



**Letitia Falk, Bruce Mathieson, UBC Okanagan,  
Centre for Species at Risk and Habitat Studies**

Supported by the Irving K. Barber  
Endowment Fund and UBCO RSG

# Is Okanagan Lake's Wildlife in danger from Kelowna's Municipal Wastewater?



Effect of the sewage contaminant coprostanol on goldfish as a model fish species.

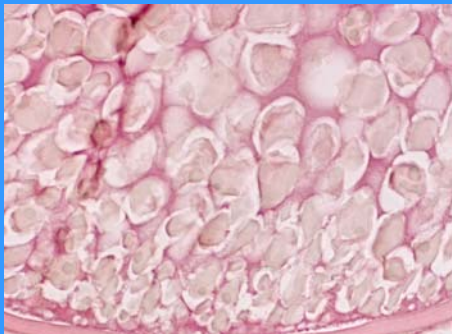
# Potential Wastewater Contaminants that can harm wildlife:

- Heavy Metals
- Oils
- Organics and Pharmaceuticals
- Phosphorus
- Nitrogen
- Coliforms



## Normal functions of estrogen:

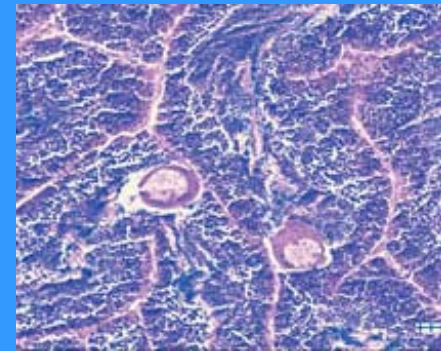
- Causes development of reproductive organs and sexual maturity
- Affects reproductive behavior
- Regulates metabolism
- Regulates muscle mass
- Regulates fluid balance



Ovary cross-section showing eggs.

## Side effects of estrogenic contamination:

- Altered sperm count
- Genital tract malformations
- Infertility
- Tumors
- Altered reproductive behavior
- Altered sex ratios

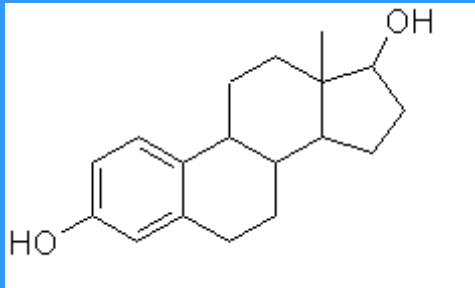


Testis cross-section showing eggs (Cyranoski 2001, Nature)

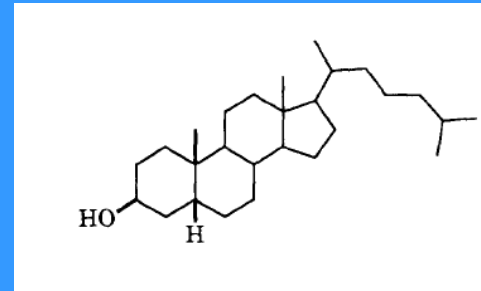


*E. coli* in the intestine break down cholesterol into the compound coprostanol, which has a similar structure to estrogen.

