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THE USE OF RAINBOW TROUT *ONCORHYNCHUS MYKISS* IN EARLY ONTOGENESIS FOR THE WATER TOXICITY ASSESSMENT

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ABSTRACT

Toxicity of some heavy metals (HM), heavy metal model mixture (HMMM), orimulsion, crude oil and water of some biotopes of Lake Druksiai was evaluated by use of the fish in all stages of development (embryos, larvae, adult fish). The rainbow trout was very sensitive test-organism to the effect of different kind pollutants and the most sensitive to the impact of pollutants were fish at early stages of development i.e. larvae and embryos. The integrated biological parameters of fish at all stages of development (survival, growth rate, and relative mass increase) were the most sensitive ones. In accordance with the sensitivity to the impact of heavy metals, animals in the earliest stages of their development were more sensitive than plants, but the plants in some cases were more sensitive to heavy metals than adult fish. Therefore, plants as well as animals – rainbow trout (Oncorhynchus mykiss) of different life stages, as test-organisms, are recommended for the inclusion of the complex biotests for the assessment of the impact of heavy metals. The complex of the most sensitive parameters investigated of rainbow trout can be successfully used for the water toxicity assessment containing different kind pollutants. The data obtained let predict the impact of pollution not only to the physiological status of aquatic organisms but as well as to their survival in natural water bodies.

KEYWORDS

Rainbow trout; Embryos; Larvae; Adult; Biological parameters; Heavy metals; Heavy metal model mixture; Crude oil; Orimulsion; Water of biotopes; Acute toxicity; Long-term toxicity

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1 INTRODUCTION

Industrial waste waters discharging into natural aquatic systems usually contain complexes of heavy metals, however majority of ecotoxicological studies on the metal toxicity evaluate effects of single metals on fish and usually on one level of biological parameters [1, 2]. Complex studies of the toxicity of effluents using different fish species and evaluating their biochemical, physiological and pathological parameters demonstrated different sensitivity of indices studied [3, 4, 5] and the authors emphasised the necessity of assessment of a complex of various level biological parameters of fish organism. Thereby contemporary approach in toxicity testing of effluents should be based on short and long-term complex studies of parameters various biological level of fish [6, 7].

Organisms more often than not are much more sensitive and quick-to-react to low concentrations ofdtoxicants than chemical and physical methods. Therefore, over the last years, while making an ecological examination and environmental monitoring, methods of biological impact assessment are widely applied and improved by using sensitive test organisms. However, the most comprehensive evaluation of sublethal toxicity of effluents is achieved when studies are performed on fish in all stages of development. Toxicity tests on fish in ontogenesis have shown more reliable detection and better quantification of heavy metal mixture [8, 9, 10] or single metal toxicity [11 12, 13, 14]. The authors proposed to use embryo-larval tests for predicting after-effects of heavy metals and their mixtures to fish organism and even to population [15, 16].

Our previous studies demonstrated the particular sensitivity of fish in early ontogenesis to different kinds of plants, e.g. orimulsion, heavy fuel oil, crude oil and even to oil spill dispersant (SIMPLE GREEN) [17, 18, 19].

The objective of our study was to evaluate the toxicity of some heavy metals (HM), heavy metal model mixture (HMMM), orimulsion, crude oil and water of some biotopes of Lake Druksiai to fish in all stages of development (embryos, larvae, adult fish); to determine and compare the sensitivity of fish in some development stages to toxicants under study with the sensitivity of different phylogenetic level organisms.

2 MATERIAL AND METHODS

2.1 Chemicals under study

Separate heavy metal stock solutions were prepared in distilled water by use of the following chemically pure (reagent grade) substances: $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ the final concentrations being recalculated according to the amount of heavy metal ions.

Heavy metal model mixture (HMMM) consisting of seven heavy metals was investigated. The formation of model mixture was carried out basing on available analytical data of average annual amounts of representative heavy metals in cooling waste waters discharging from Ignalina Nuclear Power Plant (Lithuania) into the Lake Druksiai during 1996. The stock solution of HMMM was prepared in acidified distilled water by use of the following chemically pure substances: $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 7H_2O$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $Cd(CH_3COO)_2 \cdot 2H_2O$, $MnSO_4 \cdot 5H_2O$, the final concentration being recalculated according to the amount of heavy metal ion. Concentration of HMMM solution considered to be equal to 1% was: Cu - 0.0075; Zn - 0.064; Ni - 0.0021; Cr - 0.0028; Pb - 0.0142; Cd - 0.00018; Mn - 0.0099 mg/l correspondingly.

