Sublethal effects of heavy fuel oil on rainbow trout (*Oncorhynchus mykiss*) alevins

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Sublethal effects of non-filtered and filtered heavy fuel oil (HFO) on rainbow trout were studied using the physiological parameters, behavior and growth of alevins not exposed as eggs. A long-term exposure (25 days) and two sublethal concentrations (0.09 and 0.18 g/l) of HFO were examined. It was determined that both concentrations of non- and filtered HFO strongly affected the physiological state of alevins and alterations were not dependent on time of exposure. Heart contraction rates and respiration frequency were significantly lower on days 5 through 10 and 25 of experiment. Measurements of both body weight and length showed significant reductions on days 25 of exposure, compared to controls. Swimming activity in alevins was reduced during the first 10–12 days, but the fish adapted later to low levels of HFO.

Key words: fish, alevin, heavy fuel oil, toxicity

INTRODUCTION

Oil and oil compounds incorporated into the environment often contaminate fish natal and rearing habitats and can impair the productivity of populations exposed during embryonic stages (Carls et al., 1999; Heintz et al., 1999; Heintz et al., 2000; Wertheimer et al., 1999; White et al., 1999). The threat of oil pollution to fish seldom comes just from acutely toxic concentrations that result in immediate fish kill, but also from the more subtle effects of low-level oil entrained in intertidal and subtidal sediments (Spies et al., 1996). Early life stages of fish, namely the egg and larve, are generally tolerant of short-term exposure to oil, however, long-term exposure to heavily weathered oil is very toxic to developing fish (Carls et al., 1998; Moles, Narcrose, 1998). Development of pink salmon (*Oncorhynchus gorbuscha)* incubating in gravel coated with small quantities of weathered crude oil was retarded at concentrations as low as 55.2 μg oil/g gravel and biological effects in larvae were observed when the peak total polynuclear aromatic hydrocarbons (PAH) concentration in water was as low as 4.4 μg /l (Marty et al., 1997). Toxicities in the low part per billion (ppb) range of PAH have been demonstrated in laboratory exposures of herring larvae and a number of sublethal effects, including malformations, reduced swimming ability and genetic damage were estimated (Carls et al., 1999; Rice et al., 1987). Exposed larvae were physiologically less mature and smaller. Cardiovascular malformation (edema of tube hearts) in *Menidia beryllina* elevated at the lowest dose (75 ppb) of water-soluble fraction (WSF) of crude oil tested resulted in reduced cardiac output and cessation of circulation (Middaugh et al., 1996). Rainbow trout larvae were greatly sensitive to heavy fuel oil exposure, and reduction in the physiological state and growth of fish were estimated at concentrations as low as 0.02 g/l (Stasiūnaitė, in press).

Larvae hatched in uncontaminated water may come under the influence of contaminants during larval drift by encountering an oil spill or plumes of heavily polluted river water. Information on oil compounds toxicity on larvae not exposed as eggs is not abundant and the effect is not well documented. Field observations indicated significant failure of larvae in the oiled areas (Brown et al., 1996), however, those studies not enable to investigate sublethal responses of fish to the resulting oil solutions. Only special long-term experiments are useful for determining the effects that develop slowly and can result in various pathological disturbances in future.

This study attempts to investigate the sublethal effects of heavy fuel oil on rainbow trout alevins developed from not exposed eggs.

MATERIAL AND METHODS

Toxicity tests were conducted at the Laboratory of Hydrobiont Ecology and Physiology of the Institute of Ecology. Heavy fuel oil (HFO) toxicity was investigated on rainbow trout (*Oncorhynchus mykiss)* alevins not exposed as eggs. Fish were obtained from the Žeimena fish hatchery near Vilnius. The fish were aclimated in artesian water and were transferred to the exposure chambers after four hours. The initial mean weight of fish was 62.9 ± 3.8 mg. The test-solution was prepared as follows: in a 90 l aquarium 268 g of heated HFO was mechanically mixed up with artesian water. After one day a resultant was used in tests. The experiments were initiated with alevins 7 days after hatching and ended after completion of yolk-sac resorption (total exposure time 25 days). During exposure period, the affects of two (0.09 and 0.18 g/l) concentrations of non-filtered and filtered HFO on alevin morphological (growth and yolk-sac resorption), physiological (heart rate and respiration frequency) parameters and behavior of fish were registered. The HFO was filtered in order to remove small quantitives of HFO from solution. Neither of the concentrations of non-filtered HFO affected embryonic development, but they induced sublethal responses in rainbow trout alevins hatched from treated eggs (Stasiūnaitė, in press).

