

# The Effects of Fluoride on Thyroid Hormone Signaling and Metamorphosis in *Xenopus laevis* Tadpoles

Ally Yost, Ashlyn Lisner, Kenny Cox, Dr. Marlo Jeffries

Department of Biology, Texas Christian University, Fort Worth, TX  
a.yost@tcu.edu, a.lisner@tcu.edu, kenneth.cox@tcu.edu



There has been much debate in the scientific community regarding the biological effects of fluoride. While many studies have found that fluoride alters thyroid hormone signaling, others have been unable to duplicate these results. The primary objectives of this study were to determine: 1) if exposures to fluoride alter thyroid hormone signaling in *Xenopus laevis* tadpoles and 2) if simultaneous exposures to exogenous thyroid hormone can recover fluoride-induced alterations in thyroid signaling. *Xenopus* tadpole metamorphosis is driven by thyroid hormones; therefore, changes in metamorphic rate are typically associated with alterations in thyroid hormone signaling. Tadpoles were exposed to dechlorinated water, 4 ug/L of thyroxine, 5 mg/L and 50 mg/L of fluoride, and 5 mg/L of fluoride with 4 ug/L of thyroxine. Following a 21 d exposure period, metamorphic stage, body mass and the relative expression of thyroid-hormone related genes were measured. Exposures to 50 mg/L fluoride caused alterations in metamorphosis. However, there were no significant differences in the expression of thyroid hormone related genes. These findings suggest that exposures to higher concentrations of fluoride can inhibit metamorphosis, but that this inhibition is mediated through a general toxic effect, rather than alterations in thyroid hormone signaling.



## Introduction

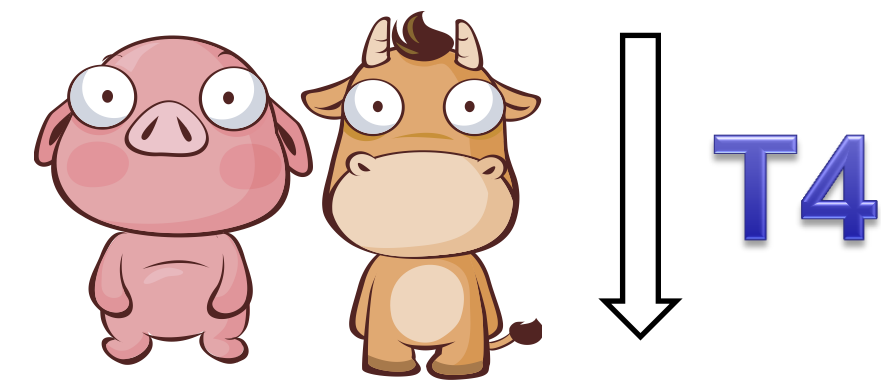
There has been controversy in the scientific community on whether or not fluoride is harmful, specifically its role as a thyroid disruptor. Some research has shown that fluoride can significantly decrease thyroid hormone levels [1, 2] while others have shown effects on other areas of the body, but not the endocrine system [3].



