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The impact of zinc on the bacterial abundance in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae

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The paper presents an estimation the effect of zinc (0.13 mg/l, 0.5 mg/l, 2 mg/l and 8 mg/l in water) on the abundance of bacteria in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae. Populations of total aerobic heterotrophic bacteria present in the intestinal tract of rainbow trout larvae, estimated using the dilution plate technique, were reduced significantly in comparison with control after exposure to 0.5 mg/l, 2 mg/l and 8 mg/l of zinc. The abundance of total heterotrophic bacteria decreased most significantly after exposure of larvae to 2 mg/l of zinc and was one order of magnitude lower in comparison with control. The same trend was observed for the abundance of proteolytic and total coliform bacteria in the intestinal tract of rainbow trout larvae after exposure of larvae to 2 mg/l of zinc. Zinc is toxic to bacteria in the intestinal tract of rainbow trout larvae, but at significantly higher concentrations in comparison with copper ions.

Key words: zinc, fish larvae, intestinal tract, bacteria

INTRODUCTION

Heavy metals are long-term contaminants with the ability to accumulate in animals, plants, soil and have no natural way to be removed. Most hazardous are toxic heavy metals: lead (Pb), manganese (Mn), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn) and their soluble compounds (Navas, Lindhotfer, 2005). Some heavy metals have no known beneficial biological function, while others play essential roles in physiological reactions. Zinc and copper ions are important in cellular metabolism, acting as co-factors in a number of important enzymes. However, they become toxic when elevated concentrations are introduced into the environment (Karan et al., 1998; McGeer et al., 2000). Zinc is a widely used heavy metal, and total zinc concentrations in European rivers range from nmoles per litre to near hundred μmoles per litre in the most polluted ones (Whitton et al., 1982), the latter comprising around 10% of 176 European rivers investigated (Stanners, Bourdeau, 1995). Zinc is the most common pollutant of Lithuanian inland waters, entering them with industrial and municipal wastewaters (Svecevičius, 1999).

For fish, two major routes of absorption are the gut and the gills. In freshwater fish, water-borne zinc is primarily absorbed via the gills, whereas uptake via the gastrointestinal tract is associated with dietary, particulate and sedimentary zinc. The mechanism of zinc uptake in the alimentary canal of freshwater fish is less studied (Glover, Hogstrand, 2002). The toxicity of zinc to fish has been widely studied, and a considerable number of experimental data are available. The physiology and toxicology of zinc in fish was investigated by Hogstrand and Wood (1996). Laboratory tests were conducted on fishes of five species common to Lithuania, to estimate their sensitivity to acute toxicity of zinc. The 96-hour median lethal concentration (96-hr LC50) values obtained from the tests ranged from 3.79 to 11.37 mg/l of zinc. The sequence of sensitivity of the tested fish species was found to be as follows: rainbow trout > three-spined stickleback > perch > dace > roach (Svecevičius, 1999). According to Clearwater et al. (2002), daily doses of 9–12 mg Zn kg^{-1} body weight d^{-1} in laboratory-prepared diets were toxic to three fish species: carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and guppy (*Poecilia reticulata*). Zinc exposure produced marked changes in the chemical composition of liver and muscle tissues of murrel (*Channa punctatus*) (Malik et al., 1998). Chronic exposure of fish to Cu, Cd or Zn ions has been shown to cause a variety of behavioural, biochemical and physiological changes including loss of appetite, reduced growth, decreased aerobic scope and mortality (McGeer et al., 2000). Although zinc and copper are required as microelements in the cells, the results of investigation of Gioda et al. (2007) have shown that the sublethal concentrations of these metals can change the biochemical parameters in fish *Leporinus obtusidens* and thus alter the normal cellular function.

Many previous studies have documented shifts in the microbial community structure under the influence of metals (Montuelle et al., 1994; Utgikar et al., 2003; Sverdrup et al., 2006). Utgikar et al. (2003) have shown that both copper and zinc ions have toxic inhibitory effects on sulphate-reducing bacteria. According to Sverdrup et al. (2006), zinc does not affect the total microbial biomass, but it had a significant effect on most of the other parameters measured. The microbial community composition was grouped differently from controls, even at the lowest concentrations of zinc added.

