DEVELOPMENTAL TOXICITY OF SODIUM IODIDE USING THE ZEBRAFISH MODEL

by

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For Yeshua and His teachings

For the laboratory as a place of worship

To the knowledge we have gained in discovering our world

To the knowledge we have yet to obtain

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NaI	Sodium Iodide
Hpf	Hours Post Fertilization
mM	Millimolar
HPT axis	Hypothalamic-Pituitary-Thyroid Axis
IDD	Iodine Deficiency Disorder
PA	Postero-Anterior
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ADHD	Attention-deficit/hyperactivity disorder
NIS	Sodium Iodine Symporter

ABSTRACT

Iodine is considered an essential nutrient as lack can cause severe metabolic and neurological issues in adults, with the added consequence of permanent developmental damage in children and infants. However, excessive iodine intake can result in similar symptoms, with a wide variance in adverse health outcomes. The safe range of iodine intake may be relatively low, with some studies suggesting the possibility of a high frequency of subclinical cases of iodine poisoning going unnoticed or misdiagnosed.

In this study, the zebrafish model was tested as an integrative whole animal model to demonstrate behavioral, morphological, and genetic responses to overt and subclinical iodine poisoning in developing humans. Zebrafish embryos were treated with sodium iodide (NaI) immediately after fertilization. Survivability was monitored every 24 hours until 120 hours post fertilization (hpf). Concentrations with no statistical significance on survival, plus the smallest dose of significant lethality were then examined using behavioral analysis at 120 hpf to compare both overt and subclinical outcomes. Morphology measurements of body length, head length, head width, brain length, swim bladder volume, jaw length, and ventral dissension were also recorded at 120 hpf. Gene expression of *slc5a5*, *tpo*, and *tshba* at 72 hpf was also measured using quantitative PCR (qPCR).

A significant decrease in survival rates were observed at 24 hpf for 25, 37.5, and 50 mM NaI treatments ($p<0.0001$). Morphological measurements taken at 120 hpf showed a significant increase in body length, head length, head width, jaw length, and swim bladder volume in the 10 mM NaI treatment group ($p<0.0001$) and a significant decrease in body length, head length, jaw length, and swim bladder volume in the 25 mM treatment group ($p<0.0001$). A ventral distension also developed near the location of the thyroid gland exclusively in the 25 mM group.

Behavioral analysis showed significant increases in movement for both the 10 mM and 25 mM treatment groups during dark phases ($p<0.0001$). The 25 mM treatment group had an increase in movement during dark phases for standard well environments ($p<0.0001$), but this did not hold true for larger well environments, instead trending towards a non-significant decrease ($p>0.05$). The 10 mM group had a significant decrease during the first light phase in standard wells ($p=0.002$), with a significant increase in the second light phase for large wells ($p=0.005$). There were no

significant changes in the expression of selected genes associated with the thyroid pathway (*slc5a5*, *tpo*, or *tshba*) across all treatment groups ($p>0.05$).

Overall, the results suggest zebrafish larvae exhibit both overt and subclinical symptoms of excess iodine intake. Future studies are needed to determine internalization, biodistribution, clearance, and further characterization of adverse outcomes along the thyroid pathway for additional exploration into subclinical thyrotoxicosis due to excess iodine intake. Researchers should express caution with time points, as the Wolff-Chaikoff effect may influence exposure windows in zebrafish.

CHAPTER 1: INTRODUCTION

The hypothalamic-pituitary-thyroid (HPT) axis acts as a regulatory gatekeeper for the regulation of development, metabolism, body temperature, digestion, breathing, and cholesterol levels (Squire, 2009). The HPT axis of the endocrine system plays an essential role in development and homeostasis, and is thus essential for humans to survive and thrive. Iodine is considered an essential nutrient because of the foundational role it serves in the HPT axis.

Iodine, or more specifically its ionized form, iodide (I^-), serves as the foundational building block in the synthesis of the two thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) within the thyroid. The ultimate role of these hormones is to maintain homeostasis via feedback regulation and promote proper developmental growth by controlling the rate of cell differentiation and gene expression (Hetzel *et al.*, 1988). Thus, imbalances in iodine intake can subsequently cause thyroid hormone imbalance, resulting in short term homeostatic issues, or long term/permanent developmental effects (Braverman and Utiger, 2005).

