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# Impact of municipal wastewater chemicals on the rainbow trout (*Oncorhynchus mykiss*) in its early development

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**Pranė Stasiūnaitė,  
Nijolė Kazlauskienė**

*Institute of Ecology,  
Akademijos 2,  
LT-2600 Vilnius, Lithuania*

The effect of 100%-treated municipal wastewater of the city of Vilnius discharged into the Neris River below Vilnius in February 1998–1999 on rainbow trout eggs and yolk-sac larvae was estimated. In order to understand the impact of the effluent on rainbow trout early life stages, their mortality and morpho-physiological parameters were studied. The tests were performed with 4–6 h eggs in 1998 and with 4–6 h eggs and eye-stage embryos in 1999 at 9–11 °C. The total exposure time was 70 days (tests with 4–6 h eggs) and 28 days (test with eye-stage embryos).

The 100%-treated effluent was found to interfere with hatching process and caused a significant decline in the number of hatching alevins. Alevins at hatch were smaller in size and grew more slowly during the pre-feeding period. The heart rate of test-embryos and alevins was not uniform through the early development and depended on the stage of development. In the test with eyed embryos the respiration frequency of treated alevins was slower, their behavior differed from that of control alevins.

Based on the above data we consider that the sublethal effects of the effluent on the rainbow trout at early stages of development can affect the density of larvae in time, survival potential, oxygen supply to tissues and organs, growth and behavior of the organisms in their further development.

**Key words:** effluent, ambient toxicity, early ontogenesis, fish

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## INTRODUCTION

Pollution of surface waters by anthropogenic chemicals is a major problem, because the contaminations are rarely limited to only one single toxicant, but typically involve a multitude of xenobiotics. Experimental results reveal that reliable predictions of mixture toxicities can be derived from the data on response concentration of single toxicants by applying two different concepts: concentration addition in cases of similarly acting mixture components and independent action for substances with dissimilar mechanisms of action [9] and other available knowledge [2, 7, 11]. The degree of hazard also depends on the rate of discharge persistence and distribution in the aquatic and bioaccumulation potential [1, 24].

Most fish will selectively accumulate toxicants from surrounding water independently of their actual concentration. Pathological effects in the organisms occur when the rate of influx exceeds the rates of detoxification and excretion.

Reproductive development of fish is a continuous process, though the ontogeny and the sensitivity of organisms to a particular physical or chemical stressor varies according to the stage of development. Field studies are difficult to monitor and not enable to investigate the sublethal effects of chemical compounds on specific life history stages. Those studies specifically focus on evaluating the growth, health and contaminant residues in the organisms. Laboratory investigation methods (especially long-term tests) are more useful, because they can induce effects similar to acute ones, in addition to the effects that develop slowly [6]. Embryo-larval stages of feral fish species including rainbow trout have been widely used in toxicity testing and usually were highly sensitive to aquatic contaminants [33, 34 and others]. Although much studies on the effect of single, pure substances on fish early development have been performed [35], data on the impact of complex combinations contained in the discharge of wastewater treatment plants are sparse. More infor-

mation is known on the effects of pulp and paper mill effluents on reproductive abnormalities, embryo and fry quality [1, 15, 22, 30, 32].

The aim of the present study was to evaluate the possible toxic effects of 100%-treated municipal wastewater of the city of Vilnius, discharged into the Neris River below Vilnius in February 1998–1999 on rainbow trout eggs and alevins.

## MATERIAL AND METHODS

The experiments were carried out on eggs and larvae of rainbow trout, *Oncorhynchus mykiss*. Freshly fertilised eggs were obtained from the Žeimena fish hatchery near Vilnius. The toxicity of 100%-treated municipal wastewater of the city of Vilnius, discharged into the Neris River below Vilnius in February 1998–1999 on rainbow trout mortality, hatching, physiological parameters and growth were investigated. Chemical and physical analysis of the municipal effluents (Table 1) were performed at the Analytical Wastewater Control Laboratory of the Vilnius Region Environmental Protection Department of the Environmental Ministry.