**ESTROGEN**

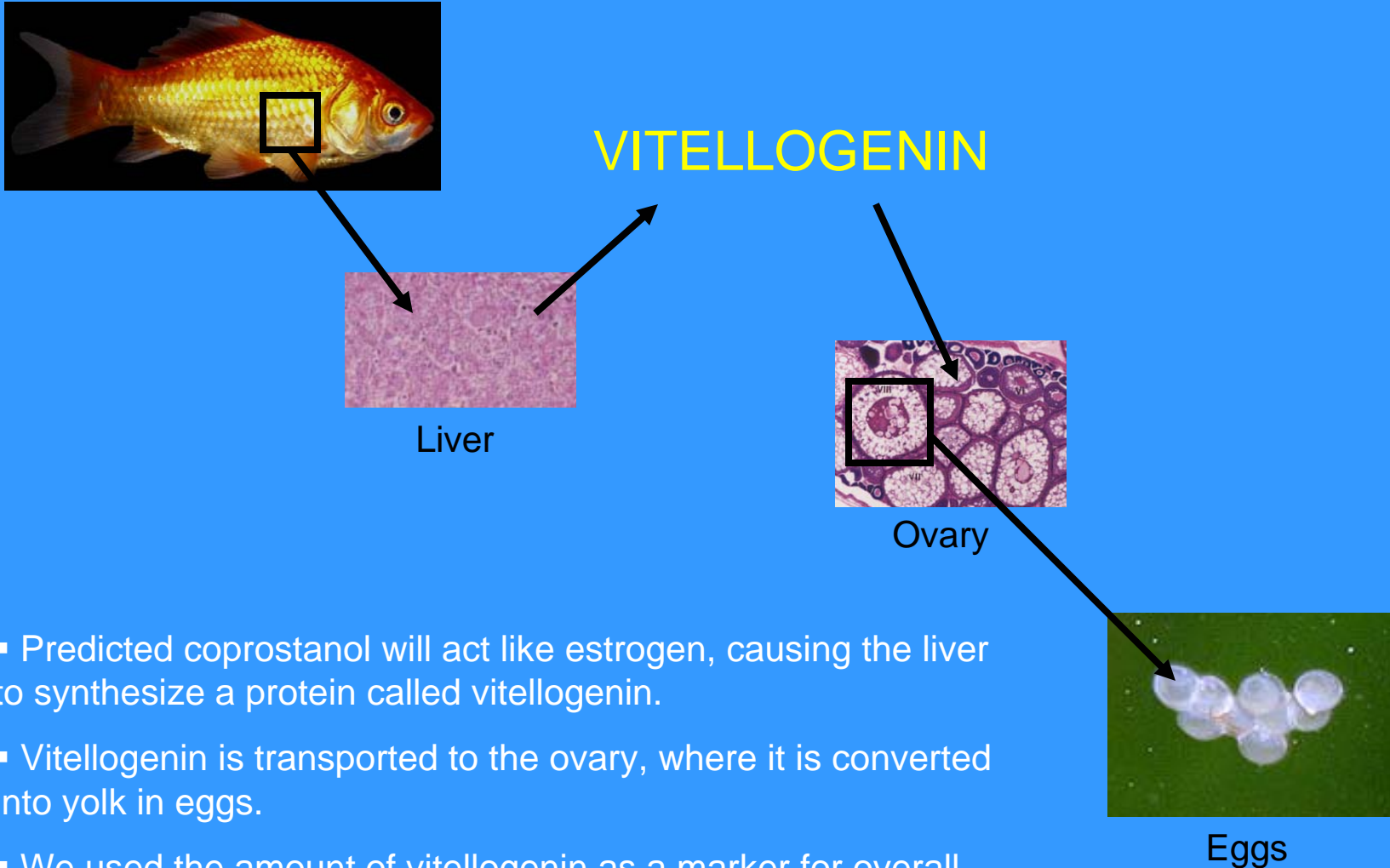


**COPROSTANOL**

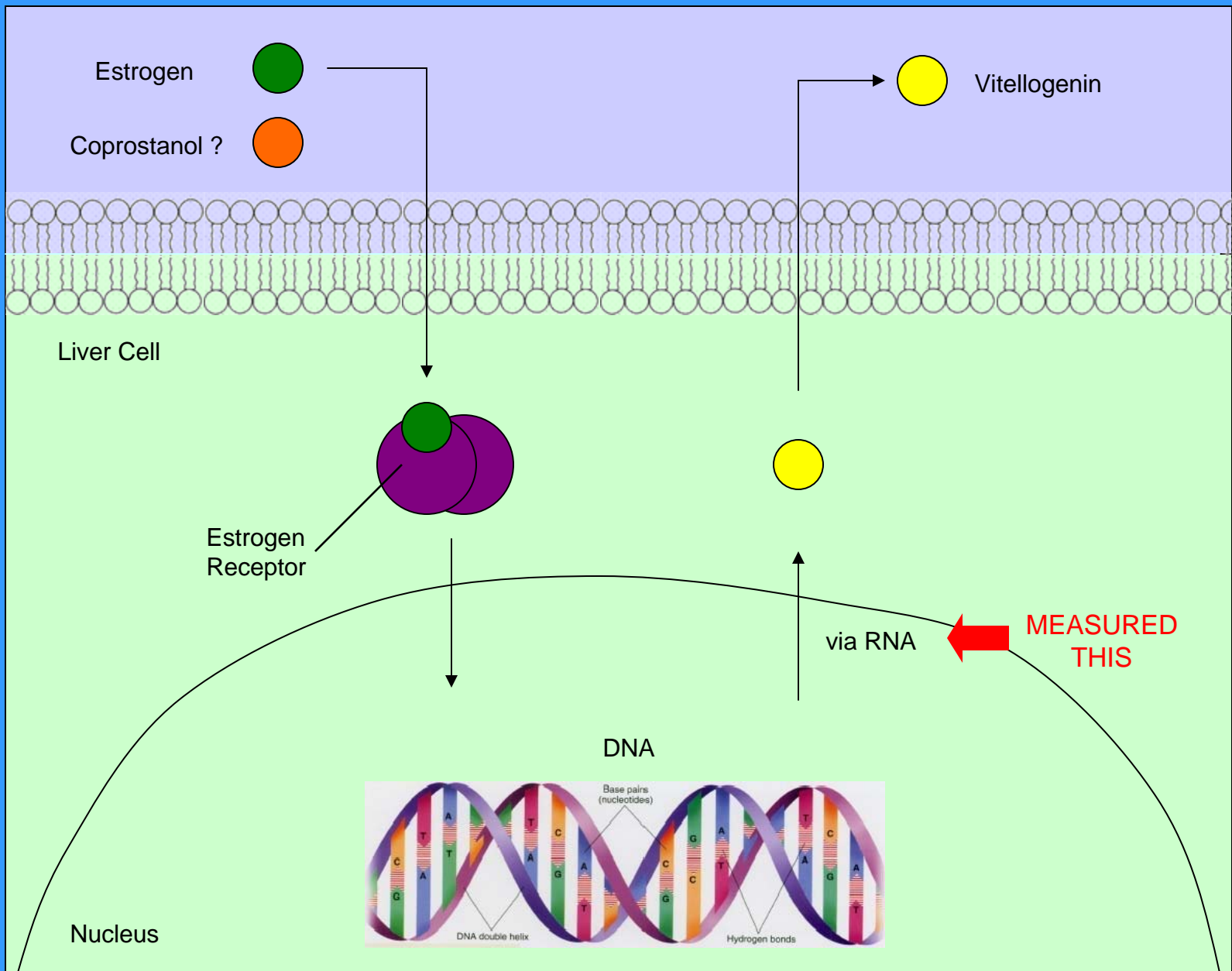


More Waste = More Coprostanol

# So what will coprostanol do?



- Predicted coprostanol will act like estrogen, causing the liver to synthesize a protein called vitellogenin.
- Vitellogenin is transported to the ovary, where it is converted into yolk in eggs.
- We used the amount of vitellogenin as a marker for overall hormone disruption.



# So what's wrong with that?

- **Making yolk is metabolically expensive**
- **Males don't usually express vitellogenin**
- **Wrong season and no eggs?**
- **May indicate other hormonal disruption**

# The Experiment:

50 Goldfish:



1. Pure water
2. Estrogen+water
3. Coprostanol+water
4. Estrogen+coprostanol+water



Liver dissected and vitellogenin expression measured.