Iodine deficiency often occurs due to a lack of regional dietary availability, and is thus often viewed and treated as a public health issue. Adults suffer significant effects from iodine deficiency, often manifesting as hypothyroidism: an underproduction of thyroid hormones. Hypothyroidism results in multiple symptoms, such as goiter, impaired mental function, fatigue, intolerance to cold, depression, and muscle aches and weakness. Due to the critical role iodine plays in the HPT axis, detrimental effects increase as age lowers. Adolescents and children additionally suffer from retarded physical development, and neonates suffer from neurocognitive impairment. Additional consequences occur *in utero*, with miscarriage, stillbirths, congenital anomalies, and Iodine Deficiency Disorder (IDD) also known as cretinism. The public health initiatives combating iodine deficiency are well justified for these reasons.

Treating this as a public health issue with "one size fits all" solutions is problematic, as the recommended daily intake of iodine for pregnant women exceeds the tolerable upper intake level for children under seven years old (Eastman and Zimmermann, 2000). Programs that introduce iodine through salt iodization can present a high risk scenario for children, as many children often exceed their recommended amount of salt intake by wide margins (Kallio *et al.*, 1998), resulting in a matched increase of iodine intake as well. In addition, it has been shown that individuals who have suffered from hypothyroidism for an extended period have a lower tolerable limit to iodine,

and become a vulnerable sub-demographic to iodine-induced disease. These nuances compound with research showing that the safe range of iodine intake is narrower than previously believed (Knudsen *et al.*, 2000).

The intuitive manifestation of excessive iodine intake is hyperthyroidism, which is the result of excessive thyroid activity and has both opposing and similar symptoms to hypothyroidism. Symptoms include intolerance to heat, weight loss, anxiety, exophthalmos, but share some symptoms, such as goiter. However, excessive iodine intake has more complex and nuanced manifestations. Due to mechanisms that are poorly understood, not all individuals will experience the same iodine induced symptoms (Pramyothin *et al.*, 2011). In some cases, excess iodine intake can appear completely asymptomatic (occult), while others can develop hypothyroidism, hyperthyroidism, or Hashimoto's disease, a permanent autoimmune disorder which causes the affected to alternate between hyperthyroidism and hypothyroidism (Leung and Braverman, 2014; Braverman and Utiger, 2005).

Sources of extreme iodine exposures can come from many sources. Dietary sources, such as foods with seaweed can result in harmfully excessive iodine intake with high variability based on culture and personal preference (Konno *et al.*, 1994). The significance of this will increase in the United States as sushi becomes more popular. This is also a factor for developmental concerns *in utero* as well, because kappa and California rolls are generally not considered to be a hazard for pregnancies due to the lack of raw fish. Water intake is also a concern, as especially deep wells have been found to have high iodine levels, resulting in endemic iodine-induced symptoms (Andersen *et al.*, 2008). In addition, high iodine doses can come from medical treatments and medications. Vitamins, which have little regulation in the United States, can have dramatically high and variable doses, while iodine medications and medical devices, such as Amiodarone or iodine based CT contrasts are doses well over one hundred times the daily upper limit (Leung and Braverman, 2014).

Overt cases of iodine-induced hypothyroidism and hyperthyroidism demonstrate obvious effects like goiter and exophthalmos and as a result, are often easily identified and remedied (Andersen *et al.*, 2008; Konno *et al.*, 1994). However, that is not the full spectrum of iodine-induced pathology. On the milder end of the spectrum are subclinical cases, which are identifiable through medical methods when screened, but are often not discovered because the symptoms are often limited to behavior (Braverman and Utiger, 2005). Symptoms of long-term excess iodine

exposures can mimic other behavioral diseases, such as Attention Deficit Hyperactivity Disorder (ADHD), which has been shown to have a high correlation with thyroid dysfunction (Chen *et al.*, 2018). In addition, the DSM-5 does not require a biological marker to confirm diagnosis as there is similarity in the language used to describe ADHD and hyperthyroidism (Table 1). As a result, it is easy for harmful iodine intake to go unreported or misdiagnosed, contributing to the prevalence of ADHD.

