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The effects of Pretilachloreon adaptation stage of Caspian roach (*Rutilusrutilus*) fingerlings

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ABSTRACT

Artificial fertilizers and mainly organophosphate herbicide are used by farmers to enhance their crop and control pests. In order to investigate the effects of Pretilachlore, fingerlings of caspian roach were exposed to a range of concentrations of toxin close to those of estuary for 96 h. Due to movement of these fingerlings in a gradient of salinity from freshwater to brackishwater some physiological parameters were also measured and compared in these environments. Cortisol levels were significantly increased in all groups exposed to Pretilachlore, and after transferring to brackishwater the amount of cortisol was increased in control group as well. K^+ and Cl^{2-} were increased in experimental groups, Na^+ changes were irregular but more changes were observed in the high concentrations of toxin. The results indicate that, fingerlings exposed to organophosphate herbicides in freshwater, may encounter some problems in physiological adaptation for entering the sea which in turn would have a negative impact on returning adults and brooders.

Keywords: Caspian roach, Biochemical parameters, Pretilachlore

INTRODUCTION

Caspian roach (*Rutilusrutilus*) is an indigenous species of the Caspian Sea fishes (Coad, 1980; Soleimani et al., 2011). It is an economically important species, which has been traditionally considered as an important source of protein by local people, and one of the main food sources for sturgeon species (Keyvanshokooh and Kalbassi, 2006). Like some other Caspian sea fishes (e.g. sturgeons) this species' population is sharply declining mainly due to over fishing, water pollution, loss of habitat and spawning grounds, and thus being categorized as "at risk" (Kiabi et al., 1999). To rehabilitate the depleted natural stocks the Iranian Fisheries Organization has the program of semi-artificial and hatchery production of valuable fish seedlings for releasing in the rivers and estuaries connected to Caspian Sea. As part of this program the fingerlings of caspian roach are released in Qare Soo River. This river meanders through the fertile agricultural plains in Golestan province, north-east of Iran and drains into the Caspian Sea. This fish is an anadromous species and therefore the fingerlings should have the ability of adaptation to variable salinity gradients from freshwater rivers to the brackishwater. The importance of both marine and

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freshwater environments in the life cycle of the fishes that have migratory reproduction system, have been studied by many scientists (Fairchild et al., 1999; Waring and Moore, 2004). During the early life stages of anadromous fishes, the freshwater conditions can have a significant impact on their survival and successful migration of spawning adults into marine environment. Exposure to different levels of toxic substances can have adverse effects on the physiology and health of aquatic organisms. Previous studies have shown the correlation between the extent of exposure of juvenile fishes to toxic contaminants in water environment, and the number of adults migrating into the sea for spawning (Fairchild et al., 1999). Due to majority of the people in north Iran are engaged in agricultural activities, amount application of synthetic fertilizers and herbicides in this area is high. Leaching and drainage of these substances into the creeks, rivers and water reservoirs will contaminate the ground and surface water, making them unsuitable for drinking and irrigation purposes, which in turn will affect the aquatic organisms. Pretilachloreis a systemic herbicide from Chloroacetamide group by selected property that is inhibiting synthesis of long chain fatty acids, which is being used extensively in rice fields to control rice pests, due to long corence period it can be regarded as a danger to the environment. Released fingerlings after location in Qare soo river estuaries, have exposed to some toxics (Shayeghy et al., 2006) such asPretilachlore.At time that fingerlingslives in freshwater and then they move towards the brackishwaters, they would undergo many complex behavioral, physiological, and biochemical changes to adopt themselves to the estuarine and eventually Caspian Sea conditions (McCormick et al., 1998). Salinity tolerance and increased hypo-osmoregulatory ability are necessary if fingerlings are to succeed survival in marine environment. Nevertheless few studies have been done to investigate the effects of freshwater pollutants on the subsequent stages of fish's life in brackishwater environment. The effects of Pretilachlore exposure on the changes in physiological conditions of caspian roach fingerlings were evaluated by measuring different parameters. Plasma cortisol is a stress indicator hormone which is indirectly increased with stressors. Electrolytes are involved in osmotic activity and provide buffer systems and mechanisms for the regulations of pH (acid–base balance). Ions of Na^+, K^+ and Ca^{2+} play a significant role in maintaining the hyper osmotic properties of freshwater fishes (Suvetha et al., 2010). In freshwater fish the physiological regulations of major electrolytes is very sensitive to environment stressors and are commonly altered in response to pollutants including herbicides (McDonald et al., 1989). These parameters were also measured to determine the extent of fingerlings ability to osmoregulation activity in brackishwater after exposure to the Pretilachlore herbicide.

