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Effects of nonylphenol on key hormonal balances and histopathology of the endangered Caspian brown trout (*Salmo trutta caspius*)

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ABSTRACT

Endocrine disruptor chemicals (EDCs) potentially pose a hazard to endangered species. Evaluation of the sensitivity of these species to EDCs could be helpful for protecting their populations. So, the present study investigated the adverse effects of nonylphenol, an EDC, on the endocrine hormones and histopathology of male and female juvenile Caspian brown trout (*Salmo trutta caspius*) following 21 days of exposure to nominal concentrations of 1, 10 and 100 μg/l. The results showed that the HSI and plasma total calcium of male and female fishes exposed to 100 μg/l nonylphenol were significantly increased compared with the control groups ($P < 0.001$). The male plasma T_3 level was significantly decreased in 10 ($P < 0.01$) and 100 ($P < 0.001$) μg/l nonylphenol. The female T_3 level increased in 1 μg/l nonylphenol concentration ($P < 0.05$). The plasma T_4 of males showed significant elevation in fishes exposed to 100 μg/l nonylphenol ($P < 0.05$), but no change for females in any of treatment groups relative to controls ($P > 0.05$). No significant effect of nonylphenol exposure was observed on male plasma TSH levels ($P > 0.05$), whereas, in females, nonylphenol at all concentrations significantly reduced TSH levels. A bell-shaped response was observed in male and female plasma GH levels. Moreover, various histopathological lesions were observed in gill and intestine tissues of fishes exposed to different nonylphenol concentrations. These results demonstrate the high sensitivity of this endangered species to even environmentally relevant concentrations of nonylphenol. Furthermore, Caspian brown trout could be used as bioindicators reflecting the toxicity of nonylphenol.

1. Introduction

Nonylphenol is a microbial breakdown product of a non-ionic surfactant, nonylphenol ethoxylate (NPE), which is widely utilized in the production of industrial and domestic cleaning agents, pesticides, paints, plastics and emulsifiers (Vincent and Sneddon, 2009). The extensive use of these products led to the discharge of NPE into the aquatic environment through municipal, agricultural and industrial wastewaters, as well as surface runoff (Mao et al., 2012). Biodegradation of nonylphenol by microorganisms under aerobic or anaerobic conditions could result in reduced concentrations of nonylphenol in water, sediment and soils (Mao et al., 2012). Alkylphenol-degrading bacteria could degrade long-chain alkylphenols through ortho-cleavage pathway and multicomponent phenol hydroxylase (Nguyen et al., 2011). Nonylphenol was shown to be both acutely and chronically toxic to a wide range of organisms, including fishes (Naderi et al., 2014; Shirdel and Kalbassi, 2014), mammals (Gan et al., 2015), crustaceans (Park and Choi, 2009), molluscs (Swedmark et al., 1971) and algae (Zaytseva et al., 2015). Endocrine disruptive effects of nonylphenol on freshwater fishes were proved in previous studies. Wu et al. (2014) reported that nonylphenol exposure resulted in significant upregulation of aromatase, estrogen receptors and vitellogenin gene expression, and elevation of hepatic and plasma vitellogenin concentration. Exposure to 4-nonylphenol in medaka (*Oryzias latipes*) embryo from 24 h postfertilization up to 104 days skewed sex ratio toward female. The mean fertility of the breeding pairs was decreased to 76% of the control pairs (Yokota et al., 2001). Intraperitoneal injection of 4-nonylphenol to juvenile rainbow trout caused significant elevation of testosterone and 17β-estradiol in plasma of females and males, respectively. In addition, 4-nonylphenol reduced plasma levels of thyroxine and triiodothyronine (Naderi et al., 2013). In developing countries, such as Iran, because of the lack of high-efficiency sewage treatment plants, large amounts of untreated sewage are released into local water resources (Tajrishy and Abrishamchi, 2005). As recently reported by Mortazavi et al. (2012, 2013), high levels of 4-nonylphenol (4-NP), up to 8.17, 10.43 and 29 μg/g dry weight in fish muscle and liver as well as sediments, respectively, were detected in the Anzali wetland. Therefore, as an endocrine disruptor, this large amount of nonylphenol could threaten the key hormonal balance and reproductive development, as well as histological structure, of aquatic animals inhabiting the southern part of the Caspian Sea, especially the endangered Caspian brown trout (*Salmo trutta caspius*).

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The Caspian brown trout, which belongs to the Salmonidae family, is an endemic species and one of the most valuable commercial fishes of the southern part of the Caspian Sea. Populations of this species have disappeared from many of its historic areas in the Caspian Sea, and its wild populations are close to extinction (Vera et al., 2011). Water pollution is one the major factors implicated in the decline of these populations (Kiabi et al., 1999). This is similar to that seen in the case of Atlantic salmon (*S. salar*), for which the historical population decline was related to the use of nonylphenol-containing pesticide (Matacil 1.8D which contained nonylphenol as primary solvent) for forest spraying (Fairchild et al., 1999). The effects exerted by Matacil 1.8D have been linked to the estrogenic potential of the 4-NP formulation. It was hypothesized that antagonistic effects of the xenoestrogen nonylphenol may influence the subsequent returns of salmon (Fairchild et al., 1999). It was also revealed in previous studies that 4-nonylphenol and 17β-estradiol could exert inhibitory effects on smoltification and the hypo-osmoregulatory physiology of Atlantic salmon (Madsen et al., 1997).

