Comparative Biochemistry and Physiology, Part C 190 (2016) 32–37

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Kidney activity increases in copper exposed goldfish (*Carassius auratus auratus*)

Sofie Moyson [a,*], Hon Jung Liew [a,b], Angela Fazio [c], Nathalie Van Dooren [a], Aline Delcroix [a], Caterina Faggio [c], Ronny Blust [a], Gudrun De Boeck [a]

[a] Systemic Physiological and Ecotoxicological Research, Department of Biology, University of Antwerp, Groenenborgerlaan 171, BE-2020 Antwerp, Belgium
[b] Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia
[c] Department of Biological and Environmental Science, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 S.Agata, Messina, Italy

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 5 August 2016
Accepted 8 August 2016
Available online 10 August 2016

Keywords:
Shubunkin
Ionoregulation
Toxicity
Swimming performance
Osmolality
Heavy metal
ATPase

ABSTRACT

In the present study, the effect of copper was examined in the common goldfish (*Carassius auratus auratus*). Fish were fasted and exposed to either a high (0.84 μM), a low (0.34 μM) or a control copper concentration (0.05 μM) for 1 and 7 days. Swimming performance was not affected by either fasting or copper exposure. Food deprivation alone had no effect on ionoregulation, but low plasma osmolality levels and plasma Na^+ were noticed in fasted fish exposed to Cu for 7 days. Both gill Na^+/K^+-ATPase and H^+-ATPase activities were undisturbed, while both kidney ATPase activities were up-regulated when challenged with the high Cu levels. Up-regulated kidney ATPase activities likely acted as compensatory strategy to enhance Na^+ reabsorption. However, this up-regulation was not sufficient to restore Na^+ to control levels in the highest exposure group.

1. Introduction

Copper is an essential nutrient for all living organisms and has numerous functions in cellular biochemistry (Burke and Handy, 2005). Increased use of heavy metals in anthropogenic activities during the last decades led to increased metal levels in aquatic environments worldwide, especially in mining and industrialised areas (Yang and Rose, 2003). The average copper level in lake and river water is 0.16 μM, but in contaminated water the concentrations can rise above 15.74 μM (ATSDR, 2004). In Flanders, as an example of an industrialised area, the average norm for environmental quality of surface waters is 0.11 μM dissolved copper, or 0.77 μM total copper. The goal to decrease copper concentrations in 2010 with a minimal of 75% compared to 1985, was not reached but between 2000 and 2010, average copper concentrations in surface waters have successfully decreased with 78% (MIRA, 2010). However, due to historic pollution, the norm for ground water quality of 1.57 μM is still occasionally exceeded (VMM, 2013). Besides industry, agriculture and aquaculture can also significantly contribute to surface water copper concentrations. Currently, increasing copper concentrations are often seen in aquaculture (Vutukuru et al., 2006) and they potentially can cause problems. Even sublethal concentrations of toxic substances can induce biochemical, physiological, morphological and genetic changes in aquatic organisms, depending on fish size and water composition (Wood, 2001). Furthermore, different fish species suffer in a varying degree from pollution. In the case of copper, gibel carp (*Carassius auratus gibelio)* appeared considerably less sensitive to aqueous Cu than common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Based on LC50 values (96 h), Cu was three times more toxic for rainbow trout (LC50: 3.3 μM) than for common carp (LC50: 10.4 μM), and seven times more toxic than for gibel carp (LC50: 22.0 μM) (De Boeck et al., 2004). It was suggested that the genus *Carassius* has a relative higher tolerance to copper compared to other freshwater species (De Boeck et al., 2004; Schjolden et al., 2007; Eyckmans et al., 2011).

Waterborne copper affects the gills of fish, the main location for gas and ion exchange, and causes mucus production, cell swelling and lifting of the epithelium (Wood, 2001). The gills are in continuous contact with the external environment and are thus a primary target for waterborne pollutants (Pandey et al., 2008). Fast copper accumulation in this organ precedes accumulation in other organs, such as liver, kidney and muscle (Grosell and Wood, 2002). This accumulation can lead to a number of adverse effects in gill tissue such as a disruption of the active uptake mechanisms for Na^+ and Cl^- (mainly through an inhibition of Na^+/K^+-ATPase activity), an increase in gill permeability, and oxidative stress (Eyckmans et al., 2010, 2011). Therefore, it

* Corresponding author.
E-mail address: Sofie.Moyson@uantwerpen.be (S. Moyson).

http://dx.doi.org/10.1016/j.cbpc.2016.08.003
1532-0456/

has been suggested that the sodium turnover rate determines the sensitivity to lethal copper exposure (Grosell et al., 2002).