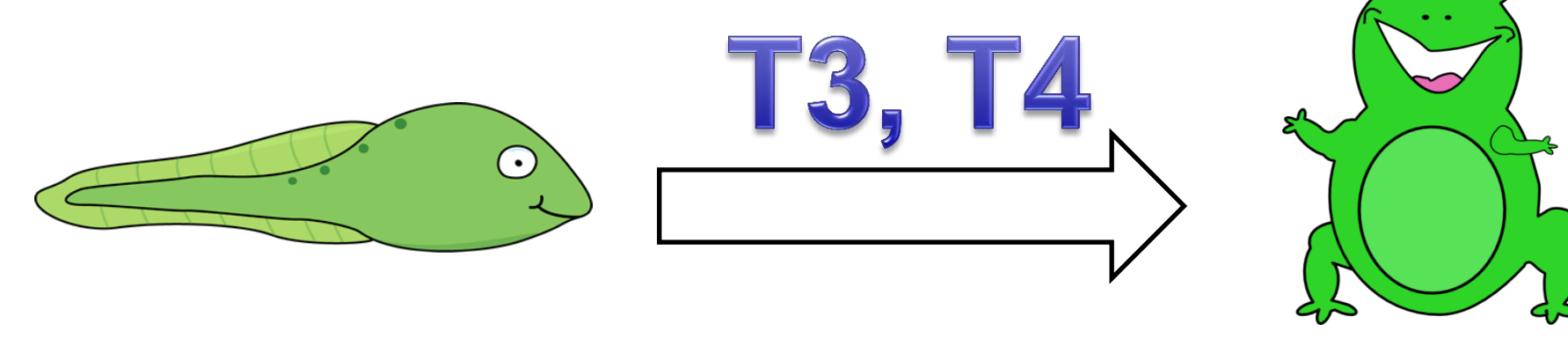
Fluoride is incorporated into drinking water in order to strengthen teeth and bone [4].



Fluoride is found in surface water due to natural and anthropogenic sources [5].



Recent studies have found that high doses of fluoride significantly decreased T4 levels in pigs and cows [1, 2]



Thyroid hormones drive metamorphosis in amphibians [6].

### Objectives

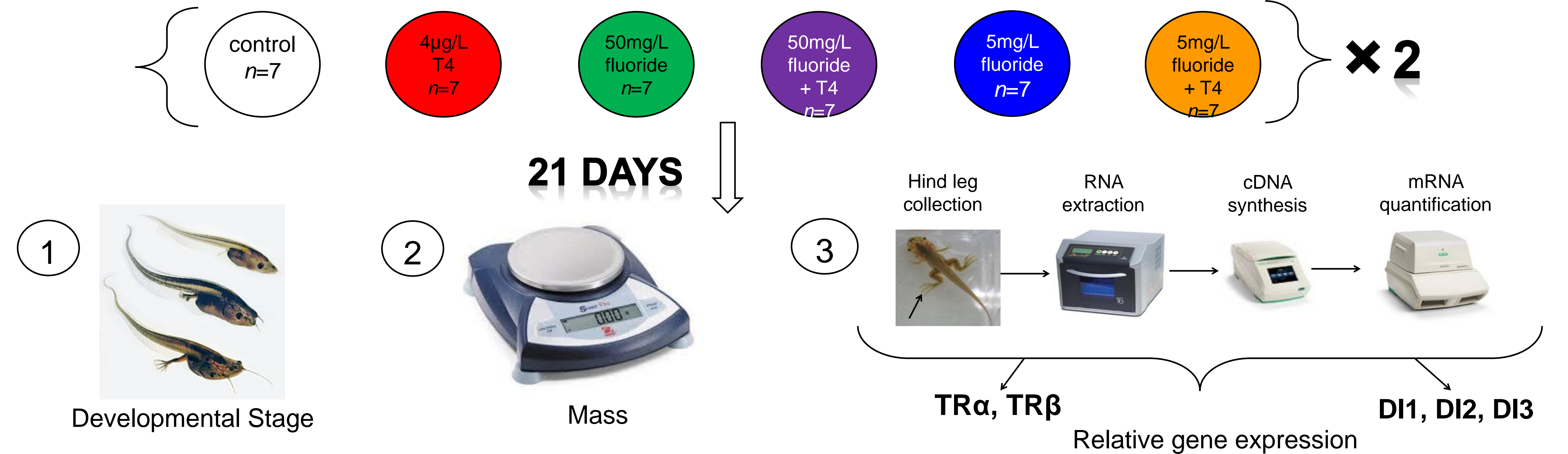
- To determine whether fluoride affects metamorphosis by evaluating its ability to alter metamorphosis in *Xenopus laevis*.
- To determine the effect of co-exposures to fluoride and thyroxine on thyroid function.