Thirty-five alevins were exposed to each concentration in duplicates. The alevins were reared in a cold and dark room at 11–12 °C. The control water was changed and the resultant from HFO production was renewed at 1-day intervals. Dissolved oxygen concentration and pH determined periodically varied between 7–7.5 mg/l and 7.2–7.5, respectively, water hardness being approximately 250 mg/l as $CaCO_3$. After 5, 10 and 25 days of exposure heart rate and respiration frequency (both in beats/min) of fish were examined for 10 individuals per batch. The behavior of fish was observed daily. At the end of experiment total dry weight (mg) and length (mm) of alevins were measured for 25 organisms per batch. The data were expressed as means ± SE. Significant differences were calculated using the Student's t test. Significance levels were set at $P \leq 0.05$.

RESULTS

Non-filtered HFO experiment

Rainbow trout alevins hatched from not exposed eggs responded quickly to HFO exposure. Low levels (0.09 and 0.18 g/l) of HFO, althought did not alter the survival of fish, were effective on heart rate (HR) and respiration frequency (RQ) of organisms. After the first 5 days of exposure the above-mentioned concentrations of HFO significantly reduced the HR and RQ of alevins (Fig. 1). Their HR decreased from 95.2 ± 3.9 to 85.2 ± 2.2 beats per minute (bpm) and RQ decreased from 91.2 ± ± 3.3 to 83.6 ± 2.3 bpm (corresponding HFO concentrations 0.09 and 0.08 g/l). In control, the mean value of the parameters studied were 116.0 ± ± 1.3 bpm and 120.0 ± 1.8 bpm, respectively. Larvae performed sluggish movements not equal to normal swimming activity. Some larvae displayed uncoordinated spurts or frantically turning circles.

With increasing the age of alevins and time of exposure (total exposure time 10 days) the toxic effect of HFO was also strong, and significant differences in mean HR and RQ between control and exposed alevins were registered (Fig. 2). After a 10-day exposure to a 0.09 g/l concentration of HFO, the mean values of HR and RQ were 101.0 ± 1.8 bpm and 96.6 ± 3.4 bpm, respectively, and 97.2 ± 1.8 bpm for HR and 97.2 ± 2.1 bpm for RQ after exposure to a 0.18 g/l concentration of HFO (in control they were 118.8 ± 2.8 bpm and 115.6 ± bpm, respectively). The behavior of larvae reversed and the swimming activity of larvae not differed from the control fish.

The same strong effect on physiological state was determined when alevins were exposed to concentration of 0.09 g/l and more of HFO for 25 days (Fig. 3). Both concentrations reduced (from 115.6 ±

