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Arsenic impacted the development, thyroid hormone and gene transcription of thyroid hormone receptors in bighead carp larvae (*Hypophthalmichthys nobilis*)

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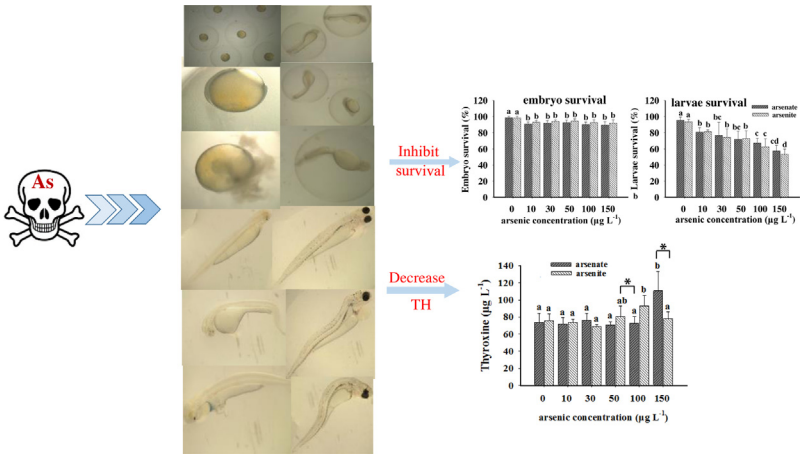
HIGHLIGHTS

- We tested arsenic toxicity on the embryos and larvae of bighead carp.
- We used low levels of AsV and AsIII from 10 to 150 μg L^{-1}.
- Arsenic slightly impacted embryo survival, but greatly decreased larvae survival.
- Arsenic increased thyroid hormone thyroxine in fish larvae by 23–50%.
- Arsenic reduced transcription of thyroid hormone receptors in fish larvae by 53–91%.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As) contamination in aquatic environment adversely impacts aquatic organisms. The present study assessed the toxicity of different As species and concentrations on bighead carp (*Hypophthalmichthys nobilis*) at early life stage, a major fish in Yangtze River, China. We measured the changes in embryo and larvae survival rate, larvae aberration, concentrations of thyroid hormone thyroxine, and transcription levels of thyroid hormone receptors (TRs) in fish larvae after exposing to arsenite (AsIII) or arsenate (AsV) at 0, 10, 30, 50, 100, or 150 μg L^{-1} for 78 h. As concentrations ≤150 μg L^{-1} had limited effect on embryo survival rate (6–8% inhibition), but larvae survival rate decreased to 53–57% and larvae aberration rate increased to 20–24% after As exposure. Moreover, thyroxine levels elevated by 23% and 50% at 100 μg L^{-1} AsIII and 150 μg L^{-1} AsV. Besides, AsIII and AsV decreased the transcriptional levels of TRα by 72 and 53%, and TRβ by 91 and 81% at 150 μg L^{-1} As. Our data showed that AsIII and AsV had limited effect on carp embryo survival, but they were both toxic to carp larvae, with AsIII showing more effect than AsV. As concentrations <150 μg L^{-1} adversely influenced the development of bighead carp larvae and disturbed their thyroid hormone homeostasis.

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1. Introduction

Arsenic (As) contamination is of environmental concern worldwide, which results from both natural processes and anthropogenic activities. Aquatic systems such as rivers and lakes have received discharge from domestic and industrial wastewaters for years. As a result, As contamination in aquatic environment is of concern in several regions around the globe. For example, elevated As concentrations in rivers and lakes have been reported, e.g., 150–180 $\mu g\,L^{-1}$ in Yangzonghai Lake, China, 8–251 $\mu g\,L^{-1}$ in Brahmaputra River, Bangladesh, and 125–145 $\mu g\,L^{-1}$ in Ramgarh Lake, India [1–3]. It is known that As exists as arsenite (AsIII) or arsenate (AsV) in the aquatic environment, and both are toxic to aquatic organisms [4,5].

Yangtze River Delta is the most industrialized and urbanized regions in China, due to the dense population and rapid economic growth in the area, various human activities have led to arsenic contamination in the river. Arsenic concentrations up to 21 $\mu g\,L^{-1}$ in Nanjing section of Yangtze River has been reported [6]. With more economic development in the region, higher As level up to 150 $\mu g\,L^{-1}$ is being projected [7], which is the USEPA provisional guideline value [8]. Due to As toxicity, As concentrations <150 $\mu g\,L^{-1}$ may also have adverse effects on aquatic organisms [9,10]. Considering As levels in Yangtze River has been increasing, it is important to understand the impact of As on aquatic organism. Bighead carp (*Hypophthalmichthys nobilis*), one of four major Chinese carps, is an important fish for human consumption in Yangtze River [11]. As a top predator in Yangtze River, it is more susceptible to As contamination than other aquatic organisms [12,13]. It is known that fish at early life stages are more sensitive to As than adult fish because of their thinner epithelial layer combined with a relatively larger body surface to volume ratio, high metabolic rate, and limited mobility [14,15]. Therefore, it is necessary to examine As toxicity on fish at early life stage.