Orimulsion was obtained from Lithuanian Thermal Power Plant. The concentration of oil hydrocarbons was measured using infrared spectroscopy in the C-H region of the spectrum after solvent extraction. As well as it was found that water-soluble-fraction (WSF) amounted only 0.009 % of total weight of orimulsion. Whereas WSF of orimulsion made very small part of it and orimulsion itself has the property to disperse in all water volume during very short period of time, no regard was paid to this fraction in toxicity tests.

The concentrations of crude oil were chosen on the ground of our previous studies, which indicated that these concentrations of substances did not cause acute (during 96 h) mortality of larvae and adult rainbow trout. Petroleum hydrocarbons were extracted into carbon tetrachloride, then they were separated on the chromatographic column and their concentrations were measured by the AN-1 analyser at wave length $1/\lambda=2930$ cm^{-1}.

The water samples of Lake Druksiai were collected at the following biotopes: 7a - the zone of the discharge of industrial waste water and storm water into Lake Druksiai; 6a - the zone of the discharge of industrial waste water and treated municipal waste water into Lake Druksiai, 1 - the zone in the Lake Druksiai far from Ignalina Nuclear Power Plant (INPP).

All tests were performed using artesian water of high quality. Average hardness of water was approximately 284 mg/l as $CaCO_3$, alkalinity was 244 mg/l as HCO_3^-, mean pH was equal to 8.0, temperature was about 10±0 5° C, and oxygen concentration ranged from 8 to10 mg/l.

2.2 Test-organisms

Rainbow trout eggs and adults (one-year-old) were obtained from Zeimena hatchery and acclimated under laboratory conditions.

The tests were started with early-eyed embryos and they were ended before active feeding of larvae. 200-400 embryos were exposed to each concentration of toxicants and two replications were done. During all the tests morphological (total body mass in mg), physiological parameters (cardio-respiratory) such as heart rate (*HR*, counts/min), *gill ventilation frequency* (*VF*, counts/min), integrated parameters (relative mass increase in %) were recorded. Long-term tests on adult fish were performed for 14-28 days. The randomly sorted fish were transferred into aquarium of 100-l capacity. The control water and toxicants test solutions were renewed every day. During testing fish were fed every day. The body lengths and weights of test fish were measured at the beginning and at the end of exposure. The tissue weights were measured at the end of exposure and tissue-somatic indices: gill-somatic (GSI) and liver-somatic (LSI) of the test fish were calculated [6]. Gill ventilation frequency (GVF, counts/min) and "coughing" rate or gill-cleaning reflex (CR, counts/min) were measured during 3-minute periods for each test fish individually and mean value for 15 fish was calculated.

All acute (96 hours) and long-term (28-70 days) toxicity tests were conducted under semi-static conditions and fish mortality observations were made at 24-hour intervals.

The Median acutely lethal concentration (LC50) values and their 95% confidence intervals were estimated by use of the trimmed SPEARMAN-KARBER method [20].

The Maximum Acceptable Toxicant Concentrations (MATC) were estimated for every parameter by defining geometric mean between the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC), the method suggested by Rand and Petrocelli [21].

The significance of all the data obtained was determined by use of STUDENT'S t-test and χ^2 with P≤0.05.

3 RESULTS AND DISCUSION

3.1 Heavy metals (HM)

The data obtained (*see Table 1*) showed that calculated 96h LC50 values for development stages of *O. mykiss* were of 0.36-0.86 mg/l of Cu, of 0.48-3.8 mg/l of Zn. Cu was found to be the most toxic. The larvae of *O. mykiss* appeared to be the most sensitive to all tested metals, followed by embryos and adult fish. The comparison of 96h LC50 values of Cu in the test-organisms showed that the given index for the larvae was about 2.4 times lower than for embryos, and 1.8 times lower for adult fish [22].

Table 1. Acute toxicity of HM to rainbow trout at all stages of development.

Stages of development	96-hour LC50 (%)	95% confidence intervalo(%)
Cu		
Embryos (eye stage)	0.86	0.80 ÷ 0.96
Larvae	0.36	0.3 ÷ 0.4
Adult fish	0.65	0.6 ÷ 0.7
Zn		
Embryos (eye stage)	1.2	1.1 ÷ 1.4
Larvae	0.48	0.4 ÷ 0.5
Adult fish	3.8	2.7 ÷ 5.4

On the basin of the obtained data, we have arranged the following sequences of metal toxicity for each development stage of *O. mykiss* and for other test-organisms. The comparison of the sensitivity of rainbow trout and other test-organisms of different phylogenetic level to heavy metals (*see Table 2*) [22].