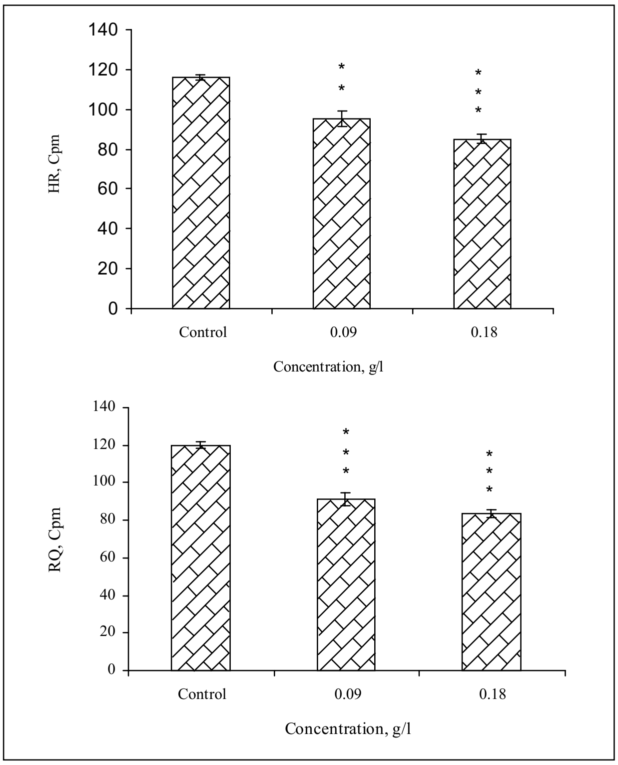

Fig. 1. Effect of non-filtered heavy fuel oil (HFO) on heart rate (HR) and respiration frequency (RQ) of rainbow trout alevins (M ± SE, n = 10, exposure time 5 days)
* Significant differences in comparison to control:
** $p < 0.01$, *** $p < 0.001$ (here and in other Figures)

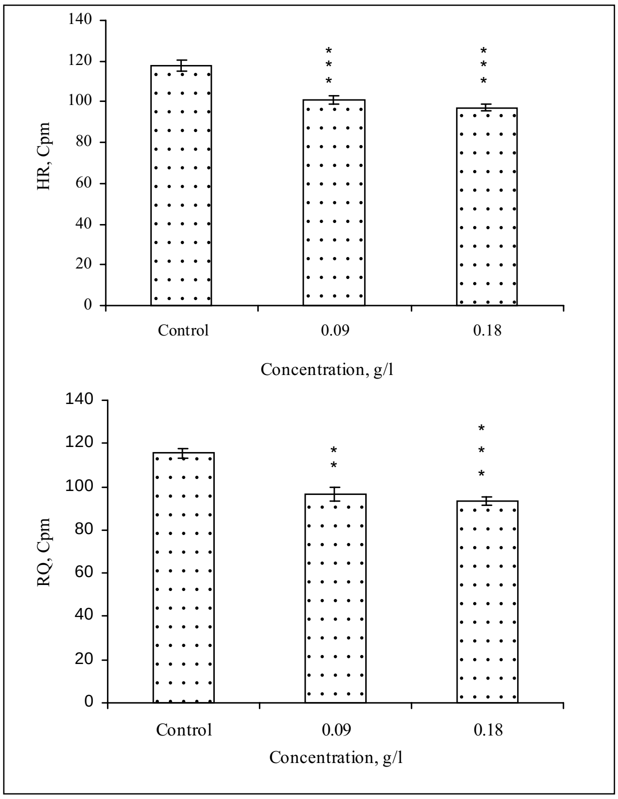

Fig. 2. Effect of non-filtered HFO on heartrate (HR) and respiration frequency (RQ) of rainbow trout alevins (M ± SE, n = 10, exposure time 10 days)

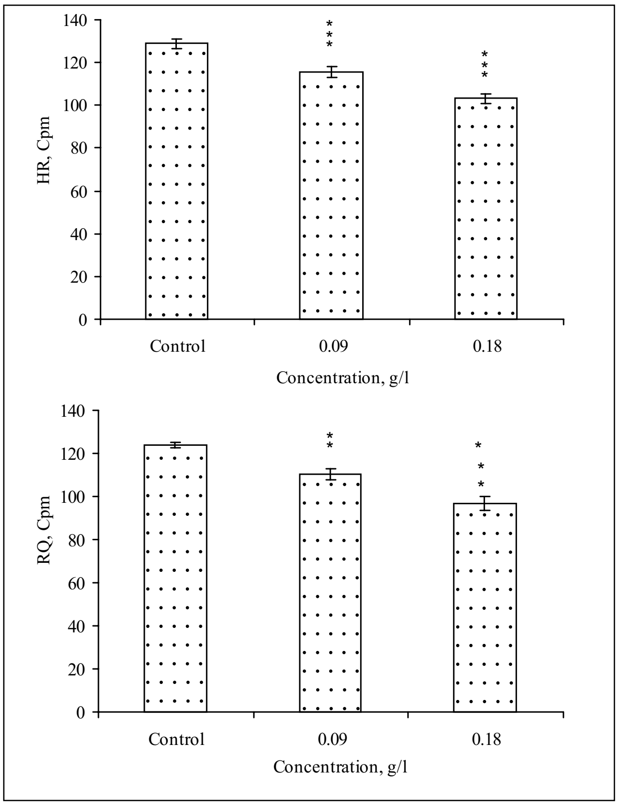

Fig. 3. Effect of non-filtered heavy fuel oil on HR and RQ of rainbow trout alevins (M ± SE, n = 10, exposure time 25 days)

± 2.7 to 103.2 ± 2.3 bpm) the HR and RQ (from 110.4 ± 2.8 to 96.8 ± 3.1 bpm) of alevins. In control alevins, the mean HR and RQ were 128.8 ± ± 2.0 and 124.0 ± 1.2 bpm, respectively.