Table 1: Language Describing Thyrotoxicosis and ADHD in Reference Texts

Thyrotoxicosis (Braverman and Utiger, 2005)	**ADHD** (American Psychiatric Association, 2013)
• "Thyrotoxicosis patients complained of anxiety, dysphoria, emotional lability, insomnia, and intellectual dysfunction. Their ability to concentrate is impaired in particular"	• "Inattention manifests behaviorally…difficulty sustaining focus"
• "Patients are restless and tremulous. Patients appear irritable, jittery, and paranoid"	• "Excessive fidgeting, taping, talkativeness"
• "Overt and subclinical thyrotoxicosis subjects reported increased anxiety and irritability and decreased vitality and activity as compared to normal. This indicates that mental disturbances are already present in patients with subclinical thyrotoxicosis"	• "Inner feelings of jitteriness, restlessness, or impatience"
• "Increased slow wave electroencephalograms"	• "Increased slow wave electroencephalograms"

For the reasons stated above, it is difficult to determine the prevalence of iodine-induced pathology. In some cases, iodine-induced diseases are temporary environmental or dietary exposures that are easily resolved once identified by correcting intake. In more extreme cases a singular dose can cause permanent effects (Leung and Braverman, 2014), and it is difficult to determine whether the individual was susceptible to exposures beforehand, or if the exposure itself resulted in subsequent susceptibility. Another confounding factor is "thyrotoxicosis without hyperthyroidism," which is an exposure to thyroid hormones that affects an individual without involving the thyroid itself (De Leo *et al.*, 2016). An example of such is "burger thyrotoxicosis", a term coined when a cluster of cases arose in the US Midwest due to bovine thyroid tissue being included in hamburger meat, subjecting all consumers to an overwhelming dose of thyroid hormones (Hedberg *et al.*, 1987). The original researcher's concern that this was actually a common occurrence that has gone mostly unnoticed has since been validated by subsequent case studies, revealing recurring burger thyrotoxicosis (Parmar and Sturge, 2003). Hashimoto's disease, even if iodine-induced initially, is an incurable autoimmune disorder that, if untreated, will cause periods of hyperthyroidism and hypothyroidism, even after the harmful exposure conditions have passed. Lastly, it is well established that poor maternal thyroid conditions, via iodine exposure or otherwise, can have neuropsychological developmental impacts *in utero* (Brent, 2007). This makes women affected with Hashimoto's a permanent confounding factor for developmental assessments, which is especially significant as Hashimoto's is five to ten times more likely to affect females than males (Staii *et al.*, 2010). Thus, with all these confounding factors, it is incredibly difficult to get accurate numbers for prevalence with overt cases, let alone subclinical cases.

However, trends can be observed and international comparisons can be made to create a general sense of position and direction. A general baseline of natural occurrences can be sensed through previously given examples. Iodine overexposure can come from dietary culture, as well as contaminated water sources, such as old deep wells that are influenced by marine waters (Konno *et al.*, 1994; Andersen *et al.*, 2008). We can also expect to see iodine exposures increase as food production regulations and oversight decrease (Hedberg *et al.*, 1987; Parmar and Sturge, 2003). In some cases, we can find links between iodine supplementation and hyperthyroidism. A longitudinal study in Switzerland monitoring the effects of iodine supplementation in a region suffering from iodine deficiency found an increase in hyperthyroidism by 27% for a year following an increase in iodine supplementation from 90 ug/day to 150 ug/day (Baltisberger *et al.*, 1995). In

other cases, iodine overdosing can also be loosely tracked by tracking one of its more robust adverse outcomes: Hashimoto's disease (Leung and Braverman, 2014). The most striking of these trends is observed as an unfortunate side effect of a well-meaning public policy. In the same time period that salt iodization was being rolled out in the United States (McClure, 1935), medical researchers compiled the amount of Hashimoto's diagnoses in a 30-year period (1930-1959) through histological or clinical means. The percentage of goiters removed specifically due to Hashimoto's disease rose from 0.1% to 13%, despite a decrease in thyroidectomies done at the Mayo Clinic during that period (McConahey *et al.*, 1962). This suggests that while overall, goiter outcomes did improve, the majority of goiters after the transition could be caused by an excess of iodine, instead of iodine deficiency. Decades later, a study found that the United States had a comparatively higher rate of both iodine consumption and Hashimoto's prevalence than Europe (Hollowell *et al.*, 2002). In this century, we see that Hashimoto's is the most common cause of hypothyroidism in the United States, with a reported prevalence of twice the prevalence of type 1 diabetes. Women are a particularly susceptible demographic, with a prevalence rate 1-2% (Staii *et al.*, 2010). Echoing the study from half a century ago, a 2010 study reported finding unexpectedly high occurrences of Hashimoto's while scanning for thyroid cancer. Over half of these Hashimoto-positive patients reported having no symptoms of hypothyroidism, suggesting a large population of subclinical thyroid pathology (Staii *et al.*, 2010), and may explain the increased rate of thyroid cancers observed in patients with slightly elevated thyroid hormones. If such a link exists between Hashimoto's and thyroid cancer, it would neatly fit in with not only the increasing incidence of thyroid cancer in the US (Chen *et al.*, 2009; Davies and Welch, 2006; Enewold *et al*., 2009), but also the world-wide data showing differences in the rate of climbing thyroid cancer incidence. A study comparing the reported incidence of thyroid cancers in countries across the world found the sharpest increase was in South Korea (Wiltshire *et al.*, 2016), which has a significantly higher average daily iodine intake (Kim *et al*., 1998).

