Long-term effect of copper on sea trout (*Salmo trutta trutta* L.) in early ontogenesis

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The chronic toxicity test was used to determine Cu^{2+} toxicity to sea trout (*Salmo trutta trutta* L.) in early stages of development (egg, larvae) as a test-object. Mortality of embryos and larvae was the criterion of toxicity. The effect of copper was analysed on three developmental periods: 1. From "eyed-egg" stage to yolk-sack resorption (58 days), 2. From the beginning of hatching to yolk-sack resorption (44 days), 3. From one-day larvae to yolk-sack resorption (38 days). It was determined that mortality of embryos and larvae depended on copper concentration, developmental stage and duration of exposition. Hatching is one of the most sensitive periods in early fish ontogenesis.

Key words: sea trout, early stages of development, copper toxicity, long-term effect, mortality

INTRODUCTION

Surface waters in Lithuania are mostly polluted with the following heavy metals: Cu, Zn, Cd, Ni, Cr, Fe, Pb, Mn. Their concentrations usually are very low, but in some extra cases exceed Maximal Permissible Concentrations (MPC) tenfold and even hundredfold [13, 14].

Acute effects of separate heavy metals (Cu, Zn, Ni, Cr, Fe) on aquatic organisms of different trophic levels (fish, crustaceans) in different developmental stages (eggs, larvae, adults) were analysed by Lithuanian specialists [8, 10]. According to these studies, the following toxicity sequences were determined: *Daphnia magna*: Cu > Cr > Zn > Ni > Fe; *Oncorhynchus mykiss* eggs: Cu > Zn > Ni > Fe > Cr; *Oncorhynchus mykiss* larvae: Cu > Zn > Ni > Fe >Cr; *Oncorhynchus mykiss* adults: Cu > Zn > Ni > > Cr >Fe [8]. Cu^{2+} was determined to be the most toxic of these five heavy metals studied to the test-objects [8]. Fish in early ontogenesis were defined to be more sensitive than adults [8, 9]. Analysis shows of literature data that the same conclusions were made in other studies regarding a decreasing sensitivity of fish to different toxicants during ontogenesis [5, 7]. Also, the most sensitive developmental stages were determined, which are blastula, gastrula, early organogenesis, hatching [19, 5, 7, 12]. Sublethal effects of copper ions on fish are widely analysed in literature, too. Sublethal concentrations of copper are known to exert a negative effect on the survival, growth, reproduction, physiological state, behaviour [15, 16, 18, 1, 11, 12, 13]. The mechanisms of copper effects on different functional systems of fish are being analysed [11]. At present, scientists are mostly interested in long-term effects of copper on early fish ontogenesis, because copper-induced functional changes and damages made during early development can greatly decrease population abundance later on [17, 7]. A number of toxicological studies are executed with rainbow trout (*O. mykiss*), carp (*Cyprinus carpio*), pike (*Esox lucius*), zebrafish (*Brachydanio rerio*) and others fish species, but the genus *Salmo* is suggested for long-term toxicity studies as one of the most sensitive test-organism groups [2].

The purpose of this study was to evaluate the long-term effect of copper (Cu^{2+}) on sea trout (*Salmo trutta trutta* L.) in early ontogenesis, depending upon developmental stage and duration of exposure.

MATERIALS AND METHODS

"Eyed-egg" stage eggs (22–24 days after fertilisation) were obtained from fish hatchery. Eggs were acclimated for 2–3 hours and later incubated in the dark at a constant temperature (9.5–10 °C). The concentration of dissolved oxygen in water was not lower than 7 mg/l, pH ~ 7.6 and water hardness ~ 250 mg/l of $CaCO_3$. Two hundred eggs or larvae (depending on the stage analysed) were exposed to each concentration in two replicates parallel with control. The test mixtures were changed every 2 days.

The experiment was performed in three parts. The effect of copper was analysed during three de-

velopmental periods: 1) from "eyed-egg" stage to yolk-sack resorption (58 days), 2) from the beginning of hatching to yolk-sack resorption (44 days), 3) from one-day larva to yolk-sack resorption (38 days). Hatching of larvae in control solution was observed on the 14th–20th days. Mortality was estimated as a part (%) from the amount of still alive test-objects.

0.2 mg/l and 0.3 mg/l concentrations of copper (Cu^{2+}) were analyzed in this study. Solutions were prepared using chemically pure CuSO4 · 5H2O. Significant differences were calculated using Student's criteria.