During the experiment (total exposure time 25 days) the growth of treated alevins was poor and was reduced under exposure to both HFO concentrations (Fig. 4). At the end of the test the mean body mass (119.6 ± 7.8 and 105.8 ± 5.1 mg) and length (22.7 ± 0.4 and 22.1 ± 0.2 mm) of the exposed fish (to 0.09 and 0.18 g/l HFO) were significantly lower as compared to the mean values of these parameters (144.0 ± 4.5 mg and 24.3 ± ± 0.3 mm, respectively) in the controls. HFO concentrations affected also yolk-sac resorption. In the control, yolk utilisation ended after 20 days of exposure, but it was resorbed 3–5 days later in the test alevins.

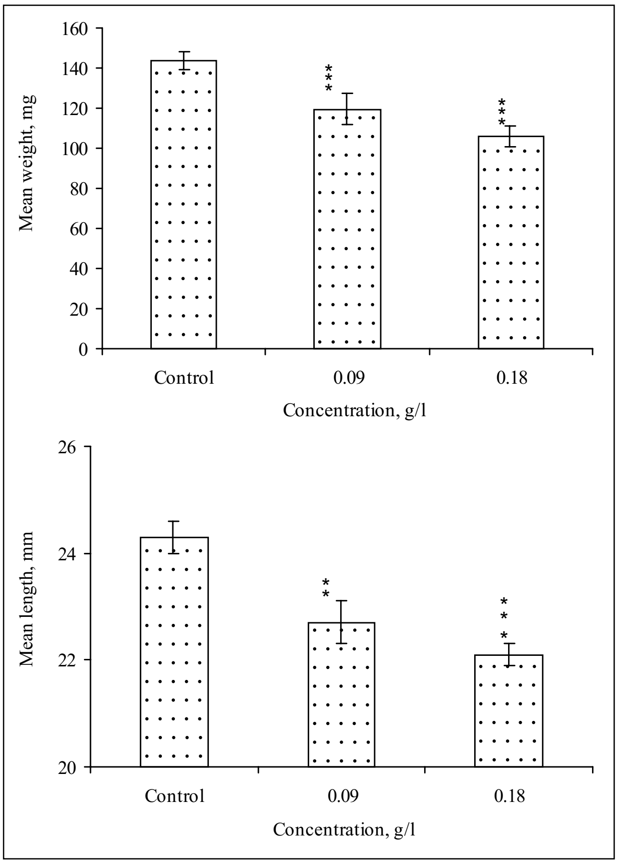

Fig. 4. Effect of non-filtered HFO on body mass and length of rainbow trout alevins (M ± SE, n = 15, exposure time 25 days)

Filtered HFO experiment

Alterations in the physiological parameters studied were registered during the first 5 days of exposure. Both concentrations (0.09 and 0.18 g/l) of HFO significantly depressed (from 99.6 ± 1.1 to 96.3 ±

± 1.3 bpm) the work of heart and reduced (from 93.2 ± 2.6 to 92.3 ± 2.2 bpm) the RQ of fish (Fig. 5). In controls, HR and RQ were on the average 116.0 ± 1.3 and 120.0 ± 1.8 bpm, respectively. Larvae were not fully immobilized, but showed only reduced swimming activity.

The same effect was pronounced in alevins reared for 10 days in HFO-contamined water. Both concentrations significantly reduced (from 106.4 ± 1.2 to 104.0 ± 1.2 bpm) the HR and RQ (from 98.4 ± ± 2.3 to 96.8 ± 2.5 bpm) of alevins (Fig. 6). The average value of those parameters in the control reached 118.0 ± 2.8 and 115.6 ± 2.3 bpm, respectively. The behavior of exposed fish reversed.