### Hypotheses

- If tadpoles are unable to reach the final stage of development, then fluoride is an inhibitor of metamorphosis.
- If genes associated with thyroid function are altered in tadpoles with inhibited metamorphosis, then fluoride mediates its inhibition by disrupting thyroid hormone signaling.

## Methods

The experiment consisted of six groups with two replicates, conducted over a 21 day time period. The groups were exposed to dechlorinated water (control), 4 ug/L of thyroxine (T4), 5 mg/L of fluoride, 50 mg/L of fluoride, 5 mg/L of fluoride with T4, or 50 mg/L of fluoride with T4. Endpoints measured were developmental stage, mass, and relative gene expression of TR $\alpha$ , TR $\beta$ , deiodinase 1 (DI1), deiodinase 2, and (DI2) deiodinase 3 (DI3). using hind limb tissue. TR $\alpha$  and TR $\beta$  are thyroid hormone receptors. DI1 and DI2 converts T4 to the active form T3, while DI3 metabolizes T4 and T3. Change in expression of these genes are indicative of changes in thyroid hormone signaling.



## Results

2-way ANOVA revealed that exposure to fluoride had a significant effect on metamorphosis. At an experimental dose of 50 mg/L of fluoride, metamorphosis was inhibited.

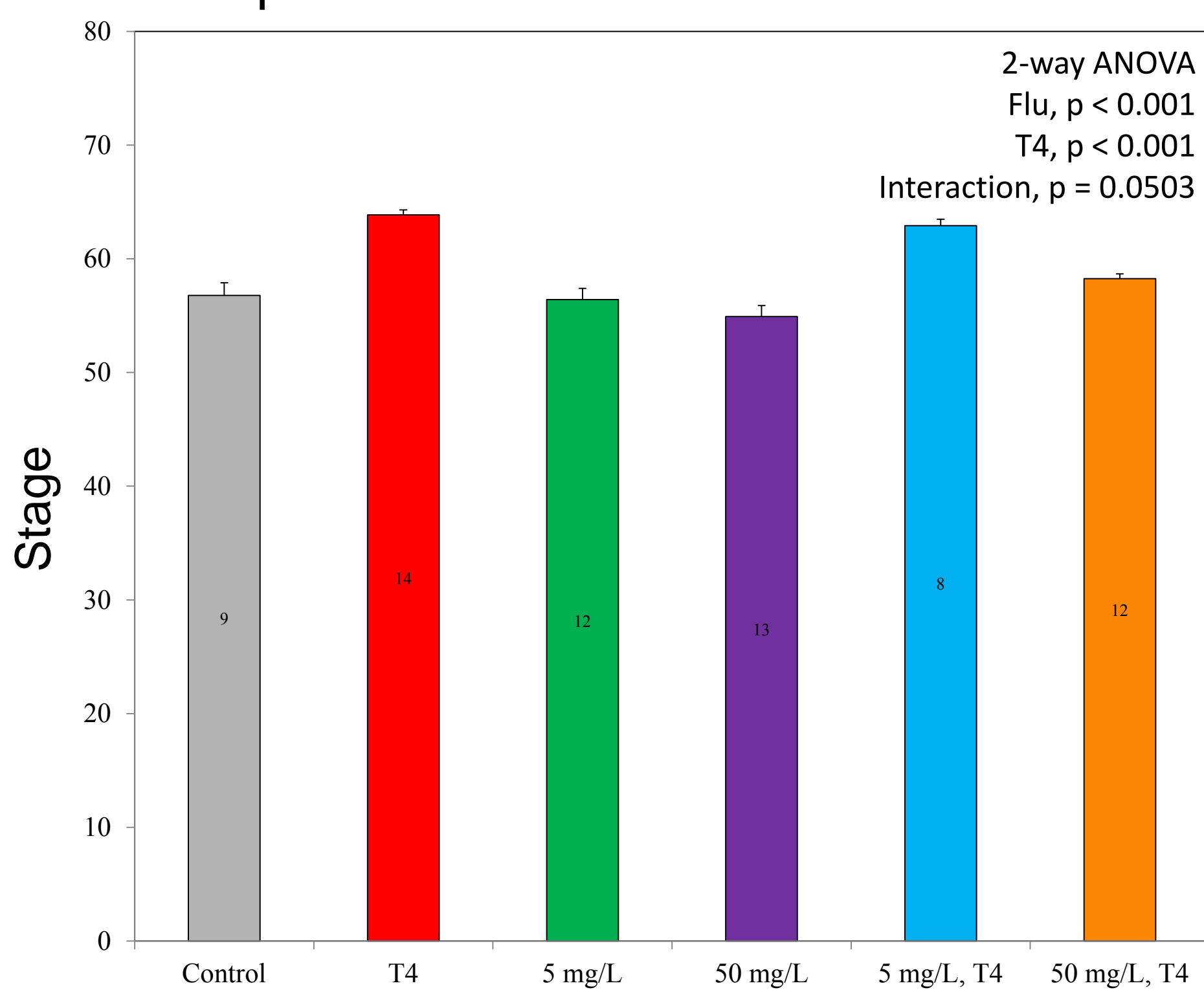


Figure 1. Final developmental stage of tadpoles exposed to dechlorinated water (control), 4 ug/L T4, 5 mg/L Flu (5 mg/L), 50 mg/L Flu (50 mg/L), 5 mg/L of Flu + 4 ug/L T4 (5 mg/L, T4), and 50 mg/L Flu + 4 ug/L T4 (50 mg/L, T4). Numbers in bars indicate *n*. Error bars indicate standard error.

2-way ANOVA revealed that exposure to 4 ug/L of T4 significantly decreased mass. Exposures to fluoride did not cause significant alterations in tadpole mass.

