THE EFFECTS OF EARLY LIFE STAGE THYROID DISRUPTION ON REPRODUCTIVE BEHAVIORS IN FATHEAD MINNOWS (PIMEPHALES PROMELAS)

by

AUSTIN REED BRYANT

Bachelor of Science, 2014
Salisbury University
Salisbury, MD

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of the requirements
for the degree of

Master of Science
May 2021

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Marlo Jeffries for all that she has done for me over the last two years here at TCU. She taught me how to be a scientific researcher and how to critically think. I would also like to thank my committee members, Drs. Matt Hale and Dr. Gary Boehm, for providing interesting feedback and thinking outside the box when it came to my project. Also, thank you to my fellow lab mate Dalton Allen for always being ready to help me at a moment's notice and always saying "you got this!" whenever I was stressing. I would also like to thank the TCU Biology department for funding my research through the SERC Grant, Adkins Fellowship, and teaching assistantship. Also, thank you to all of the members of the Jeffries Lab for helping me set up experiments, keeping my fish alive, and any other odd jobs done around the lab. Lastly, thank you to my parents, Steven and Cathie Bryant, as well as my sister Alex Bryant. You guys have always had my back no matter what.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction

Thyroid Hormone Synthesis

Thyroid hormones (THs) are produced and secreted by a discrete thyroid gland, or a series of thyroid follicles, across all vertebrates (reviewed by Hulbert, 2000). Thyroid follicles are made up of epithelial cells called thyroid follicular cells that surround a colloid-filled lumen (Blanton and Specker, 2007). Sodium iodide symporters (NIS) located in the plasma membrane of the follicular cells take up inorganic iodide from the blood and transport it into the lumen via pendrin channels where it is oxidized by thyroid peroxidase (TPO) (Zoeller et al., 2007). Thyroid peroxidase is also responsible for the iodination of tyrosine residues attached to thyroglobulin (Tg). Each thyroglobulin molecule has ~70 tyrosine residues but only a few are available for iodination (Reviewed by Citterio et al., 2019).

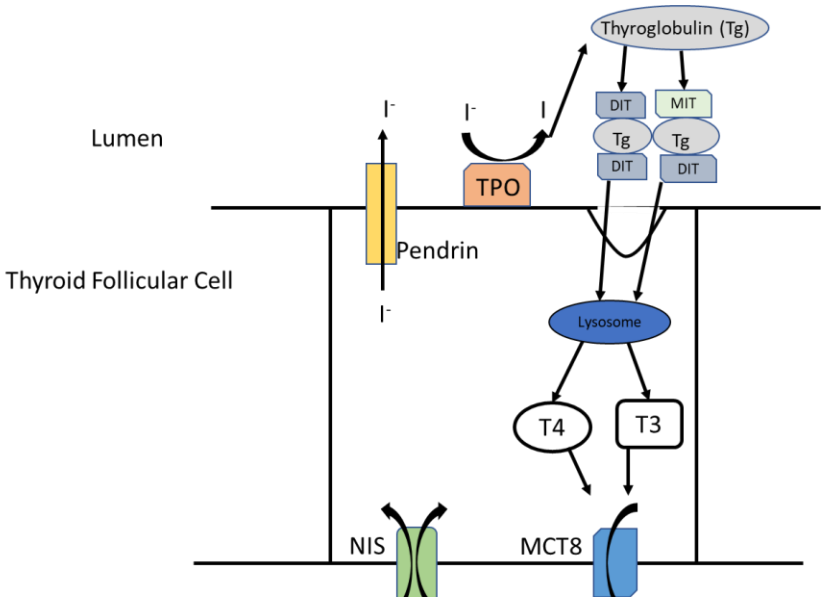

Figure 1. Thyroid hormone synthesis of thyroxine (T4). Iodide (I^-) is taken up by the sodium-iodide symporter (NIS) and transported into the thyroid follicular cell. The I^- then enters the lumen through the pendrin channels where it is oxidized by thyroid peroxidase (TPO) and added once or twice to the tyrosine residues in thyroglobulin (Tg) as to form monoiodotyrosine (MIT) or 3,5-diiodotyrosine (DIT), respectively. A coupling of an MIT and DIT on a Tg produces triiodothyronine (T3) and a coupling of two DITs on a Tg produces thyroxine (T4). Tg and the attached T3 or T4 are endocytosed into the follicular cell where they bind with lysosomes that contain enzymes to cleave T3/T4 from Tg. Thyroxine and small amounts of T3 are discharged through monocarboxylate transporters (MCT8) to circulate through the blood stream where they can interact with peripheral tissues.

The iodination of the tyrosine can occur once to produce monoiodotyrosine (MIT) or twice to produce 3,5-diiodotyrosine (DIT). Two DITs will attach to one another to form thyroxine (T4), the most common thyroid hormone. At this point, Tg and the attached T4 are endocytosed through the membrane and into the follicular cell forming a vesicle around the Tg and T4 from the membrane of the cell. The vesicle then fuses with lysosomes and enzymes in the lysosome hydrolyze Tg which frees T4, allowing for it to be transported into the bloodstream through monocarboxylate transporter 8 (MCT8; Carvalho and Dupuy, 2017). Thyroxine is the precursor to triiodothyronine (T3), the biologically active TH (Blanton and Specker, 2007) and though it can be created in the thyroid follicle cells, it is generally formed in peripheral tissues (e.g., liver, brain, gonads) where deiodinases type 1 and/or type 2 (DI1 and DI2) remove an iodine from the (5') outer ring of T4 to form T3 (Gereben et al., 2007). These peripheral tissues can utilize T3 or release it into the blood to allow other tissues to utilize it (Blanton and Specker, 2007). Thyroxine and T3 are transported throughout the blood stream by carrier proteins such as transthyretin (TTR), thyroxine-binding globulin (TBG), and albumin (Zoeller et al., 2007) and it is T3 that binds with high affinity to thyroid receptors (TRα and TRβ) that allow for certain biological effects to occur (Carr and Patiño, 2011). Thyroid hormone production is regulated by the hypothalamus-pituitary-thyroid (HPT) axis in which thyrotropin-releasing hormone (TRH) released by the hypothalamus controls the release of thyroid-stimulating hormone (TSH) from the anterior pituitary which in turn controls the release of TH from the thyroid follicles (Zoeller et al., 2007). Circulating T4 and T3, are part of a negative feedback loop which informs both the hypothalamus and pituitary to release TRH and TSH, respectively (Carr and Patiño, 2011). Therefore, having lower levels of TH will increase the output of TRH, starting the cascading effect of producing TH.

The HPT axis influences a multitudinous array of biological functions most closely associated with growth and development. Crane et al. (2004) demonstrated with fathead minnows (*Pimephales*

promelas), that peak production of T4 and T3 begins as early as 16- and 9-days post-hatch (dph), respectively, and that the thyroid follicles develop in the embryonic life stage indicating a need for THs in early life stage development. It has been shown that TH synthesis regulates metamorphosis. For example, zebrafish (*Danio rerio*) yolk-sac larvae require TH signaling for the development of cranial cartilages/extension of the lower jaw, the differentiation of digestive organs, inflation of swim bladder, and the growth of cartilaginous fins (Liu and Chan, 2002). In anurans, THs regulate important metamorphic processes such as tail resorption and hind leg lengthening (Goleman et al., 2002; Bruchholz and Hayes, 2005). Therefore, the synthesis and transport of THs throughout the body promotes standard development in vertebrates.

Thyroid Disrupting Compounds

Thyroid disrupting compounds (TDCs) are anthropogenic or naturally occurring compounds that affect TH synthesis in organisms. These TDCs can enter aquatic ecosystems from a variety of sources, such as industrial runoff, waste-water treatment effluent, and agricultural runoff (Kolpin et al., 2001). Common examples of anthropogenic TDCs are polychlorinated biphenyls (PCBs) used in pesticides, polybrominated diphenyl ethers (PBDEs) used in flame retardants, perchlorate, found in fertilizers, rocket propellant, and fireworks, and bisphenol-A (BPA) which is found in plastics and cosmetics (reviewed by Calsolaro et al., 2017). There are also non-environmental TDCs which are commonly used in laboratory settings such as propylthiouracil (PTU) and methimazole to model TDCs that inhibit TH synthesis (Crane et al., 2006; Hassan et al., 2017).

Thyroid disrupting compounds can affect the TH system in a variety of ways given how complicated and broad it is. For example, perchlorate binds to the NIS and inhibits uptake of iodide into follicular cells, reducing the production of T4 (Leung et al., 2014). Bisphenol- A has a similar structure to T3 and therefore can antagonistically bind to TRs leading to an inhibition of transcriptional activity that is influenced by T3 (Moriyama et al., 2002). PBDEs can competitively bind with TTR and limit TH

transport contributing to an alteration of thyroid hormone homeostasis (Zhao et al., 2017). Both PTU and methimazole inhibit the production of T4 by hindering TPO, which prevents the activation of iodide and the formation MIT and subsequently DIT (Van der Ven et al., 2006; Engler et al., 1982). Ultimately, there are a wide number of TDCs with numerous mechanisms of action that can inhibit thyroid hormone synthesis and signaling.

TDCs in the Aquatic Environment

There have been numerous studies focusing on TDCs in aquatic environments given their proclivity to ending up there. Within the United States, studies have shown the presence of TDCs in streams (Tan et al., 2004), lakes/sediments (Smith et al., 2001), rivers (Steinmaus, 2016) and bays (Brar et al., 2010). Globally, TDCs have been found in rivers used for drinking water in Japan (Kosaka et al., 2007), China (Shi et al., 2007) and Europe (Courderc et al., 2016). Given the varying molecular composition of TDCs they can spread and infiltrate into various environments via a number of different methods. One such method, is PBDEs can travel through water and air to distant locations far from their source, contributing to their global spread (Lema et al., 2008). PBDEs and PCBs are also known to be lipophilic and hydrophobic and can therefore be bioaccumulated and biomagnified throughout different environments (Boas et al., 2012; Bruckner-Davis, 1998). Perchlorate is extremely water-soluble; thus it is stable in water making it easily transported through surface and ground waters (Motzer, 2001). Bisphenol A has a great affinity for sorption into soil and sediments and can find its way into aquatic ecosystems through sources such as wastewater effluent, landfill leachate, and leaching from discarded BPA products (Im and Löffler, 2016). Given the widespread release and occurrence of environmental TDCs, organisms living in contaminated environments can suffer adverse effects from thyroid disruption.

Effects of TDCs

The entry of TDCs into surface waters poses a potential threat to aquatic vertebrates, including fish and amphibians, given the ability of TDCs to alter TH levels (Mukhi and Patiño, 2006; Goleman et al. 2009). Changes in TH levels are most often associated with adverse alterations in somatic development and reduced growth (Crane et al. 2005; Park et al. 2006; van der Ven et al. 2006). This is because THs have been shown to contribute to alterations in skeletal formation (Schreiber, 2006) and retardation of metamorphosis (Liu and Chan, 2002). Zebrafish develop thyroid follicles as early 72 hours post fertilization (hpf) and when zebrafish larvae were exposed to exogenous T4, paired pelvic fins grew faster and pectoral fins developed earlier as compared to control indicating THs play a role in the transition from larvae to juveniles (Brown, 1997). In a study looking at swim bladder inflation in fathead minnows, it was found that exposure to iopanoic acid, a model deiodinase inhibitor, impaired swim bladder inflation and growth as early as 6 dpf, demonstrating a need for THs early in development (Cavallin et al., 2017).

