USE OF FISH BEHAVIORAL RESPONSES IN IDENTIFYING SUBLETHAL EXPOSURE TO POLLUTANTS

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Abstract. *In present the controlling of pollution of aquatic environment is performed basically by use of analytical investigations. However, biological control of effluent toxicity before effluents are discharged into receiving waters is still seldom used. Therefore, the search into suitable, easy available and maintainable test-objects, their rapid, sensitive, and non-specific test-functions which provide information not only about lethal but also about sublethal effects of pollutants is needed. Fishes are considered to be among the best test-objects. Alterations in a number of fish behavioral responses are sensitive indicators of sublethal exposure to aquatic pollutants. These are no standardized procedures yet.*
Laboratory tests were conducted on rainbow trout Oncorhynchus mykiss. Behavioral responses, such as: leaving the nest, response to external stimuli and breathing rate in larvae as well as detection-avoidance, locomotor activity, gill ventilation frequency, and coughing rate in juveniles and adults were investigated.
Obtained data showed that fish behavioral responses can be successfully applied in solving not only theoretical but also practical problems of aquatic toxicology, such as bioassay testing for hazard assessment of separate toxicants and their mixtures, industrial and municipal wastewaters as well as natural inland waters containing complexes of pollutants of different chemical origin.

Keywords: *rainbow trout; behavior; aquatic pollutants; toxicity*

Introduction

At present the controlling of pollution of aquatic environment is performed basically by use of analytical investigations.
However, the evaluation of effluent quality by physical-chemical measurements does not provide information regarding the possible synergetic or antagonistic effects that complex effluents may have on aquatic life. Therefore it is necessary to perform biological control of effluent toxicity, before effluents are discharged into receiving waters. For rapid determination of wastewater toxicity level, bioassay testing is used.

Bioassay testing is defined as a rapid experimental evaluation of water toxicity level by use of biological objects and processes [1]. Test-objects must be easily available and maintainable. Their test-functions must be sensitive not only to acutely toxic effluent levels, but also provide information about sublethal concentrations.
Fishes are considered to be among the best test-objects. Alterations in fish behavioral responses are sensitive indicators of sublethal exposure to aquatic pollutants [2–5]. These are no standardized procedures yet.
The aims of the present study were: (1) to generalize data on behavioral responses of rainbow trout at different stages of

development (larvae, juveniles, adults) under the effect of aquatic pollutants, (2) to perform comparative analysis of their sensitivity to the effect of pollution, and (3) to evaluate suitability of the responses studied for needs of bioassay testing of the polluted waters for hazard assessment.

Materials and methods

The tests were performed on rainbow trout *Oncorhynchus mykiss* at different stages of development (larvae, juveniles, adults). Test fish were obtained from hatchery and acclimated to laboratory conditions.

The effect of separate toxicants, their mixtures as well as various natural and wastewaters was investigated.

A number of fish behavioral responses (leaving the nest, response to external stimuli, gill ventilation frequency in larvae, detection and avoidance, locomotor activity as well as gill ventilation frequency and coughing rate in juveniles and adults) were studied.

Deep-well water was used for dilution. Average hardness of the water was approximately 284 mg/l as $CaCO_3$, alkalinity was 244 mg/l as HCO_3^-, pH was from 7.9 to 8.1, temperature was maintained at 10.5 to 11.5^oC, and oxygen concentration was maintained within the range 8 - 10 mg/l.

Results and discussion

The data obtained showed that the sensitivity of fish behavioral responses to pollution varies significantly depending on fish stage of development and chemical origin of the pollutant or test water studied (Table 1).

Table 1

Behavioral responses of rainbow trout to pollutants and test waters

Test-object	Pollutant or test water	Sensitive response	Reference
Larvae	Heavy metal model mixture (Cu, Zn, Ni, Cr, Fe)	Leaving the nest; response to external stimuli	[6]
Larvae	Drūkšiai Lake water; Ignalina Nuclear Power Plant wastewater	Gill ventilation frequency	[7]
Larvae	Ammonia	Gill ventilation frequency	[8]
Larvae	Heavy metal model mixture (Cu, Zn, Ni, Cr, Pb, Cd, Mn)	Leaving the nest; response to external stimuli	[9]
Larvae	Heavy metal model mixture (Cu, Zn)	Gill ventilation frequency	[10]
Larvae	Heavy fuel oil	Gill ventilation frequency	[11]
Larvae	Treated wastewater	Gill ventilation frequency	[12]
Juveniles	Zinc	Avoidance	[13]
Juveniles	Kairiai dump filtrate	Coughing rate	[14]
Juveniles	Baltic Sea water after oil spill in Būtingė terminal in 2001	Coughing rate	[15]
Juveniles	Heavy metal model mixture (Cu, Zn, Ni, Cr, Fe)	Avoidance	[16]
Juveniles	Heavy metal model mixture (Cu, Zn, Ni, Cr, Pb, Cd, Mn)	Locomotor activity	[17]
Juveniles	Nemunas River and Curonian Lagoon water	Coughing rate	[18]
Juveniles	Hexavalent chromium	Avoidance; locomotor activity,	[19, 20]
Juveniles	Heavy fuel oil	Avoidance	[11]
Adults	Heavy metal model mixture (Cu, Zn, Ni, Cr, Fe)	Coughing rate; gill ventilation frequency	[21]
Adults	Ammonia	Coughing rate; gill ventilation frequency	[8]
Adults	Heavy metal binary mixture (Cu, Zn)	Gill ventilation frequency	[10]
Adults	Heavy fuel oil	Coughing rate; gill ventilation frequency	[11]

Overall, the most sensitive rainbow trout behavioral responses in the tests using separate toxicants or their mixtures were found to be avoidance and locomotor activity in juveniles. Low sublethal concentrations of pollutants corresponding to 0.00006 – 0.006 parts of their 96-hour LC50 values can be detected by use of these responses. [17, 19, 20]. Respiratory responses (coughing rate and gill ventilation frequency) were found to be less sensitive. By use of these responses such effective levels as 0.001 – 0.07 parts of 96-hour LC50 values can be estimated [17, 20, 11]. In spite of that behavioral data indicates that they are much more sensitive than many fish biochemical–physiological parameters [8]. Meanwhile, the least sensitive responses have been gill ventilation frequency, leaving the nest and response to external stimuli in larvae. They can be used only to detect nearly lethal (0.5 – 0.7 parts of 96-hour LC50 value) pollutant concentrations [6].

Although the literature on various types of fish behavioral responses to pollutants is abundant they are still seldom used as ecotoxicological tools in the day-to-day management monitoring of the industrial wastewaters and/or toxicity of the aquatic environment.

Conclusion

Determination of water-quality criteria is based on conventional standard acute and chronic testing procedures. Fish behavioral tests seem sensitive, rapid, ecologically significant, and they could be easily standardized. Consequently, in solving practical problems of aquatic toxicology, such as bioassay testing, fish behavioral responses can be successfully applied for hazard assessment of industrial wastewaters as well as natural inland waters containing separate pollutants of different chemical origin or their complexes.

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