Exposure time did not change the effect of HFO on HR and RQ of fish. At the end of experiment (total exposure time 25 days) alterations were registered in the HR of exposed fish (118.8 ± 2.2 bpm for 0.09 g/l HFO and 113.2 ± 1.5 bpm for 0.18 g/l HFO) and significantly differed from the control (128.8 ± 2.0 bpm). Correspondingly the RQ of treated alevins decreased from 113.2 ± 1.5 to 109.6 ± 2.5 bpm) and significantly differed from the control (124.0 ± ± 1.2 bpm) (Fig. 7).

HFO-treated fish grew slowly. After 25 days of exposure their average weight fluctuated from 126.9 ±

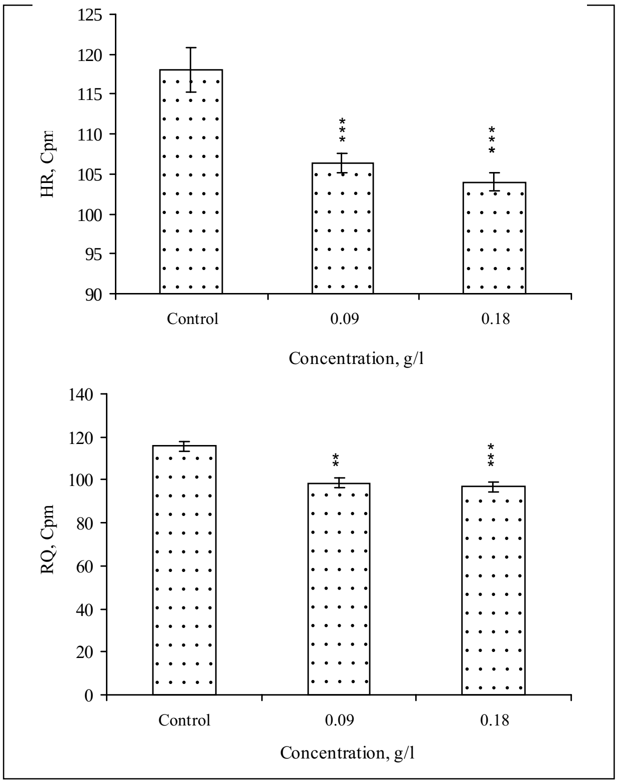

Fig. 6. Effect of filtered HFO on HR and RQ of rainbow trout alevins (M ± SE, n = 10, exposure time 10 days)

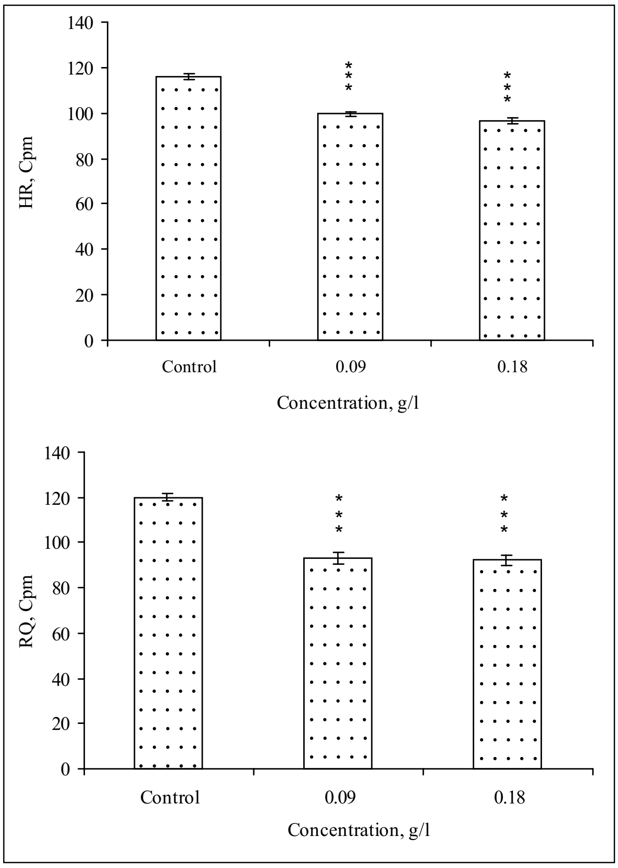

Fig. 5. Effect of filtered HFO on HR and RQ of rainbow trout alevins (M ± SE, n = 10, exposure time 5 days)