Early-life-stage exposure to TDCs can cause long term effects that have population level consequences. For example, exposures to sodium perchlorate and methimazole, skewed sex ratios towards females and if exposure continued past sexual differentiation the effect was permanent (Sharma and Patiño, 2013). Similarly, a study conducted on the African clawed frog (*Xenopus laevis*) found that exposure to perchlorate skewed phenotypic sex ratios towards females (Goleman et al., 2002b). Given that disruption of TH synthesis can impair testes differentiation, this could have negative effects on populations in the wild. In a study conducted by Bruns (2017), it was demonstrated that early-life-stage exposures to PTU reduced the reproductive output of fathead minnows by more than 50%. Furthermore, it was demonstrated that this decrease was related to the exposure history of male, not the female (Seemann, 2018), indicating that PTU induced changes unique to males are responsible for lowered reproductive output. This provides even further evidence that thyroid disruption (TD) can have lasting, population level consequences given that reproductive output was halved. Efforts to identify

these male-specific changes did not provide any evidence of delayed sexual development, altered sperm motility, decreased fertilization success, or alterations to the gonadosomatic index in response to PTU exposure (Bruns, 2017).

Neurodevelopment and Behavior

Thyroid hormones play a critical role in vertebrate physiology and contribute to the development of the nervous system (Escobar et al., 2004) as well as neurobehavioral development (Weis et al., 2001). This is typically seen through neural signaling, migration and differentiation of axons, which all contribute to neurological maturation (reviewed by Bernal, 2005). Numerous studies have found that T3 is found throughout eggs and embryos of previously studied vertebrates (reviewed by Préau et al., 2015) and that the source of this T3 is at least in part through transplacental transport or passive diffusion during embryogenesis (Obregon et al., 1984; Ayson and Lam, 1993) . Neural stem cells give rise to both neuronal and glial cells, which are crucial for neurogenesis and that the alteration of these cells can contribute to behavioral changes (Rajkowska et al., 1999) . It was established that rat neural and glial cell nuclei contained thyroid hormone receptors, TRα1, TRα2, and TRβ1, as early as gestation day 14 (Falcone et al., 1994) and given that glial cells play a role in neuroblast proliferation and myelinization, this demonstrates a need for circulating THs for proper neuronal development. Another study concluded, that when endogenous T3 was antagonized using ammonia or tetrabromobisphenol A in *X. laevis*, it significantly altered the expression of developmental genes as well as genes known to control neural stem cell function (Fini et al., 2012). Therefore, disruption of TH synthesis may impact stereotypical neuronal maturation that can affect behavior.

The presence of THs are crucial for typical brain development and it has been proposed that TH levels are important for eliciting neuroendocrine responses to environmental signals (Morgan et al., 1999). For example, zebrafish that were exposed to PBDE (i.e., BDE 49) in the embryonic stage, suffered a significant decrease in touch-escape responses (defined as distance traveled when touched with metal

probe), muscle spasms, and general lethargic movements (McClain et al., 2012). It has been shown that PBDEs can reduce the differentiation of neural stem cells into neurons, as well as inhibit neurite outgrowth (Zhang et al., 2010) indicating that the symptoms seen in the (McClain et al., 2012) study are the result of altered neurodevelopment as a result of TD. Another study that looked at zebrafish behavior found that exposure to a PCB mixture induced decreased avoidance behavior to a moving visual stimulus, demonstrating that an alteration of the motor system or the processes responsible for threat recognition (Lovato et al., 2016).

In a secondary follow up study to the Bruns (2017) and Seeman (2018) studies, Johnson (2019) wanted to determine if there had been alterations to the expression of genes related to neurodevelopment in the same fish that were used in the Bruns (2017) study by sequencing brain transcriptomes of the control and PTU-exposed fish. It was found that the PTU-exposed males had significant alteration of genes related to brain development (Johnson, 2019) which could provide a possible mechanism to the reproductive decrease seen in the Bruns (2017) study given the connection between neurodevelopment and behavior.

Study Organism

The fathead minnow has become a common aquatic species to use in ecotoxicological studies, especially for studies involving TDCs (Crane et al., 2004; Cavallin et al., 2017; Bruns, 2017). Given their ability to externally fertilize, quick transitional period from embryo to larvae, relatively quick sexual maturation period of ~4-5 months, and the ease of care, fathead minnow make excellent laboratory organisms to study (EPA, 1987; Gale and Buynak, 1982).

Mating strategies differ between species and in most cases successful mating can depend on a number of factors such as, individual dominance, promiscuity, fertilization techniques, choice of nest site, and many others (Trippel, 2003). In the case of fathead minnows, reproductive success comes generally from behaviors defined under the behavioral categories of competition or courtship (McMillan

and Smith, 1974). An example of this, is when presented with a nesting site such as a clay pot, split PVC pipe, or an overhang, males will attempt to dominate each other to gain control of the nesting site and use tactics like headbutting, tail-whipping or chasing to defend the site (McMillan and Smith, 1974). Once a male has successfully defended a nesting site they will attempt to lure or force a female under the nesting site using techniques such as chasing, butting, and lateral display amongst others (Cole and Smith, 1987). The final step in this process is to force the female to the top of the nesting site where she attaches her eggs while he fertilizes them (McMillan and Smith, 1974). This collection of behaviors represents a complex mating ritual and the inability of a male to successfully carry out these behaviors could result in a loss of reproductive output as seen in the Bruns (2017) study.

Goals and Objectives

The data gathered from the Bruns (2017), Seeman (2018), and Johnson (2019) studies indicate that early life stage thyroid disruption in fathead minnows, specifically male individuals, leads to a severe loss of reproductive output, and that genes related to neurogenesis have also be altered. Given the connection between THs and neurodevelopment it is hypothesized that fish exposed to PTU during development will display altered reproductive behaviors

The overall goal of this project was to determine if fathead minnows exhibit altered reproductive behavior resulting from early-life-stage TD induced via PTU exposure. To achieve this goal, three specific objectives were addressed. The first objective was to confirm thyroid disruption in larval fathead minnows. To accomplish this objective three methods were utilized: [1] gene expression analysis of TH related genes, [2] immunohistochemistry staining that allowed for the quantification of T4 in the thyroid follicles of fathead minnow larvae, and [3] morphometric analysis. The second objective of the study was to examine the impact of early-life-stage PTU exposure on neurodevelopment and behavior in general by assessing indicators such as feeding behavior and predatory avoidance. The final objective of this study was to determine if reproductive behaviors differed between control and PTU-

exposed males. To accomplish this objective a reproductive assay was ran that compares both courtship and competitive behaviors between control and PTU-exposed males as well as determined the overall winner of the nesting site.

Methods

Fish Care and Maintenance. All of the fish used in this project were obtained from the Texas Christian University (TCU) Environmental Toxicology Lab and procedures were approved by the TCU Institutional Animal Care and Use Committee (IACUC, Protocol #1920-3). Fathead minnow embryos were gathered by collecting breeding structures (split PVC pipes) that had eggs from TCU's fish stock and placing them in 1L beakers in incubators heated to 26± 1°C and under a photoperiod of 16-hour light: 8-hour dark until hatch.

Exposure. Newly hatched larvae were placed into three different groups, control (0 mg/L PTU), low PTU (35 mg/L), and high PTU (70 mg/L) and exposed through 39 dph. Stock solution for PTU was made daily using 6-propyl-2-thiouracil (Sigma Aldrich; St. Louis, MO). Exposures were conducted in 1 L beakers from 0 to 16 dph. Larvae were kept at a density of 42 fish per beaker and 80% water changes were performed daily.

On day 7 of the exposure, a subset of fish were sacrificed for morphometric analysis, thyroid disruption confirmation via gene expression analysis, and immunohistochemistry staining analysis. At 17 dph, larvae were moved into 9 L aquaria with an approximate density of 77 fish per tank and 66% water changes were performed daily. At 30 dph, fish were split between 12 tanks (6 control, 3 low PTU, and 3 high PTU) with an approximate density of 50 fish per tank and daily water changes continued.

At 7 dph, a sample of fish were collected to perform a feeding assay and then returned to respective 1 L beakers. On day 12 of the exposure, a random sample of fish were collected to conduct a predator avoidance assay and then returned to their respective 1 L beakers

On day 39 the exposure ended, and fish were transferred to 37 L aquaria, containing 27L of water, with a density of 50 fish per tank and 33% water changes were performed daily. At approximately 93 dph, fish were moved into 75L aquaria filled with 60L of water with a fish density of approximately 65 fish per tank. At 148 dph, fish were separated out into additional 75L tanks with a density of 33 fish per tank and were kept in these tanks till the start of the breeding assay.

Throughout the duration of this experiment, water changes were conducted daily, and the light cycle was 16-hour light:8-hour dark. From 0 to 29 dph, fish were fed *Artemia* nauplii and then transitioned to Tetramin Tropical Flakes (Tetra Holding Inc; Blacksburg, VA) with supplementary *Artemia* until fish were sizable enough to consume only flake food (approx. 39 dph). Water quality testing on pH, conductivity, ammonia, alkalinity, and hardness was performed every 4 days throughout the duration of the exposure, and then performed once every two weeks after the exposure period ended.

Thyroid Disruption Confirmation

Morphometric Analysis. On day 7 of the exposure, 10 fish per treatment were euthanized with MS-222 for mass and length measurements, and whole larvae were placed on dry ice and stored at -80°C until tissue homogenization for gene expression. Length of fish was measured using electric calipers and fish mass was measured by patting dry and weighing using digital scale.

Thyroid hormone related gene expression. Tissues that were used in the morphometric assessment were homogenized using a QSonica Q125 sonicator (Sonics & Materials Inc.; Newtown, CT) and RNA was extracted using a Maxwell 16 Instrument and a Maxwell 16 LEV simplyRNA Tissue Kit (Promega; Madison, WI) according to manufacturer's protocol (described in Sellin Jeffries et al., 2014). Total RNA concentration and purity were measured using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific; Wilmington, DE). Processed RNA samples were stored at -80°C until cDNA conversion.

Synthesis of cDNA from RNA was performed using a Bio-Rad iScript cDNA synthesis kit (Bio-Rad; Hercules, CA). Total RNA samples were diluted to a concentration of 13.33 µg/mL in nuclease free water. Next, 2 µL of qScript cDNA Supermix was combined with 8 µL of the diluted RNA in a 0.2 mL PCR

tube. The tubes were then placed in a T100 Thermocycler (Bio-Rad) under a heat cycle setting of 25°C for 5 min, 42°C for 30 min, 85°C for 5 min, and a hold at 4°C. After the cycle was completed, samples were added to 30 µL of reverse transcription buffer and stored at -80°C.

All RT-qPCR reactions were performed on a CFX Real-Time System (Bio-Rad) using PerfeCTa SYBR Green FastMix (Quantabio; Beverly, MA). Samples were loaded into 96-well plates each well containing the following: 5 µL SYBR green mix, 0.3 µL primer mix, 4.3 µL of nuclease-free water, and 0.4 µL of the cDNA sample. All plates were run under the PCR cycle: 95.0°C for 30 s, 95.0°C for 10 s, and 15 s at primer annealing temperature, where the last two steps were repeated 40 times. The primers used in these reactions were two housekeeping genes, ribosomal protein L8 (*L8)* and acidic ribosomal phosphoprotein (*arp*), as well as four thyroid hormone related genes, deiodinase 2 (*di2*), deiodinase 3 (*di3*), thyroid hormone receptor β (*trβ*), and transthyretin (*ttr*). Data for gene expression was analyzed using the standard curve method, where each target gene was normalized to the reference gene, in this case acidic ribosomal phosphoprotein (*arp*). There was not a significant difference in the expression of the reference gene between groups. For each sample, the relative expression of each target gene was calculated by dividing the mean starting quantity (SQ) of the target gene by the mean SQ of the reference gene. For easier data visualization, the relative expression determined for each sample was divided by the mean expression of the control samples.

