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Short Communication

Behavioural Responses of Medicinal Leech and Rainbow Trout Exposed to Crude Oil and Heavy Fuel Oil in Ontogenesis

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Abstract

Our study investigated the behavioural responses of medicinal leech and rainbow trout at different ontogenetic levels under the effect of crude oil (CO) and heavy fuel oil (HFO), performed comparative analysis of the sensitivity of animal responses, evaluated the specificity of these responses, and determined "safe" toxicant concentrations. Comparison of sensitivity of behavioural responses of leech and fish revealed that the most sensitive response to long-term exposure to CO was leech locomotor activity, while the most sensitive parameter to HFO was the coughing rate in juvenile fish. Our study showed that the sensitivity and specificity of behavioural responses of aquatic animals at different phylogenetic and ontogenetic levels can be successfully used to evaluate the toxicity of ambient water polluted with oil hydrocarbons.

Keywords: leech, fish, behaviour, crude oil, heavy fuel oil

Introduction

Today, the pollution of water bodies by crude oil and its products has become an acute problem all over the world. During the accidental oil spill in Būtingė Terminal (Baltic Sea, Lithuania) in 23 November, 2001, about 60 tons of crude oil were discharged into the open sea and a large water area was polluted [1]. Oil products spilled into the environment have multiple negative effects on aquatic organisms, including reducing the growth and feeding of fish. Crude oil also can cause a variety of adverse effects in early life stages of fish [2]. To detect the effects of pollutants on organisms, the behaviour of aquatic animals is a sensitive indicator of sublethal exposure to pollutants [3, 4].

Behavioural responses such as avoidance, changes in locomotor activity and feeding behaviour are regarded as integrated responses of organisms to the effects of toxicants. Furthermore, they appear to be forms of ecologically significant phenotypic adaptation, allowing them to survive in the altered environment [5, 6]. Changes in behavioural responses in the natural environment can lead to disturbances in animal migration, distribution and survival in biotopes [5, 7]. Unfortunately, investigations of this type are still scarce and in some cases totally absent. Therefore, the compilation of scientific data concerning the effects of toxicants of different chemical origin on the behaviour of aquatic animals of different phylogenetic and ontogenetic levels is required with the intention of using behavioural responses in standard toxicity tests. The purpose of the present study was to investigate the behavioural responses of medical leech and rainbow trout at different ontogenetic levels under the effect of CO and HFO; to perform comparative

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analysis of sensitivity of animal responses of two different taxonomic groups; to evaluate the specificity of these responses; and to determine "safe" toxicant concentrations.

Experimental Procedures

Toxicity tests were conducted under laboratory conditions.

Medicinal leech (*Hirudo verbana*) bred under laboratory conditions were investigated. Animals were 22-24 months old with a body weight of 5-7.5 g. Test animals were exposed to toxicants for a 20-day period. Test solutions were renewed daily at 24-hour intervals. Twenty individuals were used for each toxicant concentration. Mobility (the number of moving individuals expressed as a percent from the whole amount of tested animals), avoidance responses (the percentage of individuals escaping the test water) and cluster behaviour (gathering of animals in one cluster) were evaluated during multiple momentary recordings (every 10 min.) at 2-hour observation sessions. Changes in body shape (the percentage of individuals with the narrowing of trunk and abnormal position of suckers that occur due to the permanent contractions of certain body muscles) were recorded once a day [8].

Rainbow trout (*Oncorhynchus mykiss* Walbaum) was investigated at all stages of ontogenesis. Tests on rainbow trout larvae were started immediately following hatching and continued for 20 days. Larvae were incubated in a cold and dark room at 10±1.5ºC. Tests on juvenile rainbow trout were performed in a flow-through test apparatus consisting of 6 isolated test boxes connected to one battery with exposure duration of 1 hour [9]. Bioassay testing on juvenile rainbow trout with crude oil was not conducted. The average total length of test fish was 100 ± 10 mm and the total weight was 10 ± 2 g (mean ± SEM). Long-term (14-day) toxicity tests on adult fish were performed according to ISO 10229:1994 [10]. The average total length of test fish was 148 ± 24 mm and the total weight was 30 ± 1.2 g (mean ± SEM).

For rainbow trout, gill ventilation frequency (counts/min.), the percentage of individuals making nests, and the percentage of individuals responding to external stimuli in larvae were recorded. The following behavioural responses of juvenile rainbow trout were investigated: the latent period of detection response (in seconds), spontaneous locomotor activity (in grades) and two types of fish behavioural-respiratory response: gill ventilation frequency and coughing rate in counts per minute [9]. Gill ventilation frequency (counts/min.) and coughing rate (counts/min.) of adult fish were measured during 3-min. periods for each test fish individually and the mean value for 14 fish was calculated.