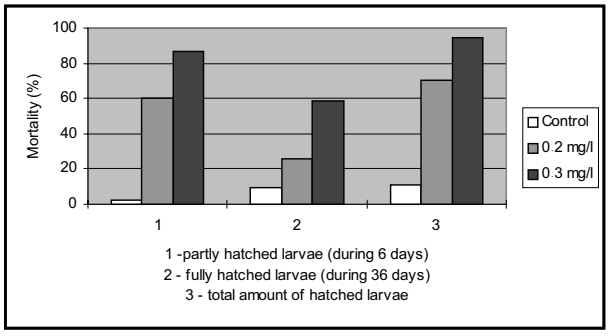

Fig. 2. Effect of Cu^{2+} on mortality of sea trout larvae (1st period)

RESULTS

1. Mortality from "eyed-egg" stage to yolk-sack resorption period (58 days). No mortality was observed in control water during the first 4 days of experiment. During the next 14 days, *i.e*. from the "eyed-egg" stage to the beginning of hatching, about 1% and during 20 days, *i.e*. from the "eyed-egg" stage to the end of hatching, 4% mortality was observed.

In comparison with these data, no mortality was observed during the first 4 days in 0.2 mg/l concentration, either, but during the next 14 days the mortality percentage was as high as 8.5% and during 20 days 26% of eggs.

In the case with eggs exposed to 0.3 mg/l of copper, 2% mortality was found during 4 days, 11% during 14 and 35.5% during 20 days (Fig. 1).

During the hatching period which lasted 6 days, mortality of partly hatched larvae reached 2.1%. After 44 days, *i.e.* from the beginning of hatching to the resorption of yolk-sack, the mortality of fully hatched larvae was 9.3%. The total percentage of dead larvae in control solution during 44 days was 10.9% (Fig. 2).

The 0.2 mg/l concentration of copper caused a 60.2% mortality of partly hatched larvae during 6 days of hatching. The mortality of fully hatched larvae during 44 days was 25.6%. Totally, there were 70.3% of dead larvae during a 44-day period.

In case of larvae exposed to a 0.3 mg/l concentration of copper, 86.5% of dead partly hatched larvae were found during the hatching period (6 days) and 58.3% of fully hatched larvae was found during a 44-day period. The total mortality of larvae during 44 days was as high as 94.6% (Fig. 2).

2. Mortality from the beginning of hatching to yolk-sack resorption (44 days). 3% of dead eggs were registered during the hatching period (6 days). The mortality of eggs increased to 20.5% at 0.2 mg/l and to 28.5% at 0.3 mg/l copper concentrations during hatching (Fig. 3).

0.2 mg/l and 0.3 mg/l of copper caused 48% and 61.7% mortality of partly hatched larvae, respectively. In comparison, only a 2% mortality was observed in control.

Mortality of fully hatched larvae during 44 days of experiment amounted to 21.5% and 54.5% in 0.2 and 0.3 mg/l of copper concentrations, respectively (9.5% in control). The total mortality during this period (44 days) was 47%, 82.5% and 11.3% in 0.2 mg/l, 0.3 mg/l of copper and control (Fig. 3).

3. Mortality from one-day larvae to yolk-sack resorption (38 days). The mortality of 1-day larvae

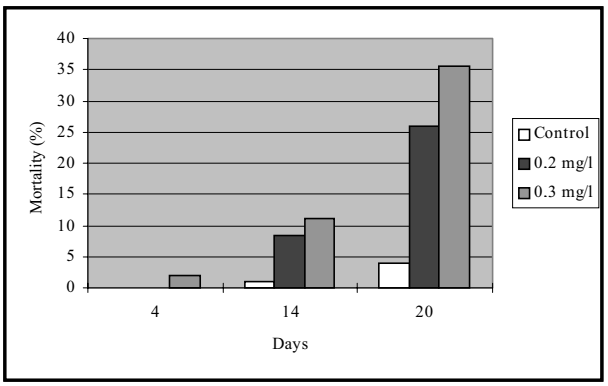

Fig. 1. Effect of Cu^{2+} on mortality of sea trout embryos (1st period)

