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# Behavioral Responses of Rainbow Trout *Oncorhynchus mykiss* to Sublethal Toxicity of a Model Mixture of Heavy Metals

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The ability of fish to detect and avoid pollutants was intensively investigated during previous decades, and a considerable amount of experimental data was compiled and reviewed (Beitinger and Freeman 1983; Giattina and Garton 1983; Atchison et al. 1987). The main deficiency of those experiments was that usually no other behavioral responses were investigated during the tests. Avoidance responses are of high ecological significance (fish migration and distribution patterns can be affected), while other behavioral responses which occur in parallel, although their ecological significance is not clear are also of interest, however. Changes in spontaneous locomotor activity and respiratory responses are sensitive behavioral indicators of sublethal toxicity in fish (Atchison et al. 1989; Little and Finger 1990; Scherer 1992). Simultaneous investigation and comparative analysis of all the above-mentioned fish behavioral responses can be of high value. This kind of information, apparently, can provide insight into the reasons for an avoidance response occurrence as well as explain the mechanisms of such fish adaptive behavior.

A previous study established that rainbow trout are able to detect and avoid low sublethal concentrations of heavy metal model mixtures of different quantitative and qualitative composition (Svecevičius 2001). The aims of the present study are: (1) to investigate spontaneous locomotor activity and respiratory responses of yearling rainbow trout in response to a model mixture consisting of seven heavy metals (HMMM) under the same controlled experimental conditions; (2) to perform comparative analysis of the sensitivity of the responses studied based on the results of avoidance and acute lethality tests; and (3) to evaluate the suitability of the most sensitive behavioral responses for needs of bioassay testing of natural and waste-waters polluted with heavy metal complexes.

## MATERIALS AND METHODS

Rainbow trout adults (one-year-old) were obtained from Žeimena (Švenčionys District, Lithuania) Hatchery. The test fish were acclimated to laboratory conditions for at least prior to testing. The fish were kept in flow-through 1000-L holding tanks supplied with aerated artesian water (minimum flow rate 1 L per 1 g of their wet body mass per day), under natural illumination and were fed commercial trout feed daily in the morning; the total amount was no less than 1 % of their wet body mass per day. The day before and during the tests the fish were not fed. The average total length of test fish was 100 ± 10 mm and the total weight was 10 ± 2 g (mean ± SEM).

The effect of HMMM was investigated. The formation of a model mixture was carried out based on available analytical data of the average annual amounts of representative heavy metals in cooling waste-water discharged from the Ignalina Nuclear Power Plant (Zarasai District, Lithuania) into Drūkšiai Lake. The stock solution of HMMM was prepared in acidified distilled water (concentrated $H_2SO_4$ was added to reach final pH = 2) by use of the following chemically pure substances: $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$, $NiSO_4{\cdot}7H_2O$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $Cd(CH_3COO)_2{\cdot}2H_2O$, $MnSO_4{\cdot}5H_2O$ («REAKHIM» Company, Russia), the final concentration being recalculated according to the amount of heavy metal ion. A concentration of the 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. Heavy metal concentrations in stock solution were 1000 times higher. All the test concentrations were expressed as parts of 96-hour LC50 which was equal to 29.3 % of HMMM derived from acute toxicity tests (Svecevičius 2001).

Artesian water was used for dilution. Its average physico-chemical characteristics included: hardness (270 - 300 mg/L as $CaCO_3$), alkalinity (244 mg/L as $HCO_3^-$), pH (7.9 - 8.1), dissolved oxygen (10 ± 1.2 mg/L) and temperature (10 ± 0.2$^0$C).

The tests were performed in a flow-through test apparatus (Figure 1) consisting of 6 isolated test boxes connected into one battery. Each test box was of 0.5-L volume, contained a separate aeration stone, isolation net and was supplied with artesian water incoming from the head box and passing through the distribution box. The flow rate (25 mL/min) of the water passing through each box was laminar. Circulating water flow was established in each test box by changing the amount of air supply into the air stone in order to induce a slight rheotaxis in the test fish for their better orientation in one direction.