In the recent years, much research has focused the effect of As on thyroid hormones in different animals. For example, Davey, et al. [16] demonstrated that 0.1–4.0 $mg\,L^{-1}$ AsIII caused shrinkage in tadpole tail (*Xenopus laevis*) by influencing its thyroid hormone using an in vivo study. Allen and Rana [17] reported that content of thyroid hormone thyroxine in rats increased from 20 to 50 $nmol\,L^{-1}$ after exposing to 40 $mg\,kg^{-1}$ AsIII body weight. Mohanta et al. [18] manifested that thyroid hormone levels were significantly reduced in guinea pigs (*Cavia porcellus*) after feeding food containing 50 $mg\,kg^{-1}$ AsIII for 11 weeks. But limited information is available on fish. Although our previous study showed that AsIII significantly affects the thyroid hormone in zebrafish [19], no data are available regarding As effect on early life stage of fish. It is known that thyroid hormones including thyroxine (T4) and triiodothyronine (T3) play an important role in the growth and metabolism of fish, especially during transitory phase from embryonic to larvae [20,21], so it is necessary to understand As effect on thyroid hormone in fish at early life stage.

To date, most studies have used high As concentrations (25–750 $mg\,L^{-1}$) to study As toxicity on fish [22,23]. However, those As concentrations are far above the typical As concentrations (21–150 $\mu g\,L^{-1}$) in the aquatic environment. In addition, most studies only tested the effect of arsenate (AsV), with few studies include arsenite (AsIII) [19,22]. To better understand the effects of As on fish, we used bighead carp at the early life stage as a test organism to determine the effects of different concentrations of AsV or AsIII (0, 10, 30, 50, 100, or 150 $\mu g\,L^{-1}$) on: (1) the development of bighead carp embryos and larvae, (2) the changes in thyroid hormone thyroxine levels in bighead carp larvae, and (3) the transcription of thyroid hormone receptor genes in bighead carp larvae. The data should shed light on the adverse impact of As on fish at early life stage in aquatic systems including Yangtze River.

2. Materials and methods

2.1. Experiment design

Arsenite ($NaAsO_2$, Sigma–Aldrich, ≥90%) and arsenate ($Na_2HAsO_4{\cdot}7H_2O$, Sigma–Aldrich, ≥98%) were dissolved in Milli-Q water to make AsIII and AsV solutions. Based on the current (up to 21 $\mu g\,L^{-1}$) and projected As level (150 $\mu g\,L^{-1}$) in Yangtze River reported by Wu et al. [6] and Jing et al. [7], AsIII and AsV concentrations of 0, 10, 30, 50, 100 and 150 $\mu g\,L^{-1}$ were used, which are referred to as $AsIII_{10}$, $AsIII_{30}$, $AsIII_{50}$, $AsIII_{100}$, $AsIII_{150}$, AsV_{10}, AsV_{30}, AsV_{50}, AsV_{100}, and AsV_{150}. The water used in this experiment had the following characteristics: pH 6.7 ± 0.5, dissolved oxygen = 6.4 ± 0.4 $mg\,L^{-1}$, conductivity = 0.252 ± 0.006 mS/cm, and total hardness = 194 ± 12.0 $mg\,L^{-1}$ $CaCO_3$ [24]. During the experiment, 50 fertilized fish eggs at the middle blastula stage were placed in Petri dishes containing different AsIII or AsV concentrations. To maintain constant AsIII and AsV concentrations, test solutions were replaced every 12 h. Water temperature and light were as following: 25 ± 1 °C and 14 light/10 dark photoperiod.

2.2. Embryo collection

Artificially fertilized eggs of bighead carp (*H. nobilis*) were obtained according to Chen, et al. [15]. Briefly, fertilized eggs were obtained by inducing ovulation of cultured brood stock with one male and one female being injected with (D-Ala6-Pro9-Net)-luteinizing hormone-releasing hormone analogue. The eggs were manually removed and artificially fertilized. Collected embryos were rinsed with embryonic rearing water and examined under anatomical lens (LAS EZ, Leica, Germany). Normally developing embryos were selected at the middle blastula stage (i.e., 5 hour post fertilization) and then transferred to plastic Petri dish (Nunc Plastics, Roskilde, Denmark) for AsIII and AsV treatments. Each incubation unit consisted of a glass Petri dish filled with 100 mL of a test solution.

2.3. Arsenic exposure experiment

It take ~30 h from embryo hatch into larvae, dead embryos were removed and counted every 8 h during the 30 h. Toxicological measurement included embryo survival, structural malformation and larvae survival. Malformations of crooked fish spine were defined as scoliosis and curvature of fish tail. Fish mortalities included coagulated embryos before hatching and dead larvae. To determine the effect of As on the content of thyroid hormone thyroxine in early life stage of bighead carp, newly hatched fish larvae were continued to expose to AsIII or AsV solutions for 48 h until carp embryo finished hatching. At the end of experiment, fish larvae were removed and stored at −80 °C for subsequent assays. Each exposure experiment was replicated six times.

2.4. Analysis of thyroxine in fish larvae

Given thyroxine (T4) content secreted by thyroid gland in fish is more than 20 times of that triiodothyronine (T3), thyroxine was used as an indicator of thyroid hormones in this study. After exposing to AsIII or AsV for 78 h, fish larvae were removed to determine the changes in the thyroxine content. Thyroxine content was measured as whole-body homogenates with samples being kept on ice during the entire procedure. Whole larvae was rinsed in 0.68% ice-cold physiological saline solution and then dried on filtrating paper. Samples were placed in Dounce homogenizer and homogenized after addition of physiological saline solution at tissue: solution of 1:9. Subsequently, whole larvae homogenates were centrifuged at 4000 *g* for 10 min at 4 °C to remove cellular debris and cartilage