# Another fish in the signaling sea: the effect of thyroid inhibition on the immune function of adult fathead minnows


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## Introduction

- Within the last few decades there has been increasing concern about chemicals in the environment capable of altering endocrine function.
- Fish are a commonly used model for exploring the effects of environmental toxins.
- The thyroid has been a specific area of interest for many studies investigating endocrine disruption, but the results have mainly focused on endpoints related to growth and development functions of thyroid hormone in fish (Figure 1).
- However, recent evidence suggests that immune function alteration can result from thyroid disruption.

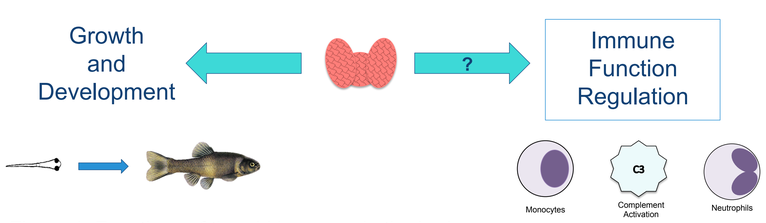



Figure 1. Functions of thyroid hormone in the body.

**The goal of this study was to determine the effects of thyroid inhibition on various aspects of immune function in fathead minnows *(Pimephales promelas)***

*Objective 1*: To determine whether exposures to the thyroid inhibitor, 6-propylthiouracil (PTU), lead to altered ability to survive bacterial infection.

*Objective 2*: To determine the effects of PTU on spleen index and differential white blood cell counts following bacterial infection.

*Objective 3*: To determine the effects of PTU on the expression of immune related genes following bacterial infection.

## Methods

***Experimental Design***

- This study was run using three trials:
  - Two trials were specifically used for mortality monitoring.
  - One trial was specifically used for tissue level and molecular level sample collection (Table 1).
- Fish in the PTU-exposed group were fed PTU-laden food for 21 days, while control fish were fed a diet free from PTU.
- Fish in control and PTU-exposed groups were injected with *Yersinia ruckeri,* a bacterial pathogen known to cause hemorrhaging.

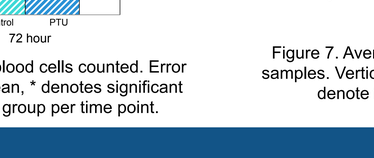



Figure 2. Pictorial description of immune genes measured (indicated in blue boxes).

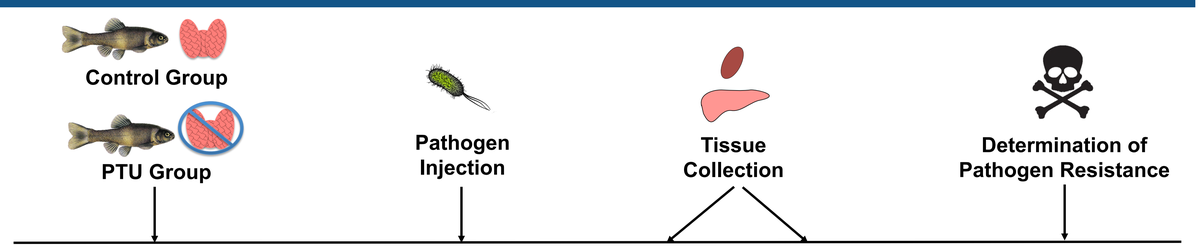



Table 1. Description of endpoints measured.

| | End Point | Function/Significance |
|---|---|---|
| Whole Organism Level | Pathogen Resistance | Survivorship monitored between groups for 14 days following pathogen exposure to understand whole organism effect |
| Tissue Level | Spleen Index | Spleen size should increase upon introduction of infection due to white blood cell proliferation within the organ; calculated as (mass of spleen ÷ mass of fish) x 100 |
| | White Blood Cell Count | White blood cells should increase in number upon introduction of infection; calculated as the percentage of blood cells that are white blood cells as counted from a blood smear |
| Molecular Level | Myeloperoxidase (*mpo*) | Enzyme used in the respiratory burst pathway to aid phagocyte interaction with pathogen (Figure 2). |
| | Interleukin 1β (*il-1β*) | Cytokine released mainly by macrophages to stimulate expression of pro-inflammatory genes. (Figure 2). |
| | Interleukin 11 (*il-11*) | Stimulates megakaryocytopoiesis and platelet production; necessary for combating the hemorrhaging associated with *Y. ruckeri* infection (Figure 2). |

## Results

- In both trials, the PTU-exposed fish exhibited a 20% decrease in survival relative to controls; however, this difference was only statistically significant in Trial 2 (Figure 3).
- This result suggests tha**t thyroid inhibition decreases the ability of the fish to fight and survive a pathogen infection.**

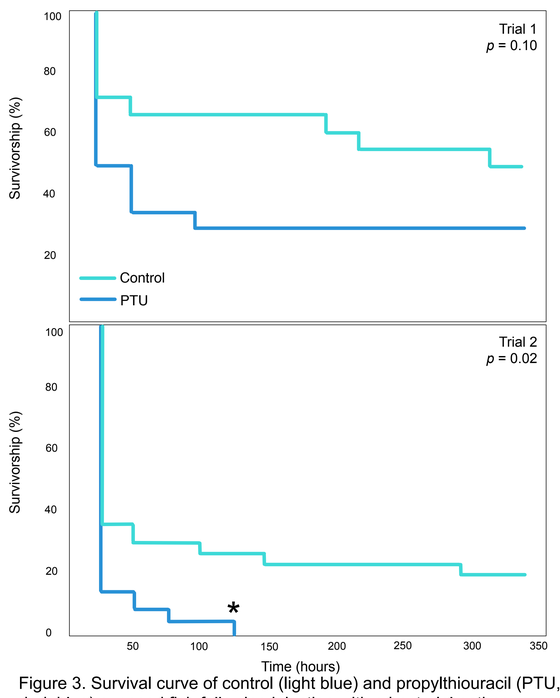



Figure 3. Survival curve of control (light blue) and propylthiouracil (PTU, dark blue) exposed fish following injection with a bacterial pathogen. * denotes a significant difference in survival. n = 30 per group.