Crude oil and heavy fuel oil. Crude oil (CO) obtained from Girkaliai bore in the Klaipėda region (company "Lietuvos NAFTA") and its heavy fuel oil product (HFO) were used as toxicants. Test concentrations of toxicants were chosen based on their 96-hour LC50 values derived from acute toxicity tests on fish [11, 12]. Nominal concentrations of CO and HFO are given in Table 1. Deep-well water was used for dilution. Average hardness of water was approximately 250 mg/l as $CaCO_3$, dissolved oxygen concentration and pH were not less than 7 mg/l and 7.9-8.1, respectively. Animals were tested under semi-static conditions. Water and test solutions were renewed at 24-h intervals. Chemical analysis of dissolved oil hydrocarbons in diluted water was performed using gas chromatography [12].

Threshold-effect-concentration (TEC) was estimated for every parameter by defining the geometric mean between the lowest-observed-effect-concentration (LOEC) and no-observed-effect-concentration (NOEC) [33].

The significance of the data obtained was determined by using Student's t-test with $P \leq 0.01$, $P \leq 0.05$.

Results and Discussion

Medicinal leech. A significant avoidance response of leeches after 20-day exposure to CO was recorded at concentrations of 0.33 g/l and higher. The calculated TEC for avoidance response was 0.23 g/l. The same parameter for HFO was 0.105 g/l (Table 1). An increase in the locomotor activity of leeches after 20-day exposure to CO was recorded at a concentration of 0.08 g/l; the calculated TEC was 0.057 g/l. Two-phase changes in mobility were observed in different concentrations of fuel oil: increased locomotor activity at low concentrations and suppressed activity at higher concentrations. An increase was recorded at a concentration of 0.005 g/l; the calculated TEC was 0.003 g/l. A significant decrease in locomotor activity was recorded at HFO concentrations of 0.16 g/l and higher. The calculated TEC was 0.106 g/l. Changes in body shape and posture of leeches after exposure to CO were recorded at concentrations of 0.33 g/l and higher. The calculated TEC was 0.23 g/l. No statistically significant changes in this parameter were found in leeches exposed to fuel oil. No cluster behaviour was observed in leeches exposed to CO. In contrast, cluster behaviour was recorded at 0.005 and higher concentrations of fuel oil. The calculated TEC was 0.003 g/l (Table 1).

Rainbow trout in ontogenesis. A significant decrease in GVF of rainbow trout larvae after a 20-day exposure to CO was recorded at 3.46 g/l and higher concentrations. The calculated TEC amounted to 2.45 g/l. The same parameter for HFO was 0.03 g/l (Table 1). The effect of 0.87 g/l and higher concentrations of CO induced a significant decrease in the number of larvae making nests (88.4% of individuals did not make nests) and in the number of larvae responding to external stimuli (84.4% showed weak or no response to external stimuli). The calculated TEC for both of these parameters amounted to 0.61 g/l (Table 1). However, the effect of a 0.43 g/l concentration of CO induced no significant alterations in behaviour patterns (98-100% of larvae made nests and showed strong response to external stimuli). The effect of 0.09 g/l and higher concentrations of HFO induced a significant decrease in the number of larvae making nests (98.6% did not make nests) and in the number of

Table 1. The estimated Threshold-Effect-Concentrations (TEC) for different behavioural parameters of the medicinal leech (*Hirudo verbana*) and rainbow trout (*Oncorhynchus mykiss*) in ontogenesis (larvae, juveniles, adults) exposed to crude and heavy fuel oil.

Behavioural parameters	Crude oil	Heavy fuel oil
	TEC (g/l)	TEC (g/l)
Medicinal leech (20-day exposure)		
Avoidance response, %	0.23	0.105
Locomotor activity, %	0.057	0.003 increase 0.106 decrease
Changes in body shape and posture, %	0.23	No significant changes
Cluster behaviour	no cluster behaviour	0.003
Rainbow trout larvae (20-day exposure)		
Gill ventilation frequency (counts/min)	2.45	0.03
Number of individuals (larvae) making nests, %	0.61	0.06
Number of individuals (larvae) responding to external stimuli, %	0.61	0.06
Rainbow trout juveniles (1-hour exposure)		
Latent period of detection response (sec)	—	0.007
Locomotor activity (grades)	—	0.075
Gill ventilation frequency (counts/min)	—	0.165
Coughing rate (counts/min)	—	0.001
Rainbow trout adults (14-day exposure)		
Gill ventilation frequency (counts/min)	0.61	0.0042
Coughing rate (counts/min)	2.45	0.0042

larvae responding to external stimuli (94.4% showed weak or no response to external stimuli). The calculated TEC for both of these parameters was 0.06 g/l (Table 1). Nevertheless, the effect 0.04 g/l concentration of fuel oil induced no significant alterations in behaviour patterns (98-100% of larvae made nests and showed a strong response to external stimuli). The coughing rate in juvenile rainbow trout increased most rapidly, followed by the latent period of detection response, locomotor activity and gill ventilation frequency. The coughing rate allowed detection of HFO at a concentration of 0.0009 g/l, whereas the latent period of detection response, locomotor activity and gill ventilation frequency occured at concentrations of 0.006 g/l, 0.075 g/l and 0.18 g/l, respectively.