Table 1: Genes chosen for thyroid hormone related gene expression including primer sequences and annealing temperatures. Abbreviations: *di2*- deiodinase 2; *di3*- deiodinase 3; *trβ*- thyroid hormone receptor beta; *ttr*- transthyretin.

Gene	Primer sequences	Annealing temp (°C)
di2	FW: AATTTTCGGATGTGGCAGAC RV: GCAGCAAACATCCTCTCCTC	54
di3	FW: TAATGAAGATGCGGGAAAGC RV: CGCCGTTTTAAAGAAGTCCA	62
trβ	FW: CAGTGAGTCCGTGGAAGACA RV: CAGGTGATGCATCGGTAATG	54
ttr	FW: CTGGTGTGTATCGGGTGGAGTT RV: GCATGAGCTTCAAACACCACAT	60
arp	FW: CTGAACATCTCGCCCTTCTC RV: GACACACACTGGCGATGTT	57

Immunohistochemistry Staining. On day 7 of the exposure 10 fish per treatment group were euthanized in MS-222 and placed in 4% paraformaldehyde overnight to fix samples. Five samples from each group were randomly chosen and washed with phosphate-buffered saline (PBS) and gradually washed with increasing methanol (MeOH)/PBS concentrations until the samples could be stored in 100% MeOH at -20°C. Samples were rehydrated using increasing concentrations of PBS/MeOH up to 100% PBS and then treated with depigmenting solution (1 mL 1M potassium hydroxide, 10 mL 30% hydrogen peroxide, 89 mL nanopure water). Following this, larvae were cracked using alternating treatments of distilled water and 100% acetone (at -20°C). Larvae were then treated with a 0.1% collagenase solution, followed by a blocking buffer (4% goat serum, 1% bovine serum albumin, 1% DMSO, 0.8% Triton X100, and 0.1% Tween-20 in PBS) for 3 hours at room temperature. Blocking buffer was removed and samples were incubated at 4°C for 4 days with a primary antibody (polyclonal rabbit anti T4) at a dilution of 1:500. Samples were then washed with blocking buffer and incubated with a secondary fluorescent antibody (Alexa Fluor 488 Green goat anti-mouse IgG1) at a dilution of 1:150 for 4 days at 4°C. The larvae are then washed and stored in blocking buffer at 4°C until imaging analysis.

Fluorescence of larvae was obtained using a Zeiss Observer Z1 microscope fitted with an LSM 710 laser (ZEISS; Oberkochen, Germany) and ZEN software for processing. Larvae samples were positioned on culture dishes (35 mm petri dish, 14 mm microwell, No. 0 coverglass, MatTek) between two trimmed cocktail straws to ensure larvae were properly positioned with ventral side facing the glass well. All images are saved as LSM files to preserve microscopy data associated with the image.

Image quantification was performed using ImageJ software (National Institute of Health) with plug-ins, StackReg and TurboReg. ImageJ was used to measure integrated density (metric for area and fluorescence) and all samples were normalized to the control group in order to analyze differences in follicular T4 content.

Behavioral Assays

Feeding Assay. At 7 dph, 20 fish from each replicate (n=60) were randomly selected for a feeding assay, the methods of which are defined in (Krzykwa and Sellin Jeffries, 2020) but are briefly mentioned below. Fish were collected before their typical morning feeding time to ensure the efficacy of the assay. The control fish were put into their own wells in a 6-well plate and 10 *Artemia* were counted and placed into each well using a plastic pipette. The whole process took ~15 min for all twenty fish in the control group. They were then placed into an incubator with a temperature range of 26± 1°C and left for one hour. As soon as the control fish were placed in the incubator the process was repeated for the low PTU fish and then once again for the high PTU fish so that assays were staggered by ~15 min each. At the end of the hour for each group the 6-well plates were removed and the remaining *Artemia* were counted and recorded. All fish at the end of the assay were returned to their respective beakers.

C-Start Assay. At 12 dph, 20 fish from each group (n=60) were randomly selected for the C-start assay, the methods of which are defined in (Krzykwa and Sellin Jeffries, 2020) but are briefly mentioned below. Replicates were put through the assay in sequential order (e.g., control, low, high, repeat) to prevent any biases in time of sampling. Individual samples were placed in petri dishes containing 20 mL

of water and positioned on a laminated 1mm grid sheet that had a haptic motor connected directly underneath to provide an external stimulus (i.e., vibration). Above the petri dish was a Fastec IL4 (Fastec Imaging; San Diego, California) high speed camera that recorded the reaction of the fish to the stimulus at 1000 frames per second. The recording was run in conjunction with FasTec FasMotion version 1.8.25 (Fastec Imaging) imaging software and videos were analyzed using ImageJ measuring body length, escape velocity, latency, total escape response and escape angle. If the fish did not respond to the stimulus, a 30 second latency period was observed before activating the stimulus again. If a fish did not respond in three attempts, they were marked as no response. All fish at the end of the assay were returned to their respective beakers.

Reproduction Assay. Once fish were sexually mature (~6 months), 32 males/group were collected and anesthetized using MS-222 in order to fin clip the males for identification. Males were then partnered with another male in a differing treatment group (i.e., control x 35 mg/L PTU, 35 mg/L PTU x 70 mg/L PTU, and control x 70 mg/L PTU) along with a single female and breeding structure in a 27L tank. This process started around 0800 and the fish were given a 3 min acclimation period before being filmed for 5 min with a GoPro camera (GoPro Inc.; San Mateo, California). Recording was done again 2 hours later for 5 min followed by a similar recording schedule the next day for a total of 20 min of film. During the assay fish were kept under optimal conditions for breeding (i.e., ~25± 1°C water temperature, 16-hour light: 8-hour dark photoperiod) and fed Tetramin flakes twice a day. Video footage was then analyzed by quantifying the number of behaviors (shown in Table 1) performed as well as the overall winner defined by the male that displayed the greater number of aggressive behaviors or spent the longest time under the breeding structure.

Table 2. Male reproductive behaviors displayed towards males and females.

Behavior	Description
Competition	
Headbutting	Male will swim towards another male hitting it with the anterior end of its snout
Chasing	Male will swim rapidly after another male
Tail-whipping	Male will position himself next to another male and laterally undulate its caudal fin, hitting the other male
Courtship	
Headbutting	Male will swim towards a female hitting it with the anterior end of its snout
Chasing	Male will swim rapidly after another female
Lateral Display	Male will position himself next to a female and without touching laterally undulate its caudal fin
Spawning	Male will cup the female and undulate his body while pushing female to ceiling of nesting site
Nest Cleaning	Male will position himself vertically under the nest site and bite or nibble the ceiling

Statistical Analysis

Statistical analysis was performed using JMP 11.2.0 software (SAS; Cary, NC). For all analyses involving comparing three or more groups a Levene's Test was used to determine unequal variance between groups. If the data had unequal variances a Wilcoxon test was used followed by a Steel-Dwass post hoc test if significance was found. For data with equal variances a one-way analysis of variance (ANOVA) was performed and if significant followed by a Dunnett's post hoc test to determine significance between groups.

A Pearson chi-square test was used to determine differences in C-start responses and the number of wins in the reproductive behavior assay. Fish pairings where there was a difference in mass greater than 30% were excluded from analysis and a *t* Test was used to determine the difference in reproductive behaviors displayed between the pairings. For all tests, statistical significance was set at $\alpha = 0.05$.

Results

Confirmation of Thyroid Disruption

Morphometric Analysis. During the exposure, there were significant differences detected in mass and length of fathead minnows at 7 dph (ANOVA, P <0.01 for both; Figure 2). For mass, there were no significant differences between the control and low-PTU fish or control and the high-PTU fish; however, the mass of the high-PTU fish was significantly less than that of the low-PTU fish. For length, there were no significant differences between the control and low-PTU group; however, the length of the high-PTU fish was significantly less that of the control and low-PTU group.

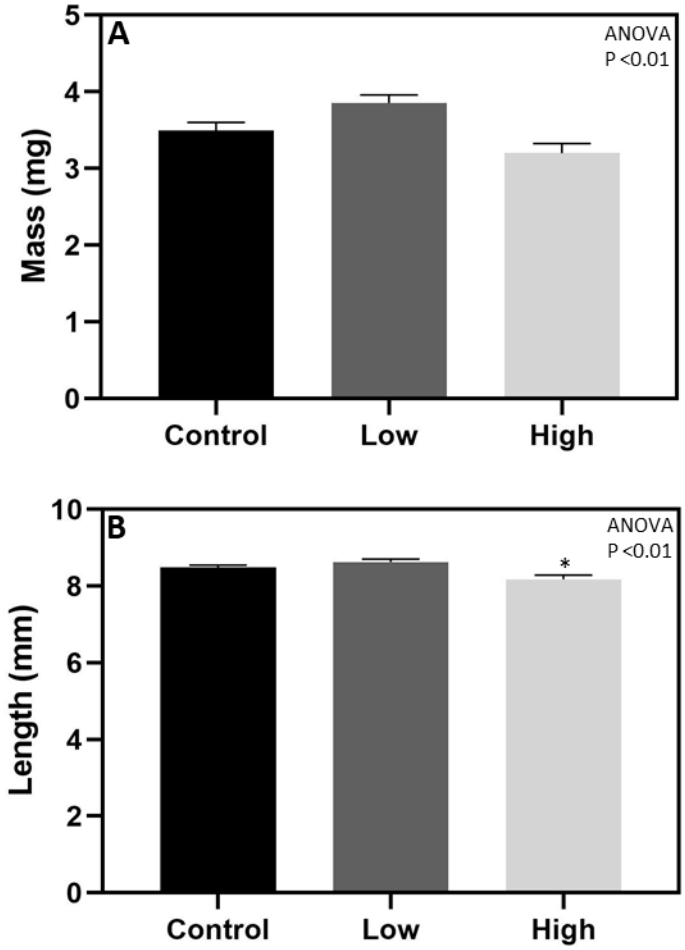

Figure 2. Average mass (A) and length (B) of fathead minnows at 7 days post hatch. Black bars represent the control (0 mg/L), dark grey bars represent the low-PTU group (35 mg/L) and light grey bars represent the high-PTU group (70 mg/L). Error bars represent standard error and * denotes significant difference between groups n= 30 per group

Immunohistochemistry Staining. On day 7 of the exposure, significant differences were detected in follicular T4 content. Specifically, there was a statistically significant 98% reduction in fluorescence in the high-PTU group compared to the control (Wilcoxon, p= 0.03; Figure 3). There was also a 47% reduction in fluorescence in the low-PTU group compared to the control group, although it was not significant.

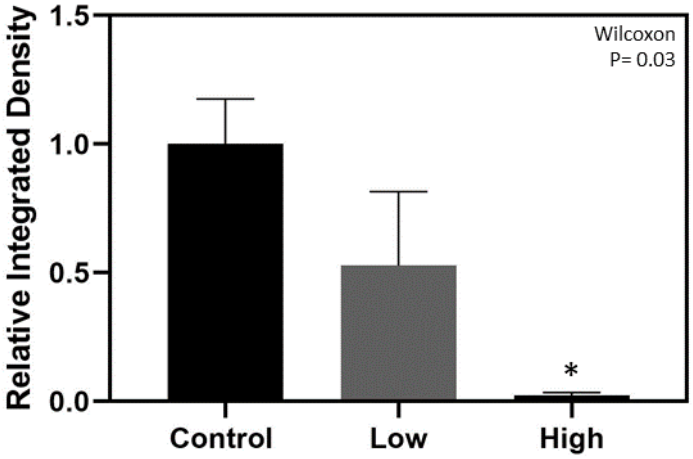

Figure 3. Relative integrated density for immunofluorescence in fathead minnows. The black bar represents the control group (0 mg/L), the dark grey bar represents the low-PTU group (35 mg/L) and the light grey bar represents the high-PTU group (70 mg/L). Error bars represent standard error and * denotes significant difference from control . n= 5 per group

Gene Expression Analysis. The results for thyroid-related gene expression appear in Figure 4. There were significant differences found between groups for all four of the genes examined. For *di2,* there was significant upregulation in the low and high-PTU group compared to the control group (Wilcoxon, P< 0.01). Significant downregulation was seen in both low and high-PTU group compared to the control group for *di3* (ANOVA, P< 0.01). The high-PTU group demonstrated significant downregulation compared to the control group for both *ttr* (Wilcoxon, P< 0.01) and *trβ* (ANOVA, P= 0.04). For *ttr*, the low-PTU group demonstrated a 55% reduction in expression compared to the control group but it was not statistically significant.