The observations of the development of a toxic effect in rainbow trout exposed to the heavy metal model mixtures of different quantitative and qualitative compositions (Svecevičius 2001) showed that this process followed certain behavioral regularities.

First of all, the test fish demonstrated evident behavioral detection of the toxicity depending on the HMMM concentration. This response was considered to be "latent period of detection response" and was defined as the period of time from the moment when the toxicant fell into the test box until the occurrence of the primary response of the test fish.

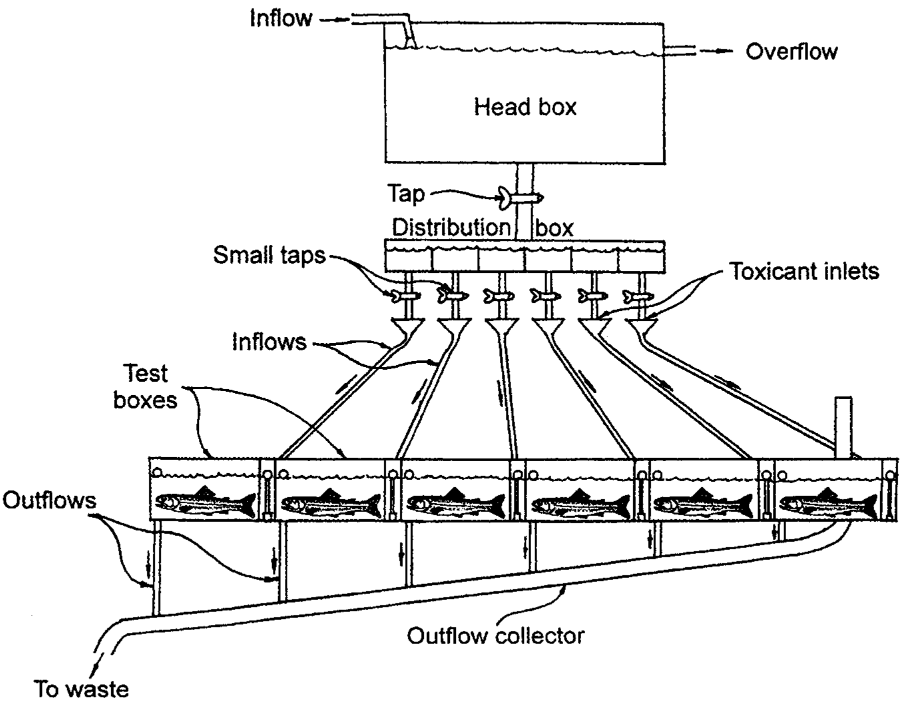


**Figure 1.** Diagram of the test apparatus.

The sudden increase in locomotor activity followed by disturbances of respiratory responses is a perturbation or alarm response.

Based on the further observations, a 5-grade fish spontaneous locomotor activity scale was established:

0 - fish is passive; it rests on the bottom; no pectoral fin movements are observed; ventilatory opercular movements are rhythmic, and fish demonstrates slight rheotaxis.

1 - fish is minimally active, little raised from the bottom; pectoral fin movements are slightly increased in frequency; ventilatory opercular movements are still rhythmic; fan-shaped dorsal fin response can be observed; fish is evidently demonstrating rheotaxis.

2 - fish activity is increased; it turns frequently, and sudden jumps can be observed; ventilatory opercular movements becomes non-rhythmic.

3 - fish is hyperactive as it tries to escape out of the box; sudden jumps out of water as well as body tremor and distinct respiratory disturbances are observed.

4 - pathological activity: erratic swimming when fish strikes one's head against the wall of the box; loss of orientation, equilibrium reflex and paralysis of respiratory apparatus followed by death.

In addition, two types of fish behavioral respiratory responses were also registered: gill ventilation frequency and coughing rate in counts per minute, respectively.

