3 ± 5.0	8
	SGR	0.81 ± 0.03	1.07 ± 0.03		0.00	0.04		0.75 ± 0.07	1.34 ± 0.02	
TN 2a	absolute	12.1 ± 0.7	17.8 ± 3.0	20	31.2 ± 0.3	303.2 ± 10.4	16	19.7 ± 1.7	124.5 ± 32.0	3
	SGR	0.76 ± 0.05	0.95 ± 0.02		0.04	0.04		0.79 ± 0.06	1.42 ± 0.03	
TN 2b	absolute	30.2 ± 0.8	292.4 ± 21.0	20	33.7 ± 0.8	471.2 ± 31.3	20	20.4 ± 0.5	143.7 ± 14.1	6
	SGR	1.02 ± 0.03	1.74 ± 0.11		0.07	0.17 ± 0.02		0.80 ± 0.07	1.46 ± 0.02	
TN 10a	absolute	19.7 ± 0.6	81.4 ± 7.9	20	30.3 ± 0.4	279.3 ± 7.9	6	18.4 ± 0.4	97.8 ± 5.2	5
	SGR	0.90 ± 0.05	1.38 ± 0.09		0.04	0.02		0.77 ± 0.05	1.35 ± 0.01	
TN 10b	absolute	23.6 ± 0.5	154.8 ± 9.7	12	34.3 ± 0.6	509.9 ± 23.1	20	19.4 ± 0.6	126.1 ± 12.2	9
	SGR	0.95 ± 0.04	1.56 ± 0.13		0.07	0.19 ± 0.01		0.78 ± 0.08	1.42 ± 0.04	
TN 20a	absolute	21.2 ± 1.4	160.1 ± 10.1	15	42.7 ± 1.9	959.7 ± 99.2	11	11.8 ± 0.9	32.6 ± 10.0	20
	SGR	0.92 ± 0.07	1.57 ± 0.10		0.13 ± 0.01	0.37 ± 0.02		0.64 ± 0.04	1.04 ± 0.02	
TN 20b	absolute	25.2 ± 0.8	201.3 ± 18.2	17	41.0 ± 1.4	790.3 ± 47.5	7	18.1 ± 0.7	92.5 ± 10.0	8
	SGR	0.97 ± 0.05	1.63 ± 0.90		0.12 ± 0.01	0.31 ± 0.02		0.76 ± 0.07	1.33 ± 0.03	
TN 100a	absolute	43.4 ± 1.4	950.4 ± 97.6	12	45.8 ± 0.6	1114.2 ± 37.9	15	17.3 ± 1.8	97.2 ± 27.8	9
	SGR	1.12 ± 0.06	2.07 ± 0.12		0.15 ± 0.02	0.41 ± 0.04		0.75 ± 0.05	1.35 ± 0.04	
TN 100b	absolute	47.7 ± 1.0	1104.0 ± 72.8	20	60.4 ± 0.7	2556.6 ± 92.6	16	19.5 ± 3.2	184.4 ± 68.9	7
	SGR	1.15 ± 0.04	2.11 ± 0.14		0.23 ± 0.02	0.64 ± 0.03		0.78 ± 0.06	1.53 ± 0.04	

[a] Initial values of BL and BW.
[b] *n* represents the number of captured fish at the end of the experiment.

toxicity (Zimmer et al., 2010) as it enables the fish maintain normal ammonia excretion when exposed to high ammonia concentrations. With feeding, low levels of ammonia may even stimulate fish growth (Morgan et al., 2001; Wood, 2004).

Water pH and temperature affect the toxicity of ammonia by regulating the relative concentrations of NH_4^+ and NH_3 (USEPA, 2013) and these vary on a diurnal basis in lakes and ponds. We did not measure diurnal variations in pH and temperature but there are good reasons to believe that diurnal variation in these variables were not of key importance for the lower toxicity in the ponds. First, pH and temperature were measured at 09:00 AM, i.e. at an intermediate level in the diurnal cycle. The levels of both are higher in the late afternoon, pH due to the daytime photosynthesis, and toxicity (un-ionized ammonia) is expectedly also higher in the afternoon than in the morning when our measurements were

