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Effect of formalin treatment on saltwater tolerance in Caspian roach (*Rutilus rutilus caspicus*)

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Abstract

The effect of formalin exposure on subsequent saltwater tolerance was investigated in Caspian roach (*Rutilus rutilus caspicus*). Fish were subjected to 0 (control), 10, 15 and 25 ppm formalin over 0 (control), 12, 24 and 168 h (4×4 treatments; 6 tanks per treatment) as well as 25, 50, 100 and 150 ppm formalin over 0.5 and 1 h (4×2 treatments; 6 tanks per treatment). Thereafter, half of each treatment's tanks (3 tanks) were subjected to formalin-free freshwater and the rests were subjected to formalin-free saltwater (12 ppt NaCl) and mortality was recorded at 0, 72 and 168 h exposure. Results showed decrease in saltwater-induced mortality with increase in formalin exposure from 12 to 168 h, particularly in fish exposed to 25 ppm formalin. However, increment in formalin exposure from 0.5 to 1 h caused increase in saltwater-induced mortality, particularly in formalin concentrations of 100 and 150 ppm. Increase in saltwater tolerance after 168 h formalin exposure might be due to adaptation mechanisms development or suppression in stress induced by formalin exposure. Decrease in saltwater tolerance after 1 h, compared to 0.5 h, formalin exposure might be due to cumulative effect of the two stressors (formalin and saltwater exposure).

Keywords: Caspian roach, formalin, saltwater, toxicity

Introduction

Caspian roach (*Rutilus rutilus caspicus*) is commercially important, has experienced a remarkable decline in the fishing yields of the Iranian Caspian Sea due to over-fishing and habitat deterioration. Therefore its artificial propagation in hatcheries to recruit its natural stocks is fulfilled for 10 years. In this program, produced Caspian roach larvae are stocked in earthen pond till reaching 1-3 g. and then released into river mouth of their origin. During releasing period, sea water is introduced to the river mouth as result of little water-flow. Also, because of little water-flow of the upper parts of the rivers, releasing of fingerlings is not applicable. Hence, they are released in the salinity of > 10 ppt which may causes osmotic shock in the fish.

Increasing of fish production in aquaculture is often accompanied by increasing incidence of fish parasites; hence it requires applying therapeutic or prophylactic drugs. Formalin is a widely-used therapy for ectoparasites, ectocommensal parasites, bacterial gill and skin disease (Speare and Ferguson, 1989; Thorburn and Moccia, 1993; Klinger and Francis-Floyd, 1998). Concentrations of 150-250 ppm over 30-60 min, and 15-25 ppm over the longer periods (depending on species and life stage) are suggested as short- and long-term formalin treatment, respectively (Stoskopf, 1988; Luzzana and Valfre, 1993; Klinger and Francis-Floyd, 1998).

Since, in the roach hatcheries, formalin is used for disinfecting of the equipments as well as medication. Application of formalin may damage gill tissue of fish (Wedemeyer, 1971; Smith and Piper, 1972) which is the vital organ of osmoregulation (Evans et al., 2005). Therefore, formalin treatment may affect internal osmotic balance of fingerlings that will release into the Caspian Sea. Hence, this study conducted to investigate the effect of formalin treatment on saltwater tolerance of Caspian roach.