Preliminary tests showed that maximal changes in responses studied were observed in all runs within 10 minutes of exposure. Further extension of the exposure caused a gradual decrease in the intensity of responses. Therefore, the test procedure was conducted as follows: test fish were placed into test boxes (one individual into each) and acclimated for 24-hour period. Following the control period, all the data were obtained by direct observation, during a 1-minute period for each parameter (locomotor activity, ventilation frequency, coughing rate). Locomotor activity was measured through momentary recordings every 10 seconds and the average value for each fish was estimated. The test period followed during which the water flow into each test box was stopped and a small amount (5 mL) of diluted stock solution of HMMM was injected through the toxicant inlet. This procedure did not change any physico-chemical characteristic of dilution water in the test boxes. The data were obtained in the same manner as during the control period within 15 minutes of exposure after the latent period of detection response was measured.

The sensitivity of the behavioral responses studied was evaluated by comparing their threshold values. Threshold-Effect-Concentration (TEC) was estimated by defining the geometric mean between the Lowest-Observed-Effect-Concentration (LOEC) and the No-Observed-Effect-Concentration (NOEC) (van Leeuwen and Hermens 1995).

Designed heavy metal concentrations were measured during blank tests. Water samples taken from the test boxes were fixed with concentrated nitric acid the total amount of heavy metals was established by atomic absorption spectrophotometry on SHIMADZU AA-6800 either with the flame or graphite furnace techniques using proprietary software. Mean measured concentrations were within 10% of target.

Overall, 12 test fish were examined for each HMMM concentration. Each of the 12 fish was tested only once. Data were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test at $p < 0.05$ through STATISTICA (Version 6.0) and GraphPad Instat™ (Version 2.04) softwares.

## RESULTS AND DISCUSSION

The data obtained showed that with an increase in HMMM concentration, the latent period of detection response rapidly decreased, while the intensity of other responses studied increased (Table 1).

**Table 1**. Behavioral responses of rainbow trout to the effect of heavy metal model mixture (HMMM) (mean ± SEM, $N = 12$)

| HMMM concentration (parts of 96-hour LC50) | Latent period of detection response (seconds) | Locomotor activity (grades) | Gill ventilation frequency (counts/ minute) | Coughing rate (counts/ minute) |
|---|---|---|---|---|
| 0 (Control) | — | 0.19 ± 0.04 | 76 ± 1.6 | 1.9 ± 0.3 |
| 0.005 | immeasurable | 0.20 ± 0.05 | 74 ± 1.3 | 1.8 ± 0.3 |
| 0.01 | 52 ± 10.3 | 0.54 ± 0.05* | 73 ± 1.8 | 1.7 ± 0.3 |
| 0.02 | 29 ± 5.5 | 0.70 ± 0.05* | 81 ± 1.4 | 2.5 ± 0.8 |
| 0.05 | 20 ± 3.0 | 0.85 ± 0.04* | 93 ±1.7* | 4.1 ± 0.9 |
| 0.1 | 14 ± 1.0 | 1.12 ± 0.04* | 95 ± 1.6* | 4.5 ± 1.3 |
| 0.2 | 10 ± 0.8 | 1.42 ± 0.05* | 97 ± 1.0* | 9.7 ± 1.9* |
| 0.5 | 6 ± 0.6 | 1.80 ± 0.05* | 99 ± 1.9* | 12.0 ± 1.5* |
| 1 | 3 ± 0.4 | 1.90 ± 0.08* | 99 ± 4.3* | 16.3 ± 2.8* |

*Note*. Asterisks (*) denote values significantly different from control ($p < 0.05$).

Locomotor activity increased most rapidly, followed by coughing rate and gill ventilation frequency. At a HMMM concentration equal to the 96-hour LC50, locomotor activity increased 10-fold, coughing rate increased 8.6 times, while gill ventilation frequency increased only 1.3 fold as compared to control. The intensity of the locomotor activity reached significance at a HMMM concentration of 0.01, gill ventilation frequency at 0.05, and coughing rate only at 0.2 parts of 96-hour LC50, respectively. Consequently, locomotor activity was found to be the most sensitive response to the effect of HMMM among the responses.