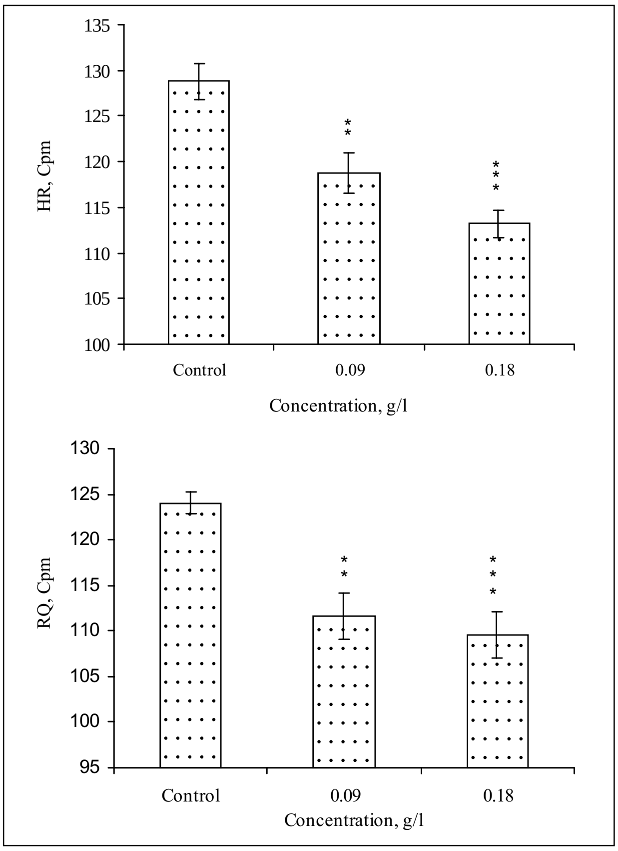

Fig. 7. Effect of filtered HFO on HR and RQ of rainbow trout alevins (M ± SE, n = 10, exposure time 25 days)

± 6.0 to 120.6 ± 6.5 mg and length decreased from 22.8 ± 0.3 to 22.6 ± 0.3 mm (0.09 and 0.18 g/l, respectively) and were significantly lower than in control (144.0 ± 4.5 and 24.3 ± 0.3, respectively) (Fig. 8). Yolk-sac utilisation was delayed by 2–4 days.

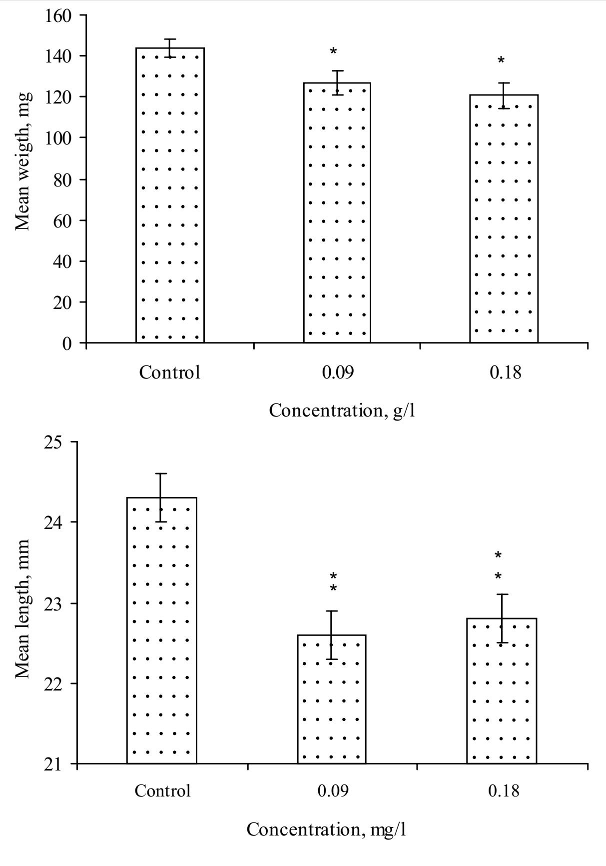

Fig. 8. Effect of filtered HFO on body mass and length of rainbow trout alevins (M ± SE, n = 15, exposure time 25 days)