Materials and methods

Two separate experiments were conducted to evaluate the effect of formalin exposure on saltwater tolerance in Caspian roach (1 ± 0.15 g). In the first experiment (long-term) fish were subjected (after 10 days acclimation) to 0 (control), 10, 15 and 25 ppm formalin (37% formaldehyde, Merck, Germany) over 0 (control), 12, 24 and 168 h (4×4 treatments; 6 tanks per treatment). Thereafter, half of the tanks per treatment (3 tanks) were subjected to formalin-free freshwater and the rests were subjected to formalin-free saltwater (12 ppt NaCl) and mortality was recorded and removed at 0, 72 and 168 h exposure. In the second experiment (short-term), fish were subjected to 25, 50, 100 and 150 ppm formalin over 0.5 and 1 h (4×2 treatments; 6 tanks per treatment). Thereafter, half of the tanks per treatment (3 tanks) were subjected to formalin-free freshwater and the rests were subjected to formalin-free saltwater (12 ppt) and mortality was recorded and removed at 0, 72 and 168 h exposure. Stocking density was ~ 2 g L-1 in both experiments. Fish were fed (Biomare, France) once a day (1% of body weight) during the acclimation period. Feeding was ceased 24 h before formalin-exposure-termination and thereafter. Continuous aeration was provided throughout the entire experiments. Water quality was as follow: temperature = 23 ± 1 ºC, dissolved oxygen > 7 ppm , pH = 7.5-7.9, total hardness = 171-179 ppm ($CaCO_3$), alkalinity = 162-170 ppm ($CaCO_3$), nitrate-N = 0.5 ppm.

Since no mortality was occurred in control group before and after salt exposure as well as in the other groups during formalin exposure (0-168 h) in the long-term experiment, data were analyzed as a 3 × 3 factorial design (3 formalin concentrations × 3 exposure periods). Data of the short-term experiment were analyzed as a 4 × 2 × 2 factorial design (4 formalin concentrations × 2 exposure periods × 2 salinities). Percentile data were transformed using the arc-sine method based on Mostellar and Youtz (1961) and analyzed by two way ANOVA and LSMeans' test using statistical software (SAS, v. 9). Significance was tested at the 0.05 level. All data were presented as means ± SD.

Results

There was no mortality after 72 h saltwater exposure, thus mortality data at 168 h were not analyzed, in both short-term and long-term experiments. No mortality was observed after before as well as 0 h after saltwater or freshwater exposure, thus data at this point was not analyzed (Fig. 1). In the long-term experiment, survival was significantly ($P < 0.01$) affected by formalin concentration, exposure period and their interaction, at both 24 and 72 h saltwater exposure. 168 h formalin treatment (all concentrations) did not affect survival after saltwater exposure (Fig. 1). 12 and 24 h exposure to 25 ppm formalin significantly deteriorated survival after saltwater exposure, compared to 10 and 15 ppm (Fig. 1). 24 h exposure to 15 ppm formalin significantly deteriorated survival, after saltwater exposure, compared to 10 ppm (Fig. 1). There was no significant difference in survival after saltwater exposure between fish treated with 15 and 10 ppm formalin over 12 h. There was significant difference in survival after saltwater exposure in fish treated with 25 ppm formalin over 12, 24 and 168 h (Fig. 1). 12 and 24 h treatment with 15 ppm formalin significantly decreased survival after saltwater exposure compared to 168 h (Fig. 1). 12 h treatment with 10 ppm formalin significantly decreased survival after saltwater exposure compared to 168 h (Fig. 1). In the short-term experiment, survival data of 0 h exposure to fresh or saltwater were very close and insignificant different, thus, they were pooled (n = 6) and expressed as a single treatment (0 h) to facilitate understanding (Fig. 2 and 3). 72 h saltwater exposure resulted significantly in lower survival compared to 0 h and 72 h freshwater exposure in following treatments: 25 ppm formalin-0.5 h (25-0.5), 50 ppm formalin-0.5 h (50-0.5), 25 ppm formalin-1 h (25-1) and 50 ppm formalin-1 h (50-1) (Fig. 2). 100 ppm formalin-0.5 h (100-0.5) and 100 ppm formalin-1 h (100-1) resulted significantly in lower survival after 72 h exposure to both fresh and saltwater, compared to 0 h (Fig. 2). 150 ppm formalin-0.5 h (150-0.5) and 150 ppm formalin-1 h (150-1) resulted significantly in lower survival after 72 h exposure to saltwater, compared to 0 h and 72 h freshwater (Fig. 2). Survival was zero in 150-1, after 72 h saltwater exposure (Fig. 2). The lowest survival at 0 h was related to 150-1 followed by 150-0.5, while the other treatments showed similar survival (100%) (Fig. 3). The lowest survival at 72 h in freshwater was related to 150-1 followed by 150-0.5, 100-1 and 100-0.5 which showed statistically similar levels (Fig. 3). The lowest survival after 72 h saltwater exposure was related to 150-1 followed by 150-0.5 (Fig. 3).