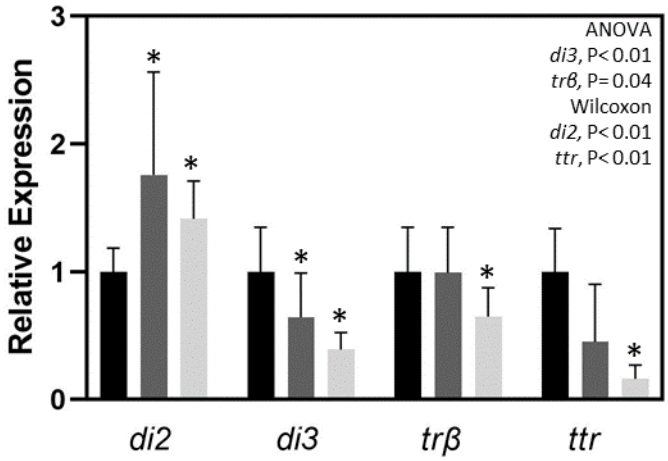

Figure 4. The normalized relative expression of thyroid-related genes (deiodinase 2, *di2;* deiodinase 3, *di3*; thyroid hormone receptor beta, *trβ*; transthyretin, *ttr*) in whole body tissue of fathead minnows at 7 days post hatch. Values represent mean fold change in propylthiouracil (PTU) exposed fish relative to control fish. Black bars represent the control group (0 mg/L), dark grey bars represent the low-PTU group (35 mg/L) and light grey bars represent the high-PTU group (70 mg/L). Error bars represent standard error and * denotes significant difference from control according to Steel or Dunnett's post-hoc test. n= 9 per group

Behavioral Assays

Feeding Assay. On day 7 of the exposure, a subset of fish was subjected to a feeding assay. There was no significant differences in the average number of artemia consumed between any of the groups (ANOVA, P= 0.07; Figure 5).

C-Start Assay. On day 12 of the exposure a C-start assay was conducted where 20 fish were randomly selected from each group and subjected to a vibrational stimulus to test predator escape response. There were no significant differences detected between the groups (Pearson Chi-square, P= 0.12; Figure 6). There were also no differences detected in the metrics that make up typical C-start behavior (ANOVA; latency, P= 0.37; velocity, P= 0.33; escape angle, P= 0.85; total escape response, P= 0.68

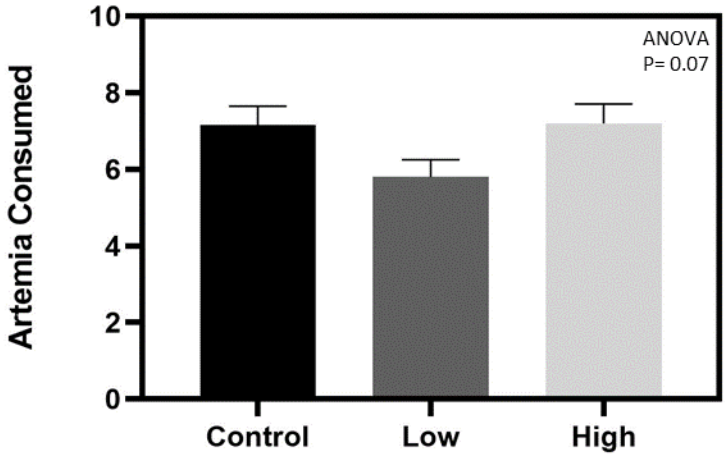

Figure 5. Average number of artemia consumed by fathead minnows at 7 days post hatch. The total number of artemia presented to the fish were 10. The black bar represents the control group (0 mg/L), t dark grey bar represents the low-PTU group (35 mg/L) and the light grey bar represents the high-PTU group (70 mg/L). Error bars represent standard error. n= 20 per group.

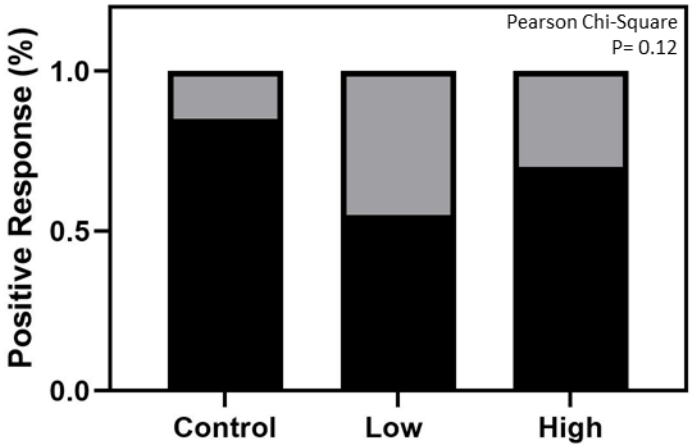

Figure 6. Percentage of positive response (displayed typical C- start response) in fathead minnows at 12 days post hatch. Black bar represents positive response and grey bar represents negative response (did not display any C-start response). n= 20 per group.

Reproduction Assay. Once fish were sexually mature, a reproductive behavior assay was performed to compare the number of behaviors related to reproduction such as competition, courtship, and nest care behaviors between the different pairings. For the control vs. low-PTU pairing, there were no significant differences detected in the number of behaviors displayed in each category. However, there was a 46% reduction in the overall number of behaviors displayed seen in low-PTU group compared to the control group, but it was not significant (*t* Test, competition, P= 0.09; courtship, P= 0.20; nest care, P= 0.21, sum of behaviors, P= 0.09; Figure 7A). Significant reductions in all behavior categories were detected in the high-PTU group compared to the control group, specifically a 71% reduction in competition behaviors, 73% reduction in courtship behaviors, and a 75% reduction in nest care behaviors (*t* Test, competition, P< 0.05; courtship, P< 0.05; nest care, P< 0.01, sum of behaviors, P< 0.01; Figure 7B). The low-PTU vs. high-PTU group displayed no significant differences in the number of behaviors between the two groups (*t* Test, competition, P= 0.18; courtship, P= 0.29; nest care, P= 0.18, sum of behaviors, P= 0.17; Figure 7C).

Win Data. In addition to quantifying the numbers of behaviors in each reproductive behavior category, winners of each trial were also recorded. In the control versus low-PTU pairing, there was no significant difference in win percentage between the two groups (Pearson chi-square, P= 0.41; Figure 8A). However, there was a significant difference in win percentage between the control vs. high-PTU group with the high-PTU group losing 82% of the trials (Pearson chi-square, P< 0.01; Figure 8B). There was no difference in win percentage in the low-PTU vs. high-PTU pairing with both groups winning 50% of the trials (Pearson chi-square, P= 1.0; Figure 8C).

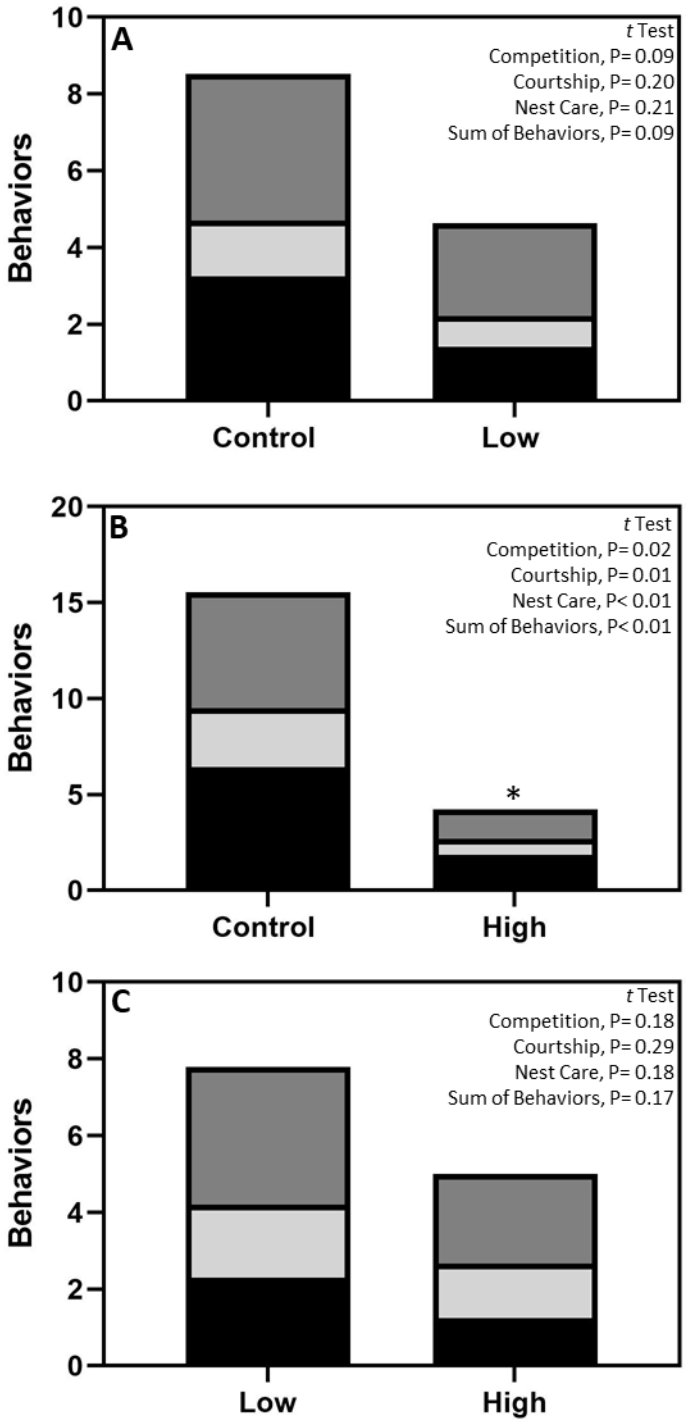

Figure 7: Average number of competition behaviors (black bar), courtship behaviors (light grey bar), and nesting behaviors (dark grey bar) between (A) control (0 mg/L) versus low-PTU (35 mg/L), n=12; (B) control (0 mg/L) versus high-PTU (70 mg/L), n=11; and (C) low-PTU (35 mg/L) versus high-PTU (70 mg/L), n=14.

