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Metabolic capacities of common carp (*Cyprinus carpio*) following combined exposures to copper and environmental hypoxia

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ABSTRACT

In aquatic ecosystems, a decline in water O_2 level is the main factor that can release heavy metal ions from top sediment layer. Therefore, hypoxia in turn, and in association with heavy metals might provide undesirable environment and impairs physiological functions of aquatic animals. To address this, metabolic capacities, including standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS) and factorial aerobic scope (FAS) of common carp were determined following exposures to different levels of water-borne Cu^{2+} as well as hypoxia. Treatments for Cu^{2+} were included: 100% (acute), 50% (sub-lethal) and 10% (chronic) of LC_{50}-96 h for immediately, 24 h and 7 days exposures respectively. Hypoxia treatments were assigned as acute for immediately, sub-lethal for 24 h and chronic for 7 days. Combined effects of treatments were also considered as acute Cu^{2+} + hypoxia, sub-lethal Cu^{2+} + hypoxia and chronic Cu^{2+} + hypoxia. While SMR of carp was reduced by chronic hypoxia, significant ($P<0.05$) increase was observed during acute hypoxia, as compared with control. The MMR and AS were significantly reduced ($P<0.05$) following all hypoxia treatments. The acute and chronic Cu^{2+} treatments showed significant ($P<0.05$) increases in SMR and MMR values. All acute and sub-lethal combined treatments showed significant ($P<0.05$) reductions in SMR, MMR and AS values, whilst chronic combined treatments showed generally increasing trends for MMR and AS. P_{Crit} was relatively reduced following all treatments except for acute and sub-lethal Cu^{2+}-treated fish that showed higher value ($P<0.05$) and no change respectively. Although all Cu^{2+} treatments increased the number of mucus cell, hypoxia treatments did not show any remarkable differences when compared with control group. In general, the results of present study reveal that hypoxia acts as limiting stressor whilst Cu^{2+} do act as loading stressors in the case of common carp metabolism. The interactive exposures mostly showing a synergist effect in all metabolic capacities with an exception for chronic treatments.

1. Introduction

Environmental hypoxia refers to any condition in which the oxygen (O_2) concentration is lower than the normal value for the living aquatic organisms. The appearance of eutrophic zones has been mainly attributed to the rapid increase in nitrogen and phosphorous flows (mostly anthropogenic) and likewise, leads to the production of algae blooms in water reservoirs. This initially triggers O_2 depletion overnight when the respiration of the vegetation is coincident with that of aquatic animals. On the other hand, the occurrence of hypoxia as a result of algae death (similar to sewage discharge) is related to microbial activities that utilize the water O_2 content (i.e., increase biochemical oxygen demand). Furthermore, hypoxic conditions are seen in the hypolimnion layer of lakes, in ambient water under frozen surface layer as well as following intensive aquaculture (Boyd and Schmittou, 1999; Nürnberg, 2004; Selman et al., 2008; Rabalais et al., 2010).

The contamination of water by heavy metals is a worldwide environmental problem. The heavy metals are constantly being entered into aquatic ecosystems through industrial effluents, mining activities and agricultural fertilizers (i.e. via leaching from the surrounding soils) (Yu, 2001). This phenomenon commonly impairs physiological, biochemical, morphological and molecular bases of aquatic animals (De Boeck et al., 1995; Scott and Sloman, 2004; Malekpouri et al., 2011). Furthermore, fish respiration might be altered following metal exposures by engendering some pathological changes in the gill tissues and therefore, disturbing the

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O_2 transfer and reducing the blood PO_2. It seems likely that this deterioration, in turn, results in a reduction of the swimming speed (Mager and Grosell, 2011) as well as changes in energy budget (De Boeck et al., 1995).

The occurrence of eutrophication and hypoxic zones, as described so far, could be seen in aquatic ecosystems and likewise, in fish farming ponds. Regarding that, many aquatic environments like south-eastern part of Caspian Sea (i.e. Gorgan Bay) are potent for O_2 depletion (Diaz and Rosenberg, 2008; Selman et al., 2008). This area is a habitat for many fish species, including cyprinids, and is undoubtedly considered to be contaminated by heavy metals during last decades (Ebadati et al., 2005). Evidences show that large amounts of heavy metals are mostly stored in the sediments and hypoxia are able to influence metals' bioavailability (Campbell, 2003).

Changes in gill ventilation in response to anoxia (and also hypoxia) have important implications for sensitivity of aquatic animals to contaminants (Hattink et al., 2005) though some aquatic species like carp are hypoxic-tolerant (Lardon et al., 2013). Metabolic suppression as well as the ability of intensifying O_2 uptake and transportation in bloodstream have been evidenced as adaptation strategies for aquatic animals to cope with hypoxia (Hochachka, 1986; Urbina and Glover, 2012). Hypoxia has adverse effect on fish growth rate and food intake due to possible direct or indirect effects on central nervous system in order to change their strategy for manage their energetic expenditure (Kramer, 1987; Medale et al., 1987; Gaulke et al., 2014). However, this capacity should be examined even when water O_2 concentration reaches critical partial O_2 tension (P_{Crit}) in combination with toxic compounds exposure.