Discussion

Everywhere there are any toxic substances, water contamination may occur due to mismanagement and ignorance. Accordingly, several studies have focused on formalin toxicity in certain species (Birdsong and Avault, Jr., 1971; Cruz and Pitogo, 1989; Reardon and Harrell, 1990). Formalin induces gill damages, including epithelial rise or separation along with gill tissue protein denaturation and hardening, in dose-dependent manner (Wedemeyer, 1971; Cruz and Pitogo, 1989; Meinelt et al., 2005). Such damages disrupt gill normal functions such as osmoregluation and gas exchange, which are stressful for fish and affect survival (Wendelaar Bonga, 1997). If fish fail to recover from a stressor agent, a subsequent normally non-fatal stressor can be fatal (Carmichael, 1984). In this study, 10-25 ppm formalin was not lethal, however, caused subsequent mortality during saltwater exposure. It might be as a result of repeated stress (formalin exposure and saltwater). Previous study on common carp (*Cyprinus carpio*) showed cumulative stress response when they subjected to crowding stress prior to saltwater exposure (Hosseini and Hoseini, 2010). Some adaptation mechanism might be developed during 168 h formalin exposure, since this group showed no significant mortality during saltwater exposure. This might be as a result of adaptation to stress caused by formalin in fish, which in turn, allowed the fish to tolerate saltwater stress in a better way. However, the other mechanisms can be involved, as well.

In the short-term experiment, mortality in saltwater following formalin treatment seems to be related to formalin-induced stress rather than gill damage. Gill damage is unlike during such short period of exposure (0.5 and 1 h). Stress response as a result of formalin exposure might intensify the adverse effects of saltwater exposure. Present results demonstrated that formalin treatment at various doses, including therapeutic ones, may deteriorate Caspian roach survival during saltwater exposure. This information should be used when this species are released into the rivers for stock rebuilding purposes, where they might face saltwater as a result of climatic and anthropogenic reasons. Exposure to 25-150 ppm formalin over 0.5-1 h, although have been suggested as the therapeutic concentrations, deteriorates saltwater tolerance in Caspian roach.

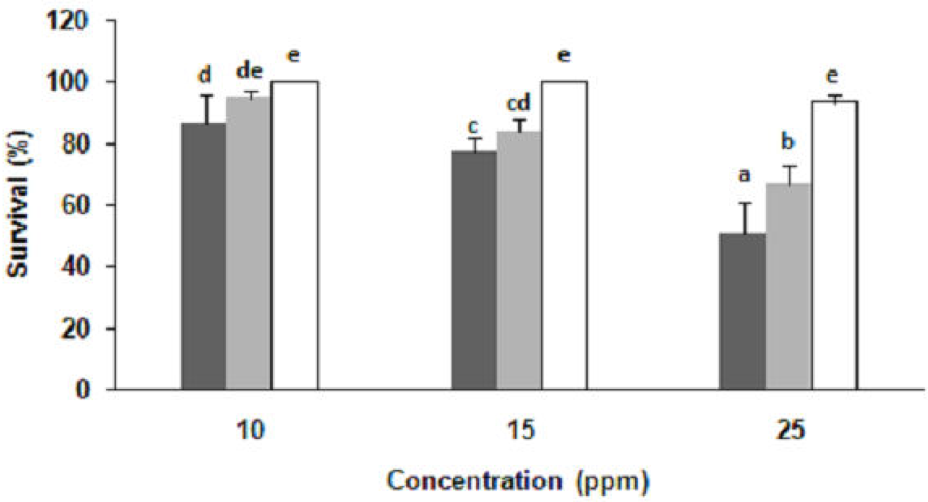

Figure 1. Effect of different formalin concentrations over 12 (black bars), 24 (grey bars) and 168 (white bars) h on survival after 72 h saltwater exposure. Different letters show significant difference among the treatments ($P < 0.05$). No mortality was observed before saltwater exposure.

