Bull. Environ. Contam. Toxicol. (2003) 71:226–233

DOI: 10.1007/s00128-003-0154-6

Bulletin of Environmental Contamination and Toxicology

Avoidance Response of Rainbow Trout *Oncorhynchus mykiss* to Heavy Metal Model Mixture after Long-Term Exposure in Early Development

G. Svecevičius

Laboratory of Hydrobiont Ecology and Physiology, Institute of Ecology, Akademijos 2, LT-2600, Vilnius, Lithuania

Received: 18 January 2003/Accepted: 20 April 2003

Fish are able to detect and spontaneously avoid many toxicants of different chemical origin (Beitinger and Freeman 1983; Giattina and Garton 1983) including heavy metals (Atchison et al. 1989) as well as their mixtures (Saunders and Sprague 1967; Hartwell et al., 1987; Hansen et al. 1999a; Svecevičius 1998; 2001). These responses occur when the choice is given to the test fish to discriminate between clean and polluted water. However, insufficient information is compiled about how avoidance response of fish can be modified in the case when they are deprived of such opportunity and constrainedly pre-exposed to toxicants for long periods of time.

At present it has already been established that fish pre-exposed to such aquatic pollutants as chromium, pulp-mill effluent, cadmium, copper and heavy metal mixtures at sublethal concentrations for several weeks or months distinctly changed their character of behavioral response and started to demonstrate preference to the same toxicant to which they had previously been exposed (Anestis and Neufeld 1986; Hartwell et al. 1987; Myllyvirta and Vuorinen 1989; McNicol and Scherer 1993; Svecevičius 1999).

Without discussing possible mechanisms, it is evident that this phenomenon is of very high ecological significance to pre-exposed fish populations, because they can be attracted to "familiar" polluted water for chemosensory impairment, sensitization, desensitization, or familiarization (McNicol and Scherer 1993). Thus the study of avoidance-preference responses of non-pre-exposed fish can not fully reflect how natural fish populations respond to pollutants. Furthermore, the information is totally lacing about how avoidance response can be modified by acclimation of fish to pollutants in their early development starting from the eggs when the avoidance of pollutants is impossible and does it cause any long-term behavioral after-effects in adult fish.

The aim of the present study was to find out whether 70-day pre-exposure of rainbow trout to model mixture consisting of seven heavy metals in early development (starting from early-eyed embryos stage and finishing yolk-sack resorbtion stage in late alevins) modifies the intensity of avoidance response in fish when they become one-year-old.

MATERIALS AND METHODS

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

The effect of model mixture (HMMM) consisting of seven heavy metals was investigated. The formation of 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 Nuclean Power Plant (Lithuania) into the Drūkšiai Lake during 1996. 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 (Svecevičius 2001).

Artesian water was used for dilution. Its average physico-chemical characteristics were: hardness was 270 - 300 mg/L as $CaCO_3$, alkalinity was 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.

Two series of separate avoidance tests were conducted on two different groups of test fish.

The first group considered as "control" consisted of common, hatchery-reared one-year-old rainbow trout. The test fish were acclimated to laboratory conditions at least for one week before starting the tests. They were kept in a 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 daily in the morning (on beef spleen mince); the total amount was no less than 1 % of their wet body mass per day. A 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).

Another group consisted of affected or pre-exposed fish. They were brought to the laboratory from the same hatchery as fertilized eggs. Seventy-day duration chronic bioassay was performed on them starting from early-eyed embryos stage till yolk-sack resorbtion stage in late alevins. Test-objects were exposed to wide range of HMMM concentrations varying from 0.2 % to 30 %. A number of various biological parameters (survival, growth, respiratory, physiological and behavioral) were recorded during the test in order to determine Maximum Acceptable Toxicant Concentration (MATC) of HMMM studied. The data obtained from this bioassay have shown that increase in total body mass of alevins at the end of the test was found to be the most sensitive parameter to the chronic effect of HMMM. Estimated MATC according to this parameter was equal to 0.5 % of HMMM (Vosylienė et al. 2002). While 96-hour LC50 of the same HMMM for adult fish was found to be equal to 29.3 % (Svecevičius 2001).

Three groups of alevins survived the test were the most numerous. Those were exposed to lower HMMM concentrations of 0.23, 0.45 and 0.9 % respectively. They were joined together, transferred into holding tanks supplied with clean artesian water and were cultivated under laboratory conditions for one year period, until they reached the same average total length and the total weight of test fish from control group. Test fish exposed to higher concentrations were strongly affected by HMMM and did not survive in clean water.