- PTU fish had significantly lower spleen indices than control fish (Figure 4) suggesting that **thyroid inhibition suppresses the increase in spleen size associated with the normal immune response**, contributing to higher mortality in PTU exposed fish.

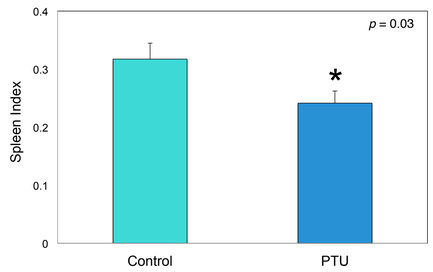



Figure 4. Average spleen index of control and propylthiouracil (PTU)-exposed fish pooled across both time points. Error bars represent standard error and * denotes significant difference.

- The PTU fish had a significantly lower percentage of white blood cells than controls (Figure 5) suggesting that thyroid inhibition **alters the white blood cell population**, which likely contributes to the decreased pathogen survival of PTU exposed fish.

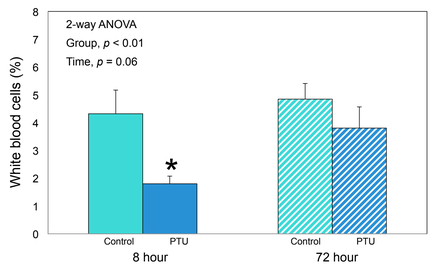



Figure 5. Average percentage of white blood cells counted. Error bars represent standard error of the mean, * denotes significant difference from 8h control. n = 6 per group per time point.

- At 8hrs, the PTU group showed a 1.9 fold decrease in *il-1β* expression relative to controls (Figure 6) suggesting that **thyroid inhibition impairs the inflammatory response needed to fight pathogens.**

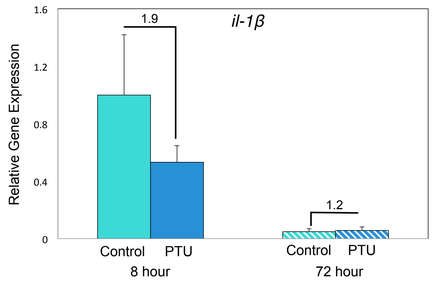



Figure 6. Average relative expression of interleukin 1β (*il-1β)* in liver. Vertical error bars represent standard error, lines over graph denote fold change relative to controls at that time point. n = 6 per group per time point.

- At 8 and 72 hrs, the PTU group showed a 3.2 and 56.4 fold increase in *il-11* expression compared to the controls, respectively (Figure 7) . This suggests that **more platelets are needed to combat hemorrhaging due to a more severe infection**, which could affect overall mortality.

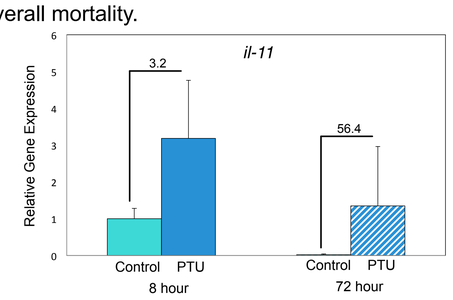
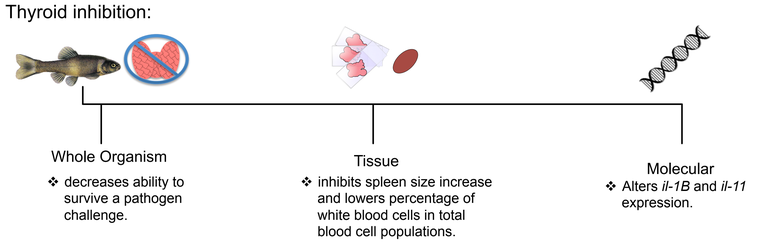



Figure 7. Average relative expression of interleukin 11 (*il-11)* in liver samples. Vertical error bars represent standard error, lines over graph denote fold change relative to control at that time point. n = 6 per group per time point.

## Conclusions

Thyroid inhibition:




***Exposure to PTU, a potent thyroid inhibitor, results in changes in immune response in adult fathead minnows.***

**Exposure to thyroid disrupting chemicals in the environment can lead to alterations in pathogen resistance. This may result in a reduced likelihood of survival in the face of a pathogen challenge.**

## Future Directions

- Collect data regarding blood differences (e.g., hematocrit) between control and PTU exposed fish to clarify the extent of effect on blood cell populations.
- Explore additional immune function related genes to narrow the mechanism by which thyroid inhibition affects the immune system.
- Utilize the endpoints identified in this study to screen environmentally-relevant thyroid disrupting compounds for immune function disruption.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