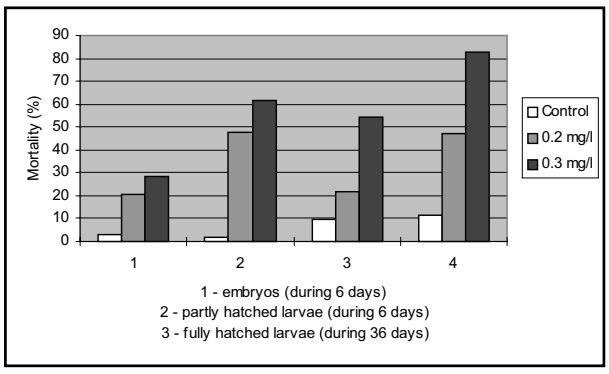

Fig. 3. Effect of Cu^{2+} on mortality of sea trout embryos and larvae (2nd period)

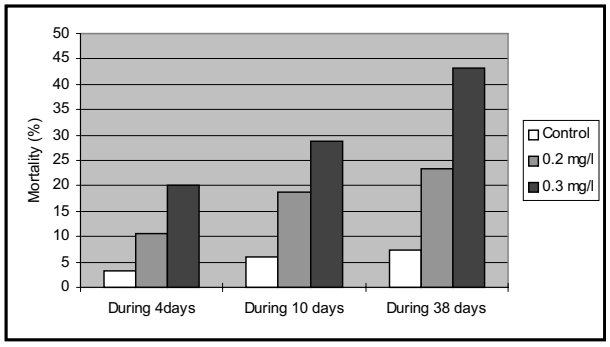

Fig. 4. Effect of Cu^{2+} on mortality of sea trout larvae (3rd period)

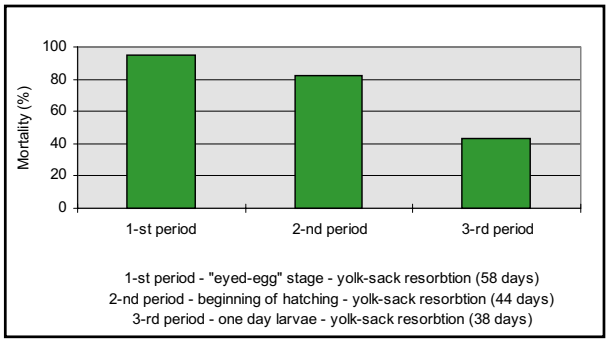

Fig. 6. Effect of 0.3 mg/l of Cu^{2+} on mortality of sea trout larvae depending on the exposed developmental stage

after 4 days of exposure in control water was 3.3%, after 10 days of 6% and after 38 days 7.3%, (Fig. 4).

At a copper concentration of 0.2 mg/l, a 10.7% mortality of 1-day old larvae was observed after 4 days, 18.7% after 10 days and 23.3% after a 38-day period of exposure.

A 0.3 mg/l copper solution caused a 20% mortality of 1-day larvae during a 4-day period, 28.7% after 10 days and 43.3% after 30 days of exposure (Fig. 4).

DISCUSSION

A chronic toxicity test was used for determination of Cu^{2+} toxicity, using sea trout (*Salmo trutta trutta* L.) in its early stages of development (egg, larvae) as a test-object. Mortality of embryos and larvae was the criterion of toxicity.

The obtained results allow to conclude that 0.2 and 0.3 mg/l concentrations of copper during a long-term test significantly increased mortality both in eggs and larvae of sea trout. (Fig. 5). According to data of other authors, copper concentrations lethal to fish ranged from 0.02 to 10 mg/l [1]. The exact values depended on water hardness, pH, fish species, developmental stage and duration of exposure [21, 8, 20, 17].

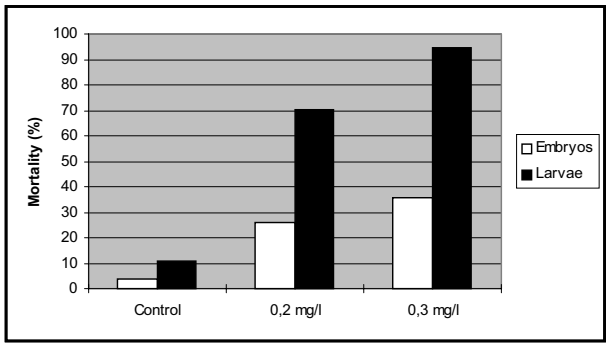

Fig. 5. Effect of Cu^{2+} on mortality of sea trout embryos and larvae

The mortality of sea trout eggs and larvae obviously depended on the developmental stage. Egg mortality was a little higher when they were exposed to copper in early "eyed-egg" stage (1st period) compared to their exposure from the beginning of hatching (2nd period). When 1-day old larvae (3rd period) were exposed to copper, the mortality was lower as compared to "eyed-egg" stage (1st period) or from the beginning of hatching (2nd period) (Fig. 6). Dependence of survival on the age of larvae was also observed while analysing the toxicity of copper in an acute toxicity test with carp [7]. Bennett and his group [3] found that fish larvae were more sensitive to than fry and juveniles to different toxicants.

