

# what we know...



## Introduction and Research Goals

There is a growing concern that pharmaceuticals and other chemicals present in the effluents (sewage discharged into a river) from municipal wastewater treatment plants enter the aquatic environment, where they may affect the fitness of fish. We conducted lab based experiments to determine the potential of effluents from different municipal wastewater plants in the Grand River Watershed to affect the reproduction of adult zebrafish.

Specifically, we investigated:

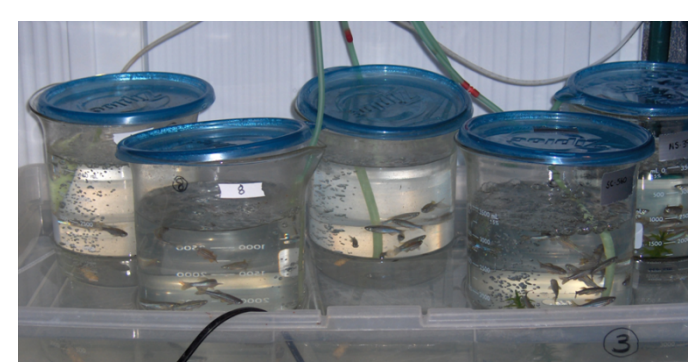
- **Whole animal reproductive performance**, based on the number of eggs spawned over a 7-day exposure period.
- **Biomarkers of reproductive function**, including the levels of sex steroid hormones in ovarian tissue and expression of selected genes in the ovary and liver.

## Background

Previous studies have shown that fish (Rainbow darter) captured downstream of the wastewater treatment plant (WWTP) in Waterloo, have altered sexual development. High numbers of fish are hermaphrodites and contain both male and female tissues. Fish collected elsewhere in the watershed are either male or female.

## Methods

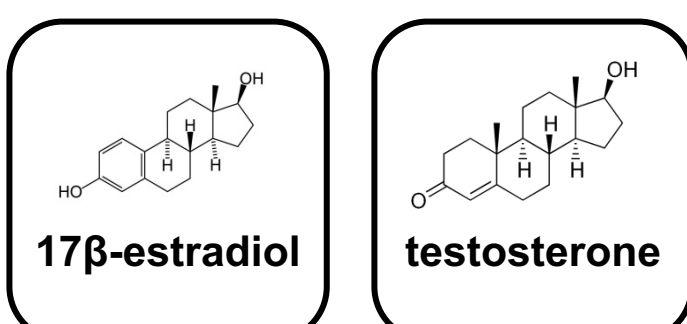
Mixed-sexed groups of zebrafish were held for 7-9 days in clean well water followed by 7 days in a 50% dilution of effluent from local municipalities (Figure 1). Eggs were collected and counted daily. Following exposure to effluent, fish were euthanized, weighed and tissues were collected for the quantification of gonadal steroid levels and expression of ovarian and hepatic genes important for reproductive processes.



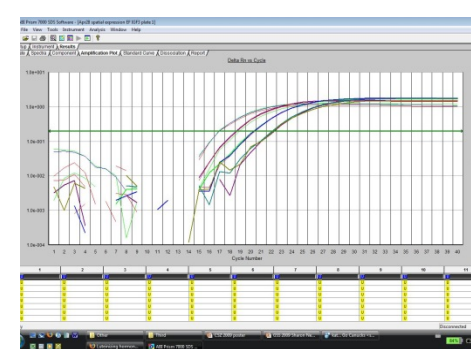
5 ♀ : 3 ♂  
7-9 d pre exposure;  
7 days 50% effluent  
6 replicates/treatment

Eggs spawned  
Ovarian weight

Ovarian steroid measurement  
using EIA



Gene expression analysis  
using qPCR



Ovarian:  
StAR  
CYP19a  
LH-R

Hepatic:  
CYP1a1  
Vtg

## Limitations

Zebrafish are not native to Canada, therefore it is possible that they do not respond to sewage effluent in a manner similar to native species. However, Zebrafish are an excellent organism in which to study reproduction as they have the ability to spawn throughout the year, unlike native species which spawn only during the spring/summer.

Fish were unable to move to an area with a lower concentration of effluent as they would in the wild.

Sewage effluent is a complex mixture of known and unknown compounds so identifying the factors affecting reproduction is difficult.

The Guelph wastewater treatment plant has a tertiary level of treatment and the effluent does not pose a hazard to fish reproduction.