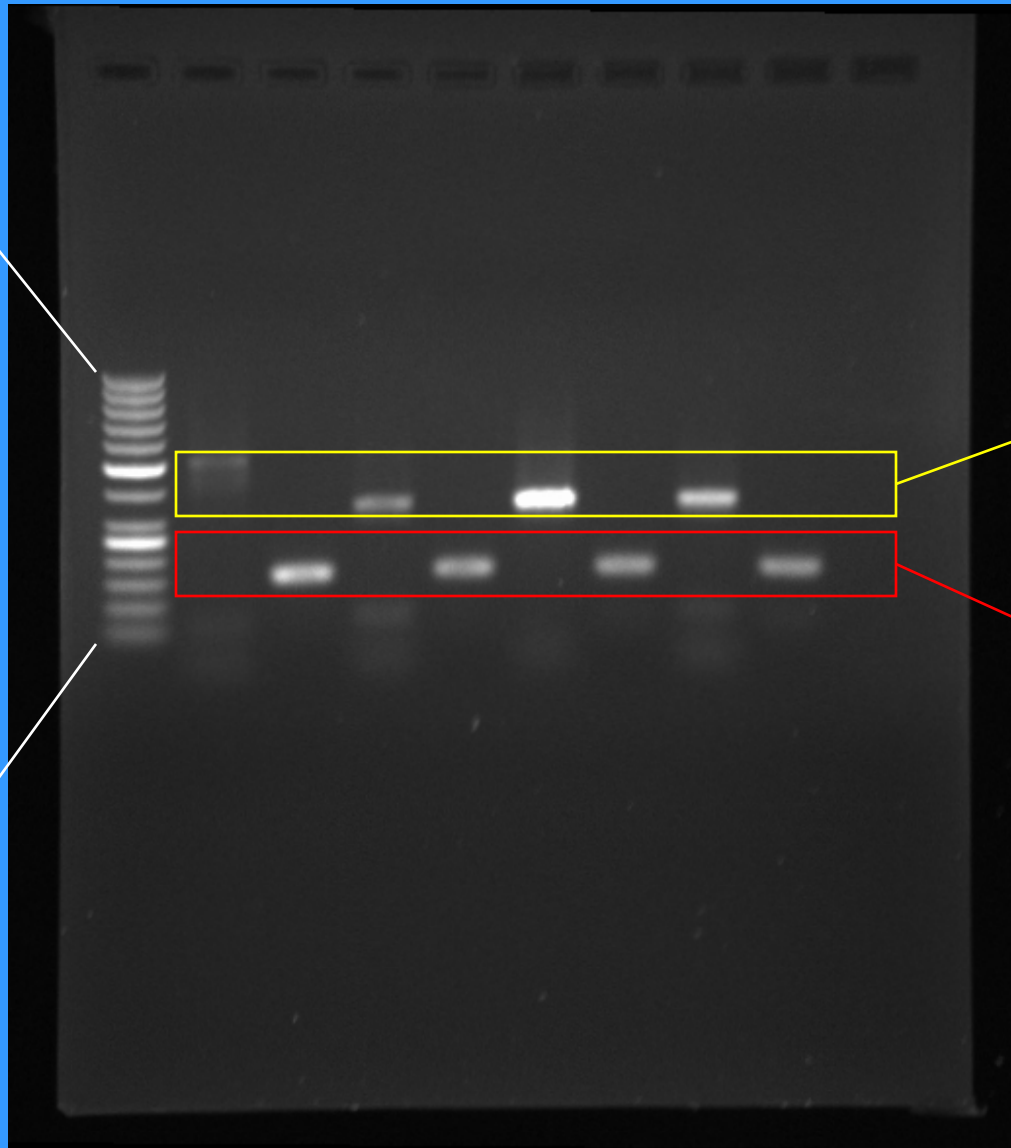
# Measuring vitellogenin expression:

- Fish euthanized in the anesthetic MS-222
- Liver Dissected
- Tissue homogenized and RNA extracted
- cDNA synthesized from RNA
- Vitellogenin and actin cDNA amplified by PCR
- Amplified DNA run on an agarose gel and photographed using GelLogic imaging system
- Photograph analyzed using KODAK 1D image analysis software to produce quantitative data