Avoidance tests were carried out by use of a flow-through U-shaped plastic steep gradient chamber of alternative preference with two parallel water streams in detail described earlier (Svecevičius 1998; 1999). The chamber was 1500 x 600 x 300 mm in size and the total flowing rate was 6 L/min. Fifteen minutes after the introduction of the test solution into one of the sections of the chamber two zones were formed: zone **a** (clean water) and zone **b** (solution).

The fish were tested in groups of ten individuals. Each group was placed in a gradient chamber and acclimated for 24 hours. The water temperature in the gradient chamber was the same as in the holding tanks. The test procedure was conducted by means of multiple momentary recordings of fish in polluted and clean water zones during 2.5-hour control and test periods. After the control period the test solution of necessary concentration was introduced into one of the chamber sections (this procedure did not change any physico-chemical characteristic of the dilution water) and the fish were allowed to choose between clean and polluted water. The nature and intensity of behavioral response were estimated by the Response index through formula:

$$\text{Response index} = 50(2 - N_T/N_C),$$

where N_C is the average number of fish in polluted zone during the control period (water is clean) and N_T is the average number of fish in the same zone during the test period (pollutant is introduced).

Each group of fish was tested only once. Every test had ten replications. The value of index 100 denoted maximal avoidance, while 0 signified maximal preference and 50 stood for indifference. The control tests have shown that the test fish preferred both sections of gradient chamber with the same probability. The results of these blank tests confirmed that data were normally distributed and had homogeneous variance. Therefore the significance of test fish behavioral responses was determined by comparing their mean Response index value to theoretically neutral response, i.e. to the value of Response index equal to 50 by use of Student's *t*-test with $p \leq 0.05$. The data obtained were analyzed statistically through GraphPad Instat™ (Version 2.04, Dennis R. Neuman, 931929S, USA) software.

At the end of the tests water samples were taken from the gradient chamber and after being fixed with concentrated nitric acid the total amount of heavy metals was established by atomic absorption spectrophotometry on AAS Varian 250 PLUS with the graphite furnace technique (Rothery 1988). The analytical data obtained confirmed that the determined heavy metal concentrations coincided with the estimated data quite satisfactorily (the error did not exceed 10%).

RESULTS AND DISCUSSION

The results of avoidance tests have shown (Table 1) that rainbow trout are able to detect and avoid very low sublethal concentrations of HMMM. Furthermore, the intensity of avoidance response was directly proportional to HMMM concentration logarithm. Therefore, the threshold avoidance concentrations were defined by use of regression analysis: a method suggested by a number of researchers (Höglund 1961; Fava and Tsai 1976; Anestis and Neufeld 1986). The point of concentration scale at which logarithmic regression intersects Response index scale at the value being equal to 50 was considered as avoidance threshold. Estimated HMMM concentrations corresponding to threshold avoidance concentrations and their 95 % confidence intervals are also presented in Table 1.

Table 1. Response indices of control and pre-exposed rainbow trout (mean ± SEM, $N = 10$) to test concentrations of HMMM solutions.

HMMM concentration (%)	Control fish	Pre-exposed fish
0.063	43 ± 2.2	43 ± 1.4
0.125	51 ± 3.0	52 ± 1.1
0.25	78 ± 1.7*	74 ± 2.0*
0.5	91 ± 1.9**	83 ± 2.3**
1	100 ± 0.0**	98 ± 0.9**
Regression equation	$Y = 103.40 + 22.22 \log X$	$Y = 98.20 + 20.14 \log X$
R^2	0.96	0.98
Avoidance threshold (%)	0.090	0.091
95% confidence interval	0.066 - 0.113	0.077 – 0.109

Note: X corresponds to HMMM concentration in %, Y corresponds to Response index. Asterisks (*) denote values significantly different from 50 ($p \leq 0.05$). Double asterisks (**) denote significant differences between mean Response indices of control and pre-exposed fish at the same concentrations of HMMM.

The data obtained have shown that both control and pre-exposed fish responded to HMMM solutions surprisingly similarly. Although pre-exposed fish less intensively avoided 0.5 % and 1 % concentrations of HMMM, no significant difference between their avoidance thresholds and their 95 % confidence intervals as well as regression line slopes was found. They fully coincided and well overlapped each other.

