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

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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

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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

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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