MATERIALS AND METHODS

Fish raring

Twelve hundred caspian roach fingerlings (average weight of 1.66 ± 0.05 gr), used in this research was supplied by the Sijowal Teleost Fishes Propagation Center (Golestan Province, Iran). They were, acclimatized to the laboratory conditions for one week in an aerated 1500 liter fiberglass container before being equally and randomly distributed into twelve 100 L tanks (four treatments, each with three repeats). Water temperature, dissolved oxygen, pH and salinity were monitored daily and maintained constant at 25.3 °C, 7.01 mg L-1, 7.8, and 3.2 ppt, respectively. The photoperiod was fixed at 14L:10D. The tanks were aerated continuously and 10% of the water volume was exchanged daily with freshwater containing the corresponding concentration of toxicant for each treatment. During acclimatization period and in brackishwater the fingerlings were fed to satiation twice a day with commercial fingerling food (Supplied by Sari Animal and Aquatic Feed Factories), and feeding was discontinued 24 h before the start of the experiment.

Sublethal toxicity experiments

Twelve hundred caspian roach were randomly divided in twelve 100 L fiberglass tanks (4 treatments by triplicate) to accomplish the 96 h (4 days) period sublethal toxicity experiment. One hundred fingerlings were placed in each tank with particular concentration of Pretilachlore that was purchased from Partonar Company. These concentrations include: 0.0 (control), 0.25, 0.5 and 0.75 mg l^{-1} of Pretilachlorethat were selected according to 1/12th, 1/6th and 1/4th of 96 h LC50 value acute toxicity that for caspian roach fingerling was reported by Khosravi Katuli et al (2013) (12 mg L-1). After 96 h of exposure, the remaining fish were shifted to herbicide-free brackishwater (salinity increase was done gradually during 12 h) and the Whole-body extract samples were analyzed at intervals of 0 h, 24 h and 96 h (4 days) after transfer to brackishwater.

Whole-body biochemical extraction and measurement

At the each sampling time, three fish per replicate were sampled (nine fish $treatment^{-1}$). Due to inadequate blood volume to accurately measure plasma biochemical parameters in small fish, whole body extracts were used in this study, in previous studies have also used the whole body extracts to measure blood biochemical factors (Ramsay et al., 2006; Prodocimo et al., 2007; Peterson and Booth, 2010). Fingerlings were removed from the tanks and to euthanasia were located immediately in the solution of Clove. For drying water bodies, fish were placed on blotting paper then immediately frozen in liquid nitrogen for 10–30 seconds, and then homogenized samples were located in gated tube for centrifuge 14000 rPM (Nakano et al., 1998; Ramsay et al., 2006). Cortisol levels were measured using radioimmunoassay (Waring et al., 1996).Whole-body Cl^{2-} by automated titration and whole-body Na^+ and K^+ by flame emission.

Statistical analysis

All experiments were carried out in triplicate and the results presented as mean of three values with standard deviation. All the data were tested for normality (Kolmogorov–Smirnov test). Analysis of variance (ANOVA) procedure followed by Duncan’ test using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference (p< 0.05) between treatment means.

Results

Caspian roach fingerlings were exposed to three different sublethal concentrations of Pretilachloreselected based on the 96 h LC50 value (Khosravi Katuli et al., 2013). No significant mortality occurred in all treatment groups throughout the study. In some cases, some abnormal behavior was observed in exposed fishes in comparison to control groups, including: Sedentary and lethargic swimming, muscle tremors and lack of response to stimulation.

Sublethal toxicity of Pretilachloreon cortisol:

Cortisol levels significantly increased in all Pretilachloreconcentration treatments ($p< 0.05$) at different sampling intervals, compared to the control groups (Fig. 1). Samples taken from treatment 2 after 24 h exposure showed the highest cortisol levels compared to other groups. At the end of toxin exposure period (96 h), when fingerlings were transferred to herbicide-free brackishwater, the amount of cortisol reversed to the pre exposure levels. However in control group the amount of cortisol increased when placed in brackishwater (Fig. 1).

Sublethal toxicity of Pretilachloreon whole-body biochemical parameters:

Mean values of the whole-body biochemical parameters of caspian roach fingerlings exposed to sublethal concentrations of Pretilachloreare presented in Fig. 1. K^+ and Cl^{2-} levels were higher in Pretilachloretreated fish at all sampling times but Cl^{2-} reduced at 24 h. In both experiments, changes in the Na^+ were not significant compared to control group. When the Pretilachlore affected fishes were transferred to herbicide-free brackishwater, the amount of K^+ depleted to even lower than pre-exposure to toxic levels, except Cl^{2-} which did not decline considerably.