These trends could be warning signs of an unaddressed public health hazard that both creates susceptibility to iodine poisoning, influences the behavior of those already susceptible, and creates serious negative health outcomes in extreme cases. It cannot be overstated that the choice to implement iodine distribution as a public health policy is beneficial to those communities served, but it is also worth investigating whether or not these policies are implemented as best as they can be, and are not exchanging one harmful health outcome for another.

Investigating subclinical thyrotoxicosis has many challenges. The same degree of iodine exposure can have hyperthyroidic, hypothyroidic, subclinical, or occult manifestations in different individuals (Braverman and Utiger, 2005), and many can tolerate overexposure with no consequences (Leung and Braverman, 2014). There are also plenty of confounding factors, such as previous iodine over-exposures, maternal pathology *in utero*, unaccounted alternative sources of iodine intake, variance amongst individual salt consumption, and insults by chemicals from other public policies, such as fluoride, which is known to obstruct thyroid mechanisms (Waugh, 2019). In addition, special considerations must be made to address the potential developmental vulnerabilities with subclinical thyrotoxicosis, while at the same time, monitoring for similar symptoms of iodine deficiency disorder. Lastly, an investigation needs to be able to distinguish between the vulnerabilities of the mother and the fetus *in utero*, which are well known to be closely linked in regards to thyroid hormones for the majority of pregnancy (Contempré *et al.*, 1993).

Thus, instead of an epidemiological or medical study, an *in vivo* laboratory study in a closed environment would be an ideal way to evade those hurdles. The zebrafish (*Danio rerio)* model is well suited for subclinical hypothyroid investigations. The zebrafish genome is mapped, and 70% of human genes have been found to have zebrafish orthologues, which increases to over 85% when looking specifically at genes associated with disease (Howe *et al.*, 2013). Due to their high fecundity and quick rate of development, large sample sizes can be quickly and economically produced, with comparative ease of discovering and isolating outlying vulnerabilities. Zebrafish are already well established as a model for developmental toxicity studies, and tools for behavioral analysis have already been developed (Stewart *et al.*, 2011; Horzmann and Freeman, 2018). In addition, zebrafish have already been demonstrated to have similar developmental vulnerabilities to a lack or excess of thyroid hormones (Liu and Chan, 2002; Kapitanova and Shkil, 2014). Lastly, the aquatic nature of zebrafish allows for consistent measurable standards of iodine dose, as well as easy separation of maternal vulnerabilities vs embryonic vulnerabilities.

The central hypothesis that was tested in this study is that zebrafish embryos exposed to different concentrations of NaI will produce effects comparable to subclinical and clinical thyrotoxicosis in humans.

The three aims of this study were as follows: The first specific aim was to identify the concentrations of NaI that would produce statistically significant mortality in zebrafish embryos. The second aim was to measure the differences in behavior in zebrafish larvae after having been

dosed with various non-lethal NaI concentrations, as well as the lowest detected concentration with significant lethality. The third aim was to measure differences in the relative expression of genes associated with the thyroid including sodium iodine symporter (*slc5a5*), thyroid peroxidase (*tpo*), and thyroid stimulating hormone (*tshba*) in zebrafish larvae after exposure to NaI. These genes were chosen to compare causes of upregulation or downregulation in thyroid gland activity. The Wolff-Chaikoff effect, a protective mechanism to shield the HPT axis from excessive iodine exposure is known to reduce expression of the sodium iodine symporter (*slc5a5*) and thyroid peroxidase (*tpo*), whereas thyroid stimulating hormone (*tshba*) production is a general systemic means of altering thyroid activity by the pituitary gland (Eng *et al*., 1999).