Table. Chemical and physical characteristics of 100%-treated municipal wastewater of the city of Vilnius discharged into the Neris River below Vilnius in February 1998–1999 (all values are mg/l unless otherwise noted)

Parameters	1998	1999
pH	7.4	7.25
Alcalinity (as HCO <sub>3</sub> <sup>-</sup> )	–	128
BOD <sub>7</sub>	43	34
Total phosphorus	2.83	2.3
Total nitrogen	14.0	17.52
Suspended solids	0.32	8.0
Mg <sup>2+</sup>	–	10.4
Ca <sup>2+</sup>	–	59
NO <sub>2</sub> <sup>-</sup>	0.30	0.22
NO <sub>3</sub> <sup>-</sup>	1.13	8.8
PO <sub>4</sub> <sup>3-</sup>	–	2.1
Cl <sup>-</sup>	–	186
NH <sub>4</sub> <sup>+</sup>	11.05	6.5
Cu	0.012	0.041
Ni	0.044	0.02
Zn	0.068	0.18
Cr	0.15	0.017
Fe	0.23	0.15
Cd	0.03	0.02
Pb	0.1	0.1
Mn	0.09	0.3
Co	0.023	–
Oil hydrocarbons	0.4	–

The experiments were performed with 4–6 h eggs in 1998 (test 1) and repeated with 4–6 h eggs (test 2) and eye-stage embryos in 1999. The tests were performed under controlled conditions and involved a long-term exposure. Total test duration was 70 days for 4–6 h eggs and 28 days for eye-stage embryos at 9–11 °C. 100–50 eggs were exposed to the effluent in duplicates. The eggs were incubated in a cold and dark room. The control water was changed and the effluent was renewed at 1-day intervals. Dissolved oxygen concentration was no less than 6.5 mg/l. The pH of control and test water was 7.3 and 7.2–7.5, respectively. The control water hardness was approximately 250 mg/l. The test water hardness was not measured.

The mortality of eggs and sac-fry was recorded daily. In experiments with 4–6 h eggs, dead embryos were placed in a saturated NaCl solution until they became transparent. After clearing, eggs were examined under a light microscope to evaluate infertilisation. 10.3–28.2% of test and 26.5–28.3% of control eggs were infertile. The initial and final biomass (total dry weight) of sac-fry was analysed. Ten newly hatched and 10–25 alevins were weighed. The data were expressed as means ± SE. Significant differences were calculated using the unpaired Student's test.

## RESULTS

**Tests with 4–6 h eggs.** Mortality of rainbow trout eggs was not the predominant response to the effluent studied through cleavage, formation of layers and morphogenesis in comparison to control (7.6 and 1.5%, respectively). Differences in development rates between effluent-treated eggs and controls were not detected. Rainbow trout embryo test organisms and control ones began to hatch 34 days after the start of fertilisation. The control embryos hatched in 7–8 days. The hatching of test embryos was delayed to 12 days and a significant increase in larvae mortality occurred as a result of yolk-sac abnormalities, retarded growth and development. The mortality of abnormal treated and control larvae reached 41.7% and 22.5%, respectively. Part of embryos (up to 4.4% in control and 12.1% in test) remained in envelope and never hatched. The hatching in test objects and controls reached 38.6% and 71.6%, respectively.

The impact of the effluent on yolk-sac larvae mortality after hatching was less pronounced. By the end of experiment (30 days after hatching) the mortality of exposed as well as control alevins was 11.6% and 7.4%, respectively. The data on mortality are summarised in Fig. 1.

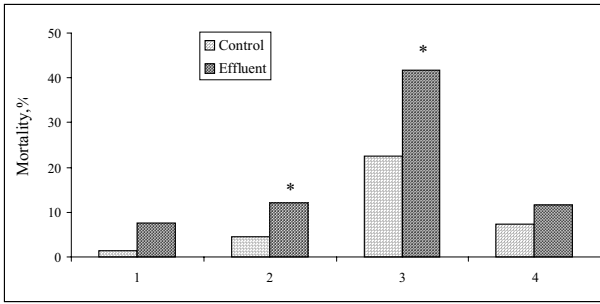


Fig. 1. Impact of effluent on rainbow trout mortality. Exposure 4–6 h after fertilisation, test 1: 1 – embryo till hatching, 2 – unhatched embryo, 3 – alevin during hatching, 4 – alevin 30 days after hatching. \* Significantly different from control ( $P < 0.05$ )

The effluent evoked an impairment of the normal heart rate of rainbow trout embryos, 1- and 10-day-old alevins (Fig. 2). The heart work of embryos was depressed slightly before hatching and the embryos often showed a tonus-like tail movements, while the heart rate of 1- and 10-day alevins was deranged more significantly.