The relationship between HMMM concentration and behavioral response intensity was non-linear (Figure 2). Regression analysis was conducted and the following descriptive models were applied: Reciprocal Logarithm Fit for latent period of detection response, Logistic Model for gill ventilation frequency and Exponential Association (3) for locomotor activity and coughing rate.

Comparison of the threshold values for the studied responses indicates almost the same sensitivity level of locomotor activity and avoidance response (Table 2), apparently due to their reliance on the same category of behavioral responses as described by Scherer (1992). Increase in locomotor activity can be initiated through chemosensory irritation, since it was established that the fish olfactory system is involved in the formation of an avoidance response to heavy metals (Brown et al. 1982; Svecevičius 1991). Furthermore, gill ventilation frequency and coughing rate are intimately associated with respiratory demands and gill irritation or blockage, and locomotor activity may reflect a more non-specific stress response resulting in changes in blood cortisol and glucose levels (Wedemeyer and McLeay 1981; Scherer et al.1986).

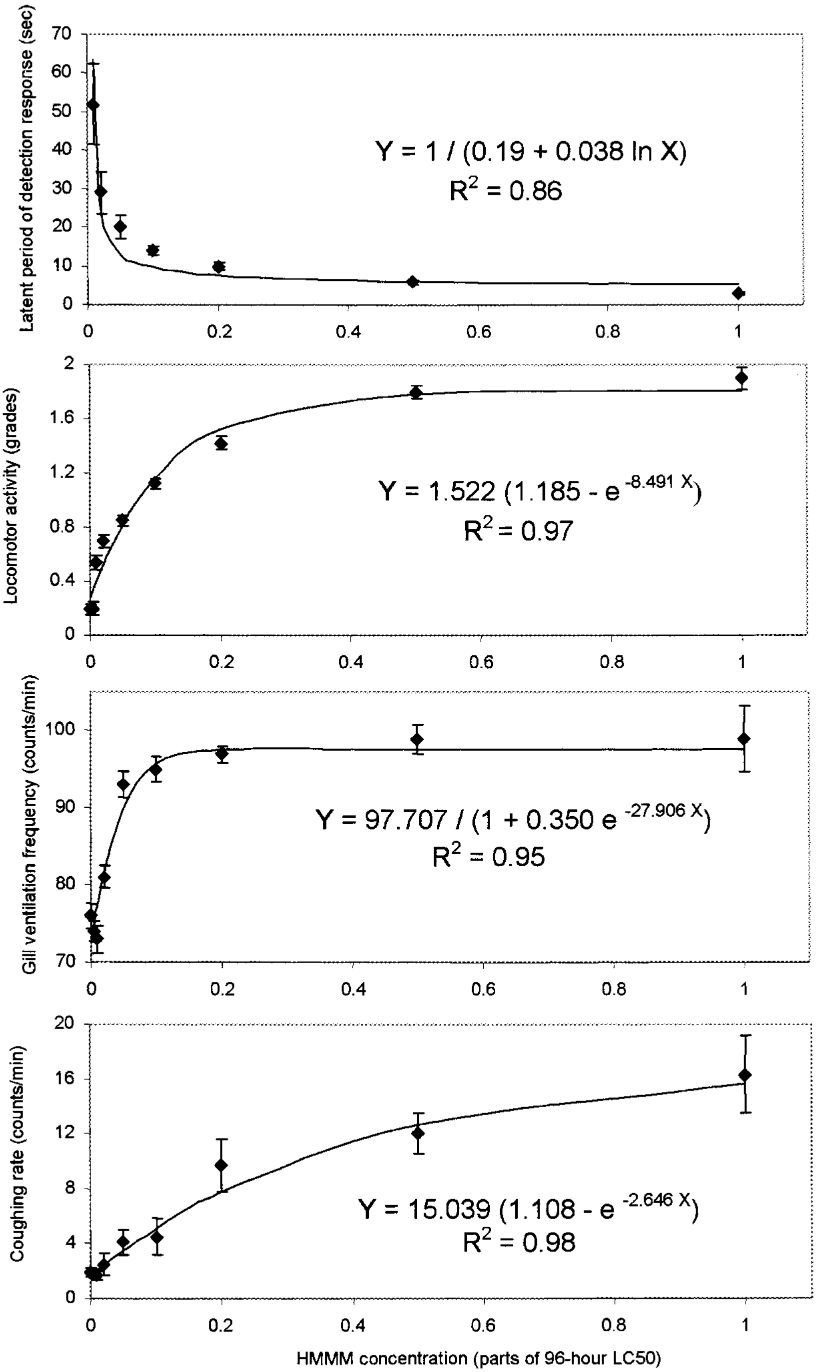


**Figure 2.** Relationship between intensity of rainbow trout behavioral responses and HMMM concentration.

**Table 2.** Comparison of rainbow trout behavioral response threshold concentrations with acutely lethal concentration

| Behavioral response | Threshold-Effect-Concentration (TEC) (HMMM in %) | Part of 96-hour LC50 |
|---|---|---|
| Locomotor activity | 0.21 | 0.007 |
| Gill ventilation frequency | 0.93 | 0.032 |
| Coughing rate | 4.14 | 0.14 |
| Avoidance response | 0.18 | 0.006 |

*Note.* TEC for avoidance response was derived from data of the previous study (Svecevičius 2001). NOEC = 0.125 % and LOEC = 0.25 % of HMMM.

Heavy metals at sublethal levels are known to elevate spontaneous locomotor activity in fish. Little and Finger (1990) summarized the effects of pollutants on swimming behavior of fish. For example, activity is increased at 0.006-0.009 mg/L of copper in brook trout (*Salvelinus fontinalis*), at 0.05 mg/L of copper in catfish (*Arius felis*) and 0.1 mg/L of copper and cadmium in largemouth bass (*Micropterus