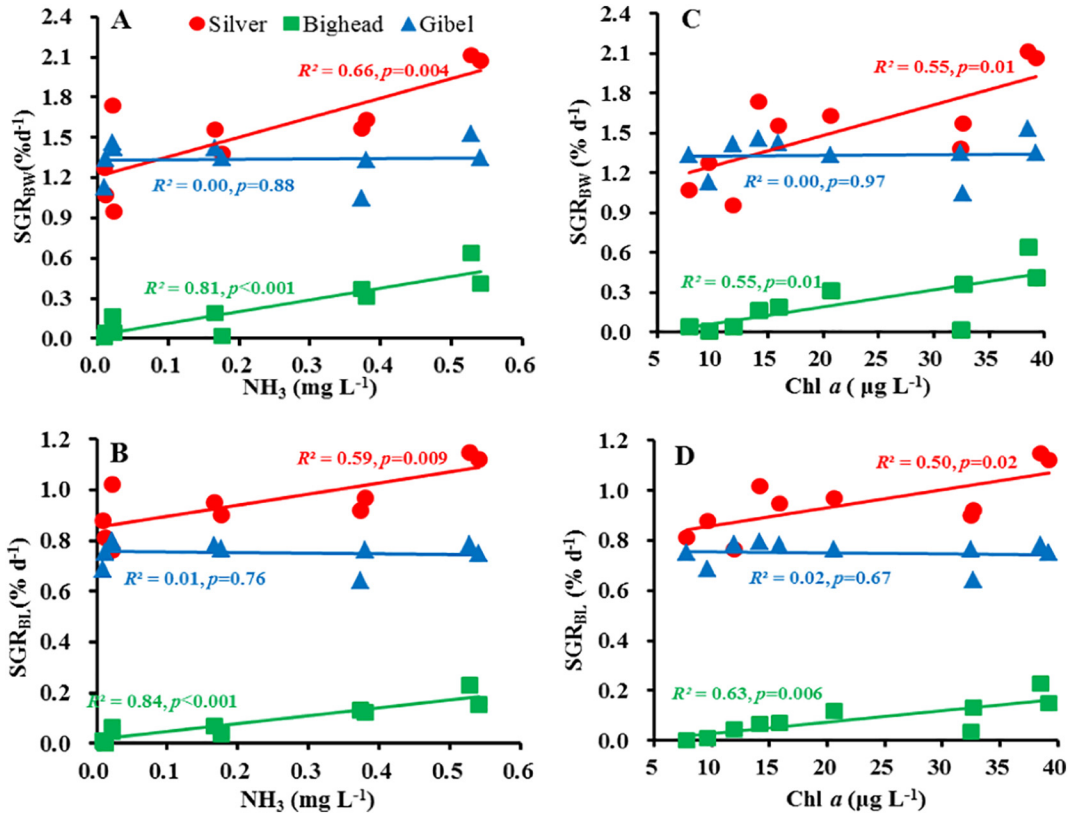

Fig. 6. Relationships between annual mean un-ionized ammonia (NH_3) and phytoplankton chlorophyll *a* (Chl *a*) with specific growth rates derived from body weight (SGR_{BW}) and body length (SGR_{BL}) for the three cyprinids (silver carp, bighead carp, and gibel carp) after one-year exposure in the ponds ($n = 10$).

performed. Hence, the determined values for ammonia underestimate the real maximum concentrations in the ponds, which most probably occurred in the afternoon.

Tolerance of fish to ammonia toxicity may change with size and be higher for large organisms (Hasan and Macintosh, 1986; Zhou et al., 1986; Zheng et al., 1998). In our study, the bighead carp individuals were much larger in the pond experiments (mean 260 g) than in the acute toxicity test (5 dph), while silver carp were similar for the two systems (0.6 g vs. 5 dph); gibel carp were smaller in the pond experiments (mean 0.8 g) than in the acute toxicity test (1.96 g). The observed similar recapture rates irrespective of N loading and high growth rate of the three fish species in the ponds, including the small-sized gibel carp, suggest that differences in size between typical lab experiments and our field experiment were not of key importance for the observed higher tolerance to ammonia toxicity under natural ecosystem conditions.