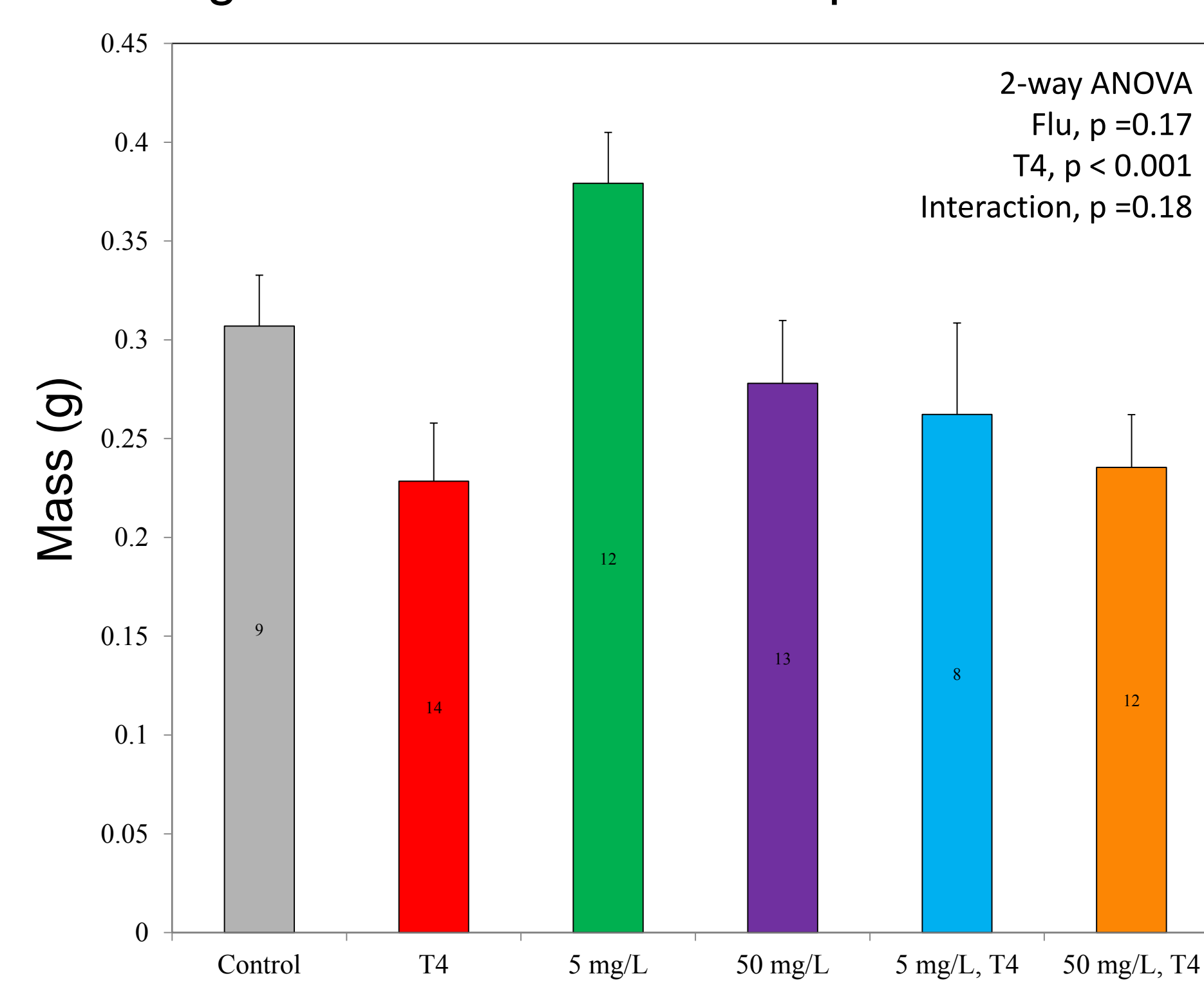


Figure 2. Mean final body mass of tadpoles exposed to dechlorinated water (control), 4 ug/L T4, 5 mg/L Flu (5 mg/L), 50 mg/L Flu (50 mg/L), 5 mg/L of Flu + 4 ug/L T4 (5 mg/L, T4), and 50 mg/L Flu + 4 ug/L T4 (50 mg/L, T4). Numbers in bars indicate *n*. Error bars indicate standard error.

There were no significant changes in the expression of any of the thyroid-related genes measured.

	Control	T4	5 mg/L	50 mg/L	5 mg/L, T4	50 mg/L, T4
TR $\alpha$	0.404±0.11	0.293±0.03	0.185±0.04	0.493±0.18	0.227±0.04	0.358±0.07
TR $\beta$	0.583±0.18	0.679±0.06	0.879±0.16	0.397±0.14	0.775±0.11	0.685±0.21
DI1	0.609±0.17	0.634±0.08	0.602±0.09	0.665±0.18	0.950±0.21	0.823±0.25
DI2	0.393±0.12	0.347±0.09	0.510±0.07	0.350±0.14	0.304±0.03	0.401±0.06
DI3	0.446±0.14	0.378±0.09	0.530±0.08	0.439±0.17	0.326±0.09	0.569±0.06

Table 1. Relative gene expression TR $\alpha$ , TR $\beta$ , DI1, DI2, and DI3 of tadpoles exposed to dechlorinated water (control), 4ug/L of T4, 5 mg/L Flu (5mg/L), 50 mg/L fluoride (50 mg/L), 5mg/L Flu + T4 (5 mg/L, T4) and 50mg/L Flu + T4 (50 mg/L, T4). All p-values >0.05.

## Conclusions

- Fluoride inhibited metamorphosis at the 50 mg/L level; however, there were no significant changes in the expression of thyroid-related genes.
- This indicates that fluoride does not inhibit metamorphosis through disruption of thyroid hormone signaling.
- Rather, the results of this study suggests that fluoride-induced alterations in metamorphosis are a result of general toxicity.
- Overall, the evidence presented here does not support the hypothesis that fluoride is a thyroid hormone disrupter.

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