Investigations of the impact of heavy metals on test-organisms of different phylogenetic level showed that in most cases Cu was the most toxic metal to both plants and animals, but Zn, which is one of the least toxic metals to plants, was very toxic to animals. Summing up we can say that it is impossible to single out the most sensitive test-object among those studied that could be universal for the toxicants or at least for their groups.

Table 2. The sensitivity of rainbow trout and other test-organisms of different phylogenetic level to heavy metals.

Test-organisms	Heavy metals
O. mykiss: (LC50):	
Embryos	Cu> Zn >Ni >Fe >Cr
Larvae	Cu> Zn> Ni>Fe >Cr
Adult fish	Cu> Zn> Ni >Cr >Fe
L. sativum: (EC50)	Cr(VI)> Cu>Ni> Zn
S. polyrrhiza: (EC50)	Cu = Cr(VI)> Ni> Zn >Fe (II)
Tradescantia: (EC50)	Ni> Cu = Cr(VI)> Zn
Daphnia magna (LC50)	Cu> Cr >Zn >Ni >Fe

3.2 Heavy metal model mixture (HMMM)

Acute toxicity tests were conducted in order to determine basic toxic characteristics of HMMM studied. The results of acute toxicity tests are presented in (*see Table 3*) However, no significant difference between sensitivity of embryos and adult fish to HMMM was found [23].

Table 3. Acute toxicity of HMMM to rainbow trout at all stages of development

Stages of development	96-hour LC50 (%)	95% confidence intervals (%)
Embryos (eye stage)	26.4	22.8 ÷ 30.6
Larvae	9.5	8.1 ÷ 11.2
Adult fish	29.3	26.5 ÷ 32.4

Mortality of aquatic animals is the most significant parameter in toxicology [21]. Comparison of HMMM concentrations induced mortality of test fish as shown in (*see Table 3*) demonstrated that the most sensitive to HMMM were larvae then followed embryos and adult fish. These data confirmed our earlier conclusions that embryos are more resistant to impact of heavy metal mixtures as compared to larvae stage [12]. Probably embryos chorion as a barrier saves the developing organism from the external harmful impact (including heavy metals), meanwhile larvae of fish, which lost a chorion, are very sensitive to heavy metals as they experience their direct negative effect [24, 12]. It should be noted that 96 hour LC50 of HMMM to embryos and adult individuals did not differ significantly: 26.4 % - embryos, 29.3 % - adult fish, respectively.

Adult fish were found to be rather sensitive to heavy metal mixture studied as compared to embryos. The statistical evaluation of biological parameters revealed marginally significant difference between embryos and larval MATC values. Only changes in gill ventilation frequency of adult fish were registered at lower concentrations of HMMM. MATC to this parameter was equal to 0.87 % of HMMM, that extremely significantly differed from MATC estimated to embryo heart rate as shown in (*see Table 4*). Meanwhile no differences were found between

MATC's of HMMM calculated to ventilation frequencies of larvae and adult fish that confirmed higher sensitivity of larvae and adult fish to external effects as compared to embryos. MATC's of HMMM calculated to respiratory parameters of adult fish t ranged from 1.77 to 3.54% of HMMM [23].

Table 4. Maximum Acceptable Toxicant Concentrations (MATC) of HMMM to biological parameters of rainbow trout at all stages of development

Stage of development	MATC (%)
Embryos	
Heart rate	9.3
Larvae (20-day old)	
Heart rate	2.7*
Gill ventilation frequency	2.7*
Total body mass at the end of tests	0.49*
Adult fish	
Gill-somatic index	0.87*
Gill ventilation frequency	0.87*

* Values significantly different from controls (P<0.05)

The comparison of the physiological responses of rainbow trout exposed to HMMM reveal their sensitivity differences depending on fish stage of development. MATC calculated to such parameters as disturbances in heart rate of embryos, lower heart rate and slowing of ventilation frequency of larvae ranged from 2.7 to 9.3 % of HMMM as shown in (*see Table 4*). We may presume that as a one negative consequence of disturbances in functioning of respiratory system of organism in early ontogenesis was a decrease in mass of larvae at the end of yolk resorbtion. MATC to this parameter was very low (0.49 % of HMMM). Our data confirmed that integrated biological parameters like survival, growth rate, development or reproduction indices are the most sensitive to various environmental impacts [25].

