Reproductive and histopathological effects of municipal wastewater effluent exposure in male and female fathead minnows

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Municipal Wastewater Effluent (MWWE)

- Municipal wastewater treatment plants (MWTP) responsible for removing contamination from water
- Treated effluent still contains a number of contaminants
- Endocrine active compounds in MWWE are of particular concern

Sexual characteristics
Behaviour
Fecundity and hatching success
MWWE: The situation in Saskatchewan

Increase in population (urban)

Receiving environments often small water bodies

Outdated removal systems at MWTP

high risk for aquatic organisms
Regina WWTP
Low flow/volume receiving environment
Wascana Creek
Water quality does not meet guidelines
Low $\rightarrow$ No dilution
High risk site

Saskatoon WWTP
High flow/volume receiving environment
South Saskatchewan River
More advanced treatment facility
Greater dilution
Low risk site
Assessment of Environmental Impacts of Municipal Effluents (AIME)

**In Vitro Studies with Validated Bioassays**

**Chemical Analytical Studies**

**In Vivo Studies with Native Species (FHM)**

FHM Reproductive Assays

Wild Fish Study (In Stream)
Objectives

1. Identify and compare potential effects of MWWEs from Regina and Saskatoon on the reproductive output of male and female fathead minnows

2. Examine the transcriptional changes of reproductive genes to determine by which mechanism of action the effluents may be affecting minnows
Test Species – Fathead Minnow

- Native to Saskatchewan ecosystems
- Well understood biology and reproductive patterns
- Excellent lab species
  - Tolerance to wide range of water conditions
  - Established guidelines and protocol
- Established endpoint measurements for endocrine disruption
  - Vitellogenin, fecundity, secondary sex characteristics
- US-EPA 2009 Fathead minnow 21-day reproductive bioassay
Methods- Exposure

- Conducted at the University of Lethbridge
- Fish transferred to culture tower system
  - 4:2 female to male per tank
- Establish baseline fecundity + fertility (2 weeks)
- Dilution for high treatment group determined by preliminary 96h LC50 assay
  - 50%, 75%, and 100%
Tower 1: Regina

Tower 2: Saskatoon

0% (x 4) 10% (x 4) 50% (x 6)

50% water change
Eggs collected/counted
% Fertilization recorded
Mortalities recorded

21 days

Liver, Gonad, Brain:
Gene Expression

Gonads:
Histopathology

Blood: Steroid
Hormone Levels

Secondary Sex Characteristics

Morphometrics
There was no significant effect of Saskatoon MWWE on fathead minnow fecundity.

Regina MWWE caused a significant decrease in fecundity at both effluent concentrations.
Somatic Indices

Regina Effluent

Saskatoon Effluent

*
Gonad Histology - Male Fathead Minnows

Increased Spermatogonia

Testicular Degeneration
# Gonad Histology - Male Fathead Minnows

<table>
<thead>
<tr>
<th>Source</th>
<th>Regina</th>
<th>50 %</th>
<th>Saskatoon</th>
<th>50 %</th>
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<tbody>
<tr>
<td>Effluent</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number of males examined</td>
<td>8</td>
<td>11</td>
<td>8</td>
<td>11</td>
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<tr>
<td><strong>↑ proportion of spermatogonia</strong></td>
<td></td>
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</tr>
<tr>
<td>Non-remarkable</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Grade 1</td>
<td>-</td>
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<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Grade 2</td>
<td>-</td>
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<tr>
<td>Testicular degeneration</td>
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<td>5</td>
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<tr>
<td>Grade 1 (10% of observed tissue)</td>
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<td>Incidence of testis-ova</td>
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<td>Interstitial cell hyperplasia/hypertrophy</td>
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## Gonad Histology - Female Fathead Minnnows

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<td>Change in gonadal staging</td>
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<td>Non-remarkable</td>
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<tr>
<td>Stage 1</td>
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Females – Change in Oocyte Staging

Saskatoon Females

Regina Females

Stage 1

Stage 2

Stage 3

Maturation
Sex Steroid Hormone Analysis

**Females**

- **Regina E2**
  - Control: ng/mL
  - 10% Effluent: ng/mL
  - 50% Effluent: ng/mL
- **Saskatoon E2**
  - Control: ng/mL
  - 10% Effluent: ng/mL
  - 50% Effluent: ng/mL

**Males**

- **Regina 11-KT**
  - Control: ng/mL
  - 10% Effluent: ng/mL
  - 50% Effluent: ng/mL
- **Saskatoon 11-KT**
  - Control: ng/mL
  - 10% Effluent: ng/mL
  - 50% Effluent: ng/mL
Gene Expression - Regina

ERa Female Liver

VTG Female Liver

AR Female Liver

ERa Male Liver

VTG Male Liver

AR Male Liver
Gene Expression- Saskatoon

ERa Female Liver

VTG Female Liver

AR Female Liver

ERa Male Liver

VTG Male Liver

AR Male Liver
Conclusions

- Regina MWWE caused a significant decrease in fecundity at both effluent concentrations
- No effects on somatic indices or secondary sex characteristics at either site
- Exposure to MWWE from both sites resulted in changes in gonad histopathology in males → indicating delayed maturation?
- Changes in gene expression suggest endocrine activity in effluents from both sites
  - transcript analysis in gonad and brain
Acknowledgements

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