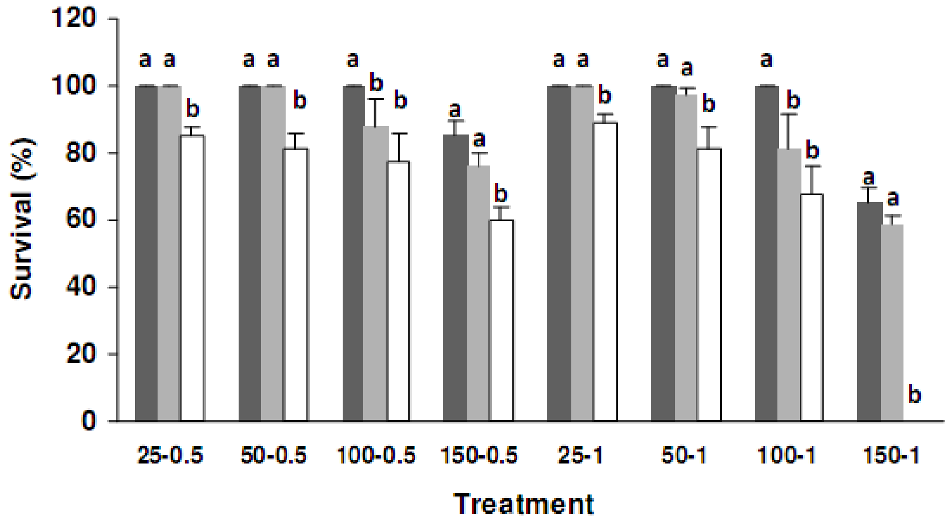

Figure 2. Effect of different formalin treatment on survival after fresh or saltwater exposure [survival at 0 h (black bars), 72 h freshwater (grey bars) and 72 h saltwater (white bars)]. Different letters show significant difference among each treatment, separately (P < 0.05).

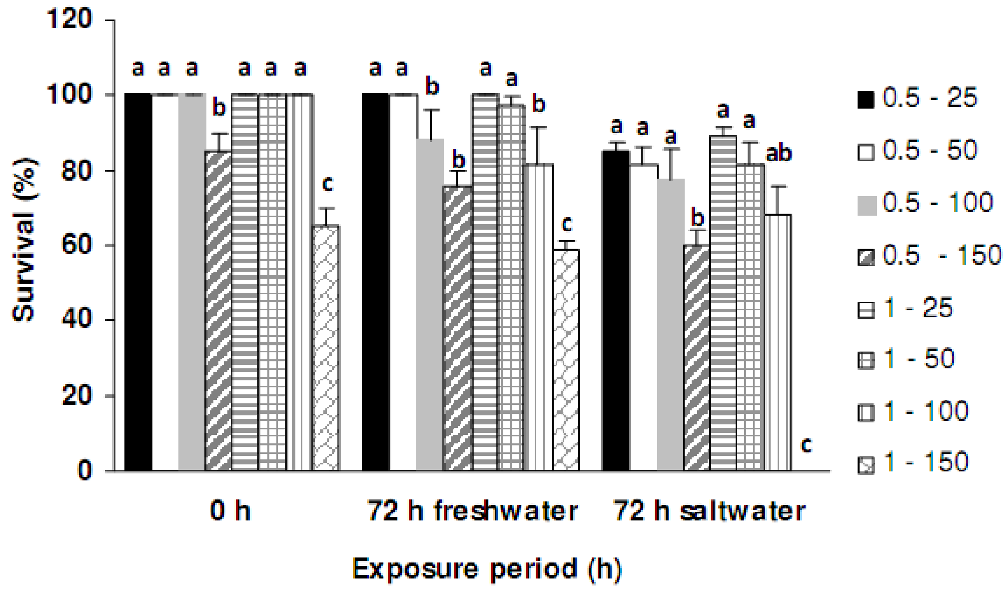

Figure 3. Effect of period of exposure to fresh or saltwater on survival in different formalin treatments. Different letters show significant difference among each exposure period, separately (P < 0.05).

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