In previous research (Svecevičius 1999), in order to determine fish behavioral adaptation capabilities to heavy metals and their mixtures adult rainbow trout were pre-exposed for 3 months to sublethal concentrations of two different model mixtures consisting of five heavy metals (Cu, Zn, Ni, Cr, Fe) and two copper concentrations of 0.1 and 0.2 mg/L. Toxicity of the test solutions under study ranged from 0.15 to 0.3 parts, of 96-hour LC50 value, derived in acute toxicity tests. During the 3-month test period, every month, the behavior of fish was investigated. Responses of the pre-exposed fish were compared to those of control fish responses. The experimental results of this study have shown that control rainbow trout were capable to avoid test solutions spontaneously with almost maximal intensity of response. However, pre-exposed fish significantly preferred all concentrations of test solutions. Furthermore, the intensity of preference response was also very high. Unfortunately, test fish responses after recovery period when they were transferred into clean water to the same toxicants to which they had previously been exposed, were not investigated.

The data obtained in above-mentioned study were found to be in close agreement with some of literary data concerning behavioral responses of fish pre-exposed to pollutants. Thus rainbow trout pre-exposed to sublethal concentrations of chromium for 7-20 weeks demonstrated much higher avoidance thresholds of chromium than the control fish (Anestis and Neufeld, 1986). The authors suggested that such fish behavior in natural environment might be determined as a "physiological trap" and might affect their behavioral patterns, if fish previously were pre-exposed to specific toxic substances. Hartwell et al. (1987) pre-exposed fathead minnows *Pimephales promelas* from 3 to 9 months to a single concentration of fly ash slurry consisting of chromium, copper, arsenic and selenium. Pre-exposed fish demonstrated only mild avoidance at concentrations which control fish strongly avoided while showing strong attraction to high concentrations of the slurry. Similarly, vendace *Coregonus albula* avoided bleached kraft mill effluent (BKME), although fish pre-exposed to BKME for 1 week preferred contaminated water (Myllyvirta and Vuorinen 1989). Lake whitefish *Coregonus clupeaformis* pre-exposed to cadmium for 3 weeks expressed noticeable preference to water containing sequentially increasing sublethal cadmium concentration (McNicol and Scherer 1993).

The mechanism of such behavior of fish is not clear. However, there is no doubt that such fish responses are directly connected with changes in fish chemoreceptor sensitivity to pollutants. At present it has already been established that fish olfactory system is involved in forming avoidance response to individual heavy metals (Brown et al. 1982; Svecevičius 1991). Moreover, heavy metals can affect fish olfactory system directly inducing structural and functional alterations (Rhenberg and Schreck 1986; Baatrup 1991; Blaxter and Ten Hallers-Tjabbes, 1992; Julliard et al. 1993; Saucier and Astic, 1995; Hansen et al. 1999b).

Recovery of morpho-functional alterations in the fish olfactory system after such kind of effects was demonstrated as well. Thus, Hara et al. (1983) suggested a 12 week rehabilitation period for biologically affected fish after pre-exposure to a pollutant. Anestis and Neufeld (1986) have found that rainbow trout population pre-exposed to critical chromium concentration of 0.8 mg/L for 7-20 weeks followed by acclimation for 7 days in clean water demonstrated a functional recovery of their chemoreceptive capacity within that period of time, since their respective avoidance curves were similar to the control fish populations.

The data obtained in the present study as well as other studies evidently confirm the suggestion that fish acclimation of such kind to heavy metals is of temporary character and test fish recover functionally after particular period of time independently of whether they were pre-exposed to these substances in early development or in considerably later life periods.

This kind of information can throw the light on fish adaptation capabilities to pollutants as well as explain some fish behavioral responses in the natural environment. For example, while fish are known to avoid pollutants in the field, attraction to pollutants has not been reported (McNicol and Scherer 1993). The question is whether this pattern can be extended to pollutants of other chemical origin or it can be applied only to heavy metals. Therefore further, more exhaustive investigation of the mechanisms by means of which fish can adapt to pollutants is needed.