Our earlier investigations demonstrated that copper and a mixture of five metals (Cu, Zn, Ni, Cr, Fe) decreased the abundance and reduced the diversity of bacteria in the digestive tract of rainbow trout (Mickėnienė, Šyvokienė, 1998, 2001).

It is generally believed that the processes of bacterial colonisation in early developing fish larvae are complex and depend upon the bacterial flora of eggs, live feed and larval rearing water (Ringø, Birkbeck, 1999). Now it seems acceptable that the primary transient microflora will become established in the larval stage of fish and will develop into persistent flora in the juvenile stage or after metamorphosis.

The aim of this work was to examine the impact of zinc on the abundance of bacteria in the intestinal tract of rainbow trout larvae.

MATERIALS AND METHODS

The experiment was performed in the Experimental Aquarium of the Institute of Ecology of Vilnius University. Rainbow trout (*Oncorhynchus mykiss*) larvae were obtained from a fish hatchery. Fish were acclimated in artesian water and were transferred to exposure chambers after four hours. The chronic experiment was initiated with 9-day-old larvae and finished after the completion of yolk-sac resorption (total exposure time 20 days). The initial mean weight of fish was 81.2 ± 2.04 mg.

Chemically pure zinc sulphate ($ZnSO_4 \cdot 5H_2O$) dissolved in distilled water was used as a stock solution. The required concentration was calculated according to the amount of zinc. Toxicity of zinc concentrations ranging from 0.13 mg/l to 8.0 mg/l was assessed on the abundance of bacteria in the intestinal tract of rainbow trout larvae.

Fifty larvae were exposed to each concentration in duplicates. Larvae were reared in a cold and dark room. Control water was changed and test-solutions were renewed daily.

Water specifications during the period of investigations were as follows: the maintained temperature ranged from 11.4 to 13.9 °C, dissolved oxygen varied from 8.6 to 9.7 mg/l, pH measured about 7.8 and hardness about 250 mg/l as $CaCO_3$. At the end of the experiment (20 days after the start of exposure), the abundance of bacteria in the intestinal tract of 10 fish larvae per batch was analysed.

Populations of aerobic and facultative anaerobic heterotrophic bacteria in the intestinal tract of rainbow trout larvae were estimated using the dilution plate technique. In animals of such a small size, dissection is unworkable. Therefore, the isolation of bacteria from the intestinal tract of larvae was reduced to the preliminary procedures for surface bacteria removal. The majority of the remaining bacteria were treated as being of intestinal origin. Ten fish larvae were washed three times in autoclaved water, thereafter; animal surfaces were treated with 70% ethanol, washed again three times in autoclaved water and homogenized.

The homogenates of animals were weighed aseptically and placed into test tubes. Then nine volumes of diluents (sterile physiological solution) were added and diluted up to 10^6. Subsamples of 0.05 ml of the dilution (at least 3 dilutions expected to give 30 to 200 CFU – colony forming units) were placed on three different nutrient media. The plated media were incubated at 20 °C (except MacConkey agar plates which were incubated at 37 °C) for 5 days. The media chosen were: soya peptone agar (for isolation of total heterotrophic bacteria), milk agar (for proteolytic bacteria as a separate group of heterotrophic bacteria): 1 l distilled water, 20 g agar, 40 ml skimmed milk; MacConkey agar (for isolation of total coliform bacteria). Bacterial colonies appearing on each plate were counted, and counts of viable bacteria (CFU g^{-1}) of larvae homogenate were obtained.

The differences in bacterial counts were assessed using the one-way analysis of variance (ANOVA) technique. In all cases, treatments were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Zinc is a widely used heavy metal, and in the opinion of Paulsson et al. (2000) the toxicity of zinc to aquatic life therefore seems to be strongly underestimated.