Although the observed mortality presumably was low and growth was high, the histological endpoints in liver and gills clearly showed a response to the ammonia concentrations. These changes in liver and gills could be a protective and adaptive consequence, or a damage of high ammonia exposure. Our results therefore suggest that the histological changes were rather a defense to external stress. Similar results have been obtained in ammonia exposure experiments using other fish species. For example, augmentation of fat storage in liver was suggested as a survival strategy of Nile tilapia (*Oreochromis niloticus* L.) at elevated ammonia levels (Benli et al., 2008). Gill re-modelling has been widely suggested as an adaptive strategy to cope with stressful environments. An exposure test of goldfish, *Carassius auratus* (highly ammonia-resistant cyprinid), suggested that the posterior sides of the lamellae may play a role in the inter-lamellar cell mass´ capacity to hinder branchial ammonia excretion (Perry et al., 2010b; Smith et al., 2012). Thickening of filaments and lamellae in the form of enlargement of inter-lamellar cell mass as observed in our study has also been found for common carp, *Cyprinus carpio* (a less ammonia-sensitive cyprinid), clearly for goldfish, and although less clear and very slowly (Sinha et al., 2014) even for rainbow trout, *Oncorhynchus mykiss* (highly ammonia sensitive). Histological changes probably affect chronic parameters like physiological condition or fitness of fish (see e.g. Sokolova et al., 2012), which were not determined in this experiment.

4.2. Different responses among species to ammonia exposure

The acute toxicity tests showed different 96-h LC_{50} among species: gibel carp (0.73 mg NH_3 L^{-1}, 120.3 mg TAN L^{-1}) > silver carp (0.35 mg NH_3 L^{-1}, 30.8 mg TAN L^{-1}) > bighead carp (0.33 mg NH_3 L^{-1}, 22.3 mg TAN L^{-1}). The underlying mechanism is unclear. In addition to the natural variation among species, the size (life stage) of the test fish may be a factor. In this study, the higher LC_{50} of gibel carp was probably caused by the use of juvenile gibel carp contrary to the application of 5 dph larvae of silver carp and bighead carp. For many fish, tolerance to ammonia toxicity tends to increase with body size. For example, for grass carp (*Ctenopharyngodon idella* Val.) the 96-h LC_{50} of NH_3 increased from 0.57 mg L^{-1} through 0.61 mg L^{-1} to 0.68 mg L^{-1} when the juvenile sizes increased from 0.04 g through 0.17 g to 3.24 g (Zhou et al., 1986). The same trend was found for common carp (*Cyprinus carpio* L.) and many other species such as fathead minnow (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*) (Hasan and Macintosh, 1986; Zheng et al., 1998). Appendix A in the USEPA study (USEPA, 2013) contains a compilation of data on acute toxicity of ammonia to aquatic animals.

The 96-h LC_{50} TAN of gibel carp (120.3 mg L^{-1}) in this study ranked 14th among the 27 fish genera included in the criteria of USEPA (2013), being closest to that (110.0 mg L^{-1}) for *Cyprinella* sp. (Cyprinidae). The 96-h LC_{50} TAN of silver carp (30.8 mg L^{-1}) and bighead carp (22.3 mg L^{-1}) are lower than that (51.9 mg L^{-1}) of mountain whitefish, *Prosopium williamsoni*, the most sensitive fish included in the criteria. This does not necessarily mean that silver carp and bighead carp are more sensitive than mountain white fish as much larger fish (63 g–177 g) were used in the tests for mountain whitefish than the 5 dph larvae in this study (Thurston and Meyn, 1984).

In our chronic test, all the three cyprinids showed similar responses in histology and survival to ammonia exposure, whereas the response of growth rates differed between species. Contrary to gibel carp, both silver carp and bighead carp showed clearly increasing growth rates with increasing ammonia concentrations. This contrast may be explained by differences in the diet of the species. Both silver carp and bighead carp are planktivores (Cremer and Smitherman, 1980), and their growth rates depend naturally on the amount of plankton available. Gibel carp are omnivores (Xie et al., 2001) and their growth does therefore not rely significantly on the amount of phytoplankton. The suggested explanations are confirmed by the correlations calculated between phytoplankton chlorophyll *a* (Chl *a*) and the growth variables of the three fish species; added ammonia promoted the growth of pelagic silver carp and bighead carp by stimulating the development of phytoplankton (and likely also zooplankton) and thus food production for these fish. Obviously, this was not the case for the more benthic gibel carp, despite that some of the phytoplankton biomass will eventually reach the bottom when they sink to the sediment or die.

5. Conclusion

We conclude that: (1) fish might be more tolerant to high ammonia concentrations in natural aquatic ecosystems than under laboratory conditions; (2) fish adaptation and environmental mediation may reduce the toxicity of ammonia, although marked histological changes were found in our study; (3) planktivores and omnivores differ in growth responses to ammonia exposure, probably because of secondary effects related to food availability; (4) stimulation of phytoplankton with added ammonia results in increased growth of planktivores; and (5) the previous regulatory limits on ambient ammonia may be higher than required from lab tests. Field-scale chronic toxicity tests covering full life histories of fish and other aquatic organisms are therefore encouraged in order to optimize determination of the effects of ammonia in natural environments.

