The Effects of Thyroid Disruption on Reproductive Function in Fathead Minnows

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Introduction

- The endocrine system is made up of the hormone signaling pathways in an organism. Compounds that disrupt normal hormone function are called endocrine disrupting chemicals (EDCs).
- It has been shown that alterations in thyroid signaling can lead to alterations in reproductive function. However, research into thyroid disruption usually does not take this into account (Fig. 1; Duarte-Guterman et al. 2014. *Gen Comp Endocr* 203:69-85).
- Results of thyroid-reproduction interaction differ between species and are not known for the fathead minnow (*Pimephales promelas*). This is an issue as this species is commonly used in testing for endocrine disruption.

The objectives of this study were to:
1) **assess the effects of chemically induced thyroid disruption on the thyroid signaling pathway**
2) **determine the reproductive outcomes of thyroid disruption in the fathead minnow**

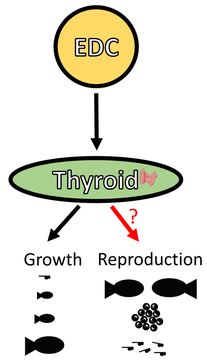

Figure 1: Research on the effects of thyroid disruption have traditionally only measured endpoints associated with thyroid function like growth. However, thyroid disruption may also have reproductive effects.

Methods

- Adult fathead minnows were exposed to thyroxine (T_4) or propylthiouracil (PTU) to promote and inhibit thyroid function, respectively. Doses were Low PTU (2500 µg PTU/g food), High PTU (5000 µg PTU/g food), Low T_4 (50 µg T_4/g food), and High T_4 (100 µg T_4/g food).
- Fish were fed dosed food during a 21-day breeding assay. Fecundity (# eggs per female per day), fertilization success, and 48-hour egg survival were monitored.
- At the end of the study, secondary sexual characteristics (Fig. 2) were measured, tissues were collected for gene expression analysis (Table 1) and gonadosomatic index (GSI) was calculated.

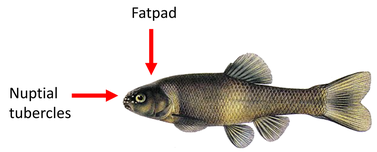

Figure 2: Red arrows indicate secondary sexual characteristics (tubercles and fatpad) of male fathead minnows.

Table 1: Subset of genes analyzed during gene expression analysis for tissues of fish in the breeding study

Target	Rationale
3ß-hydroxysteroid dehydrogenase	Testosterone/estrogen synthesis pathway
Aromatase	Converts testosterone to estrogen
Estrogen receptor α	Mediates estrogen signaling
Deiodinase II	Activates inactive thyroid hormone
Brain transcription element-binding protein	Expressed in response to thyroid hormone

Objective 1 Results: Thyroid disruption

- There were significant changes in expression of *deiodinase 2* in brains of PTU exposed females (Fig. 3) and livers of T_4 exposed females (Fig. 4). There was 4-fold increase in *brain transcription element-binding protein* expression in brains of T_4 exposed males (Fig. 5).
- These changes in gene expression suggest that the thyroids of these fish were successfully disrupted.

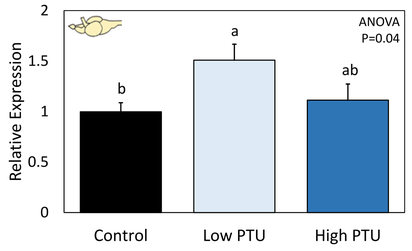

Fig. 3: Relative expression of *deiodinase 2* mRNA in the brains of PTU exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

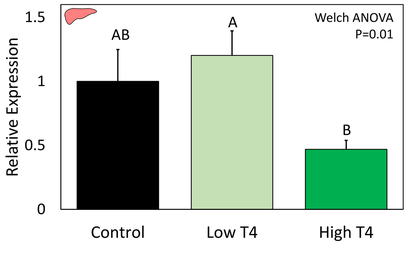

Fig. 4: Relative expression of *deiodinase 2* mRNA in the liver of T_4 exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

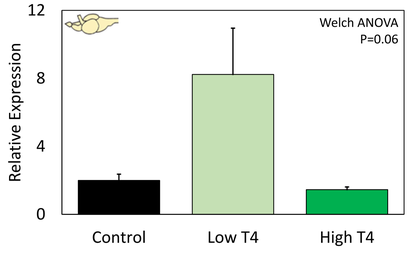

Fig. 5: Relative expression of *brain transcription element-binding protein* mRNA in the brain of T_4 exposed males after the 21-day breeding assay. Error bars represent one standard error.0

Objective 2 Results: Reproductive function

- There were no significant differences in fecundity, fertilization success, or secondary sexual characteristics. There were significant differences in 48-hour embryo survival (Fig. 6A and 6B). There were also significant differences in GSI of T_4 exposed females (Fig. 7).