A significant decrease in gill ventilation frequency of adult fish after a 14-day exposure to CO was observed at concentrations of 0.87 g/l and higher. The calculated TEC was 0.61 g/l, while for the coughing rate of fish this parameter amounted to 2.45 g/l. Both gill ventilation frequency and coughing rate of adult rainbow trout exposed to 0.006 g/l and higher concentrations of HFO were significantly elevated. The calculated TEC for both of these parameters was the same and accounted for 0.0042 g/l (Table 1).

The present study was the first step in evaluating and comparing the impact of CO and HFO on behavioural responses of aquatic animals of different phylogenetic and ontogenetic levels. According to Little et al. [13], behavioural measurements may be useful indicators of sublethal contamination of the aquatic environment and can be successfully used in biomonitoring or in the prediction of hazardous chemical impacts on natural populations.

The behavioural parameters of medicinal leeches determined in the present study showed that after a 20-day exposure to CO, the most sensitive parameter was the locomotor activity of leeches (TEC=0.057 g/l). Quite sensitive parameters were also avoidance response and changes in body shape: the estimated TEC for both of these parameters was 0.23 g/l. Under the effect of HFO, the most sensitive parameters were cluster behaviour (TEC=0.003 g/l) and an increase in locomotor activity (TEC=0.003 g/l). Accordingly, the behavioural responses of medicinal leeches appear to be sensitive indices for evaluating the toxicity of oil hydrocarbons on aquatic animals. The advantages of using medicinal leeches in other toxicity studies have already been discussed in our previous papers: the simplicity of keeping animals under laboratory conditions and unsophisticated measuring of various behavioural and physiological parameters [8, 14-16, 34].

The present study demonstrates that the behavioural responses of larvae and adult rainbow trout induced by treatment with CO were recorded at the same concentration of the toxicant, whereas the behavioural responses of juvenile and adult fish exposed to HFO were found to be more sensitive as compared to those of larvae (Table 1). Our previous studies showed a different sensitivity of physiological parameters of rainbow trout at different stages of development to a heavy metal model mixture, HFO and CO [2, 11, 17]. These data are in agreement with the findings of a variety of studies that have shown that fish in early life stages and other aquatic organisms are the most sensitive to pollutants [18-21]. Several authors have demonstrated that the sensitivity of aquatic organisms to toxicants varied depending on their life stages, the nature of toxicants, duration of exposure, concentrations, and different ways of entering the organism [2, 19-22, 34].

The present study demonstrated that coughing rate is the most sensitive response to the effect of HFO among fish behavioural responses, although the latent period of detection response (a specific indicator) was recorded at the same sensitivity level, allowing determination of HFO at a low concentration range of 0.005-0.01 g/l (Table 1).

All experimental data obtained for juvenile rainbow trout are similar to the results of a previous study conducted with a heavy metal model mixture [9].

The results of the present study also confirm the data on the estimated Baltic Sea water toxicity level after the oil spill in Būtingė Terminal by use of fish behavioural responses, where it was found that coughing rate is the most sensitive non-specific behavioural response most suitable for toxicity testing of seawater containing oil hydrocarbons [1]. It is evident that all rainbow trout responses studied are sensitive behavioural indicators of low sublethal oil hydrocarbon toxicity and meet the criteria as rapid bioassay tools for early warning systems for pollution as discussed by Cairns and van der Schale, and van der Schale et al. [23, 24]. Van der Schale et al. [25] considered that fish behavioural parameters (gill ventilation frequency, cough rate, ventilatory depth and movement) can be useful as standard toxicity tests for benchmark concentrations. However, the data from the present study allow us to confirm that such fish responses as coughing rate and detection response, apparently, can be used for detecting oil hydrocarbon at evidently safe, even background levels. According to Cairns et al. and Diamond et al. [26, 27], fish respiratory behaviour can be successfully used in identifying chronically safe, sublethal toxicant concentrations to fish.

A comparison of sensitivity of behavioural responses of medicinal leeches and rainbow trout revealed that the most sensitive response to long-term exposure to CO was leech locomotor activity (TEC = 0.057 g/l), while the most sensitive parameter to short-term exposure to HFO was found to be coughing rate in juvenile fish (TEC = 0.001 g/l). Behavioural responses (avoidance response, locomotor activity, cluster behaviour) of leech were less sensitive (TEC ranged from 0.105 to 0.003 g/l). According to Woltering and Viljoen [28, 29], the sensitivity of organisms to pollutants varies among assorted species, populations and life stages. Currently, multispecies Freshwater Biomonitor Systems have been proposed and used to assess behavioural responses of aquatic organisms to environmental pollutants or municipal wastewater [30-32].

Our studies on sensitivity and specificity of behavioural responses at different phylogenetic and ontogenetic levels of aquatic animals (leeches and fish) can be successfully used to evaluate the toxicity of ambient water polluted with oil hydrocarbons.

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