In many salmonid species, the estrogenic-like behavior of nonylphenol has been shown (Meucci and Arukwe, 2005; Xie et al., 2005). Nevertheless, only a few studies have evaluated the effects of nonylphenol on thyroid and growth hormone homeostasis and non-gonadic histopathology in fishes (Robertson and McCormick, 2012; Naderi et al., 2013; Naderi et al., 2014). However, the current study is the first to examine the effect of nonylphenol on brown trout.

Thyroid hormones have a pivotal function in development, metabolism, growth, differentiation, metamorphosis and reproduction in fishes, as in other vertebrates (Carr and Patiño, 2011). Hence, the effects of contaminants on thyroid function have received much recent attention. However, in fishes, the mechanisms for disturbing thyroid hormones are still unclear (Guo and Zhou, 2013). GH, known as somatotropin, contributes to diverse physiological functions, such as growth stimulation, lipid metabolism, behavior, energy partitioning, osmoregulation, gonadal development, steroidogenesis and smoltification in teleosts. Consequently, a disruption in GH balances caused by exposure to pollutants could disturb many physiological processes, including the normal reproductive development of fishes (McCormick et al., 2005).

In the present study, plasma levels of thyroid-related hormones (TSH, T_3 and T_4) and GH as vital hormones contributing to key physiological processes were used as endocrine biomarkers. In addition, gill and intestine histopathology, as suitable non-endocrine toxicant biomarkers, and plasma total calcium, as a surrogate measure for plasma vitellogenin, were also evaluated in Caspian brown trout following exposure to both environmentally relevant and higher levels of nonylphenol. To our knowledge, this is the first study comparing the endocrine hormones of sexually immature male and female fish in response to nonylphenol exposure.

2. Materials and methods

2.1. Ethical note

The protocol of this study was approved by the Research Council of the Faculty of Natural Resources and Marine Sciences of Tarbiat Modares University (152/D/11,197, 29–01-2012).

2.2. Fish

Juvenile Caspian trout weighting 40 ± 10 g (1^+ year, n = 100) were procured from a local commercial hatchery and brought to the laboratory, and maintained in a 1000-l tank containing dechlorinated tap water (pH 7.9–8.2; dissolved oxygen 9 ± 0.3 mg/l; salinity 0.4 ppt; electrical conductivity (EC) 800 μS/cm; total hardness 102 mg/l $CaCO_3$). The fishes were acclimatized to their new surroundings for 1 week before the exposure period, and fed twice daily with commercial trout pellets at a ratio of 2% of the total body weight per day. During the adaptation and exposure period, fishes were kept in a 12:12 (light:dark) photoperiod at a mean temperature of 13 °C.

2.3. Chemicals

Nonylphenol (analytical standard, PESTANAL®, Riedel-de Haën, CAS number: 84,852–15-3) was purchased from Sigma-Aldrich corporation, Seelze, Germany. Absolute ethanol was purchased from Merck Company. Before experimentation, nonylphenol was dissolved into absolute ethanol to prepare a stock solution.

2.4. Physicochemical parameters of the water

Physicochemical parameters of the water, including, salinity, EC, pH and dissolved oxygen, were determined by the Consort multiparameter analyzer (Consort C863, Belgium). Based on a colorimetric method, total hardness was quantified using the Palintest Hardicol test and a Palintest Photometer (Palintest 8000, UK).

2.5. Experimental design and chemical exposure

Nominal exposure concentrations of nonylphenol (1, 10 and 100 μg/l) were prepared by diluting the stock solution to reach the desired concentrations of nonylphenol in the exposure tanks. The exposure concentrations were designed according to the nonylphenol LC_{50} value determined in our previous study (Shirdel and Kalbassi, 2014). Based on the value obtained for 96 h LC_{50}, three nominal concentrations of nonylphenol [1 (~0.5% of LC_{50}), 10 (~5% of LC_{50}) and 100 (~50% of LC_{50}) μg/l] were used for the subacute semi-static in vivo exposure conditions in the current study. Fishes were randomly allocated to five treatments in duplicate (Lammer et al., 2009; Modesto and Martinez, 2010) (ten 300-l tanks containing 100 l water) in which each replicate contained ten fish. In addition to the nonylphenol treatments (1, 10 and 100 μg/l), two control treatments [water control and solvent control (0.01% (*v*/v) ethanol)] were also used. The concentration of ethanol in the solvent control was similar to that in the nonylphenol treatments (10 ml ethanol per 100 l of water). The total volume of water and exposure solution in the tanks was renewed twice per day. The experimental period lasted for 21 days. The survival and behavior of the fishes during the experiment were also assessed.

2.6. Determination of nonylphenol concentrations in the test waters

Concentrations of nonylphenol in the test waters were measured according to the method described by Yamamoto et al. (2001). Briefly, 500 ml of water from each exposure concentration was sampled immediately after the preparation of exposure solutions. Fifty grams of sodium chloride was added to the water samples, and then acidified with hydrochloric acid (pH ≤ 3). Liquid–liquid extraction was used to extract water samples. The samples extracted twice with 50 ml of dichloromethane by shaking for 5 min. The extracts were dried by anhydrous sodium sulfate, and then concentrated to 5 ml using rotary evaporator, transferred to a test tube and evaporated to 1 ml under nitrogen flow. The concentrated extract was analyzed by a gas chromatograph/mass spectrometer (HP Agilent 7890A, 5975C). About 1 μl of each extract was injected into the GC system through a capillary column (HP-5ms; 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) in a splitless mode. The actual concentrations of nonylphenol in the test waters immediately after the preparation of exposure solutions were 0.95 ± 0.34, 8.72 ± 2.03 and 91.67 ± 11.72 μg/l, respectively for 1, 10 and 100 μg/l nominal concentrations. Nonylphenol was not detected in the control groups.