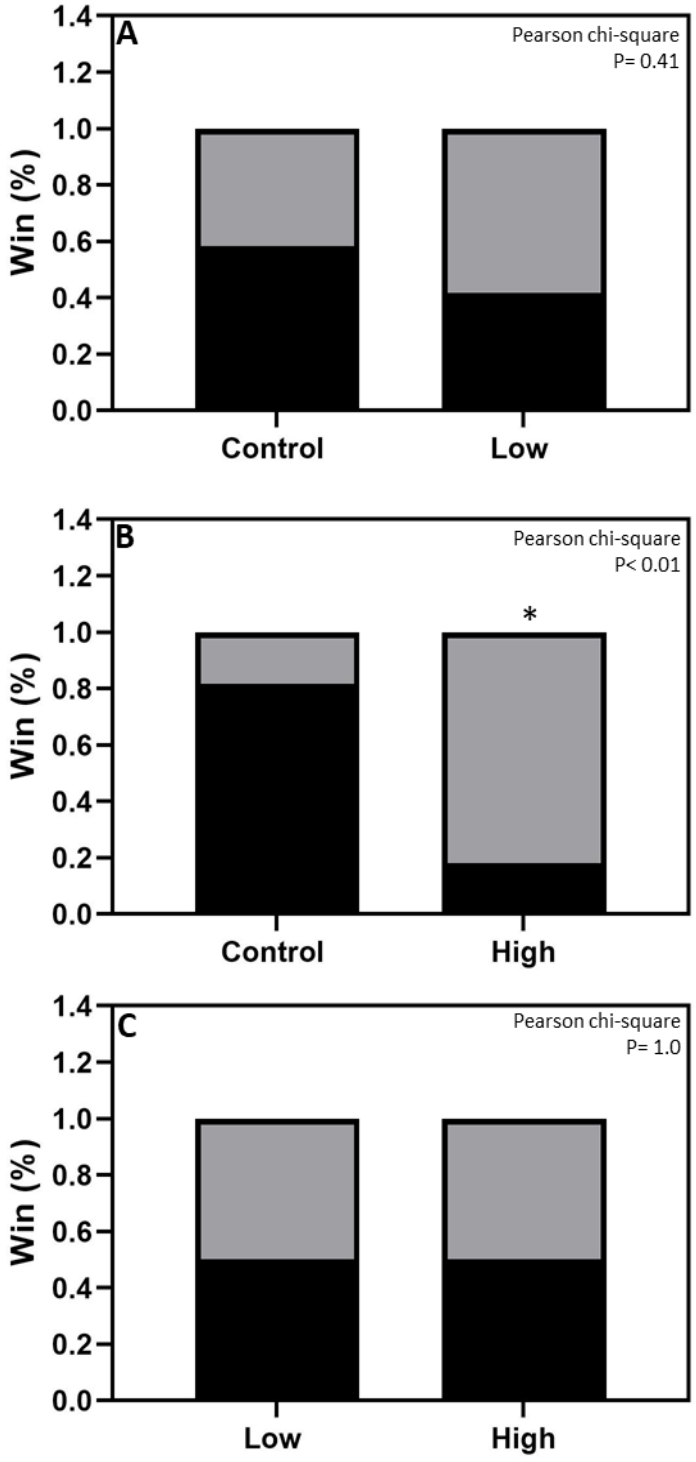

Figure 8. Percentage of wins (one male displayed a greater number of reproductive behaviors than the other) between (A) control (0 mg/l) versus low-PTU (35 mg/L), n=12; (B) control (0 mg/l) versus high-PTU (70 mg/L), n=11; and C) low-PTU (35 mg/L) versus high-PTU (70 mg/L), n=14. Black bars represent win percentage, grey bars represent losing percentage and asterisks (*) denotes significant differences.

Discussion

Confirmation of Thyroid Disruption

Morphology. Thyroid hormones are known to be an important factor in growth and development of teleosts (Power et al., 2001), therefore, measurements such as mass and length are used as biomarkers for TD (Crane et al., 2006; Macaulay et al., 2017). In this study, 7 dph high-PTU fish were significantly shorter in length than the control group and the low-PTU group. It was also demonstrated that the high-PTU group displayed significantly lower mass compared to the low-PTU group. Other studies have similarly demonstrated that TD significantly impacts length and mass (Van Der Ven et al., 2006; Chen et al., 2010) therefore, these findings suggest that TD occurred as early as 7 dph.

Immunohistochemistry. There are multiple indices that are used to measure TD and one of the most sensitive ones is follicular thyroid content represented by T4 (McNabb et al., 2004). In recent years immunohistochemistry techniques have been used to assess the T4 content in thyroid follicles of zebrafish (Radula and Babin, 2009). In this study, immunohistochemistry was utilized to quantitatively assess the amount of T4 located within the thyroid follicles of control and PTU exposed fathead minnows by measuring integrated density. The results of this assay indicate a dose dependent response of diminished fluorescence in the fish exposed to PTU relative to the control group (Figure 3). The high-PTU group demonstrated significantly lower fluorescence compared to the control, and although the low-PTU group exhibited a 2-fold decrease compared to the control group, it was not statistically significant. Given the fact that PTU is known to lower T4 content in thyroid follicles of zebrafish (Schmidt and Braunbeck, 2011), due to PTU acting as a TPO inhibitor, it can be deduced that TH synthesis was successfully disrupted in PTU exposed fish.

Thyroid related gene expression. Significant alterations were seen in the expression of genes related to TH synthesis. There was a significant downregulation in *trβ* in the high-PTU group. Thyroid hormone receptor β is a nuclear receptor that binds T3 and acts as a transcription factor to activate TH target genes (Zoeller et al., 2007). Considering that T3 production is contingent on the presence of T4

and DI2 (Kuiper et al., 2005) and the fact IHC analysis showed reduced T4 concentrations, the downregulation seen in the high PTU group could be explained by a reduced amount of circulating T3

There was significant upregulation of *di2* in both the low- and high-PTU groups of fish. The purpose of DI2 is to convert T4 to T3 (Bianco and Kim, 2006); therefore, the upregulation seen could represent a compensatory mechanism intended to increase concentrations of T3, even in the face of decreased T4 production. Similar results have been previously reported among zebrafish exposed to the polybrominated diphenyl ether, DE-47, a known thyroid disruptor (Yu et al., 2010), as well as perchlorate (Zhao et al., 2013).

For *di3*, there was a significant dose-dependent downregulation in both PTU-exposed groups compared to the control group. The main function of DI3 is to inactivate T3 to 3,5-diiodo-L-thyronine (T2), an inactive product that is metabolized by continued deiodination, in order to rid the body of excess thyroid hormones and maintain thyroid hormone homeostasis (Bianco and Kim, 2006). The observed downregulation of *di3* could be due to lowered circulating T3 concentrations. The body would want to compensate for lowered T3 concentrations by limiting the amount of metabolization that is occurring to T3 in order to allow for the maximization of T3 reaching target tissues.

Dose-dependent alterations were seen in the relative expression of *ttr*; however, statistically significant differences in expression were only observed between fish in the high-PTU group and the control group. Transthyretin is a transport protein that allows for the travel of T4 and T3 throughout the blood (Carr and Patiño, 2011). One potential explanation behind the observed downregulation is that due to a decrease in overall TH concentration, less TTR is needed for transport. A secondary, and more likely reason for lowered TTR that could explain the downregulation is a compensatory mechanism that would increase the amount of free TH to enter cells (Campinho et al., 2012). Only free THs can enter cells, therefore, greater importance is placed in having a higher amount of circulating THs that can interact with target tissues.

Given the results of the three methods used to confirm thyroid disruption (i.e., growth analysis, immunohistochemical labeling of thyroxine, and thyroid hormone related gene expression), it can be concluded that early life stage thyroid disruption was successful in fathead minnows exposed to PTU and that it was displayed in a dose-dependent manner.

Early Life Stage Behavior Assays

Feeding Assay. Feeding represents a broad endpoint used to assess the overall wellbeing of organisms due to the complex physiological nature of prey recognition and capture (Portugues and Engert, 2009). Previous studies have demonstrated altered foraging behavior as a result of TD. Examples include mummichogs (*Fundulus heteroclitus*) experiencing TD from a polluted site demonstrated decreased foraging behavior compared to mummichogs from a non-polluted site (Smith and Weis, 1997), triclosan decreased foraging behavior in *D. rerio* (Wirt et al., 2018), and exposure to DDT diminished prey capture of *X. laevis* towards live prey (South et al., 2019). The ability to feed depends strongly on visuomotor behaviors which rely heavily on multisynaptic pathways that link visual perception to muscle action in the periphery (Gahtan et al., 2005). In many teleost species, vision is critical for recognizing and acquiring prey (Hairston et al., 1982), meaning proper formation of the eyes is crucial to survival in these species. In addition to proper eye development, general nervous system development which can affect locomotion is also necessary for successful feeding. Thyroid disruption has been shown to reduce neuronal migration, delay neuron proliferation, impair development of dendrites, delay myelination, and reduce axonal guidance (Reviewed by Prezioso et al., 2018), all of which are necessary components of central nervous system development. In a previous study, zebrafish that were exposed to tetrabromobisphenol A, a known thyroid disruptor, demonstrated neurodevelopmental toxicity via a downregulation of genes related to central nervous system development, and subsequently decreased average swimming speed and locomotor activity, indicating that improper nervous system development affects locomotor activity. Given the fact that thyroid

disruption has been shown to alter eye development and locomotion, which are key components of feeding, fish exposed to PTU in this assay might have altered feeding behavior.

In this assay, there were no significant alterations in the feeding behavior of fish exposed to PTU, regardless of concentration, compared to the control group. A potential explanation for this is that neuron formation associated with eye development was not significantly altered enough that it would lead to a difference in prey capturing ability. Neuronal development is comprised of many specific processes such as axonogensis, neuron differentiation, neuron projection extension, and dendrite morphogenesis (Reviewed by Prezioso et al., 2018). During neuronal development, axons will form synaptic connections with dendrites of other neurons, which allows for the exchange of information (Reviewed by Stiles and Jernigan, 2010). The observed lack of statistically significant differences in prey capture between the control and PTU exposed groups suggests that neuron formation was either not altered at all or not altered enough to induce changes in eye development and/or the nervous system signal transduction required for normal locomotion. A previous study did find that larval zebrafish exposed to PTU for 5 days post fertilization altered eye size and their visual capacities, as seen in a dose-dependent decrease in their optokinetic response (i.e., a compensatory ocular motor reflex; Baumann et al., 2016). This effect was noted at an exposure concentration of 100 mg PTU/L, but not at 50 mg PTU/L; thus, it is possible that the concentrations of PTU used in this study (35 and 70 mg/L) were not high enough to elicit any significant alterations to proper neuronal growth that could potentially affect eye development and locomotion.

C-start Assay. A well-studied innate predator escape response that is conserved across teleost species is known as C-start. This is a fast-start escape response where the fish will form a C-shape with its body and quickly swim in a direction away from a predator (Domenici and Blake, 1997; Painter et al., 2009). This fast-start reaction is controlled by the Mauthner cells located in the hindbrain of teleosts (Eaton et al., 2001). Mauthner cells are part of the reticulospinal system, and their purpose is to receive sensory (i.e., visual, auditory, and vibrational) input and relay that information through axons to the

spinal cord, where spinal motoneurons become activated to initiate the C-start response (Grantyn et al., 1992). Considering successful C-start relies on information being relayed by a series of neurons starting in the hindbrain and continuing down the spinal cord, any significant alteration of this pathway could result in a change to the C-start escape response. Previous studies have demonstrated that abnormal neurodevelopment leads to altered behavior, specifically predator escape response (Oliveri et al., 2015; Shan et al., 2015). Therefore, if alterations are seen in C-start behavior it could be indicative of abnormal neurodevelopment.

In the present study, there were no significant alterations in C-start responses between the control and PTU-exposed groups as measured by the number positive responses. There are multiple measurements that quantify the total C-start behavior such as, latency in reaction time, velocity, and escape angle (McGee et al., 2009), but there were no significant differences in these metrics between the control group and the PTU-exposed groups. This indicates that the concentrations of PTU used in the present study did not impact the ability of larval fish to react to the vibrational stimulus, nor to successfully complete the C-start maneuver.