The data obtained have shown that fish especially at early stages of development are very sensitive to HMMM impact and our data let predict negative possibilities for survival of future generations of more sensitive fish species that will experience even low level impact of these pollutants. The changes in physiological parameters of embryos exposed to HMMM such as heart rate slowing may induce negative consequences in respiratory process, development and hatching success. Decrease in survival of population is a possible after-effect [26].

3.3 Orimulsion

Acute toxicity tests were conducted in order to determine basic toxic characteristics of orimulsion. The results of acute toxicity tests are presented in (*see Table 5*). The data obtained have shown that the most sensitive to acute toxic effect of orimulsion were larvae, then followed embryos and adult fish.

Table 5. Acute toxicity of orimulsion to rainbow trout at all stages of development.

Stage of development	96-hour LC50 (g/l)	95% confidence interval (g/l)
Embryos (eye stage)	0.1	0.09 — 0.12
Larvae	0.06	0.05e— 0.07
Adult fish	2.22	2.02e— 2.43

The primary contact of test fish with toxic solutions of orimulsion induced their hyperactivity, although after 24 hours fish became less active. The gills of dead fish were plugged with mucous and small particles of bitumen. It is evident that fish kills occurred due to dispersed and soluble parts of orimulsion [17]. Estimated Maximum Acceptable Toxicant Concentrations (MATC) of total orimulsion for all biological parameters of rainbow trout studied in long-term tests are presented in (*see Table 6*).

Orimulsion (stable emulsion of natural bitumen and water) has the property to disperse in all water volume during short period of time. Its toxic effect on rainbow trout can be characterised by combined effects of dispersion and water-soluble-fraction. Toxicity tests have shown that the most sensitive to acute toxic effect of orimulsion were larvae, then followed embryos and adult fish. Duration of exposure significantly decreased the value of LC50 in adult fish. Acute toxic effect of orimulsion to rainbow trout can be characterised by very narrow toxic effect zone and the sharp boundary between lethal and sublethal concentrations.

Table 6. Long-term effects of total orimulsion to rainbow trout at all stages of development

Parameters	MATC , g/l
Embryos	
Heart rate after 5-day exposure	0.019
Larvae	
Gill ventilation frequency after 20-day exposure	0.008
Heart rate after 20-day exposure	0.017
Total body mass at the end of the tests (60-day exposure)	0.004
Relative mass increase at the end of the tests (60-day exposure)	0.0017
Hatching duration	0.07
Amount of hatched larvae	0.008
Yolk-sac resorbtion	0.004
Exit off the nest	0.004
Response of larvae to external stimuli	0.017
Adult fish	
28-day LC50 (95 % confidence interval)	0.26 (0.21e — 0.32)
Specific growth rate (*r*)	0.09

Maximum Acceptable Toxicant Concentration (MATC) of 0.0017g/l of total orimulsion to fish was derived from long-term tests based on of the most sensitive parameter of rainbow trout larvae (relative mass increase at the end of the test) [17]. Fish at early stages of development are especially sensitive to impact of oil hydrocarbons [27]. We may presume that as a one negative consequence of disturbances in functioning of cardiorespiratory system of organism in early ontogenesis (decrease in heart rate and gill ventilation frequency) was a decrease in mass of larvae at the end of yolk resorbtion [17].

3.4 Crude oil

The exposure of adult fish to crude oil (1610 mg/l) did not affect their survival. Larval mortality rate increased and at the end of tests it was approximately ~ 36%. Crude oil significantly ($P<0.05$) decreased the average body mass, HR and GVF of larvae at the end of the tests (*see Table 7*). GVF of adult fish decreased from 93.3±3.6 (after a 1-day exposure) to 73.0±2.0 count/min after a 4-day exposure [19].

Table 7. The effects of crude oil on biological parameters of larvae of rainbow trout at the end of exposure

Compounds	Parameters		
	Average body mass (mg)	HR (counts/min)	GVF (counts/min)
Crude oil	98.9±4.9*	80.6±4.2*	102.6±4.1*
Control	124.0±4.2	121.1±5.4	126.3±7.0

* Values significantly different from controls ($P<0.05$)

Crude oil did not affect adult fish survival, but larval mortality increased at the end of the tests. Significant alterations in cardio-respiratory parameters of larvae and respiratory indices of adult fish confirmed the specific toxic effect of oil on this system. Oil coats gills of aquatic organisms, inducing pathological lesions on respiratory surfaces, and, thus, causes problems with oxygen supply and respiration [28].