Additionally, copper is believed to inhibit ammonia excretion (Beaumont et al., 1995a, 1995b, 2000a, 2000b, 2003), by which increased ammonia levels can lead to reduced swimming capacity by depolarising muscle cells (McKenzie et al., 2003). A possible explanation for this is that copper may act to disrupt the enzymes (e.g. carbonic anhydrase) and/or transporters such as Na^+/K^+ ATPase involved the Na^+/NH_4^+ exchange mechanism (Zimmer et al., 2012). Another possibility is that copper can inhibit the bidirectional transport of ammonia by binding to peptide residues, suggesting Rh glycoproteins as potential targets for Cu toxicity (Lim et al., 2015). A recent study suggests that Rhcg-a transcript level declined following Cu exposure which might account for Cu induced ammonia efflux inhibition (Sinha et al., 2016) supporting the former. In a comparative study, it was seen that common carp (*Cyprinus carpio*) and gibel carp (*Carassius auratus gibelio*) reacted to sublethal copper exposure (1 μM) with an immediate decrease in swimming speed, while rainbow trout (*Oncorynchus mykiss*) displayed a delayed response, and clear increases in plasma and muscle ammonia were observed (De Boeck et al., 2006).

A wide range of toxicological responses to copper has been reported in several tissues (gills, liver, intestine and muscle) and plasma for various species (De Boeck et al., 2004, 2006, 2010; Ebrahimpour et al., 2011). However, not much is known about the effect of Cu toxicity at sublethal level on the kidney as secondary ionoregulatory organ. The present research was conducted to investigate the ionoregulatory responses in both gill and kidney of fish exposed to sublethal Cu concentrations. We chose to work with shubunkin (*Carassius auratus auratus*), a goldfish variety, since the gibel carp, another *Carassius auratus* subspecies, showed the clearest increase in kidney Cu accumulation in contrast with common carp and rainbow trout (De Boeck et al., 2006). *Carassius auratus auratus* is the most common ornamental fish species worldwide and is thought to be the domesticated form of gibel carp, which is an invasive species in the waters of the Benelux. Furthermore, this species also provides an excellent research model to understand the response to environmental challenges (e.g. Lushchak et al., 2001; Sinha et al., 2012; Kong et al., 2013).

In the present study, goldfish were exposed to either a high (0.84 μM) or a low (0.34 μM) sublethal copper concentration for 1 day or 7 days. The exposure doses were based on an earlier study (De Boeck et al., 1995), where these doses immediately decreased MO_2 in common carp, but allowed recovery at the lower exposure level. To reduce the effects of feeding on ammonia accumulation and excretion, experimental fish were fasted during the exposure, in parallel with fasted control fish. Our first aim was to investigate the effect of copper exposure on swimming performance. We hypothesised that, due to a decreased ammonia excretion, swimming capacity would be reduced at the high exposure concentration. A second aim was to examine the resulting plasma osmolality and Na^+ levels of fish exposed to different Cu levels. We expected to observe reduced plasma Na^+ levels, a reduced plasma osmolality and an increased plasma ammonia concentration. Finally, a third goal was to compare sublethal Cu effects on ionoregulatory responses in the gill and kidney of goldfish, by measuring Na^+/K^+ ATPase and H^+-ATPase activity. An initial reduction of gill ATPase followed by a recovery was expected. We further hypothesised that kidney ATPase might be activated to compensate for the potential reduction in ionoregulatory capacities at the gills. Finally, it was expected that fasting would not have any significant effects on the measured parameters, as earlier studies in our lab showed that one week of fasting only had minor effects on goldfish (Liew et al., 2012, 2013).