C-start begins with taking in visual, auditory, and vibrational stimuli and transducing them into musculoskeletal activation through the Mauthner cells (Eaton and Hackett, 1984) meaning, that for the control and PTU exposed groups their ability to receive these cues and translate that information through the Mauthner cells to the spine was not significantly altered. There is also the possibility that even if the Mauthner cells were partially altered, there are compensatory mechanisms in place to achieve the same escape response. In a previous study, Mauthner cells were removed from larval zebrafish and the authors found that the fish were still able to respond to stimulus cues albeit more slowly (Liu and Fetcho, 1999). This indicates that fish can still react to external cues via other pathways, which have a longer processing time than the Mauthner cell pathway. Overall, the lack of significant alterations between control and PTU exposed groups in both the feeding and C-start assay indicates that

processes involved with neural development had not been impacted enough at the time to illicit any significant changes.

Reproductive Behavior Assay

Fathead minnow reproduction involves intense bouts of behaviors which are split into two main categories; the first being competition where males display aggressive behaviors to each other in to gain and defend a nesting site, and the second being courtship and cleaning behaviors where the male cleans the nest and display behaviors to the female to lure her into the nesting site (McMillan and Smith, 1974). In this study, a reproductive behavioral assay was used to quantify the number of behaviors performed between the pairings (i.e., control vs. low-PTU, control vs. high-PTU, and low-PTU vs. high-PTU) as well as the male that won for each trial.

For the win data, the only pairing for which significant differences were observed was the control vs high-PTU pairing. High-PTU males won in 18% of trials, while control males won 82% of the time. The low proportion of wins among high-PTU males can be explained by significant differences in the number of competition behaviors displayed by the high-PTU fish in this assay. For a fish to have won the trial, they needed to gain control of the nesting site, which can be accomplished by outcompeting other males through the display of aggressive competition behaviors. Three specific behaviors were quantified for competition (i.e., headbutting, chasing, and tail-whipping). The data shows that of those three behaviors only chasing was significantly reduced in the high-PTU group compared to the control, although there were declines in the number of behaviors displayed in headbutting and tail-whipping, but they were not significant.

One possible mechanistic explanation behind the reduction in competition behaviors is related to the upregulation of genes involved in the catecholaminergic system. Johnson (2019) demonstrated that PTU exposed fish showed significant upregulation of *comt*. The role of the *comt* gene is to produce catechol-O-methyltransferase which is responsible for breaking down dopamine (Männistö et al., 1992).

Dopamine has been shown to play a crucial role during neurodevelopment. A previous study demonstrated the presence of dopamine receptors, D1 and D2, in the brain during embryonic development indicating that dopamine plays a role in neurogenesis (Jung and Bennett, 1996). Using tyrosine-hydroxylase antibodies, it has been shown that dopaminergic tracts spread throughout the brain and spinal cord of zebrafish as early as 18 hpf and continue to spread throughout the embryonic life stage (McClean and Fetcho, 2004). In part, this spread of dopaminergic tracts come from progenitor cells that can differentiate into dopaminergic neurons (Noisa et al, 2015). In a study looking at the effect of dopamine on progenitor cells in mice at embryonic day 15, it was found that dopamine plays a role in in the proliferation and differentiation of progenitor cells in the striatum (Popolo et al., 2004), a mammalian brain region homologous to the subpallial structure of teleosts, which is associated with learning, motor control, and behavior (Tay et al., 2011; Reviewed by Vaz et al., 2019). Thus, a lack of dopamine during early development, as suggested by the upregulation of *comt* seen in the Johnson (2019) study, could alter the development of specific brain regions associated with these processes.

In addition to alterations seen in the expression of *comt*, Bruns (2017) showed that *bteb* expression had been significantly downregulated in PTU-exposed fish. This is unsurprising given the fact that *bteb* transcription relies on TH receptor activation (Denver et al., 1999). The primary role of BTEB is to influence neurite development; therefore, fish exposed to PTU might have had altered neurological function (Denver et al., 1999). When organs (e.g., brain and central nervous system) are developing in early life stage vertebrates it represents a sensitive period for exposure to thyroid disruptors (Reviewed by Batista and Hensch, 2019). It is during this period that thyroid disruption can exert organizational effects (i.e., structural changes that happen in early development that become permanent) that can alter brain circuitry, which can affect growth, reproduction, and behavior (Reviewed by Rosenfeld et al., 2017). In the present study there were no alterations in the early life stage behavioral tests between control and PTU-exposed fish; however, there were differences in the competition behaviors which

potentially indicates that thyroid disruption during the larval stage potentially elicited organizational effects that were not seen until the fish had reached maturity.

Along with the decrease in competition behaviors in the control vs. high-PTU group, there were also significant decreases in both courtship and nest care behaviors with a 73% and 75% decrease, respectively. These results provide a potential explanation for the 50% decrease in the fecundity of high-PTU exposed fish as seen by Bruns (2017). Fecundity is a measurement of both clutch size and spawning frequency, with clutch size being in part a metric of ovarian function (Spence and Smith, 2006) and spawning frequency being the result of male behavior.

One of the results of the Bruns (2017) study showed significant downregulation in the expression of *arom*, which is responsible for converting testosterone to estrogen (Nathan et al., 2001). Estrogen has been shown to play crucial roles during neural development in vertebrates. This is demonstrated because cytochrome P450 (*cyp19b)* which is the gene that encodes for aromatase found primarily in the brain of teleosts, is expressed in zebrafish <7 hpf, suggesting that estrogen is a necessary component of neural development (Sawyer et al., 2006). In fact, through immunohistochemistry, the expression of estrogen receptors have been associated with areas of the brain such as the hypothalamus, preoptic area (POA), and hippocampus which are areas of the brain that regulate reproductive behaviors (Shughrue et al., 1997).

Estrogen initiates two concurrent processes in the brain: 1) masculinization, a process which makes the brain capable of manufacturing male-specific behaviors and 2) defeminization, a process by which female-specific behaviors are stripped from the brain (Brand et al., 1991; Houtsmuller et al., 1994). Estrogen has been shown to play a role in axonal regeneration, which disrupts neuronal repair and plasticity, and cell proliferation of the pallium, thalamus, and preoptic area of zebrafish larvae (Nasri et al., 2021). One area of particular interest, considering it is heavily organized by estradiol, is the POA which is part of the hypothalamus (Reviewed by Lenz et al., 2012). In the electric fish (*Brachyhypopomus guaderio*), the POA becomes activated in male fish during reproduction (Pouso et al., 2019) and in male

rats, ablation of the POA lead to a reduction in the motivation to mate and the physical act of copulation (Christensen et al., 1977), indicating that the POA plays an important role male sexual behavior. If these brain regions were altered in the fish exposed to PTU, it could provide a possible explanation behind the reductions seen in the courtship behaviors.

The downregulation in the expression of *arom* in the Bruns (2017) study, likely leads to a decrease in circulating estrogen and subsequent reductions in estrogen receptor expression, as estrogen receptors are autoregulated meaning a decrease in circulating estrogen levels induces a decrease in estrogen receptor expression (Varriale and Tata, 1990). This is corroborated by Bruns (2017), in which there was a significant down regulation of estrogen receptor alpha in fathead minnows exposed to PTU. Given this and the known role of estrogen in the masculinization and defeminization of the brain, it is plausible that the lack of courtship behaviors observed in the high-PTU group stems from alterations in estrogen signaling.

Conclusion

In the present study, early life stage thyroid disruption in fathead minnows was confirmed through morphometric analysis, gene expression of thyroid hormone related genes, as well as quantification of follicular T4 content. It was hypothesized that early life stage thyroid disruption would alter reproductive behaviors in adult male fathead minnows. When looking at the analyses of reproductive behaviors, there was a clear and significant reduction in the display of reproductive behaviors by PTU-exposed males compared to the control males. The lack of reproductive behaviors displayed by PTU-exposed males links the findings of the Bruns (2017) and Johnson (2019) studies, and now there is now a mechanistic connection between early life stage thyroid disruption, altered neurodevelopment, reduction in reproductive behaviors, and decreased fecundity. However, there were no significant alterations in early life stage behavioral assays between the PTU-exposed groups and the control group. This could represent the onset of organizational effects produced at the larval stage or a

latency period in the observable affects that are the result of abnormal neurodevelopment. Therefore, future studies would be necessary to determine if behaviors of males are altered throughout the life span in response to early life stage thyroid disruption.

References

Anne McNabb FM, Larsen CT, Pooler PS. 2004. AMMONIUM PERCHLORATE EFFECTS ON THYROID FUNCTION AND GROWTH IN BOBWHITE QUAIL CHICKS. Environ Toxicol Chem. 23(4):997.

Ayson FG, Lam TJ. 1993. Thyroxine injection of female rabbitfish (Siganus guttatus) broodstock: changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effect on larval growth and survival. Aquaculture. 109(1):83–93.

Batista G, Hensch TK. 2019. Critical Period Regulation by Thyroid Hormones: Potential Mechanisms and Sex-Specific Aspects. Front Mol Neurosci. 12:77.

Baumann L, Ros A, Rehberger K, Neuhauss SCF, Segner H. 2016. Thyroid disruption in zebrafish (Danio rerio) larvae: Different molecular response patterns lead to impaired eye development and visual functions. Aquatic Toxicology. 172:44–55.

Bernal J. 2005. Thyroid Hormones and Brain Development. In: Vitamins & Hormones. Vol. 71. Elsevier. p. 95–122.

Berry DL, Rose CS, Remo BF, Brown DD. 1998. The Expression Pattern of Thyroid Hormone Response Genes in Remodeling Tadpole Tissues Defines Distinct Growth and Resorption Gene Expression Programs. Developmental Biology. 203(1):24–35.

Bianco AC, Kim BW. 2006. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest. 116(10):2571–2579.

Blanton ML, Specker JL. 2007. The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. Crit Rev Toxicol. 37(1–2):97–115.

Boas M, Feldt-Rasmussen U, Main KM. 2012. Thyroid effects of endocrine disrupting chemicals. Molecular and Cellular Endocrinology. 355(2):240–248.

Brand T, Kroonen J, Mos J, Slob AK. 1991. Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations. Hormones and Behavior. 25(3):323–341.

Brar NK, Waggoner C, Reyes JA, Fairey R, Kelley KM. 2010. Evidence for thyroid endocrine disruption in wild fish in San Francisco Bay, California, USA. Relationships to contaminant exposures. Aquatic Toxicology. 96(3):203–215.

Brown DD. 1997. The role of thyroid hormone in zebrafish and axolotl development. Proc Natl Acad Sci U S A. 94(24):13011–13016.

Bruns PC. 2017. The reproductive effects of thyroid disruption in the fathead minnow (*Pimephales promelas*). (Unpublished master's thesis). Texas Christian University, Fort Worth, TX.

Buchholz DR, Hayes TB. 2005. Variation in thyroid hormone action and tissue content underlies species differences in the timing of metamorphosis in desert frogs. Evolution & Development. 7(5):458–467.

Calsolaro V, Pasqualetti G, Niccolai F, Caraccio N, Monzani F. 2017. Thyroid Disrupting Chemicals. Int J Mol Sci. 18(12).

Campinho MA, Morgado I, Pinto PIS, Silva N, Power DM. 2012. The goitrogenic efficiency of thioamides in a marine teleost, sea bream (Sparus auratus). General and Comparative Endocrinology. 179(3):369–375.

Carr JA, Patiño R. 2011. The hypothalamus–pituitary–thyroid axis in teleosts and amphibians: Endocrine disruption and its consequences to natural populations. General and Comparative Endocrinology. 170(2):299–312.

Carvalho DP, Dupuy C. 2017. Thyroid hormone biosynthesis and release. Mol Cell Endocrinol. 458:6–15.

Cavallin JE, Ankley GT, Blackwell BR, Blanksma CA, Fay KA, Jensen KM, Kahl MD, Knapen D, Kosian PA, Poole ST, et al. 2017. Impaired swim bladder inflation in early life stage fathead minnows exposed to a deiodinase inhibitor, iopanoic acid. Environmental Toxicology and Chemistry. 36(11):2942–2952.