Acknowledgements

The research was supported by the State Key Laboratory of Freshwater Ecology and Biotechnology (2016FBZ09, 2014FB14, and 2011FBZ14) and the Science and Technology Support Program of Hubei Province (2015BBA225). Hai-Jun Wang was supported by the Youth Innovation Association of Chinese Academy of Sciences (2014312). Erik Jeppesen was supported by the MARS project (Managing Aquatic ecosystems and water Resources under multiple Stress) funded under the 7th EU Framework Programme, Theme 6 (Environment including Climate Change), (603378), AQUACOSM (Network of Leading European AQUAtic MesoCOSM Facilities Connecting Mountains to Oceans from the Arctic to the Mediterranean) (731065) and CLEAR (a Villum Kann Rasmussen Centre of Excellence project). We thank Xu-Fang Zeng for her technical support with chemical analysis of water samples and Anne Mette Poulsen for editions.

References

Admiraal, W.I.M., Botermans, Y.J.H., 1989. Comparison of nitrification rates in three branches of the lower river Rhine. Biogeochemistry 8, 135–151.

American Public Health Association (APHA), American Water Works Association, Water Pollution Control federation, 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. American Public Health Association, Washington, DC, USA, p. 1268.

Benli, A.C.K., Köksal, G., Özkul, A., 2008. Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): effects on gill, liver and kidney histology. Chemosphere 72, 1355–1358.

Camargo, J.A., Álonso, A., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int. 32, 831–849.