It was revealed that in the intestinal tract of control rainbow trout larvae counts of total heterotrophic bacteria amounted up 6.5 log CFU g^{-1}. According to Ringø and Birkback (1999), gut bacteria concentration in the intestinal tract of fish larvae increased soon after hatching, and the colonization of larvae intestine seems to follow a two-step pattern. Populations of aerobic heterotrophic bacteria present in the gastrointestinal tract of turbot larvae, estimated using the dilution plate technique, increased approximately from 4×10^2 bacteria larva^{-1} on day 3 post-hatching to approximately 10^5 bacteria fish^{-1} on day 16 post-hatching (Ringø & Vadstein 1998).

Our data showed that after exposure of rainbow trout larvae to 0.13 mg/l of zinc the abundance of total heterotrophic bacteria in the intestinal tract of larvae was at same level as in control (Fig. 1).

In our experiments, the density of total heterotrophic bacteria in the intestinal tract of larvae was reduced significantly in comparison with control after exposure to 0.5 mg/l, 2 mg/l and 8 mg/l of zinc. The abundance of total heterotrophic bacteria decreased most significantly after exposure of larvae to 2 mg/l of zinc and was one order of magnitude lower in comparison with control.

Our earlier investigations demonstrated that long-term exposure (3 months) of the rainbow trout (10.8–12.2 cm in total length and 25.0–30.0 g in total weight) to a heavy metal (0.1 and 0.2 mg/l of copper) influenced the abundance of bacteria in the digestive tract. The densities of heterotrophic bacteria in the digestive tract after exposure to 0.1 mg/l of copper decreased two times compared to control (Mickėnienė, Šyvokienė, 1998). The abundance and diversity of bacteria in the digestive tract of rainbow trout decreased under exposure to a mixture of five metals (Cu, Zn, Ni, Cr, Fe) (Mickėnienė, Šyvokienė, 2001). After 48 hours of exposure to 100% of a heavy metal mixture, the abundance of heterotrophic bacteria in the digestive tract of fish (17–18 cm in total length and 42–48 g in total weight) decreased 7 times compared to that of control. After a 96-hour exposure, the abundance of heterotrophic bacteria in the digestive tract of the rainbow trout decreased even more. The heavy

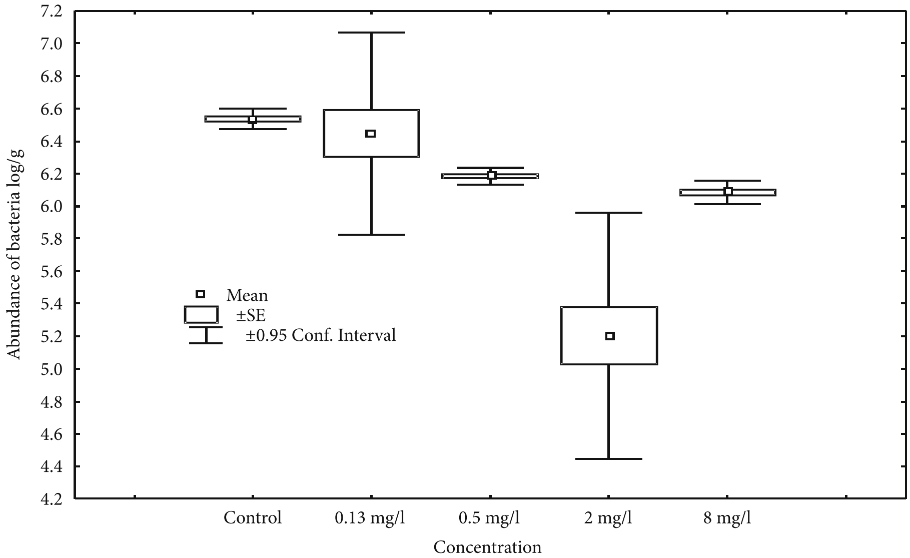

Fig. 1. Impact of zinc on the abundance of total heterotrophic bacteria in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae

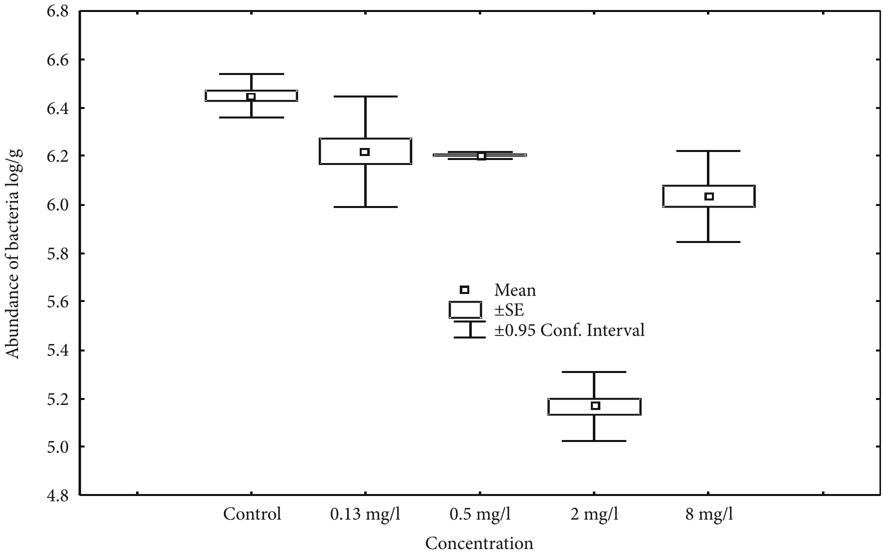

Fig. 2. Impact of zinc on the abundance of proteolytic bacteria in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae

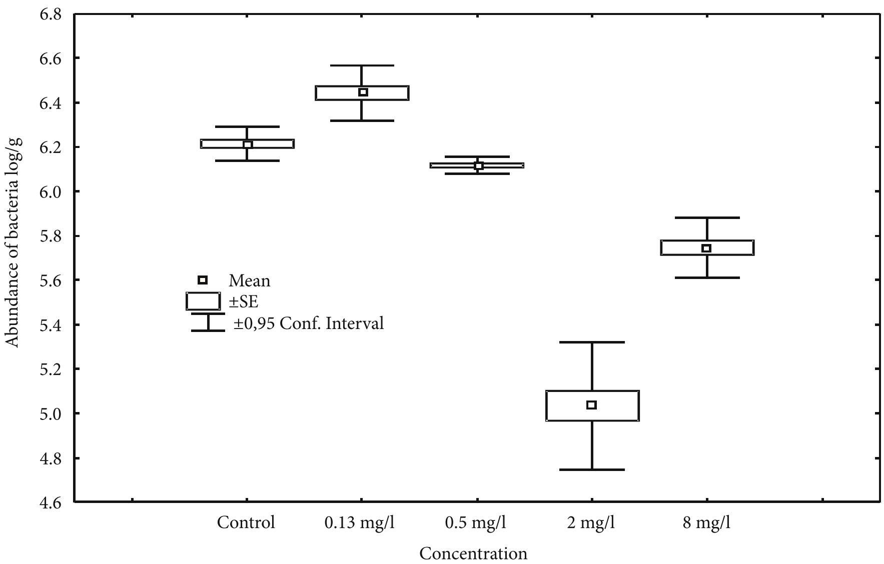

Fig. 3. Impact of zinc on the abundance of total coliform bacteria in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae

metal mixture reduced the diversity of bacteria in the digestive tract of rainbow trout. Bacteria of the genera *Flavobacterium*, *Micrococcus* and of the family *Enterobacteriaceae* disappeared from the bacteriocenosis of the digestive tract.

Šyvokienė et al. (2006) found that the density of total heterotrophic bacteria was reduced significantly in comparison to that of control after exposure to 0.21–0.28 part of 96-hour LC50 of an equitoxic copper–zinc mixture, but bacterial counts were not significantly different from each other. The abundance of total heterotrophic bacteria in the intestinal tract of fish was two orders of magnitude lower compared to that of control fish.

The total heterotrophic bacterial densities increased in the intestinal tract of rainbow trout larvae after exposure to 8 mg/l of zinc in comparison with exposure to 2 mg/l of zinc.