DNA Ladder

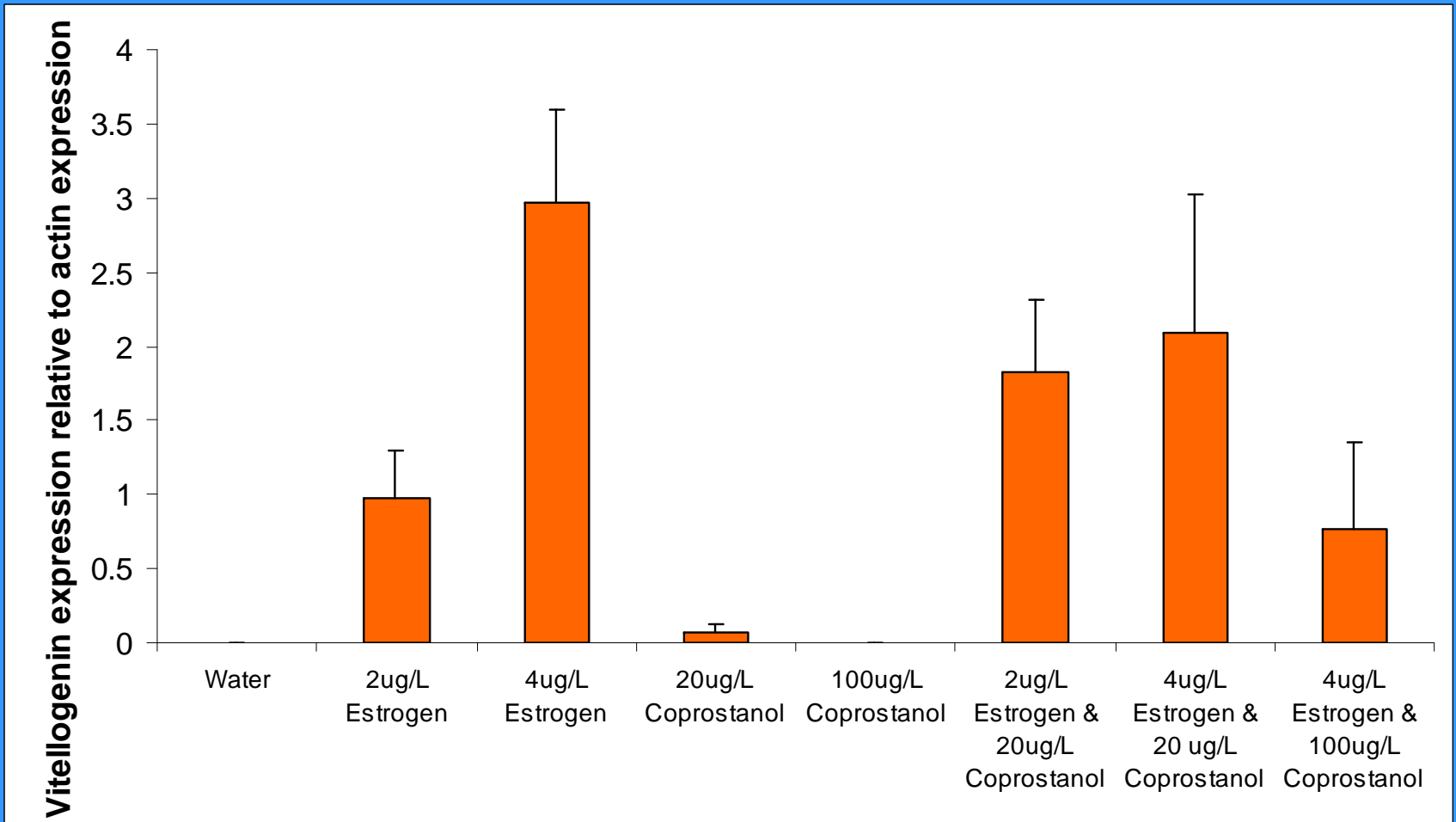
1000 bp  
900 bp  
800 bp  
700 bp  
600 bp  
500 bp  
400 bp  
300 bp  
200 bp  
150 bp  
100 bp  
50 bp



Vitellogenin  
330 bp

Actin  
190 bp

# Results:



\* Treatment significantly affected expression ( $p=0.0001$ )

Sex did not significantly affect expression ( $p=0.8509$ )

Weight did not significantly affect expression ( $p=0.2593$ )

# Explanation:

Treatment type, but not sex or weight significantly affected vitellogenin expression.

Estrogen stimulates vitellogenin expression in a dose-dependant manor.

Coprostanol interferes with the normal estrogen-induced stimulation of vitellogenin gene expression in a dose-dependant manor.

Coprostanol may both increase and decrease estrogenic stimulation of vitellogenin expression.

Being able to produce both increased and decreased expression, is not uncommon among hormone receptors.

Coprostanol may bind to estrogen receptors, but induce a weaker response than estrogen.

In combination, coprostanol may compete with estrogen for the estrogen receptors, lessening the estrogenic response.

Organic pollutants in our water must be carefully monitored, due to their hormone-disrupting potential.

What next?



## Acknowledgements:

Thank you to...

- The Irving K. Barber Endowment Fund
- The Centre for Species at Risk and Habitat Studies
- Dr. Bruce Mathieson, UBC Okanagan
- Brian McAuley and Kelowna Wastewater Treatment Facility

Questions?