Exposure to municipal wastewater effluent influences fecundity and hormone-signaling pathways in fathead minnows (Pimphales promelas)

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BACKGROUND
- Municipal wastewater effluent (MWWE) is a growing concern in prairies.
- Increasing urban populations, small receiving water bodies, outdated treatment technologies and contains complex mixture of chemicals.
- In Southern Saskatchewan, Canada, there exists two distinct scenarios of treatment of MWWE and risk to receiving environments (Figure 1).
- Chemicals in MWWE have potential to disrupt endocrine signaling pathways involved in reproduction and development → manifest at the population level.
- Short-term reproductive bioassays with fish are a useful tool for assessing functional effects of endocrine disruption (i.e. fecundity, see characteristics, biochemical changes).

Gene expression analysis of key pathways in reproduction (e.g. steriodogenesis) can provide additional insight into mechanisms underlying functional responses.

METHODS
- Effluents were collected end of pipe at Saskatoon and Regina treatment plants and transported to University of Lethbridge for exposure.
- Exposure of fathead minnows (FHM) followed the US-EPA Short-term Reproductive Bioassay (EPA/R01/R-01/067) and endpoints were measured as indicated in Figure 2.
- Expression (mRNA) of key genes along the hypothalamic-pituitary-gonadal-liver axis (HPG) was measured in males and females by qPCR:
  - ERα: estrogen receptor α (ERa), FSHr, aromatase (A), stenogonadotropin (VTG), heat shock protein 70 (HSP70), glutathione (GST), cytochrome P450 1A (CYP1a), cytochrome P450 3a (CYP3a).
  - GONADS: follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), steroidalogenic acute regulatory protein (StAR), 17β-hydroxysteroid dehydrogenase (17βHSD), aromatase (A), CYP1a.
  - BRAIN: estrogen receptor α (ERa), ESR1, aromatase (A), aromatase B (CYP19b).

OBJECTIVES
- Identify and compare functional effects and transcriptional responses in male and female fathead minnows exposed to MWWEs from Regina and Saskatoon.

RESULTS - Fecundity

Figure 3. Cumulative egg production of FHM exposed to Regina and Saskatoon MWWEs. Exposure to Regina MWWE caused a significant decrease in fecundity at both 10% and 50% effluent, while exposure to 50% Saskatoon MWWE resulted in an increase in egg production. Asterisks denote significant difference (p<0.05; one-way ANOVA).

RESULTS - Gene Expression

Figure 8. Gene expression in FHM exposed to Regina MWWE. Significant decrease in estrogen receptor observed in females. Asterisks denote significant difference relative control (p<0.05; one-way ANOVA) set at 2 (red dashed line).

Figure 2. Schematic of exposure design and endpoints measured in adult male and female fathead minnow after 21 d exposure to MWWE.

Figure 6. Representative photos of histopathological criteria observed in testes of male FHM. Increased spermatogenesis: (A) Regina 0% MWWE = non-remarkable, (B) Regina 50% MWWE = Grade 1. Testicular degeneration: (C) Regina 0% MWWE = non-remarkable, (D) Regina 50% MWWE = Grade 1. Increased incidence of spermatogenesis and testicular degeneration suggest potential delayed maturation.

Figure 7. Representative photos of histopathological criteria observed in ovary of female FHM. Gonadal staging: (A) Regina 0% MWWE = stage 3 female ovary, (B) Regina 50% MWWE = stage 1 female ovary. Oocyte atresia: (C) Regina 0% MWWE = non-remarkable, (D) Regina 50% MWWE = Grade 1. Increased incidence of oocyte atresia observed at 50% exposure. No change in gonadal staging.

Figure 4. Frequency of proportion of spermatogenesis and testicular degeneration for testes of male FHM after 21 day exposure to Regina and Saskatoon MWWE. MH=Non-remarkable

Figure 5. Frequency of oocyte atresia and gonadal staging for ovaries of female FHM after 21 day exposure to Regina and Saskatoon MWWE. MH=Non-remarkable

RESULTS - Histopathology

Figure 9. Cumulative # of eggs. Regina Effluent-Treatment 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500 8000 8500 9000 9500 10000 Time (Days) Regina Effluent

Figure 10. Cumulative # of eggs. Saskatoon Effluent-Treatment 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500 8000 8500 9000 9500 10000 Time (Days) Saskatoon Effluent

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