Chen T-H, Cheng Y-M, Cheng J-O, Chou C-T, Hsiao Y-C, Ko F-C. 2010. Growth and transcriptional effect of dietary 2,2’,4,4’-tetrabromodiphenyl ether (PBDE-47) exposure in developing zebrafish (Danio rerio). Ecotoxicology and Environmental Safety. 73(3):377–383.

Christensen LW, Nance DM, Gorski RA. 1977. Effects of hypothalamic and preoptic lesions on reproductive behavior in male rats. Brain Res Bull. 2(2):137–141

Citterio CE, Targovnik HM, Arvan P. 2019. The role of thyroglobulin in thyroid hormonogenesis. Nat Rev Endocrinol. 15(6):323–338.

Cole KS, Smith RJF. 1987. Male courting behaviour in the fathead minnow, Pimephales promelas. Environ Biol Fish. 18(3):235–239.

Couderc M, Marchand J, Zalouk-Vergnoux A, Kamari A, Moreau B, Blanchet-Letrouvé I, Le Bizec B, Mouneyrac C, Poirier L. 2016. Thyroid endocrine status of wild European eels (Anguilla anguilla) in the Loire (France). Relationships with organic contaminant body burdens. Science of The Total Environment. 550:391–405.

Crane HM, Pickford DB, Hutchinson TH, Brown JA. 2004. Developmental changes of thyroid hormones in the fathead minnow, Pimephales promelas. Gen Comp Endocrinol. 139(1):55–60.

Crane HM, Pickford DB, Hutchinson TH, Brown JA. 2005. Effects of ammonium perchlorate on thyroid function in developing fathead minnows, Pimephales promelas. Environ Health Perspect. 113(4):396–401.

Crane HM, Pickford DB, Hutchinson TH, Brown JA. 2006. The effects of methimazole on development of the fathead minnow, Pimephales promelas, from embryo to adult. Toxicol Sci. 93(2):278–285.

Denver RJ, Pavgi S, Shi Y-B. 1997. Thyroid Hormone-dependent Gene Expression Program for *Xenopus* Neural Development. J Biol Chem. 272(13):8179–8188.

Domenici null, Blake null. 1997. The kinematics and performance of fish fast-start swimming. J Exp Biol. 200(Pt 8):1165–1178.

Eaton RC, Hackett JT. 1984. The Role of the Mauthner Cell in Fast-Starts Involving Escape in Teleost Fishes. In: Eaton RC, editor. Neural Mechanisms of Startle Behavior. Boston, MA: Springer US. p. 213–266.

Eaton RC, Lee RKK, Foreman MB. 2001. The Mauthner cell and other identified neurons of the brainstem escape network of fish. Progress in Neurobiology. 63(4):467–485.

ENGLER H, TAUROG A, DORRIS ML. 1982. Preferential Inhibition of Thyroxine and 3,5,3'-Triiodothyronine Formation by Propylthiouracil and Methylmercaptoimidazole in Thyroid Peroxidase-Catalyzed Iodination of Thyroglobulin*. Endocrinology. 110(1):190–197.

Falcone M, Miyamoto T, Fierro-Renoy F, Macchia E, DeGroot LJ. 1994. Evaluation of the ontogeny of thyroid hormone receptor isotypes in rat brain and liver using an immunohistochemical technique. European Journal of Endocrinology. 130(1):97–106.

Fini JB, Mével SL, Palmier K, Darras VM, Punzon I, Richardson SJ, Clerget-Froidevaux MS, Demeneix BA. 2012. Thyroid Hormone Signaling in the Xenopus laevis Embryo Is Functional and Susceptible to Endocrine Disruption. Endocrinology. 153(10):5068–5081.

Gahtan E, Tanger P, Baier H. 2005. Visual Prey Capture in Larval Zebrafish Is Controlled by Identified Reticulospinal Neurons Downstream of the Tectum. J Neurosci. 25(40):9294–9303.

Gale WF, Buynak GL. 1982. Fecundity and Spawning Frequency of the Fathead Minnow—A Fractional Spawner. Transactions of the American Fisheries Society. 111(1):35–40.

Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A, Bianco AC. 2008. Cellular and Molecular Basis of Deiodinase-Regulated Thyroid Hormone Signaling1. Endocrine Reviews. 29(7):898–938.

Goleman WL, Carr JA, Anderson TA. 2002. Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratios in developing *Xenopus laevis*. Environ Toxicol Chem. 21(3):590–597.

Goleman WL, Urquidi LJ, Anderson TA, Smith EE, Kendall RJ, Carr JA. 2002. Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in Xenopus laevis. Environmental Toxicology and Chemistry. 21(2):424–430.

Grantyn A, Hardy O, Oliver E, Gourdon A. 1992. Relationships beteen Task-Related Discharge Patterns and Axonal Morphology of Brainstem Projection Neurons Involved in Orienting Eye and Head Movements.

Hairston NG, Li KT, Easter SS. 1982. Fish vision and the detection of planktonic prey. Science. 218(4578):1240–1242.

Hassan I, El-Masri H, Kosian PA, Ford J, Degitz SJ, Gilbert ME. 2017. Neurodevelopment and Thyroid Hormone Synthesis Inhibition in the Rat: Quantitative Understanding Within the Adverse Outcome Pathway Framework. Toxicol Sci. 160(1):57–73.

Houtsmuller EJ, Brand T, de Jonge FH, Joosten RNJMA, van de Poll NE, Slob AK. 1994. SDN-POA volume, sexual behavior, and partner preference of male rats affected by perinatal treatment with ATD. Physiology & Behavior. 56(3):535–541.

Hulbert AJ. 2000. Thyroid hormones and their effects: a new perspective. Biol Rev Camb Philos Soc. 75(4):519–631.

Im J, Löffler FE. 2016. Fate of Bisphenol A in Terrestrial and Aquatic Environments. Environ Sci Technol. 50(16):8403–8416.

Johnson A. 2019. Connecting developmental thyroid disruption to impaired reproductive success in fathead minnows (*Pimephales promelas*). (Unpublished master's thesis). Texas Christian University, Fort Worth, TX.

Jung AB, Bennett JP. 1996. Development of striatal dopaminergic function. I. Pre- and postnatal development of mRNAs and binding sites for striatal D1 (D1a) and D2 (D2a) receptors. Developmental Brain Research. 94(2):109–120.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999−2000: A National Reconnaissance. Environ Sci Technol. 36(6):1202–1211.

Kosaka K, Asami M, Matsuoka Y, Kamoshita M, Kunikane S. 2007. Occurrence of perchlorate in drinking water sources of metropolitan area in Japan. Water Res. 41(15):3474–3482.

Krzykwa JC, Sellin Jeffries MK. 2020. Comparison of behavioral assays for assessing toxicant-induced alterations in neurological function in larval fathead minnows. Chemosphere. 257:126825.

Kuiper GGJM, Kester MHA, Peeters RP, Visser TJ. 2005. Biochemical Mechanisms of Thyroid Hormone Deiodination. Thyroid. 15(8):787–798.

Leet JK, Lesteberg KE, Schoenfuss HL, Olmstead AW, Amberg JJ, Ankley GT, Sepúlveda MS. 2013. Sex-Specific Gonadal and Gene Expression Changes throughout Development in Fathead Minnow. Sex Dev. 7(6):303–307.

Lema SC, Dickey JT, Schultz IR, Swanson P. 2008. Dietary Exposure to 2,2′,4,4′-Tetrabromodiphenyl Ether (PBDE-47) Alters Thyroid Status and Thyroid Hormone–Regulated Gene Transcription in the Pituitary and Brain. Environ Health Perspect. 116(12):1694–1699.

Leung AM, Pearce EN, Braverman LE. 2014. Environmental Perchlorate Exposure: Potential Adverse Thyroid Effects. Curr Opin Endocrinol Diabetes Obes. 21(5):372–376.

Liu KS, Fetcho JR. 1999. Laser Ablations Reveal Functional Relationships of Segmental Hindbrain Neurons in Zebrafish. Neuron. 23(2):325–335.

Liu Y-W, Chan W-K. 2002. Thyroid hormones are important for embryonic to larval transitory phase in zebrafish. Differentiation. 70(1):36–45.

Lovato AK, Creton R, Colwill RM. 2016. Effects of embryonic exposure to polychlorinated biphenyls (PCBs) on larval zebrafish behavior. Neurotoxicology and Teratology. 53:1–10.

Macaulay LJ, Chernick M, Chen A, Hinton DE, Bailey JM, Kullman SW, Levin ED, Stapleton HM. 2017. Exposure to a PBDE/OH-BDE mixture alters juvenile zebrafish (Danio rerio) development. Environ Toxicol Chem. 36(1):36–48.

Männistö PT, Ulmanen I, Lundström K, Taskinen J, Tenhunen J, Tilgmann C, Kaakkola S. 1992. Characteristics of catechol O-methyltransferase (COMT) and properties of selective COMT inhibitors. p. 291–350.

McClain V, Stapleton HM, Tilton F, Gallagher EP. 2012. BDE 49 and developmental toxicity in zebrafish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 155(2):253–258.

McGee MR, Julius ML, Vajda AM, Norris DO, Barber LB, Schoenfuss HL. 2009. Predator avoidance performance of larval fathead minnows (Pimephales promelas) following short-term exposure to estrogen mixtures. Aquatic Toxicology. 91(4):355–361.

McHenry M j., Feitl K e., Strother J a., Van Trump W j. 2009. Larval zebrafish rapidly sense the water flow of a predator's strike. Biology Letters. 5(4):477–479.

McLean DL, Fetcho JR. 2004. Ontogeny and innervation patterns of dopaminergic, noradrenergic, and serotonergic neurons in larval zebrafish. J Comp Neurol. 480(1):38–56.

McMillan VE, Smith RJF. 1974. Agonistic and Reproductive Behaviour of the Fathead Minnow (Pimephales promelas Rafinesque). Zeitschrift für Tierpsychologie. 34(1):25–58.

Mcnabb FMA, Larsen CT, Pooler PS. 2004. Ammonium perchlorate effects on thyroid function and growth in bobwhite quail chicks. Environmental Toxicology and Chemistry. 23(4):997–1003.

Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K. 2002. Thyroid Hormone Action Is Disrupted by Bisphenol A as an Antagonist. The Journal of Clinical Endocrinology & Metabolism. 87(11):5185–5190.

Morreale de Escobar G, Obregon MJ, Escobar del Rey F. 2004. Role of thyroid hormone during early brain development. Eur J Endocrinol. 151 Suppl 3:U25-37.

Motzer WE. 2001. Perchlorate: Problems, Detection, and Solutions. Environmental Forensics. 2(4):301–311.

Mukhi S, Patino R. 2006. Effects of Prolonged Exposure to Perchlorate on Thyroid and Reproductive Function in Zebrafish. Toxicological Sciences. 96(2):246–254.

Nasri A, Mezni A, Lafon P-A, Wahbi A, Cubedo N, Clair P, Harrath AH, Beyrem H, Rossel M, Perrier V. 2021. Ethinylestradiol (EE2) residues from birth control pills impair nervous system development and swimming behavior of zebrafish larvae. Science of The Total Environment. 770:145272.

Nathan L, Shi W, Dinh H, Mukherjee TK, Wang X, Lusis AJ, Chaudhuri G. 2001. Testosterone inhibits early atherogenesis by conversion to estradiol: Critical role of aromatase. Proceedings of the National Academy of Sciences. 98(6):3589–3593.

Noisa P, Raivio T, Cui W. 2015. Neural Progenitor Cells Derived from Human Embryonic Stem Cells as an Origin of Dopaminergic Neurons. Stem Cells International. 2015:e647437.

