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Sensitivity of brown trout reproduction to long-term estrogenic exposure

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ABSTRACT

A decline in brown trout (*Salmo trutta fario*) catches has been reported in Switzerland, but at present the causative factors have not been clearly identified. Estrogen-active endocrine disrupters (EEDs) have been suggested as one possible explanation, since they are widespread in the aquatic environment and often found at elevated concentrations.

In the present study the effects of long-term estrogenic exposure on the reproductive capability of brown trout were investigated. Adult fish were continuously exposed to an environmentally relevant mixture of the natural estrogens estrone (E1), 17β-estradiol (E2) and the xenoestrogen 4-nonylphenol (NP); the average measured concentrations over the entire exposure time ($n = 9$) were 14.0 ng/l (Min 8.1 and Max 20.6) for E1, 2.1 ng/l (Min 1.3 and Max 4.1) for E2 and 111.0 ng/l (Min 106.7 and Max 115.9) for NP. A solvent control served as negative control, and up to 10-fold higher mixture concentration than the environmentally relevant concentration served as positive control. The fish were exposed for 150 days from the onset of gonadal recrudescence until sexual maturation. Plasma vitellogenin (Vtg) was significantly induced by both concentrations of the estrogenic mixture, whereas effects on growth and fertility were only observed in fish exposed to the high mixture treatment. Fertilization success and offspring hatchability in brown trout exposed to the high mixture treatment were significantly reduced to 9% and 6%, respectively. Developmental time from fertilization until hatching, the percentage of larvae with malformations and survival of larvae, however, were not affected.

The results suggest that a combination of estrogen-active compounds at environmentally relevant concentrations would not adversely affect those parameters of brown trout reproductive capability measured in this study. Plasma Vtg in male brown trout appeared to be more sensitive to (xeno)estrogen exposure than the measured reproductive effects.

1. Introduction

A decline in freshwater fish populations has been observed in many parts of the world (Duncan and Lockwood, 2001; Keiter et al., 2006) with little evidence for the causes. In Switzerland a decline of brown trout (*Salmo trutta fario*) catches has been reported since the 1980s (Burkhardt-Holm et al., 2005). Brown trout are the ecologically important fish species in the (pre-)alpine rivers of Switzerland and play an important role in recreational and professional fishing. It is a sensitive cold water fish requiring high quality of water and habitat (Elliott, 1994; Armstrong et al., 2003). One possible explanation for the decline in brown trout catches could be an impaired water quality due to chemical pollution. More specifically, the presence of endocrine disrupting chemicals (EDCs) might pose a risk to brown trout populations as these environmental contaminants have the potential to affect development and reproduction of fish. Of particular relevance may be EDCs with estrogenic activities, i.e., compounds which are able to activate estrogen receptors, as such these compounds have been found to affect reproduction of feral fish in many areas of the world (Purdom et al., 1994; Sumpter and Jobling, 1995; Kime et al., 1999).

Estrogen-active endocrine disruptors (EEDs) enter river water to a considerable part through effluent discharge from sewage treatment plants (STP), when natural and synthetic estrogens, as well as industrial chemicals such as alkylphenol polyethoxylates are incompletely removed (Belfroid et al., 1999; Ternes et al., 1999;

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Johnson et al., 2005). Chemical analysis showed that the estrogenicity in river water is caused predominantly by the natural estrogens estrone (E1) and 17β-estradiol (E2) and by the xenoestrogen 4-nonylphenol (NP) (Ternes et al., 1999; Johnson et al., 2005). In Swiss rivers, average estrogenicities of ≤2 ng/l (E2 equivalent) have been reported, although the concentrations show high daily and seasonal fluctuations reaching up to 7 ng/l in the summer (Vermeirssen et al., 2005).

The question arises whether the estrogenic contamination may have adverse consequences on reproductive capabilities of brown trout populations, thereby possibly contributing to the observed decline (Burkhardt-Holm et al., 2005). Few studies have reported consequences of environmentally relevant estrogenic exposure on salmonid reproduction. In 2-month experiments with EE2-exposed male and non-exposed female rainbow trout (*Oncorhynchus mykiss*) decreased embryonic survival (Brown et al., 2007) and impaired embryonic development (Schultz et al., 2003) of offspring were detected. Continuous short-term (2 months) exposure of adult rainbow trout to NP (Lahnsteiner et al., 2005a) also affected the embryonic development. Non-continuous NP exposure of adult rainbow trout for 10 days per month over a 4-month period resulted in reduced hatching rate (Schwaiger et al., 2002). Whether results obtained with rainbow trout can be transferred to brown trout, the indigenous salmonid species in Switzerland, remains uncertain. Previous comparative studies pointed to a higher toxicant sensitivity of brown versus rainbow trout (Schmidt-Posthaus et al., 2001), but it is uncertain whether these observations apply for EDCs as well.