CHAPTER 2: MATERIALS AND METHODS

2.1 Zebrafish Husbandry

Embryos were obtained from a breeding colony of SPF 5-D strain laboratory zebrafish (*Danio rerio*). The adult colony resided in a Z-Mod System (Aquatic Habitats, Apopka, FL) on a daily light-dark cycle of 14 and 10 hours, respectively. Water conditions were monitored twice daily to ensure a constant temperature of 28℃, a pH range of 7.0-7.3, and conductivity at a range of 470-550 µS. Embryos were harvested from adult zebrafish in accordance with established protocols (Westerfield, 2007, Peterson *et al*., 2009). Adult fish were fed a blend of brine shrimp (Artemia franciscana; Artemia International LLC., Fairview, Texas), Golden Pearls 500–800 µm (Artemia International LLC., Fairview, Texas), and Zeigler adult zebrafish food (Zeigler Bros Inc., Gardners, PA). Zebrafish were bred according to established protocol (Westerfield, 2007) with the notable exception of the practice of draping breeding tanks with an aluminum foil ceiling to block direct contact with the light source to increase authenticity of dawn environments and natural hormone activity. Embryos were collected immediately after the breeding period, rinsed with filtered water, and randomly sorted into groups for experimentation. Dosing occurred within one hour after fertilization. After dosing, all embryos were incubated at 28℃ until the time point of data collection. All fish were treated humanely to prevent suffering and stressors. All protocols were approved by the Purdue University Animal Care and Use Committee.

2.2 Water Quality Parameters and Chemical Treatment Regimen

All water used in treatments originated from a RO water system. Marine salt (Seachem Marine Salt) and sodium bicarbonate were then added to ensure all treatments done were within a pH range of 7.0-7.3 and conductivity range of 500-550 µS. Biological replicates were embryos produced from separate mating events. A total of 50 embryos were randomly sorted into 100 mm x 20 mm polystyrene petri dishes and dosed promptly after fertilization with 20 mL of aforementioned water with the appropriate dosing of Sodium Iodide (NaI) (CAS#7681-82-5). Embryos within a dish were considered as subsamples. A NaI solution of 250 mM was prepared and diluted to 0, 1, 10, 25, 37.5, and 50 mM. Iodine and iodide concentrations in dosing solutions (including control treatment of prepared fish water only) were tested using the Seachem Multitest®

Iodine & Iodide test kit (Seachem Laboratories, Madison GA, USA) to determine background concentrations in control treatment and to confirm test treatment concentrations. Accuracy of the kit was confirmed using the provided reference test material. All data are reported in this study as mM NaI.

2.3 Mortality and Hatch Rate Changes Resulting from Exposure to NaI

Mortality was observed every 24 hours through 120 hours post fertilization (hpf) among six biological replicates (N=6) with 50 subsamples per treatment dish. Confirmation of mortality in inactive embryos was determined by a lack of a heartbeat via inspection with a dissection microscope. All dead embryos were promptly removed upon discovery to reduce exposure to additional mortality factors.

Hatch rate, considered an expression of stress, was also recorded. Hatch rate was defined as the percentage of hatched embryos to all viable embryos in a given petri dish. Hatch rate was recorded in 24-hour intervals up to 120 hpf.

2.4 Morphological Assessment

Subsamples were treated with the following doses of NaI concentrations: 0, 1, 10, or 25 mM. 37.5 and 50 mM dosing groups were not included due to low mortality in previous studies. Mortality and hatching were observed every 24 hours up to 120 hours post fertilization (hpf).

Larvae were euthanized at 120 hpf via anesthetic overdose of 0.4 mg/mL tricaine-S (ethyl m-aminobenzoate methanesulfonate; Western Chemical Inc., Ferndale. WA). Morphological images were taken via microscope photography in posteroanterior and lateral positions as described previously (Horzmann *et al.*, 2017). Images were later analyzed using the Aperio ImageScope program (version 12.4.0.5043, Leica Biosystems Pathology Imaging 2003-2018). The posteroanterior measurements included were: body length, head length, swim bladder length, and swim bladder width (Figure 1). The intent of the posteroanterior body length measurement was to determine the development of the larval equivalent of the axial skeleton. As such, the measurement excluded the caudal fin to avoid any possible confounding factors that could affect the axial appendicular ratio. Lateral measurements included brain length, swim bladder depth, jaw length (as defined by the distance between the anterior tip of the Meckel's cartilage to the posterior

portion of the palatoquadrate), and soft tissue distension ventral to the palatoquadrate (Figure 2). All measurements were taken with the entire specimen being perpendicular to the base floor to alleviate interference with head widening. Swim bladder volume measurements were taken as a function of swim bladder length, width, depth, and was calculated as a spheroid with the formula of $V=(4/3)\pi abc$. Lastly, "ventral distension", which measured the expansion of soft tissue around the aorta by measuring the distance between the jaw line, and the ventral edge of the soft tissue was measured. These measurement methods were performed across three replicates with each treatment group consisting of 20 subsamples per treatment per replicate.