Obregon MJ, Mallol J, Pastor R, Escobar GMD, Rey FED. 1984. l -THYROXINE AND 3,5,3’-TRII000- l -THYRONINE IN RAT EMBRYOS BEFORE ONSET OF FETAL THYROID FUNCTION. Endocrinology. 114(1):305–307.

Oliveri AN, Bailey JM, Levin ED. 2015. Developmental exposure to organophosphate flame retardants causes behavioral effects in larval and adult zebrafish. Neurotoxicol Teratol. 52(0 0):220–227.

Painter MM, Buerkley MA, Julius ML, Vajda AM, Norris DO, Barber LB, Furlong ET, Schultz MM, Schoenfuss HL. 2009. ANTIDEPRESSANTS AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS AFFECT PREDATOR AVOIDANCE BEHAVIOR OF LARVAL FATHEAD MINNOWS (PIMEPHALES PROMELAS). Environ Toxicol Chem. 28(12):2677.

Park J-W, Rinchard J, Liu F, Anderson TA, Kendall RJ, Theodorakis CW. 2006. The thyroid endocrine disruptor perchlorate affects reproduction, growth, and survival of mosquitofish. Ecotoxicology and Environmental Safety. 63(3):343–352.

Popolo M, McCarthy DM, Bhide PG. 2004. Influence of Dopamine on Precursor Cell Proliferation and Differentiation in the Embryonic Mouse Telencephalon. Dev Neurosci. 26(2–4):229–244.

Portugues R, Engert F. 2009. The neural basis of visual behaviors in the larval zebrafish. Current Opinion in Neurobiology. 19(6):644–647.

Pouso P, Cabana Á, Goodson JL, Silva A. 2019. Preoptic Area Activation and Vasotocin Involvement in the Reproductive Behavior of a Weakly Pulse-Type Electric Fish, Brachyhypopomus gauderio. Front Integr Neurosci.

Power DM, Llewellyn L, Faustino M, Nowell MA, Björnsson BT, Einarsdottir IE, Canario AV, Sweeney GE. 2001. Thyroid hormones in growth and development of fish. Comp Biochem Physiol C Toxicol Pharmacol. 130(4):447–459.

Préau L, Fini JB, Morvan-Dubois G, Demeneix B. 2015. Thyroid hormone signaling during early neurogenesis and its significance as a vulnerable window for endocrine disruption. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms. 1849(2):112–121.

Prezioso G, Giannini C, Chiarelli F. 2018. Effect of Thyroid Hormones on Neurons and Neurodevelopment. Horm Res Paediatr. 90(2):73–81.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA. 1999. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry. 45(9):1085–1098.

Raldúa D, Babin PJ. 2009. Simple, Rapid Zebrafish Larva Bioassay for Assessing the Potential of Chemical Pollutants and Drugs to Disrupt Thyroid Gland Function. Environ Sci Technol. 43(17):6844–6850.

Rosenfeld CS, Denslow ND, Orlando EF, Gutierrez-Villagomez JM, Trudeau VL. 2017. Neuroendocrine disruption of organizational and activational hormone programming in poikilothermic vertebrates. Journal of Toxicology and Environmental Health, Part B. 20(5):276–304.

Sawyer SJ, Gerstner KA, Callard GV. 2006. Real-time PCR analysis of cytochrome P450 aromatase expression in zebrafish: Gene specific tissue distribution, sex differences, developmental programming, and estrogen regulation. General and Comparative Endocrinology. 147(2):108–117.

Seemann M. 2018. Investigating the Causes of Reproductive Impairment Following Thyroid Disruption in the Fathead Minnow (Pimephales promelas). (Unpublished undergraduate honor's thesis). Texas Christian University, Fort Worth, TX.

Schmidt F, Braunbeck T. 2011. Alterations along the Hypothalamic-Pituitary-Thyroid Axis of the Zebrafish (Danio rerio) after Exposure to Propylthiouracil. J Thyroid Res. 2011:376243.

Schreiber AM. 2006. Asymmetric craniofacial remodeling and lateralized behavior in larval flatfish. Journal of Experimental Biology. 209(4):610–621.

Sellin Jeffries MK, Kiss AJ, Smith AW, Oris JT. 2014. A comparison of commercially-available automated and manual extraction kits for the isolation of total RNA from small tissue samples. BMC Biotechnol. 14(1):94.

Shan SD, Boutin S, Ferdous J, Ali DW. 2015. Ethanol exposure during gastrulation alters neuronal morphology and behavior in zebrafish. Neurotoxicology and Teratology. 48:18–27.

Sharma P, Patiño R. 2013. Regulation of gonadal sex ratios and pubertal development by the thyroid endocrine system in zebrafish (Danio rerio). Gen Comp Endocrinol. 184:111–119.

Shi Y, Zhang P, Wang Y, Shi J, Cai Y, Mou S, Jiang G. 2007. Perchlorate in sewage sludge, rice, bottled water and milk collected from different areas in China. Environment international. 33:955–62.

Shughrue PJ, Lane MV, Merchenthaler I. 1997. Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. Journal of Comparative Neurology. 388(4):507–525.

Smith GM, Weis JS. 1997. Predator-prey relationships in mummichogs (Fundulus heteroclitus (L.)): Effects of living in a polluted environment. Journal of Experimental Marine Biology and Ecology. 209(1):75–87.

South J, Botha TL, Wolmarans NJ, Wepener V, Weyl OLF. 2019. Assessing predator-prey interactions in a chemically altered aquatic environment: the effects of DDT on Xenopus laevis and Culex sp. larvae interactions and behaviour. Ecotoxicology. 28(7):771–780.

Spence R, Smith C. 2006. Mating preference of female zebrafish, Danio rerio, in relation to male dominance. Behavioral Ecology. 17(5):779–783.

Steinmaus CM. 2016. Perchlorate in Water Supplies: Sources, Exposures, and Health Effects. Curr Environ Health Rep. 3(2):136–143.

Stiles J, Jernigan TL. 2010. The Basics of Brain Development. Neuropsychol Rev. 20(4):327–348.

Tay TL, Ronneberger O, Ryu S, Nitschke R, Driever W. 2011. Comprehensive catecholaminergic projectome analysis reveals single-neuron integration of zebrafish ascending and descending dopaminergic systems. Nature Communications. 2(1):171.

Trippel EA. 2003. Estimation of Male Reproductive Success of Marine Fishes. Journal of Northwest Atlantic Fishery Science. 33.

Varriale B, Tata JR. 1990. Autoinduction of estrogen receptor is associated with FOSP-1 mRNA induction by estrogen in primary cultures of Xenopus oviduct cells. Molecular and Cellular Endocrinology. 71(3):R25–R31.

Vaz R, Hofmeister W, Lindstrand A. 2019. Zebrafish Models of Neurodevelopmental Disorders: Limitations and Benefits of Current Tools and Techniques. International Journal of Molecular Sciences. 20(6):1296.

van der Ven LTM, van den Brandhof E-J, Vos JH, Power DM, Wester PW. 2006. Effects of the Antithyroid Agent Propylthiouracil in a Partial Life Cycle Assay with Zebrafish. Environ Sci Technol. 40(1):74–81.

Weis JS, Smith G, Zhou T, Santiago-Bass C, Weis P. 2001. Effects of Contaminants on Behavior: Biochemical Mechanisms and Ecological Consequences: Killifish from a contaminated site are slow to capture prey and escape predators; altered neurotransmitters and thyroid may be responsible for this behavior, which may produce population changes in the fish and their major prey, the grass shrimp. BioScience. 51(3):209–217.

Wirt H, Botka R, Perez KE, King-Heiden T. 2018. Embryonic exposure to environmentally relevant concentrations of triclosan impairs foraging efficiency in zebrafish larvae. Environmental Toxicology and Chemistry. 37(12):3124–3133.

Yu L, Deng J, Shi X, Liu C, Yu K, Zhou B. 2010. Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic–pituitary–thyroid axis of zebrafish larvae. Aquatic Toxicology. 97(3):226–233.

Zhang C, Liu X, Chen D. 2010a. Role of brominated diphenly ether-209 in the differentiation of neural stem cells in vitro. Int j dev neurosci. 28(6):497–502.

Zhang C, Liu X, Chen D. 2010b. Role of brominated diphenly ether-209 in the differentiation of neural stem cells in vitro. International Journal of Developmental Neuroscience. 28(6):497–502.

Zhao X, Peng S, Xiang Y, Yang Y, Li J, Shan Z, Teng W. 2017. Correlation between Prenatal Exposure to Polybrominated Diphenyl Ethers (PBDEs) and Infant Birth Outcomes: A Meta-Analysis and an Experimental Study. Int J Environ Res Public Health. 14(3).

Zhao X, Wang S, Li D, You H, Ren X. 2013. Effects of perchlorate on BDE-47-induced alteration thyroid hormone and gene expression of in the hypothalamus–pituitary–thyroid axis in zebrafish larvae. Environmental Toxicology and Pharmacology. 36(3):1176–1185.

Zhou T, John-Alder HB, Weis JS, Weis P. 2000. Endocrine disruption: thyroid dysfunction in mummichogs (Fundulus heteroclitus) from a polluted habitat. Marine Environmental Research. 50(1–5):393–397.

Zhu B, Zhao G, Yang L, Zhou B. 2018. Tetrabromobisphenol A caused neurodevelopmental toxicity via disrupting thyroid hormones in zebrafish larvae. Chemosphere. 197:353–361.

Zoeller RT, Tan SW, Tyl RW. 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. Crit Rev Toxicol. 37(1–2):11–53.

VITA

Personal
Austin Reed Bryant, born September 6, 1992 in Baltimore, MD to Steven and Cathie Bryant
Graduated from C. Milton Wright High School in Bel Air, MD in May 2010

Education
M.S. Biology, Expected Graduation May 2021
B.S. Biology, Salisbury University, Graduated May 2014

Awards and Funding
Science and Engineering Research Center (SERC) Grant. Investigating the connections between early life stage thyroid disruption and long-term behavioral effects. $1,006. 2020
Adkins Fellowship. Investigating the connections between early life stage thyroid disruption and long-term behavioral effects. $3,600. 2020

Teaching Experience
Contemporary Issues in Biology, Laboratory. A study of biology spanning contemporary issues in the biological sciences from the cellular and molecular to the ecosystem and biosphere levels of biological organization. 2019-2020
Introductory Biology 2, Laboratory. A laboratory introduction to the diversity, evolution, anatomy, and physiology of eukaryotic organism (protists, fungi, plants, and animals.) 2020-2021

ABSTRACT

THE EFFECTS OF EARLY LIFE STAGE THYROID DISRUPTION ON REPRODUCTIVE BEHAVIORS IN FATHEAD MINNOWS (PIMEPHALES PROMELAS)

By Austin Reed Bryant, M.S., 2019
Department of Biology
Texas Christian University

Thesis Advisors: Drs. Marlo Sellin Jeffries, Matt Hale, and Gary Boehm

Changes in thyroid hormones levels have been associated with alterations in somatic development and growth. However, recent studies have shown that alterations in thyroid hormone levels during early life stage development can lead to long-term changes in reproduction. Specifically, fathead minnows that have been exposed to propylthiouracil (PTU) experienced a 50% reduction in fecundity. The purpose of this study was to determine if early life stage thyroid disruption led to an alteration of reproductive behaviors in male fathead minnows. To accomplish this, larval fathead minnows were exposed to PTU and reproductive behaviors were quantified. Results showed that PTU-exposed fish demonstrated significantly fewer reproductive behaviors than those in the control group. This data provides an explanation for the previously observed 50% decrease in fecundity in the fathead minnows exposed to PTU and provides further evidence that early life stage thyroid disruption can interfere with the display of key and ecologically-relevant behaviors later in life.