3.5 Water of various biotopes of Lake Druksiai

Our data (*see Table 8*) show that during the study period water of biotopes 1 was not toxic to rainbow trout embryos and larvae: 7.7-10.2 % of embryos and 10.6-12.6 % of larvae died (in the control 7.6-8.3 % and 9.4-10.4 %, respectively), the physiological indices of embryos and larvae were approximately to those of the control (*see Table 9*). The function of the cardiorespiratory system in this water was at the control level: HR of the embryos - 96.8 counts/min., of the larvae - 106.4 counts/min; GVF of the larvae was 128.4 counts/min. The average body mass of larvae was 74.8 mg, its relative mass increase at the end of the tests (60-day exposure) being 77% [29].

Table 8. The effect of the water of various biotopes Lake Druksiai to rainbow trout embryos and larvae.

Lake Druksiai biotopes	Mortality of embryos,e%	Mortality of larvae,e%
1	7.7	10.6
6a	20.3	28.6
7a	10.6	18.6
Control	8.3	10.4

In the water of Lake Druksiai biotopes were observed changes in the physiological state of larvae (*seeeTable 9*). The HR of the embryos in the water of both biotopes 6a and 7a was approximately similar to those in the control. Meanwhile, we observed changes in the cardiorespiratory system of larvae: HR and GVF increased, the average body mass was less than in the control, relative body mass increase at the end of the tests (60-day exposure) being only 40-47% (80% in the control)e

Table 9. The effect of the water of various Lake Druksiai biotopes to the physiological parameters of rainbow trout embryos and larvae.

Lake Druksiai biotopes	Embryos	Larvae			
	Parameters				
	HR, counts/min	HR, counts/min	GVF, counts/min	Average body mass, mg	Relative mass increase at the end of the tests (60-day exposure)
1	96.8±2.4	106.4±2.8	128.4±2.2	74.8±2.4	77
6a	99.4±2.2	132.4±3.6*	142.2±2.6*	64.2±2.2*	45
7a	94.4±1.8	122.6±2.6*	138.2±2.4*	68.4±1.8*	47
Control	98.4±2.4	110.4±4.2	126.4±3.6	76.4±1.8	80

* Values significantly different from controls ($P<0.05$)

The results allow conclude that the waters of the Lake Druksiai biotopes 6a, 7a were not favorable for the normal growth and development of larvae. The observed changes show that the INPP effluents bring into Lake Druksiai toxicants that cause alterations in the physiological systems of test-organisms, slowing their growth and development and even causing their death.

The water patterns from some biotopes of Lake Druksiai (biotopes 6a, 7a) produced toxic effect to organisms as well. Therefore the chemical substances entering with different origin waste waters predetermine the toxicity of the Water Lake Druksiai observed in our experiments. INPP wastewaters consist of many components, such as heavy metals, radionuclides, alkalies and solutions diluted with acids, weak organic acids, oil products etc. The concentration of each component in various biotopes of reservoir can be different and changes constantly. Amount of heavy metals in lake Druksiai increased after INPP operational start: copper, zinc, manganese, chromium, cadmium, nickel, iron increased from two and half to forty times in some water biotopes of lake Druksiai [8, 30]. The data obtained testing of the water of some biotopes of Lake

Druksiai have shown that all the discharged waters entering in the lake are more or less harmful to rainbow trout (*O. mykiss*) embryos and larvae.

4 CONCLUSIONS

Our study demonstrated that the fish (rainbow trout) was very sensitive test-organism to the effect of different kind pollutants (some heavy metals, heavy metal model mixture, orimulsion, crude oil and water of some biotopes of Lake Druksiai. The most sensitive to the impact of pollutants were fish at early stages of development i.e. larvae and embryos. The integrated biological parameters of fish at all stages of development (survival, growth rate, and relative mass increase) were the most sensitive ones. In accordance with the sensitivity to the impact of heavy metals, animals in the earliest stages of their development were more sensitive than plants, but the plants in some cases were more sensitive to heavy metals than adult fish. Therefore, plants as well as animals (O. mykiss) of different life stages, as test-organisms, are recommended for the inclusion of the complex biotests for the assessment of the impact of heavy metals. The complex of the most sensitive parameters investigated of rainbow trout can be successfully used for the water toxicity assessment containing different kind pollutants. The data obtained let predict the impact of pollution not only to the physiological status of aquatic organisms but as well as to their survival in natural water bodies.

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