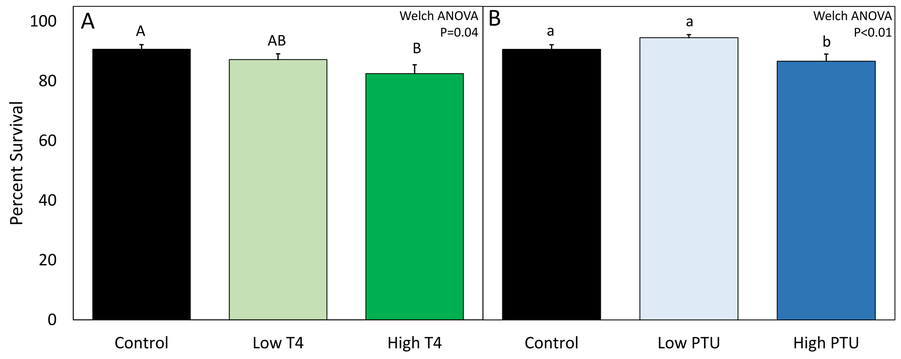

Fig. 6: 48-hour survival of eggs collected from T_4 (A) and PTU (B) breeding pairs during the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

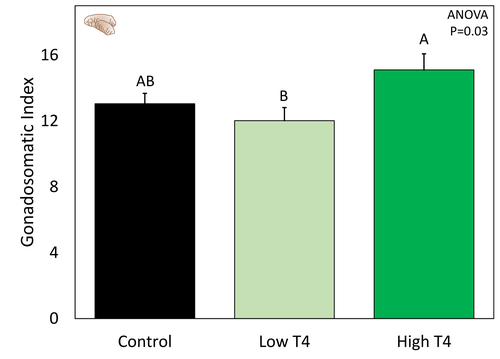

Fig. 7: Gonadosomatic index of T_4 exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

- There were significant differences in expression of *estrogen receptor α* (Fig. 8) in liver and *3ß-hydroxysteroid dehydrogenase* in gonad of males exposed to T_4 (Fig. 9). There were also significant differences in *aromatase* expression in gonads of females exposed to PTU (Fig. 10).

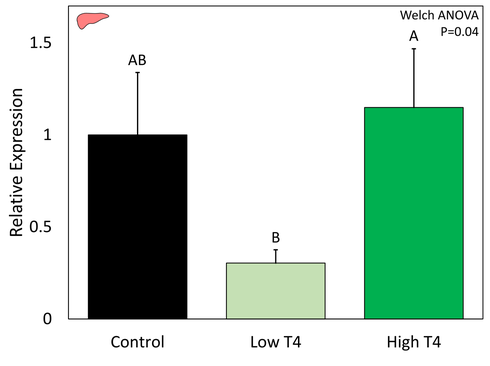

Fig. 8: Relative expression of *estrogen receptor α* mRNA in the liver of T_4 exposed males after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

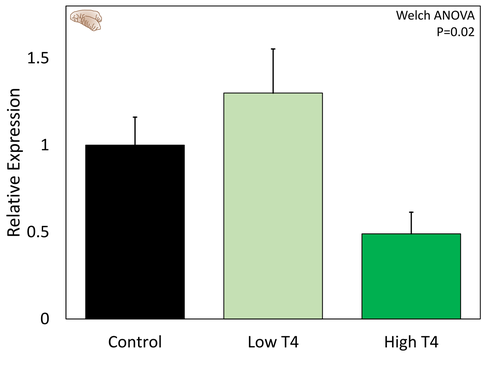

Fig. 9: Relative expression of *3ß-hydroxysteroid dehydrogenase* mRNA in the gonad of T_4 exposed males after the 21-day breeding assay. Error bars represent one standard error. Although a significant difference was detected, a Steel-Dwass post-hoc test was unable to detect specific differences.

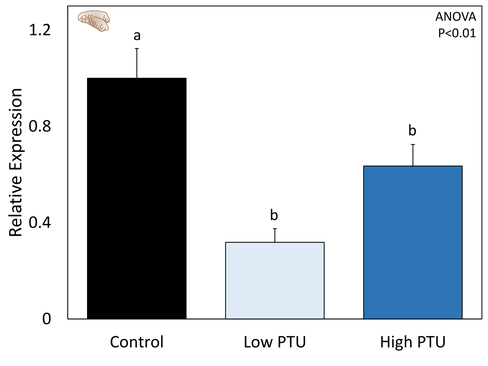

Fig. 10: Relative expression of *aromatase* mRNA in the gonad of PTU exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Conclusions

- As a whole, the results show that thyroid disruption in adult fathead minnows can lead to reproductive alterations.
- Reproductive endpoints should be considered along with those traditionally associated with the thyroid in future research on thyroid disrupting EDCs.
- Taking HPT-HPG crosstalk into account may help identify mechanisms of EDCs that cause reproductive alterations but that do not appear to directly impact the HPG axis.

Acknowledgments: TCU Adkins Fellowship and the other members of the Jeffries lab.