Analysis of the results shows that the survival greatly depends not only on the exposed developmental stage, but also on the duration of exposure. The toxic effect of copper on eggs and larvae increases with prolonging the duration of exposure (Figs. 1, 4). Similar results were presented by McKim [15, 18] and other authors.

To summarize the obtained results, we can conclude that the effect of Cu^{2+} differs depending on developmental stage. Partly and fully hatched larvae during the hatching period were found most sensitive, 1-day old larvae were less sensitive, and "eyedegg" stage eggs were least sensitive to copper (Fig. 7). The same situation was observed and reported by us and by other autors [6, 7, 16, 17, 12]. Also, it was found that 0.2 mg/l of copper decreased the hatchability of larvae [4]. Copper-exposed embryos usually were degenerated and died in the beginning of hatching [7]. Thus, we can state that hatching is one of the most sensitive periods in early fish ontogenesis. The other frequently mentioned sensitive periods are gastrula and early organogenesis [19, 17, 7]. The damage caused by the toxicant during these periods greatly decreases the vitality of hatched larvae [5].

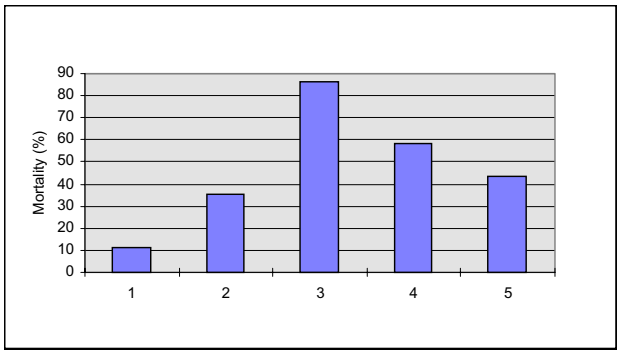

Fig. 7. Effect of 0.3 mg/l of Cu^{2+} on mortality of sea trout larvae depending on developmental stage
1 – eggs during early "eyed-egg" stage – beginning of hatching
2 – eggs during early "eyed-egg" stage – end of hatching
3 – partly hatched larvae during period of hatching
4 – fully hatched larvae during beginning of hatching – yolk-sack resorption period
5 – 1-day old larvae during beginning of hatching – yolk-sack resorption period

Thus, the hatchability and survival of sea trout in early development chronically (38–58 days) exposed to 0.2 or 0.3 mg/l of copper can have a great impact on the health status of sea trout in the following stages of development.

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ILGALAIKIS VARIO POVEIKIS ŠLAKIUI (*SALMO TRUTTA TRUTTA* L.) ANKSTYVOJE ONTOGENEZĖJE

S a n t r a u k a

Letaliniu testu siekta nustatyti vario toksiškumą, panaudojus test-objektą – šlakį (*Salmo trutta trutta* L.) ankstyvose jo vystymosi stadijose (ikrai, lervos). Toksiškumo kriterijus buvo embrionų ir lervų mirtingumas. Vario poveikis tirtas trimis etapais: 1. Nuo „akutės" stadijos iki trynio rezorbcijos (58 paros); 2. Nuo ritimosi pradžios iki trynio rezorbcijos (44 paros); 3. Nuo vienadienių lervų iki trynio rezorbcijos (38 paros). Nustatyta, kad embrionų ir lervų mirtingumas priklauso nuo vario koncentracijos, nuo paveiktos vystymosi stadijos ir nuo poveikio ekspozicijos. Ritimosi periodas yra vienas jautriausių periodų ankstyvoje žuvų ontogenezėje.

Raktažodžiai: šlakis, ankstyvos vystymosi stadijos, vario toksiškumas, ilgalaikis poveikis, mirtingumas