REFERENCES

Anestis I, Neufeld J (1986) Avoidance – preference reactions of rainbow trout (*Salmo gairdneri)* after prolonged exposure to chromium (VI). Wat Res 20:1233-1241

Atchison GJ, Henry MG, Sandheinrich MB (1987) Effects of metals on fish behavior: A review. Environ Biol Fish 18:11-25

Baatrup E (1991) Structural and functional effects of heavy metals on the nervous system, including sense organs, of fish. Comp Biochem Physiol 100C:253-257

Beitinger TL, Freeman L (1983) Behavioral avoidance and selection of fishes to chemicals. Res Rev 90:35-55

Blaxter JHS, Ten Hallers-Tjabbes CC (1992) The effect of pollutants on sensory systems and behavior of aquatic animals. Netherlands J Aquat Ecol 26:43-58

Brown SB, Evans RE, Thompson BE, Hara TJ (1982) Chemoreception and aquatic pollutants. In: Hara TJ (ed) Chemoreception in Fishes. Dev Aquacult Fish Sci 8:362-393

Fava J, Tsai C (1976) Immediate behavioral reactions of blacknose dace (*Rhinichthys atratulus*) to domestic sewage and its toxic constituents. Trans American Fish Soc 105:430-441

Giattina JD, Garton RR (1983) A review of the preference-avoidance responses of fish to contaminants. Res Rev 87:43-90

Hansen JA, Woodward DF, Little EE, DeLonay AJ, Bergman HL (1999a) Behavioral avoidance: A possible mechanism for explaining abundance and distribution of trout species in a metals impacted river. Environ Toxicol Chem 18:313-317

Hansen JA, Rose JD, Jenkins RA, Gerow KG, Bergman HL (1999b) Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: Neurophysiological and histological effects on the olfactory system. Environ Toxicol Chem 18:1779-1991

Hara TJ, Brown SB, Evans RE (1983) Pollutants and chemoreception in aquatic organisms. In: Nriagu JO (ed) Aquatic Toxicology, Advances in Environmental Science and Technology. Wiley, New York, p 249-306

Hartwell SI, Cherry DS, Cairns J Jr (1987) Avoidance responses of schooling fathead minnows (*Pimephales promeles*) to a blend of metals during a 9-month exposure. Environ Toxicol Chem 6:177-187

Höglund LB (1961) The reactions of fishes in concentration gradients. Fisheries Board, Swedish Institute of Freshwater Research, Drottningholm, Report 43:1-147

Julliard AK, Saucier D, Astic L (1993) Effects of chronic low-level copper exposure on ultrastructure of the olfactory system in rainbow trout (*Oncorhynchus mykiss*). Histol Histopathol 8: 655-672

McNicol RE, Scherer E (1993) Influence of cadmium pre-exposure on the preference-avoidance responses of lake whitefish (*Coregonus clupeaformis*), to cadmium. Arch Environ Contam Toxicol 25:36-40

Myllyvirta TP, Vuorinen PJ (1989) Avoidance of bleached kraft mill effluent by pre-exposed *Coregonus albula* L. Wat Res 23:1219-1227

Rhenberg BC, Schreck CB (1986) Acute metal toxicology of olfaction in coho salmon: Behavior, receptors and odor-metal complexation. Bull Environ Contam Toxicol 36:579-586

Rothery E (1988) Analytical methods for graphite tube atomizer. Mulgrave. Victoria. Australia

Saucier D, Astic L (1995) Morpho-functional alterations in the olfactory system of rainbow trout (*Oncorhynchus mykiss*) and possible acclimation in response to long-lasting exposure to low copper levels. Comp Biochem Physiol 112A:273-284

Saunders RL, Sprague JB (1967) Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon. Water Res 1:419-432

Svecevičius G (1991) The role of olfaction in avoidance reactions to pollutants by vimba *Vimba vimba* (L.). Ekologija 4:3-8

Svecevičius G (1998) Fish community reactions to heavy metal gradients. Proc Latvian Acad Sci Sec B 52:150-152

Svecevičius G (1999) Fish behavioral adaptation capabilities to heavy metals. In: Lovejoy DA (ed) Heavy Metals in the Environment: An Integrated Approach. Institute of Geology, Vilnius, p 275-278

Svecevičius G (2001) Avoidance response of rainbow trout (*Oncorhynchus mykiss*) to heavy metal model mixtures: A comparison with acute toxicity tests. Bull Environ Contam Toxicol 67: 680-687

Vosylienė MZ, Kazlauskienė N, Svecevičius G (2002) Complex study into the effect of a heavy metal model mixture on biological parameters of rainbow trout (*Oncorhynchus mykiss*). Environ Sci Pollut Res (Online First) [DOI: http://dx.doi.org/10.1065/espr2002.02.109]