## THE REPRODUCTIVE EFFECTS of municipal wastewater effluents

### Research Findings

- In studies conducted in 2013 and 2015, a 50% dilution of water collected immediately downstream of the wastewater treatment plant in Waterloo, Ontario resulted in a significant reduction (50 and 85% in the two years) in the numbers of eggs spawned (Figure 2).
- In contrast, effluent collected downstream of two different sewage treatment plants (Kitchener and Guelph, Ontario), as well as an upstream reference site on the Grand River (Hespeler) and the laboratory control water had no effects on spawning success (Figure 2).
- Neither measurement of ovarian steroid hormone levels (not shown) nor the expression of selected genes involved in steroid hormone synthesis revealed consistent site differences in the response to the different municipal effluents (Figure 3).

Figure 1. Sites of wastewater treatment plants and level of treatment.

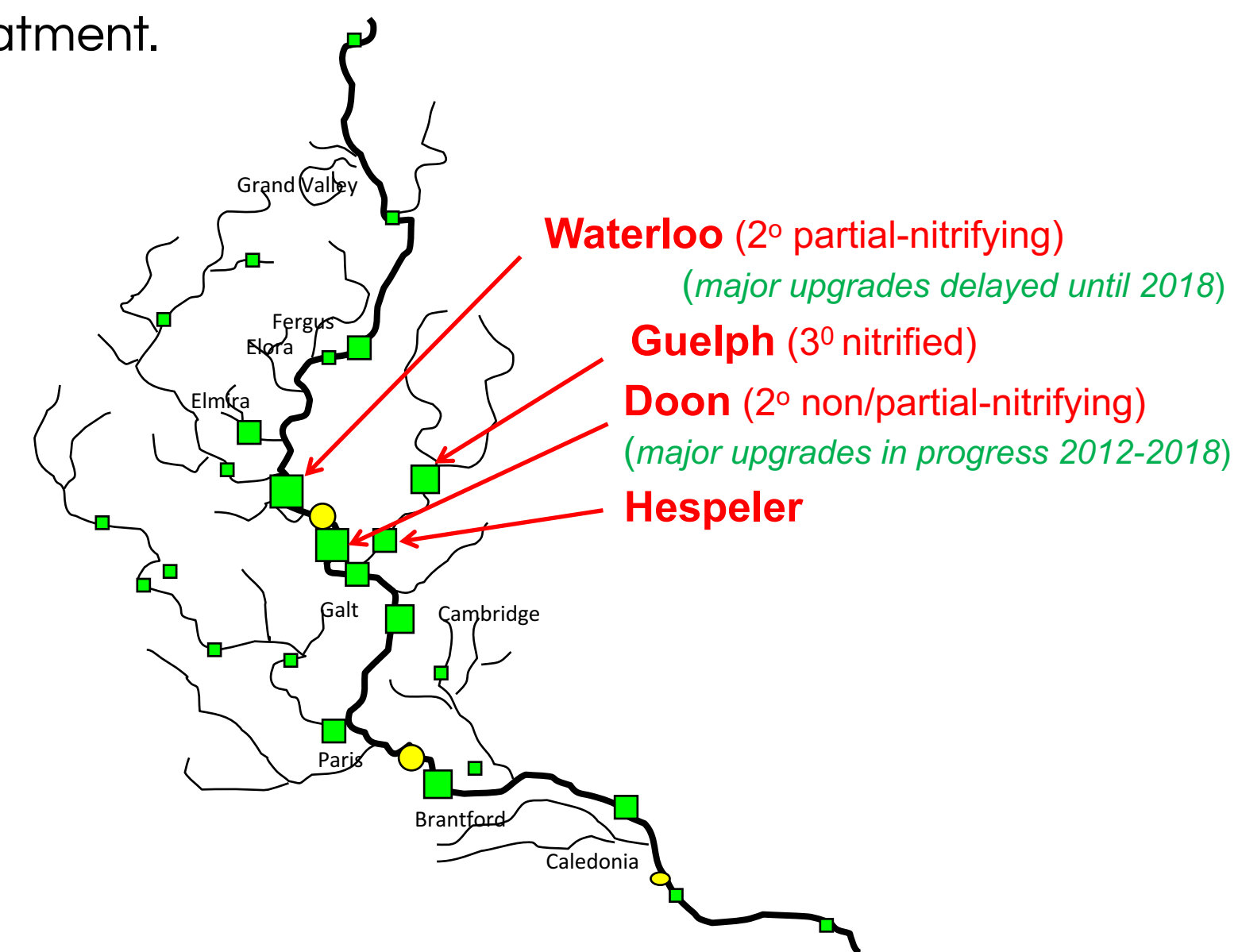
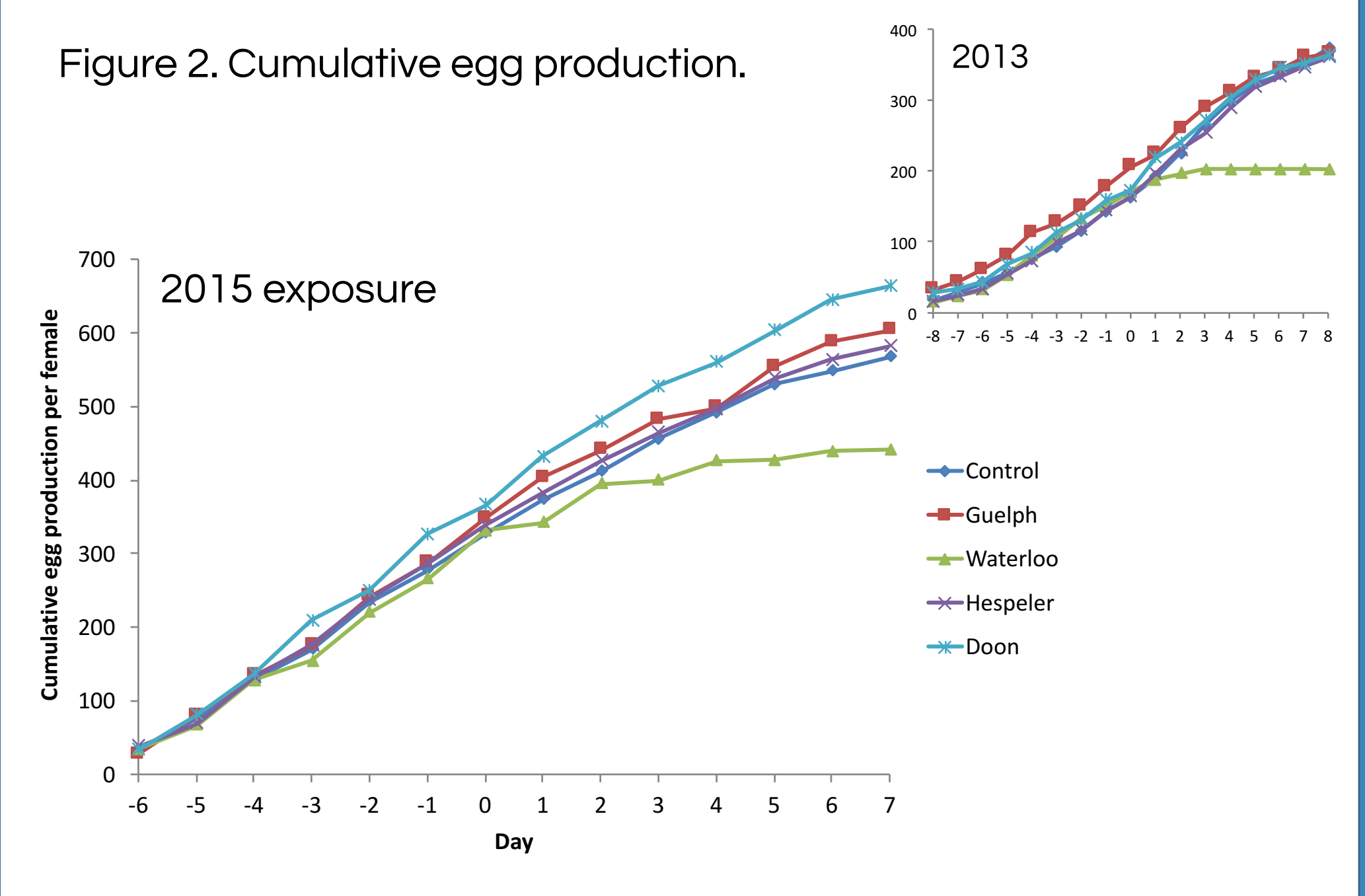
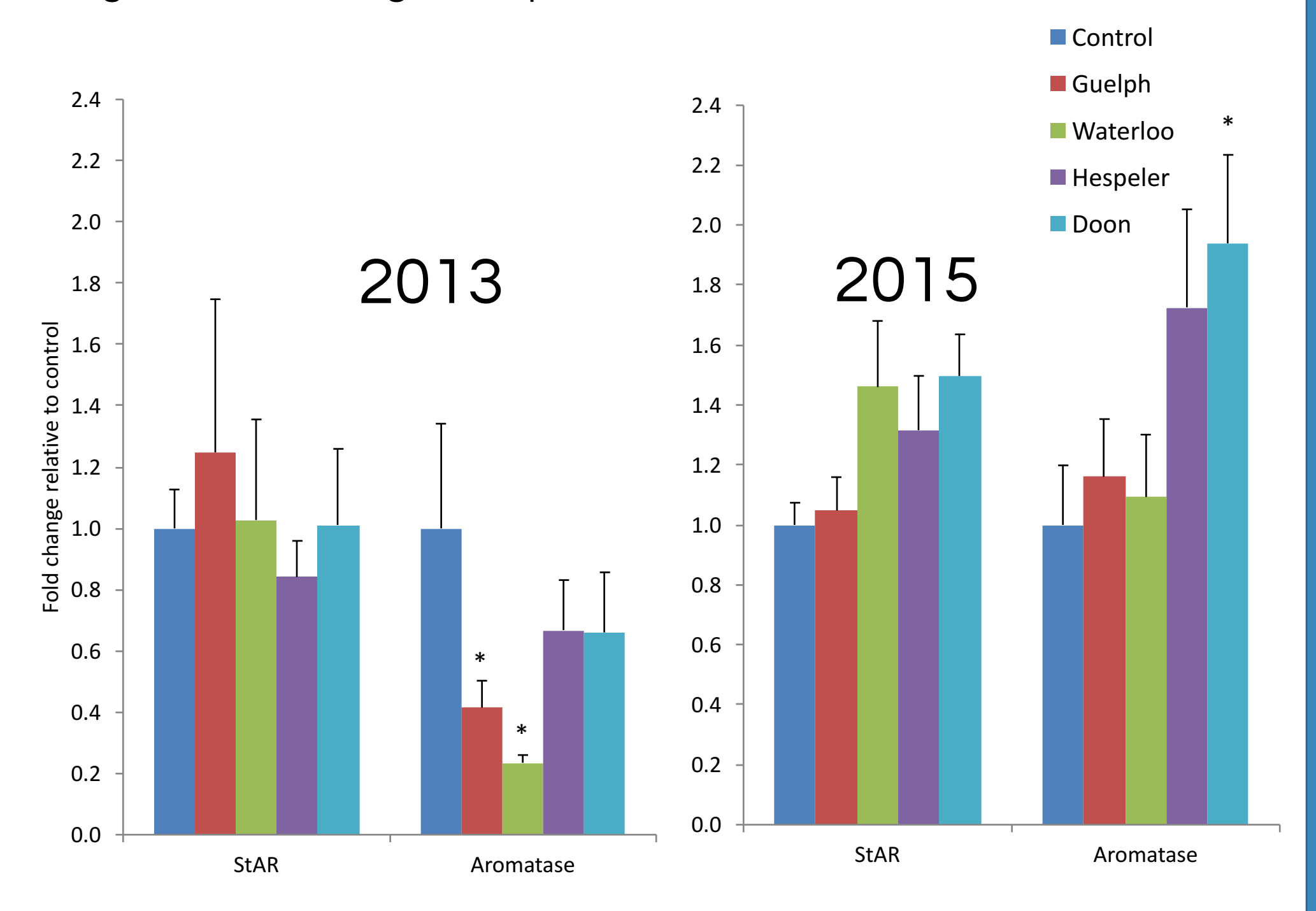


Figure 2. Cumulative egg production.



- Cumulative egg production reduced in zebrafish exposed to Waterloo WWTP effluent

Figure 3. Ovarian gene expression.



- Ovarian gene expression did not consistently change with treatment

### Conclusions

- Spawning success may be a reliable marker to assess the potential effects of municipal wastewater effluent on fish reproduction. Biomarkers of response such as steroid hormone levels or gene expression had less predictive ability.
- While pharmaceuticals such as ethinylestradiol, venlafaxine, ibuprofen and carbamazepine were present in each of the effluents, their presence was not predictive of effects on spawning success. Rather, high concentrations of ammonia detected in the Waterloo effluent are suspected to be responsible for the reproductive impairment.
- These studies also show that some but not all municipal wastewater treatment plants in the Grand River Watershed may represent a significant hazard to fish reproduction.
- The tertiary treatment effluent receives at the Guelph WWTP is effective at removing ammonia and constituents that may affect reproduction. Major renovations are being made at the Waterloo WWTP which would address its output of high concentrations of ammonia.

### Researchers

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