Lloyd (1961) showed greater toxicities of lead (Pb^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) at lower O_2 concentration. The author interpreted this with greater ventilation of fish following hypoxia. In another experiment, hypoxia led to lower survival in rainbow trout, *Oncorhynchus mykiss* when treated with the same concentration of Zn^{2+} (Lloyd and Herbert, 1962). Additionally, fathead minnows, *Pimephales promelas* exposed to higher Cu^{2+} concentrations were less tolerant to low O_2 levels (Bennett et al., 1995). Therefore, environmental hypoxia can aggravate the toxicity of metals in aquatic animals and reduce their survival.

As described earlier, there is great deal of investigation that characterized the effect of either hypoxia and/or heavy metal exposures on aquatic organisms. However, studies that integrate biological responses of combined effects of hypoxia and chronic, sub-lethal and acute concentrations of pollutants on fish are still quite rare. Since different levels of hypoxia may trigger different level of physiological responses (Herbert and Steffensen, 2005), and the occurrence of hypoxic condition and heavy metal pollutants are quite frequent in natural condition (Ivanina et al., 2011), we aimed to examine individual fish response to both single and combined stressors i.e., hypoxic condition and Cu^{2+} exposures. Regarding that, we conducted a series of experimental studies to address the interactive effects of hypoxia and Cu^{2+} on O_2 consumption and gill histopathology in common carp, *Cyprinus carpio*. Metabolic capacities, including standard and maximum metabolic rates (SMR and MMR) as well as absolute and factorial aerobic scope (AS and FAS) were monitored as valid indicators for elucidating the impact of stressors on *C. carpio*. Gill histology was also carried out to determine any possible injuries and the probable role of mucus secreting cells in O_2 consumption properties.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of reagent grade and were supplied from Merck Chemical Company (Germany) unless otherwise stated. $CuSO_4 \cdot 5H_2O$ was purchased from Panreac, Spain.

2.2. Fish maintenance

Common carp (*C. carpio* **L.**) were obtained from a local supplier. The healthy specimen kept in a recirculating system to acclimate to our experimental condition for at least 2 weeks. Fish were fed daily *ad libitum* with standard diet for carp (31.3% crude protein, 11.6% crude fat, 11.7% ash). Water O_2 concentration and temperature were maintained at $>5.8\ mg\ l^{-1}$ ($>80\%$ of air saturation) and 20–22 °C, respectively.

2.3. Experiments

The respirometry experiment was conducted by juvenile *C. carpio* based on two preliminary tests, i.e. determinations of median lethal concentration (LC_{50}) of Cu^{2+} and hypoxia tolerance as follows:

2.3.1. LC_{50} calculation

A total of 49 specimens (BW = 18.0 ± 2.34 g, TL = 11.2 ± 0.78 cm) were randomly assigned to perform acute toxicity test of Cu^{2+}. According to protocol suggested by OECD, No 203, a semi-static condition was applied (OECD, 1992). To do this, six geometric serial concentrations (23.1 up to 85.7 $mg\ l^{-1}$ for Cu^{2+}) and a control group were provided. Each group experimental concentration contained 7 fish. The fish were exposed to the serial concentration of Cu^{2+} and the mortality rate was assessed and freshwater was renewed daily for a period of 96 h. The fish did not feed throughout the experiment. At the end of experiment, all data were subjected to analysis of probit regression to calculate LC_{50}-96 h value for the further treatments.

2.3.2. Hypoxia tolerance

The experiment examined the threshold of low dissolved O_2 (DO) on normoxia-acclimated fish to indicate hypoxia tolerance. We have considered two different experiments; the first experiment was modified from method suggested by Fu et al. (2014). Briefly, each individual fish ($n = 10$, BW = 19.1 ± 2.71 g, TL = 11.5 ± 0.67 cm) was transferred to a 4 l glass aquarium and acclimated for a period of 24 h prior to experiment. An appropriate mesh screen was placed below the waterline to prevent possible access of fish to water–air interface. Nitrogen bubbles were injected to the water to reduce O_2 level rapidly from 7.2 (100% of air saturation, 171 mmHg for PO_2) to 5.0 (71%, 120 mmHg), 2.5 (37%, 62 mmHg) and then to 1.2 $mg\ l^{-1}$ (21%, 36 mmHg). Following 1 h holding at each mentioned O_2 level, the water O_2 level was lessened slowly at a rate of 0.1 $mg\ l^{-1}\ min^{-1}$ until the loss of equilibrium (LOE) was observed.

The second experiment was carried out for each individual fish ($n = 8$, BW = 23.601.45 g, TL = 12.460.45 cm) by a step-wise decrease (0.1 $mg\ l^{-1}\ min^{-1}$) in water O_2 level from normoxia until the LOE was observed. The test equipment and condition were similar to previous described protocol.

2.3.3. Treatments' setup

In the O_2 consumption experiments, *C. carpio* ($n = 78$) were subjected to different levels of Cu^{2+} and hypoxia on single and combined bases. To address this, 10 treatment groups (8 fish/