Discussion

The life of juvenile fishes that migrate from freshwater to the seawater environment is closely related to previous stages of their life in fresh water (Waring and Moore, 2004). Rivers, in which fingerlings fish spend adjustment period, might be contaminated with various pollutants such as pesticides, herbicides and fertilizers. Qare soo River estuary which is the natural nursery habitat and consequently the physiological adjustment place of caspian roach fingerlings is variably polluted with some toxicants (Shayeghy et al., 2006) such asPretilachlore herbicide residues. Fishes are very susceptible to the environmental pollution of water, therefore, contaminants such as Pretilachlore might cause significant damages to certain physiological and biochemical processes in fishes and other aquatic organisms (Banaee et al., 2011; John, 2007). This research investigated the effects of Pretilachlore within the concentration range (based on the 96 h LC50 value) that was close to estuaries concentration too. In general physiological and biochemical parameters measurements can be used as a useful tool to identify the environmental risks. In ourexperiments, we measured some whole-body factors as biomarkers to demonstrate the physiological condition of the fingerlings. Cortisol is a hormone secreted from the Hypothalamo-pituitary gland-internal axis (HPI) and used in many studies as the stress indicator (Flik et al., 2006; Gagnon et al., 2006; Sepici-Dinçel et al., 2009). The report found that cortisol levels in fish are varied in accordance with the changing seasons (Pottinger, 1998) it is released in the faced with stressor and thus levels of this hormone sudden rise in blood. In this study after 24 h exposure to Pretilachlore, the cortisol levels of whole-body extract, reached to the highest levels in all treatments, showing a significant increase compared to the control groups (Fig. 1). After 96 h (four days) when all fingerlings in three experimental groups were transferred to brackishwater the cortisol levels reduced sharply, except in control groups which showed a quantitative increase in cortisol levels (Fig. 1). The former sharp decline could be due to the elimination of the stressor factor, but the later elevation can be attributed to the stress inflicted due to the transfer of the fishes from the fresh water into the much more saline brackishwater. Sepici-Dinçel et al., (2009) and Waring and Moore, (2004) found that amount of cortisol levels in the fishes that exposed to cyfluthrin and atrazine respectively, were significantly higher than the control group. Alterations in ionic regulation in animals can be affected by environmental stressors. In this study, in caspian roach fingerlings which were exposed to sublethal concentrations of Pretilachlore, K^+ and Cl^{2-}

(except 24 h) levels elevated significantly compared to the control group (Fig. 1). When the fingerlings were exposed to herbicide we expected a decline in the whole-body electrolytes levels because of the haemodilution process. Na^+ changes were irregular but more changes were observed in the high concentrations of toxin. Suvetha et al., (2010) found that electrolytes levels decreased in *Cyprinuscarpio* exposed to pesticide. Cause of the rise electrolyte levels may be due to raised passive efflux of ions across the gills and also the restraint of active ion uptake by the chloride cells of the gills can more contribute to the negative ion balance of the blood (WendelaarBoonga and Lock, 1991). However, it is not clear exactly why it happens but probably this is related to the particular stages of fingerlings life, because in the course of life they live in a hypo-osmotic environment but their bodies are physiologically ready to adapt to brackishwater conditions. Similar works were also reported by Das and Mukherjee, (2003), who found serum ionic levels like Na^+ and Mg^{2+} in the blood of *Labeorohita* fingerlings exposed to cypermethrin were significantly increased compared to control groups. Again measurement of electrolytes in fingerlings, determine physiological status of them after transferring to the toxin-free brackishwater. Watson and Beamish, (1980) reported that electrolyte levels in fish exposed to pesticides have been able to return to their initial concentrations. In our experiments we observed that after transferring of fish to brackishwater chloride levels decreased (Fig. 1). Coho salmon smolts were exposed to the fungicide Didecyldimethyl Ammonium Chloride in freshwater and then transferred to sea water for challenge in the 24 h, the results showed a decrease in osmoregulation ability in this smolts (Johnston et al. 1998).

Conclusion

In summary, the osmoregulation capacity in fingerlings exposed to Pretilachlore was reduced in both the river and the brackishwater environments. This is due to tissue destruction and irregularities in the electrolytes of whole-body extract. Changes in the amount of cortisol hormone levels due to stressful conditions were also confirmed. Although no significant mortality was observed in fingerlings which were transferred to brackishwater, the destructive effects on osmoregulation capability in fingerlings fish can affect the survival rate and eventually the number of adult fish and brooders returning to the river.