One-day old alevins were significantly smaller in size compared to control. At the end of experiment, 30 days after hatching, the growth of effluent-treated alevins was reduced, the amount of yolk remain-

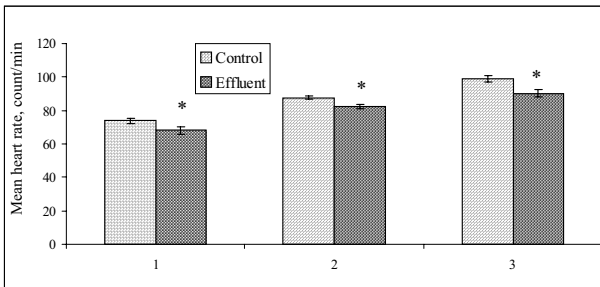


Fig. 2. Impact of effluent on mean heart rate of rainbow trout,  $n = 10$ . Exposure 4–6 h after fertilisation, test 1: 1 – embryo, 2 – 1-day-old alevin, 3 – 10-day-old alevin. \* Significantly different from control ( $P < 0.05$ )

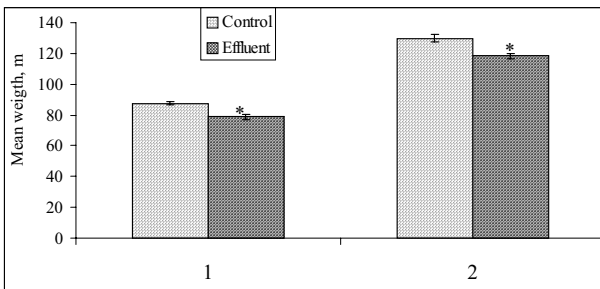


Fig. 3. Impact of effluent on mean weight of rainbow trout. Exposure 4–6 h after fertilisation, test 1: 1 – 1-day-old alevin,  $n = 10$ , 2 – 10-day-old alevin,  $n = 25$ . \* Significantly different from control ( $P < 0.05$ )

ing in their organisms was greater than in control. The effect of the effluent on the mean weight of 1-day- and 30-day-old alevins is shown in Fig. 3.

The toxicity of the effluent for 4–6 h eggs was determined repeatedly in February 1999 (test 2). The effects of the effluent on rainbow trout embryos was similar to those observed in 1998 (test 1). They were expressed in a prolonged incubation period, reduced number of normal larvae, however, embryo mortality till hatching was higher than in test 1. During the prefeeding period the effect of effluents on the mortality of alevins was not significant. The mortality of controls was 5.2% and of test organisms 9.5% (Fig. 4).

Disturbances in heart rate of embryos and alevins and reduction in size were also noted (Figs. 5 and 6). The effluent depressed the heart work of late embryos, but stimulated the heart work of alevins after hatching. The individual heart beat frequency of 10-day-old alevins varied from 70–80 to 120 beats per minute, whereas variations in the mean value were not significant compared to control. One-day old alevins were smaller in size and the growth of fish was poor through 30 days after hatching.