The aim of the present study was to examine the effects of a low-dose, long-term exposure to EEDs on the reproductive capability of brown trout. To this end, brown trout were exposed over the full period of gonadal maturation (150 days) to an environmentally relevant mixture of three estrogen-active substances present in Swiss rivers, namely E1, E2 and NP (Aerni et al., 2004; Rutishauser et al., 2004; Johnson et al., 2005; Vermeirssen et al., 2005). The ratios of the substances used in the mixture were comparable to those measured in the field (Aerni et al., 2004; Johnson et al., 2005). Two concentrations of this mixture were applied, the first was designed to reflect elevated environmental concentrations of each chemical in Swiss rivers (E1:E2:NP as 20:2:400); the second used up to 10-fold higher concentrations (E1:E2:NP as 100:10:4000) to act as a positive control.

As endpoints we measured growth, hepatosomatic index, gonadosomatic index, fertilization success, embryonic development, hatchability, survival and malformations of offspring. Further, concentrations of the estrogenic biomarker vitellogenin (Vtg) (Kime et al., 1999; Larsson et al., 1999; Thorpe et al., 2001) were measured in the plasma of exposed male fish to quantify the estrogenic potency of low- and high-treatment group.

2. Materials and methods

2.1. Brown trout

Mature brown trout of both sexes (3–4 years old) were obtained from a private fish hatchery in Switzerland (Mändli, Liestal, Switzerland) and transferred to the laboratory at Lake Lucerne. The fish were acclimated to freshwater of Lake Lucerne for 14 days, starting August 22, 2006.

On 5 September 2006, 60 brown trout per concentration (20 individuals per tank, three tanks per concentration) were randomly distributed to the experimental tanks. Fish were individually tagged by injection of a passive integrated transponder tag (PIT tags length 12.50 mm, diameter 2.07 mm, RFID radio frequency identification, biomark, ID, USA) into the peritoneal cavity. Total length (mm) and total wet weight (g) were measured for each fish and blood was sampled from the caudal vein of three to four males per tank for vitellogenin analysis.

To reduce or prevent aggressive behavior, six stainless steel tubes (length 50 cm, diameter 10 cm) were placed within each tank to provide shelter. Every 2–3 days, brown trout were fed with pellets (HOKOVIT HO-6507 bio 4.8 mm, H.U. Hofmann AG, Bützberg, Switzerland); the feeding stopped 2 days before the end of the experiment.

For handling and sampling, the fish were anaesthetized in a mixture of 0.5 ml clove oil dissolved in 10 ml alcohol and diluted with 15 l water.

2.2. Experimental design

Brown trout were exposed to environmentally low concentration (Mix_{low}) and high concentration (Mix_{high}) mixtures of estrone (Sigma–Aldrich, Buchs, Switzerland, E9750, 99% purity), 17β-estradiol (Sigma–Aldrich, Buchs, Switzerland, E8875, 98% purity) and 4-nonylphenol (Acros Organics, 99% purity) for 150 days until the onset of spawning. During the exposure the fish were held outdoor in 1000-L stainless steel tanks under natural light conditions and at an average water temperature of 6.9 °C (Min 4.9 °C; Max 13.3 °C). A flow through system (flow meter, Emerson Process Management Brooks Instrument, Baar, Switzerland) was used for the estrogenic exposure with a total flow rate of 10 l/min using lake water (Fig. 1), which was tested prior to the start of the estrogenic exposure. Stock solutions of chemicals in 99.8% ethanol (Sigma–Aldrich, Buchs, Switzerland) were diluted in lake water and dispensed over a Hamilton diluter (Hemotec, Gelterkinden, Switzerland) into tanks to provide the following nominal concentrations: solvent control (C, ethanol), low concentration (Mix_{low}) with 20 ng/l E1, 2 ng/l E2 and 400 ng/l NP, and high concentration (Mix_{high}) with 100 ng/l E1, 10 ng/l E2 and 4000 ng/l NP. Three replicates per concentration C, Mix_{low} and Mix_{high} were used. To equilibrate the actual EED concentrations in the tanks the dispensing system was started 7 weeks before addition of the brown trout.

Water samples were taken from each tank at 09:00 during the acclimation period (three sample points), and then every 3 weeks during the exposure (six sample points). For the first four sampling times, test chemical concentrations were analyzed separately for each tank to have a measure of the variance between the replicates. For later samples a pool of the three replicates per concentration was made and then this pooled sample was analyzed. Concentrations of each of the three chemicals within the estrogenic mixture were then measured using LC/MS/MS (Vermeirssen et al., 2005).

For all materials (water pipes, chemical tubes, tanks, fish shelters or bottles for dosing solutions) stainless steel, glass or Teflon® was used.

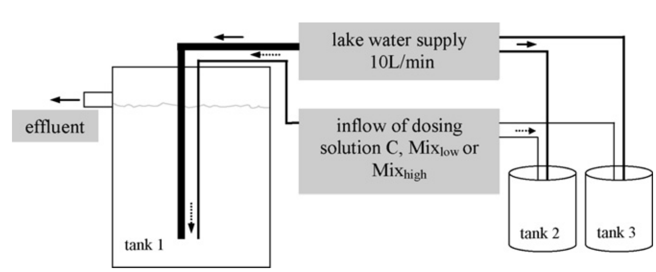

Fig. 1. Stainless steel 1000-L tanks. Lake water and the inflow dosing solutions of C (control), Mix_{low} (low concentration) or Mix_{high} (high concentration), with three replicates per concentration are shown (tanks 1–3).