salmoides*). In bluegill (*Lepomis macrochirus*) hyperactivity was induced by sublethal concentrations of 0.1 mg/L of cadmium and zinc and at 0.05 mg/L of chromium. However, contrary to those data Ellgaard et al. (1995) using an observational technique have found hypoactivity in goldfish (*Carassius auratus*) induced by nickel at sublethal concentrations of 25-75 mg/L.

Atchison et al. (1987) summarized the effects of heavy metals on the respiratory behavior of fish and concluded that coughing rate, directly observed or quantified, was often the more sensitive measure of effect than gill ventilation rate. Overall those rates increased at 0.005-0.15 mg/L of cadmium, at 0.002-0.025 mg/L of chromium, 0.009-0.048 mg/L of copper and 0.144-2.55 mg/L of zinc depending on fish species and testing conditions.

The data of the present study probably only partly confirms the statement on coughing sensitivity. Although coughing rate increased more rapidly, it was found to be less sensitive than gill ventilation frequency according to TEC.

It is evident that all the rainbow trout responses studied are sensitive behavioral indicators of low sublethal heavy metal toxicity and meet the criteria as rapid bioassay tools for early warning systems for pollution as discussed by Cairns and van der Schale (1980). Such responses as locomotor activity and avoidance response, apparently, allow detecting of heavy metals at evidently safe, even background levels. Respiratory responses were found to be less sensitive, but also can be successfully used in bioassay testing of treated industrial and municipal effluents before they are discharged into receiving waters. According to Cairns et al. (1982) fish respiratory behavior can be successfully used in identifying chronically safe toxicant concentrations to fish. The detection response was found to be highly specific and is usually disregarded by researchers as a behavioral parameter. However, it can be very informative and a rapid tool in getting the answer (in few seconds) on water toxicity level.

I believe that these results will be useful in developing an automatic system for rapid detection of sublethal heavy metal pollution, with a view to its adoption for routine industrial monitoring.

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