Figure 1: Example of posteroanterior measurement technique of larvae: Measurements include head length and width, swim bladder length and width, and full body length

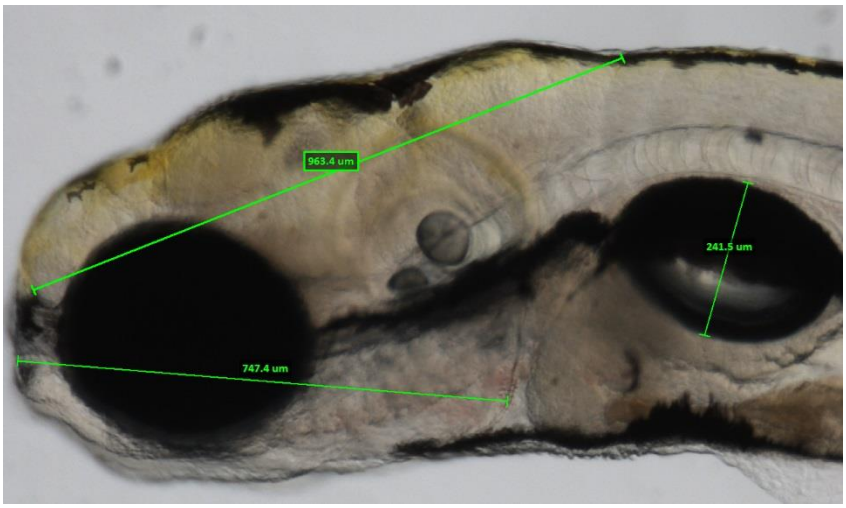


Figure 2: Example of lateral measurement technique of zebrafish larvae: Measurements include brain length, jaw length, and swim bladder depth

2.5 Behavior Assessment

Nine replicates of each treatment group consisting of 24 subsamples per treatment per replicate were analyzed for differences in behavior using the Noldus DanioVision Observation Chamber (Noldus Information Technology, Wageningen, Netherlands) as previously described (Horzmann *et al.*, 2017; Horzmann *et al.*, 2018). Each larva was placed in a 1cm x 1cm well in a 96 well plate. Water temperature was maintained at 28°C via continuous flow surrounding the 96 well plate. After a 10-minute acclimation period of darkness movement data was recorded by an overhead infrared camera for 50 minutes of alternating 10 minute periods of light and dark, beginning with a dark period (Figure 3). The DanioVision system then tallied movement behavior and divided data into the following endpoints: total distance moved, velocity, and time spent moving. Lastly, excessive iodine intake could be linked with loss of fine motor control (Aakre *et al.*, 2017). To address this as a potential confounding factor, an additional behavior study using larger, circular wells with a 2 cm diameter was also performed to compare with standardized 96 well plates. This study used 13 replicates consisting of 4 subsamples per treatment per replicate.