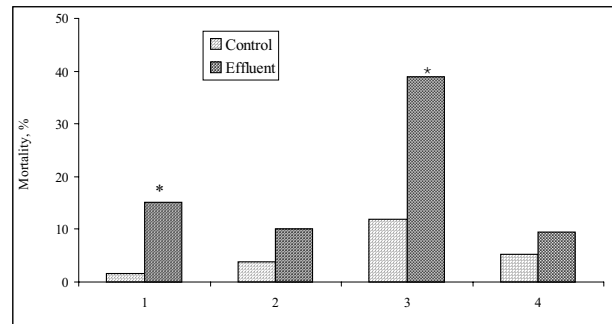


Fig. 4. Impact of effluent on rainbow trout mortality. Exposure 4–6 h after fertilisation, test 2: 1 – embryo till hatching, 2 – unhatched embryo, 3 – alevin during hatching, 4 – alevin through 30-day after hatching. \* Significantly different from control ( $P < 0.05$ )

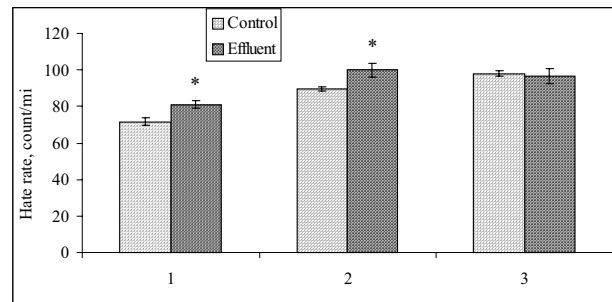


Fig. 5. Impact of effluent on mean heart rate of rainbow trout,  $n = 10$ . Exposure 4–6 h after fertilisation, test 2: 1 – embryo, 2 – 1-day-old alevin, 3 – 10-day-old alevin. \* Significantly different from control ( $P < 0.05$ )

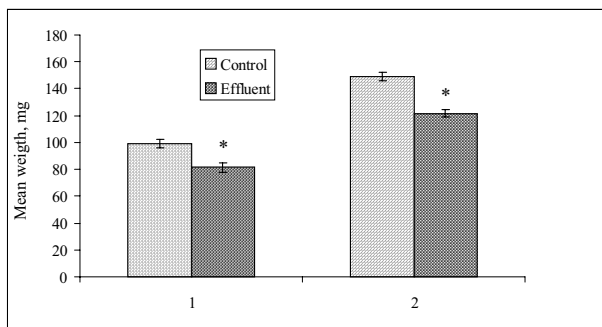


Fig. 6. Impact of effluent on mean weight of rainbow trout. Exposure 4–6 h after fertilisation, test 2: 1 – 1-day-old alevin, n = 10, 2 – 10-day-old alevin, n = 25. \* Significantly different from control ( $P < 0.05$ ).

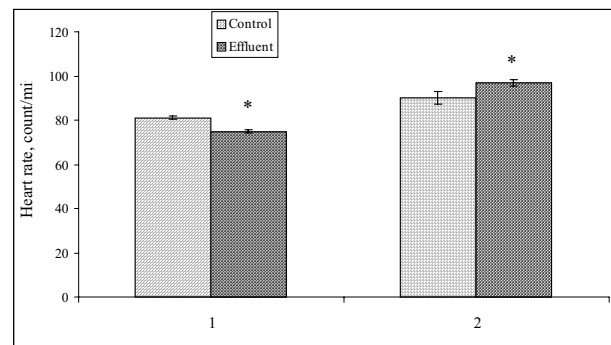


Fig. 8. Impact of effluent on mean heart rate of rainbow trout, n = 10. Exposure of eyed-egg stage: 1 – embryo, 2 – 10-day-old alevin. \* Significantly different from control ( $P < 0.05$ ).

The effluent affected the yolk-sac utilisation and body pigmentation almost in 60% of test alevins.

**Tests with eye-stage embryos.** In the case of eyed embryos, exposure was initiated 26 days after fertilisation. No significant difference in mortality was found between exposed and control eggs. The mortality reached 18% in the exposed and 14% in the control organisms. The development rates of test and control embryos were not uniform. The development of effluent-treated embryos was delayed, and test embryos began to hatch 3–4 days later than controls. Hatching was completed within 8–9 days in experiment and during 5 days in control. Hatchability in test embryos was significantly lower, because many embryos were unable to perform complete hatching and died partially emerged from the chorion or half-hatched. Only 41.5% of the organisms hatched in the wastewater and 89.50% in control. The toxicity of the effluent was not observed during alevin development. The mortality of 10-day-old organisms reared in the discharge and in the control water was similar (up to 10–12%) (Fig. 7).