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Figure captions

Fig. 1 Whole-body cortisol, sodiume, potassume and chloride levels in caspian roach (*Rutilus rutilus*) fingerlings during and after exposure to sub-lethal concentrations of Pretilachlore(0, 0.25, 0.5 and 0.75 mgl^{-1}) in fresh water and brackishwater. Significant differences were characterized by alphabetical letters (One-way ANOVA). Values are expressed as mean ± S.D, n = 9.

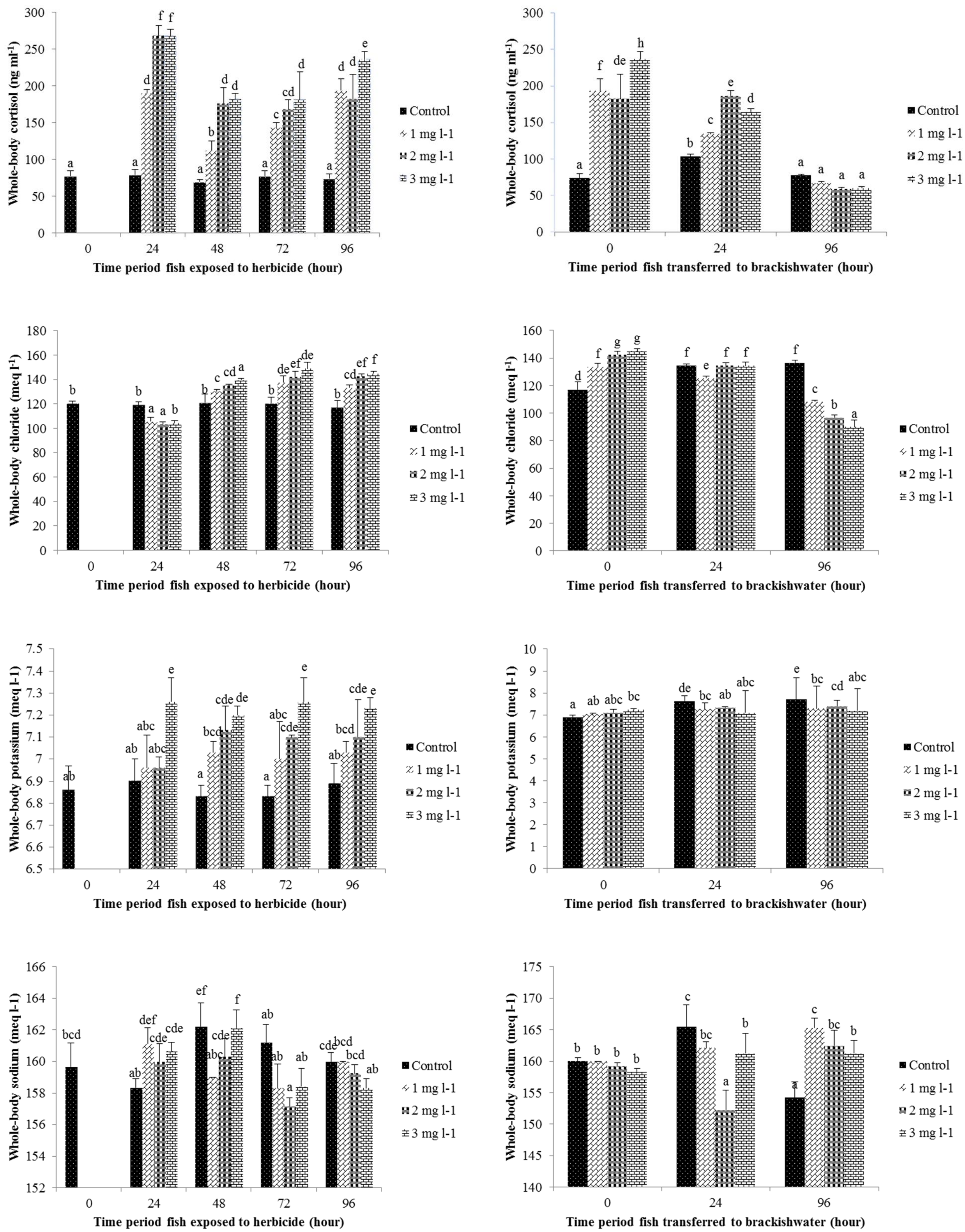
Whole-body cortisol (ng ml-1)
Whole-body chloride (meq l-1)
Whole-body potassium (meq l-1)
Whole-body sodium (meq l-1)
Time period fish exposed to herbicide (hour)
Time period fish transferred to brackishwater (hour)
Control
1 mg l-1
2 mg l-1
3 mg l-1

Fig 1.