The same trend was observed for the abundance of proteolytic bacteria in the intestinal tract of rainbow trout larvae, which decreased even after exposure of larvae to the lowest test concentration of zinc – 0.13 mg/l, but the difference was not significant (Fig. 2). Proteolytic bacterial densities, like the densities of total heterotrophic bacteria, decreased most significantly after exposure of larvae to 2 mg/l of zinc. The abundance of proteolytic bacteria in the intestinal tract of rainbow trout decreased four times after exposure to 0.1 mg/l of copper, and they disappeared after exposure to 100% of a heavy metal mixture (Mickėnienė, Šyvokienė, 1998, 2001). Mickėnienė et al. (2007) found that total heterotrophic and proteolytic bacteria in the intestinal tract of rainbow trout larvae were reduced significantly in comparison with control larvae after exposure to 0.03, 0.06 0.13 mg/l of copper. Handy et al. (2000) showed that copper was mostly absorbed in the middle and hind intestine of the African walking catfish (*Clarias gariepinus*). A heavy metal accumulated in fish digestive tract affects the bacterioflora and consequently changes the abundance of bacteria. According to Sverdrup et al. (2006), one of the most pronounced changes in soil under the effect of zinc was the increase of fungi and a decrease of bacteria with increasing the concentrations of zinc. The results reported by earlier mentioned authors also prove that bacteria are sensitive to zinc ions.

The abundance of total coliform bacteria increased after exposure of larvae to the lowest test concentration of zinc (0.13 mg/l), but the difference was not significant (Fig. 3). After exposure of larvae to 2 mg/l of zinc, the abundance of total coliform bacteria in the intestinal tract decreased most significantly, as did the abundance of total heterotrophic and proteolytic bacteria.

Cultivable total heterotrophic as well as proteolytic and total coliform bacterial densities increased upon exposure to the highest test concentration of zinc (8 mg/l), possibly as a result of the release of organic carbon from the poisoned copper-sensitive organisms.

Zinc is toxic to bacteria in the intestinal tract of rainbow trout larvae, but at significantly higher concentrations in comparison with copper ions. The density of total heterotrophic bacteria in the intestinal tract of rainbow trout larvae was reduced significantly in comparison with control after exposure to 0.03, 0.06, 0.13 mg/l of copper (Mickėnienė, Šyvokienė, Stasiūnaitė, 2007). A similar influence of zinc on the density of bacteria was observed at 2 mg/l.

It is well known that fish harbor the communities of microorganisms in the intestinal tract that also fulfil the necessary functions (Sugita et al., 1997, 2002). Correspondingly, xenobiotics that inhibit bacterial abundance and qualitative composition in the intestinal tract of fish larvae, as well as their activities, will have negative effects on food assimilation efficiency and antibacterial abilities against fish pathogens. This may slow down their further development and disturb their vital functions. An insight into the response of bacterioflora in the intestinal tract of fish to xenobiotics may help to explain variations in growth. Fish larvae with well-developed microflora in their digestive tract have a better chance of adapting to changing nutritional substrates, because a wide spectrum of enzymes produced by bacteria is involved in digestion.

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CINKO POVEIKIS BAKTERIJŲ GAUSAI VAIVORYKŠTINIO UPĖTAKIO (O*NCORHYNCHUS MYKISS*) ŽARNYNE

Santrauka

Ištirtas cinko vandenyje poveikis bakterijų gausai vaivorykštinio upėtakio lervų žarnyne. Bendrų heterotrofinių bakterijų, išskirtų praskiedimo metodu, gausa upėtakio lervų žarnyne sumažėjo paveikus lervas 0,5 mg/l, 2 mg/l ir 8 mg/l cinko. Bendrų heterotrofinių bakterijų gausą lervų žarnyne labiausiai paveikė 2 mg/l cinko, nes jų gausa sumažėjo 10 kartų. Tokia pati tendencija veikiant lervas 2 mg/l cinko nustatyta proteolitinių ir bendrų žarnyno bakterijų gausai. Nustatyta, kad cinkas mažiau toksiškas upėtakio lervų žarnyno bakterijoms, lyginant su variu.

Raktažodžiai: cinkas, žuvų lervos, žarnynas, bakterijos