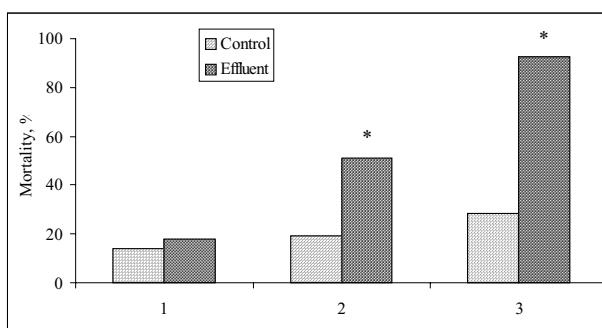


Fig. 7. Impact of effluent on rainbow trout mortality. Exposure of eyed-egg stage: 1 – embryo, 2 – alevin during hatching, 3 – alevin (hatching 10 days after hatching). \* Significantly different from control ( $P < 0.05$ ).

Chronic effluent toxicity in embryos and 10-day-old alevins leads to heart work disturbances. The heart rate was significantly depressed in embryos, but significantly stimulated in alevins (Fig. 8). The respiration frequency of alevins was slower than that of control (on average  $83.1 \pm 2.7$  curt/min and  $104.0 \pm 3.2$  curt/min, respectively). The growth of the alevins after hatching was not identical, either. Many of the alevins did not swim at all but were lying on the bottom, displaying uncoordinated spurts or frantically turning circles. The mean body mass in the exposed 10-day-old organisms was  $72.7 \pm 2.6$  mg compared to  $94.4 \pm 2.6$  mg in the controls.

## DISCUSSION

From the data obtained it is evident that the chemical materials and chemical compounds entering the inland water from water treatment plants are environmental contaminants with high ecotoxicological risks for aquatic organisms, including fish species. The effect of municipal effluents on rainbow trout was modified by the protection of the developmental stage. The lower toxicity of municipal effluents to younger embryonic stages can probably be explained by the protection of these life stages by the chorion which acts as a barrier for chemicals and they cannot reach the target organs [20, 25, 31]. On transition from embryo to alevin, the organisms become more sensitive to living conditions than those that had completed morphogenesis. The effect of effluents on hatching such as prevention of hatching and the mortality of non-hatched eggs observed after regular hatching may be caused by incomplete dissolution of the chorion and by oxygen deficiency in the growing embryo. Those effects can be negative for the density of larvae in time and space and may desynchronize food availability at the time of first feeding [26].

In agreement with literature data, most experiments on the effects on environmental contaminants on the early development of fish were conducted with heavy metals applied separately. However, only a few investigations were done on the dynamics of heavy metals in mixtures that may produce unexpected effects which are not pronounced in single substances. In our previous studies [28], the effect of 8 heavy metals in mixture was very strong on rainbow trout hatching and similar to the effect noted for the effluent. The concentrations of metals in the mixture were adequate to mean annual levels of heavy metals found in discharges from the Vilnius City into the Neris River in 1996–1997. The effect of the contents of five common heavy metals found in the industrial discharges from the city of Vilnius into the Neris River during 1990–1991 in mixture was also significant on hatching and other parameters of rainbow trout [29]. Studies on the effects of other environmental contaminants on fish hatching are also sparse. The petroleum hydrocarbons (water-soluble fraction of crude oil) are known to delay hatching of rainbow trout [10, 14] and *Theagra chalcogramma* [5]. High concentrations of petroleum hydrocarbons can sometimes exert narcotic effects on late ready-to-hatch embryo [5 and others]. Chlorine reduced the hatching of striped bass (*Morone saxatilis*), white perch (*Morone americana*) and blueback herring (*Alosa aestivalis*). Their larvae were shorter at hatching, larval development was inhibited, and in the herring abnormal larvae were observed [21]. Exposure of rainbow trout eggs and alevins to un-ionized ammonia caused mortality at or after hatching, retarded larval growth, inhibited yolk-sac absorption [3].