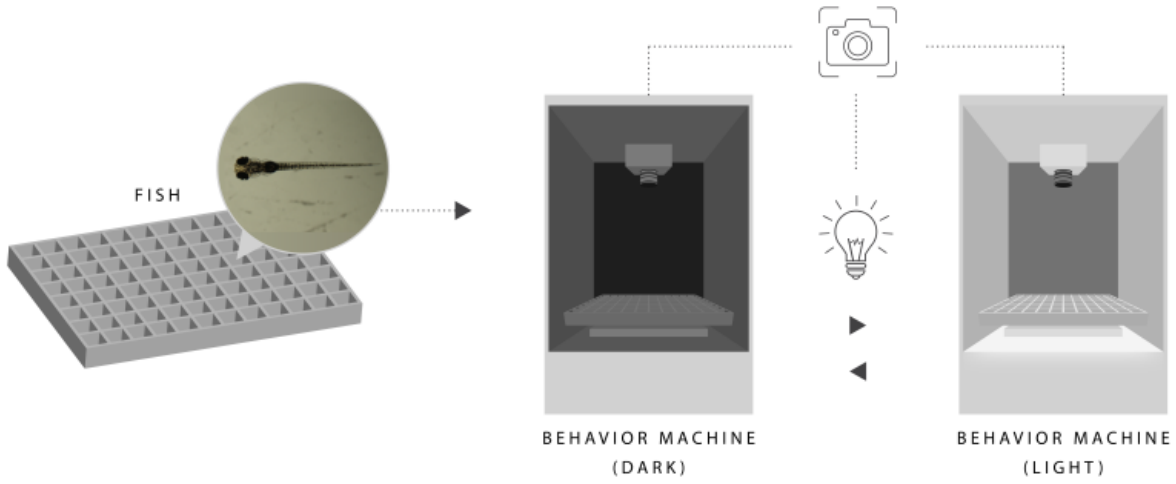


Figure 3: Graphic of behavioral study design: Larvae were placed within small wells, (1cm x 1cm) and placed within a light proof enclosure. An infrared camera recorded movement during periods of darkness and light. Temperature was maintained by partially submerging the plate in temperature controlled water, which actively cycled through a heater in a separate installation

2.6 Gene Transcript Response Assessment

Zebrafish embryos were exposed to 0, 1, or 10 mM NaI as described through 72 hpf. Immediately following the exposure period, 50 embryos were pooled into one sample and homogenized in Trizol® Reagent (Life Technologies, Carlsbad, CA) within an airtight screw-top tube and flash frozen in liquid nitrogen. RNA was isolated and purified according to the protocol outlined by Peterson and Freeman (2009). A NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) was used to confirm purity and quantity. RNA was then converted to cDNA using the Super Script® First-Strand Synthesis System for RT-PCR (Life Technologies, Carlsbad, CA). qPCR was performed targeting solute carrier family 5 member 5 (*slc5a5*), thyroid peroxidase (*tpo*), and thyroid stimulating hormone subunit beta a (*tshba*) following MIQE guidelines (Bustin *et al.*, 2009) and using the BioRad iQ SYBR Green Supermix kit and BioRad CFX96 Real-Time System thermal cycler as previously described (Horzmann *et al.*, 2018). Gene specific primers were designed using the Primer3 website (Table 2). These genes were chosen to compare causes of upregulation or downregulation in thyroid gland activity. Common reference genes were tested for consistent expression and *β-actin* was chosen due to superior consistency. Each sample was run in triplicate and weighed as a technical replicate within the BioRad system software, and were calculated using a standard curve and normalized to *β-actin*. This procedure was repeated across 4 replicates with each treatment group consisting of 50 subsamples per treatment per replicate.

Table 2: Primers used for Gene Transcript Analysis

NCBI Accession Number	Gene Name	Primer Sequence	
NM_001089391.1	*solute carrier family 5 member 5*	Forward:	GTCTGTCTCTATGGCTTGCTGT
	(*slc5a5)*	Reverse:	CTGTGGTGTTGAGTGTTGCATT
XM_021467270.1	*thyroid peroxidase*	Forward:	CCTAAAGTGATTGGCCAGGAGT
	(tpo)	Reverse:	GCGAACACGTTAGACACTGATG
NM_181494.2	*thyroid stimulating hormone subunit beta a*	Forward:	GCAGATCCTCACTTCACCTACC
	(tshba)	Reverse:	GCACAGGTTTGGAGCATCTCA
NM_181601	*β-actin*	Forward:	CTAAAAACTGGAACGGTGAAGG
	(actb2)	Reverse:	AGGCAAATAAGTTTCGGAACAA

2.7 Statistics

SAS 94 software was used to perform a probit analysis to determine the LC50 for mortality in the 24 hpf survival tally. Quantitative data of survival, hatching, morphological, and genetic response studies were analyzed with an ANOVA using SAS 94 software (SAS institute Inc., Cary, NC) to compare treatment groups at each observational time point. When the outcome was statistically significant, a Least Significant Difference (LSD) test at $\alpha = 0.05$ was performed to determine differences between treatment groups. A repeated measures ANOVA was applied to analyze phasic behavior data.

CHAPTER 3: RESULTS

3.1 Confirmation of Iodine/Iodide Concentrations

Iodine can be present in water sources. Iodine/iodide concentrations were tested in the control treatment to ascertain background concentration and in each treatment dosing solution to confirm treatment concentration (Table 3). Iodine/iodide concentration in the control treatment was less than 0.01 ppm and as such had minimal influence on expected concentrations of dosing solutions, which were all near but slightly less than expected concentrations.

Table 3: Iodine/iodine concentrations in treatment dosing solutions.