DISCUSSION

In this study, chronic tests were used for the determination of heavy fuel oil (HFO) toxicity to rainbow trout alevins hatched from non-exposed eggs. Physiological parameters, growth and behavior of fish were the criteria of HFO toxicity, and heart rate and respiration frequency as well as the growth were the most sensitive parameters studied. The response of physiological parameters to both non-filtered and filtered HFO was rapid and did not depend on the concentration tested and on the duration of exposure. The same strong effect was registered on days 5, 10 and 25 of exposure. Besides, the response of physiological parameters of alevins exposed to HFO resembled that of fish hatched from eggs incubated in HFO-polluted water (Stasiūnaitė, in press). To our knowledge, the effect of HFO on the physiological state of other fish species was not investigated, however, not abundant experimental data demonstrated that oil compounds exert a strong effect on the heartbeat of yolk-sac larvae. Petroleum hydrocarbons decreased larval activity and reduced the heart rate of pike *Esox lucius* larvae (Hakkila, Niemi, 1973). The effect was observed after 2 days. Larvae of herring *C. harengus* and plaice *Pleuronectes platessa* showed narcosis within 20 min when they were treated with 8 mg/l of oil dispersant (Wilson, 1972; 1974). Also, under the influence of oil/dispersant mixtures (0.01/0.005 ml^{-1}) a narcotic effect on herring larvae was found. These mixtures (>0.5 mg/l) as well as the other petroleum hydrocarbons alone (Kuhnhold, 1969; Stene, Lonning, 1984) induced impaired swimming and prey catching behavior. The effect of oil and dispersants on larval activity may influence the survival of fish directly, because the anesthetized larvae are more susceptible to predation than others (Rosenthal, Alderdice, 1976). Besides, slower movements of larvae and uncoordinated swimming decrease the number of encounters with food particles (Westernhagen, 1986). In our study the reduced swimming activity of rainbow trout larvae was reversible. The effect of HFO was registered during the first 10–12 days, however, later the behavior of fish recovered.

One of the major effects of HFO on rainbow trout alevins was reducing larval growth. Lowered rate of yolk-sac utilisation also occurred and was considered to be one of the reasons for the slow growth of the fish. Both concentrations of non-filtered and filtered HFO inhibited the growth and the effect was pronounced on the total body mass and length of fish. Reduction in larval growth in oil-polluted waters was measured in Pacific salmon *O. gorbuscha* when alevins were exposed for 10 days (Rice et al., 1975) and in pike *E. lucius* larvae when they were reared in oil-contaminated water (>0.1 mg/l) (Vuorinen, Axell, 1980). Larval size and condition (i.e. the amount of yolk reserves) are important factors influencing successful inition of feeding. Larvae that are not able to use these reserves for differentiation to the feeding stage will perish. Under natural conditions, growth reduction in early development can result in a delay in sexual maturity. Contaminants accumulated in the body of fish can be transferred to the eggs during oogenesis, making the probable effects more severe.

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Pranė Stasiūnaitė

SUBLETALUS MAZUTO POVEIKIS VAIVORYKŠTINIO UPĖTAKIO (*ONCORHYNCHUS MYKISS*) LERVOMS

S a n t r a u k a

Nefiltruoto ir filtruoto mazuto poveikis vaivorykštinio upėtakio lervoms, išsiritusioms iš mazutu nepaveiktų ikrų, buvo tirtas įvertinant fiziologinių rodiklių ir elgsenos pokyčius, taip pat lervų augimą ilgą laiką (25 paras) veikiant žuvis subletalių 0,09 ir 0,18 g/l koncentracijų mazutu. Nustatyta, kad abiejų koncentracijų nefiltruotas ir filtruotas mazutas reikšmingai slopino širdies susitraukimų ir kvėpavimo dažnį. Pokyčiai buvo vienodi 5–10 ir 25-ą poveikio parą. Mazutu paveiktos lervos augo lėtai, vidutinė jų kūno masė ir ilgis statistiškai patikimai skyrėsi nuo kontrolinių lervų. Žuvų elgsenos pokyčiai registruoti 10–12 parų, tačiau vėliau žuvys prisitaikė prie mazuto poveikio.

Raktažodžiai: žuvys, lervos, mazutas, toksinis poveikis