Heart rate, size of alevins at hatch, and growth of organisms after hatching were the other parameters investigated in this study. Significant effects of effluents on heart beat frequencies may induce negative consequences for respiratory process, development, “viable hatch” and supply of tissues and organs with oxygen [8]. The environmental pollutants such as nitrite are accumulated by freshwater fish in the blood to the levels exceeding the environmental concentration. Nitrite enters the erythrocyte and oxidizes haemoglobin to form methaemoglobin which is unable to bind oxygen [4]. Changes in embryonic heart rate were also caused experimentally by other pollutants such as petroleum hydrocarbons and heavy metals [16, 17, 23, 27].

Growth was also an important indicator of the effects of effluents. Larval size and condition (*i. a.* amount of yolk reserves) are important factors influencing the successful initiation of feeding. The growth of fish is associated with many biochemical phenomena that occur in a somewhat regulated pat-

tern [19]. These biochemical changes may be indicators of many manifestations that occur later, including growth reduction. Under natural conditions, growth reduction in early development can result in a delay in sexual maturity.

In the environment, eggs would be exposed to the pollutants even before the time of spawning. Contaminants accumulated in the body of the adult fish would be transferred to the eggs during oogenesis making the probable effects on embryonic development more severe. Abnormal blastomere morphology has been observed in eggs collected from plankton or broodstock [13, 18]. Studies with a mechanical pulp mill effluent indicated that pre-exposure of brown trout parental fish affected the quality of eggs and milt and therefore had a negative impact on egg hatchability and fry growth [12]. In other words, in nature the concentrations of pollutants are not stable so that cause-and-effect linkages vary as well. Therefore it is necessary to evaluate the direct link between the initial and the final effects of environmental pollutants on the organisms, *i.e.* to estimate the hazard of toxicants for the reproduction endpoints and for the quality of the second generation.

## CONCLUSIONS

1. The rainbow trout egg stage was relatively sensitive to the effluent studied and very sensitive during hatching.
2. The effluent significantly affected the heart rate of rainbow trout embryos and alevins through a period of 30 days and 10 days after hatching (tests with 4–6 h eggs and eye-stage embryos, respectively).
3. The growth of the organisms was poor till hatching and through the pre-feeding period.

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Pranė Stasiūnaitė, Nijolė Kazlauskienė

**KOMUNALINIŲ NUOTEKŲ POVEIKIS ANKSTYVAM  
VAIVORYKŠTINIO UPĖTAKIO (*ONCORHYNCHUS  
MYKISS*) VYSTYMUISI**

**S a n t r a u k a**

Įvertinta išvalyto (100%) Vilniaus miesto komunalinio vandens, išleisto į Neries upę žemiau Vilniaus 1998–1999 m. vasario mėn., įtaka vaivorykštinio upėtakio ikrams ir lervoms. Siekiant nustatyti nuotekų poveikį ankstyvoms vaivorykštinio upėtakio vystymosi stadijoms, analizuota mirtingumas ir morfofiziologiniai požymiai. Tyrimai atlikti 1998 m. su ikrais, apvaisintais prieš 4–6 val., 1999 m. – su tokiais pat ikrais ir „akutės“ stadijos embrionais. Poveikis tęsėsi atitinkamai 70 parų ir 28 paras.

Nustatyta, kad dėl (100%) išvalytų komunalinių nuotekų sutriko embrionų ritimasis ir statistiškai patikimai sumažėjo išsiritusių lervų skaičius. Nuotekose išsiritusios lervos buvo mažesnės ir augo lėčiau pasyvios mitybos eigoje. Testembrionų ir lervų širdies darbas buvo skirtingas ir priklausė nuo vystymosi stadijos. Tyrimuose su „akutės“ stadijos embrionais test-lervos kvėpavo lėčiau, elgsena skyrėsi nuo kontrolinių.

Tyrimų duomenų pagrindu manytume, kad subletalinis nuotekų poveikis ankstyvam vaivorykštinio upėtakio vystymuisi gali turėti neigiamos įtakos lervų tankiui, gyvybingumui, organų ir audinių aprūpinimui deguonimi, augimui ir elgsenai jiems toliau vystantis.

**Raktažodžiai:** nuotekos, toksiškumas, ankstyva ontogenezė, žuvis