Camargo, J.A., Álonso, A., Salamanca, A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58, 1255–1267.
Chesterikoff, A., Garban, B., Billen, G., 1992. Inorganic nitrogen dynamics in the river seine downstream from Paris (France). Biogeochemistry 17, 147–164.
Constable, M., Charlton, M., Jensen, F., McDonald, K., Craig, G., Taylor, K.W., 2003. An ecological risk assessment of ammonia in the aquatic environment. Hum. Ecol. Risk. Assess. 9, 527–548.
Cremer, M.C., Smitherman, R.O., 1980. Food habits and growth of silver and bighead carp in cages and ponds. Aquaculture 20, 57–64.
Dodds, W.K., López, A.J., Bowden, W.B., Gregory, S., Grimm, N.B., Hamilton, S.K., et al., 2002. N uptake as a function of concentration in streams. J. N. Am. Benthol. Soc. 21, 206–220.
Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32, 2379–2383.
Environment Canada, 2001. Priority Substances Assessment Report: Ammonia in the Aquatic Environment. Minister of Public Works and Government Services Canada, Ottawa, ON, Canada.
Finlay, J.C., Small, G.E., Sterner, R.W., 2013. Human influences on nitrogen removal in lakes. Science 342, 247–250.
Finney, D.L., 1971. Probit Analysis. Cambridge University Press, London.
Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Golterman, H.L., Clymo, R.S., Ohmstad, M.A.M., 1978. Methods for physical and chemical analysis of fresh waters. Handbook 8, second ed. Blackwell Scientific Publications, Oxford, pp. 111–117.
Hall, R.O., Tank, J.L., 2003. Ecosystem metabolism controls nitrogen uptake in streams in grand Teton National Park, Wyoming. Limnol. Oceanogr. 48, 1120–1128.
Hasan, M.R., Macintosh, D.J., 1986. Acute toxicity of ammonia to common carp fry. Aquaculture 54, 97–107.
ISO 5664, 1984. Water Quality; Determination of Ammonium; Distillation and Titration Method.
ISO 6777, 1984. Water Quality; Determination of Nitrite; Molecular Absorption Spectrometric Method.
ISO 7150–1, 1984. Water Quality; Determination of Ammonium. Part 1. Manual Spectrometric Method.
ISO 7890–3, 1988. Water Quality; Determination of Nitrate. Part 3. Spectrometric Method Using Sulfosalicylic Acid.
Jha, R., Ojha, C.S.P., Bhatia, K.K.S., 2001. Refinement of predictive reaeration equations for a typical Indian river. Hydrol. Process. 15, 1047–1060.
Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 2243–2246.
Mackin, J.E., Aller, R.C., 1984. Ammonium adsorption in marine sediments. Limnol. Oceanogr. 29, 250–257.
Morgan, I.J., McDonald, D.G., Wood, C.M., 2001. The cost of living for freshwater fish in a warmer, more polluted world. Glob. Chang. Biol. 7, 345–355.
Moss, B., Jeppesen, E., Søndergaard, M., Lauridsen, T.L., Liu, Z.W., 2013. Nitrogen, macrophytes, shallow lakes and nutrient limitation – resolution of a current controversy? Hydrobiologia 710, 3–21.
Mulholland, P.J., Tank, J.L., Sanzone, D.M., Wollheim, W.M., Peterson, B.J., Webster, J.R., Meyer, J.L., 2000. Nitrogen cycling in a forest stream determined a ^{15}N tracer addition. Ecol. Monogr. 70, 471–493.
Murphy, J., Riley, P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Passell, H.D., Dahm, C.N., Bedrick, E.J., 2007. Ammonia modeling for assessing potential toxicity to fish species in the Rio Grande, 1989–2002. Ecol. Appl. 17, 2087–2099.
Perry, S.F., Schwaiger, T., Kumai, Y., Tzaneva, B., Braun, M.H., 2010b. The consequences of reversible gill remodeling on ammonia excretion in goldfish (*Carassius auratus*). J. Exp. Biol. 213, 3656–3665.
Peterson, B.J., Wollheim, W.M., Mulholland, P.J., 2001. Control of nitrogen export from watersheds by headwater streams. Science 292, 86–90.
Rosenfeld, J.K., 1979. Ammonium adsorption in nearshore anoxic sediments. Limnol. Oceanogr. 24, 356–364.
Sinha, A.K., Matey, V., Giblen, T., Blust, R., De Boeck, G., 2014. Gill remodeling in three freshwater teleosts in response to high environmental ammonia. Aquat. Toxicol. 155, 166–180.
Smith, A.A., Zimmer, A.M., Wood, C.M., 2012. Branchial and extra-branchial ammonia excretion in goldfish (*Carassius auratus*) following thermally induced gill remodeling. Comp. Physiol. Biochem. A 162, 185–192.
Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15.
Thurston, R.V., Meyn, E.L., 1984. Acute toxcity of ammonia to five fish species from the northwest United States. Technical Report No. 84-4. Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT.
USEPA [United States Environmental Protection Agency], 1999. 1999 Update of Ambient Water Quality Criteria for Ammonia. Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, Washington, D.C., USA.
USEPA [United States Environmental Protection Agency], 2013. 2013 Update of Ambient Water Quality Criteria for Ammonia. Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, Washington, D.C., USA.
Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of Earth's ecosystems. Science 277, 494–499.
Wood, C.M., 1993. Ammonia and urea metabolism and excretion. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Ann Arbor, MI, pp. 379–425.
Wood, C.M., 2004. Dogmas and controversies in the handling of nitrogenous wastes: is exogenous ammonia a growth stimulant in fish? J. Exp. Biol. 207, 2043–2054.
Wootton, R.J., 1990. Ecology of teleost fishes, fish and fisheries. second ed. Vol. 24. Chapman and Hall, London; New York.
Xie, S., Zhu, X., Cui, Y., Wootton, R.J., Lei, W., Yang, Y.X., 2001. Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. J. Fish Biol. 58, 999–1009.
Young, R.G., Huryn, A.D., 1999. Effects of land use on stream metabolism and organic matter turnover. Ecol. Appl. 9, 1359–1376.
Yu, Q., Wang, H.Z., Li, Y., Shao, J.C., Liang, X.M., Jeppesen, E., Wang, H.J., 2015. Effects of high nitrogen concentrations on the growth of submersed macrophytes at moderate phosphorus concentrations. Water Res. 83, 385–395.
Zheng, Y.H., Hu, X.W., Liu, Z.D., Qin, S.G., 1998. An acute toxicity of ammonia on fry of carp. Southwest China Agric. Sci. 11, 80–85 (in Chinese with English abstract).
Zhou, Y.X., Zhang, P.Y., Zhou, R.Z., 1986. The acute and subacute toxicity to ammonia on grass carp (*Ctenopharyngodon idella*). Acta Hydrobiol. Sin. 11, 32–39 (in Chinese with English abstract).
Zimmer, A.M., Nawata, C.M., Wood, C.M., 2010. Physiological and molecular analysis of the interactive effects of feeding and high environmental ammonia on branchial ammonia excretion and Na+ uptake in freshwater rainbow trout. J. Comp. Physiol. B. 180, 1191–1204.