2. Materials and methods

2.1. Fish maintenance

Goldfish, *Carassius auratus auratus*, of the shubunkin colour variety were obtained from a local fish supplier (Aqua Hobby, Heist op den Berg, Belgium). The fish were kept in the aquaria facilities of the laboratory of 'Systemic Physiological and Ecotoxicological Research' at the University of Antwerp with softened Antwerp City tap water (17 °C, pH 8.2 ± 0.4, water hardness 297 ± 11 $mg \cdot L^{-1}$ CaCO3, 33 $mg \cdot L^{-1}$ Na, 44 $mg \cdot L^{-1}$ Cl, 60 $mg \cdot L^{-1}$ Ca, 7.1 $mg \cdot L^{-1}$ Mg). Water quality was checked daily by using Standard Tetra Test Kits (Visocolor, Machery-Nagel, Germany) and values remained $<0.1\ mg \cdot L^{-1}$ of NH_3/NH_4^+; $<0.03\ mg \cdot L^{-1}$ of NO_2^- and $<25\ mg \cdot L^{-1}$ of NO_3^-. Water was filtered through biological filters containing wadding, lava stones (0.8–16.0 mm) and activated carbon (charcoal). About 80% of the water was replaced twice a week. Fish were fed at 2% body weight (BW) with commercial pellets ('Hikari Staple', Kyorin Food Ind. Ltd., Japan) twice a day.

One day before the experiment, 56 fish with a body mass of 12.18 ± 0.41 g (mean ± S.D.) were transferred from their maintenance tanks into a temperature-controlled room set at 17 °C with a photoperiod of 14L:10D. Fish were randomly distributed into seven 50–60 L glass aquaria, filled with 40 L well aerated water, with a density of 8 fish per aquarium. A bio-filter, filled with lava stones and wadding, ensured the water quality and aeration. Black plastic shielding minimized visual disturbance. Water quality was monitored as mentioned above and about 80% of the water was statically replaced every two days.

2.2. Experimental design

The experimental setup consisted of 3 control groups: 1 fed control group and 1 fasted control group at day 1 and 1 fasted control group at day 7. Four exposed groups of goldfish were exposed to either 0.34 μM copper (LCu) or 0.84 μM copper (HCu) for 1 day or for 7 days and fish were fasted during the exposure. A copper sulphate solution ($CuSO_4 \cdot 5H_2O$ RPL, Leuven, Belgium) was added to the water of the experimental groups. After each water replacement the water was again spiked with copper stock solution. Copper concentrations (measured by High Resolution Inductively Coupled Plasma Mass Spectrometer ICP-MS, Element XR, Thermofisher Scientific, Bremen, Germany) were monitored daily and were on average 0.05 ± 0.01 μM (control), 0.34 ± 0.02 μM (LCu) and 0.84 ± 0.19 μM (HCu).

2.3. Critical swimming speed, U_{crit}

After 1 and 7 days, U_{crit} of both exposed and control groups was determined. Eight fish from the same group were placed 14 h prior to the experiment in individual separate Blazka-style swimming respirometers with a known volume (≈4.1 L). During this acclimatization period, fish were oriented using a water velocity of $10\ cm \cdot s^{-1}$. The head tank provided a continuous flow of air-saturated water through each flume at a rate of $4\ L \cdot min^{-1}$. Water had the same copper concentration as during the exposure period. The total content of the recirculation system was approximately 450 L. After the acclimatization period, water velocity was increased with $5\ cm \cdot s^{-1}$ every 20 min, until fish fatigued. Fatigue was determined by the fact that fish could no longer maintain position against the current and were swept and held against the mesh screen. Then, speed was briefly lowered to allow fish to restart swimming. Fish were considered totally fatigued when they were swept downstream for the second time within the same 20 min interval (Tudorache et al., 2007). At this point, the performance test was terminated. U_{crit} was calculated as $U_{crit} = U_i + [U_{ii}(T_i/T_{ii})]$, where U_i is referred as the highest velocity sustained for the whole interval, while U_{ii} is the velocity increment ($5\ cm \cdot s^{-1}$), T_i is the time elapsed at fatigue velocity and T_{ii} is the interval time (20 min) (Brett, 1964; De Boeck et al., 2006). The absolute values ($cm \cdot s^{-1}$) were converted to relative swimming speeds in body lengths per second ($BL \cdot s^{-1}$) by factoring the absolute values with body length (fork length). The biometric parameters body mass and fork length did not significantly differ between groups (Table 2).