Sodium Iodine Dosage (mM)	Theoretical content of NaI in water (ppm)	Theoretical content of I,I^- in water (ppm)	Measured content of I,I^- in water (ppm)
0 mM NaI	0 ppm NaI	0 ppm I	>0.01 ppm I
1 mM NaI	149.9 ppm NaI	126.9 ppm I	102.4 ppm I
10 mM NaI	1499 ppm NaI	1269 ppm I	1024 ppm I
25 mM NaI	3747 ppm NaI	3172.6 ppm I	2560 ppm I
37.5 mM NaI	5621 ppm NaI	4759 ppm I	3840 ppm I
50 mM NaI	7495 ppm NaI	6345 ppm I	5120 ppm I

3.2 Changes in Survival and Hatch Rate as a Result of Exposure to NaI

Premature hatching is a sign of stress, so hatch rate was recorded along with survival rate. Embryos exposed to NaI had the highest shift in mortality within 24 hpf. Significant increases in mortality were found in 25 mM, 37.5 mM, and 50 mM groups, and each of these groups had a statistically significant grouping (Figure 4). Mortality after 24 hpf was rare, with the exception of the 37.5 mM group, which lost one embryo each day at the 48, 72, and 120 hpf time points (Figure 5). The 37.5 mM group suffered a unique spike in mortality at the 96 hpf time point; however, with some replicates losing as many as seven (of the original 50) embryos. The LD50 at 24 hpf was calculated to be 26.6 mM NaI.

Hatch rate was also recorded at 24 hour intervals. The only significant increase in hatch rate occurred for the 37.5 mM group at 48 hpf ($p=0.0016$).

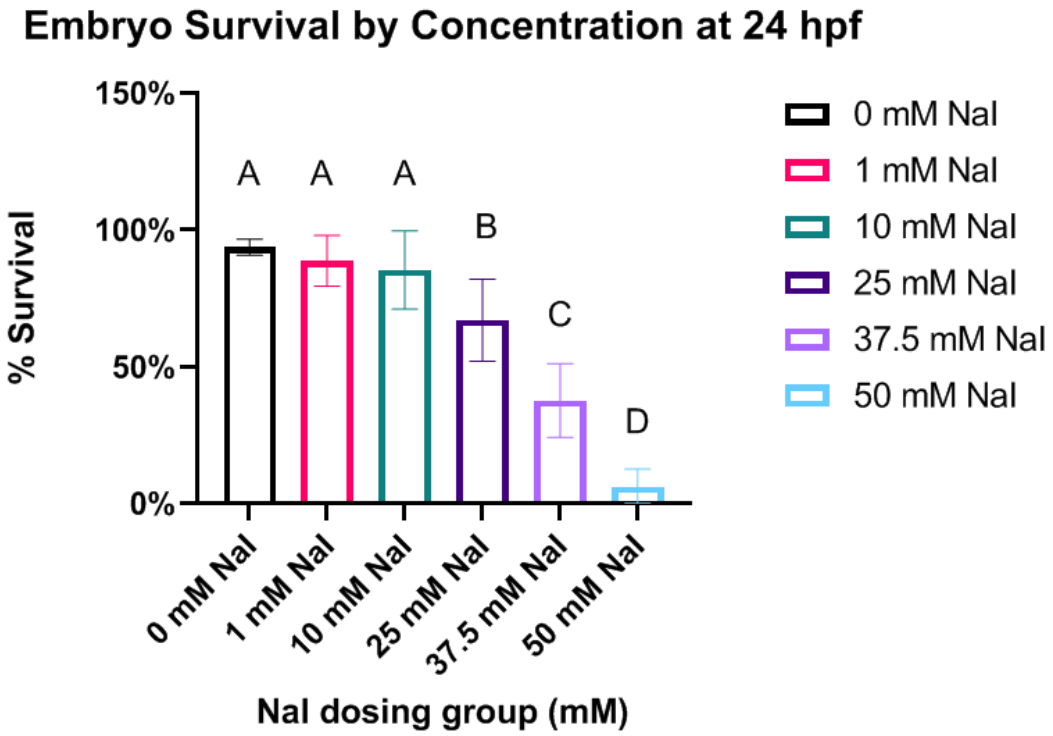


Figure 4: Survival rates following NaI exposure at 24 hpf: Zebrafish exposed to NaI had statistically significant mortality at 25, 37.5, and 50 mM. Error bars represent standard deviation. N=6 with 50 subsamples per treatment per replicate to total 300 embryos per treatment group. Different letters indicate different significant groups at $p<0.05$.

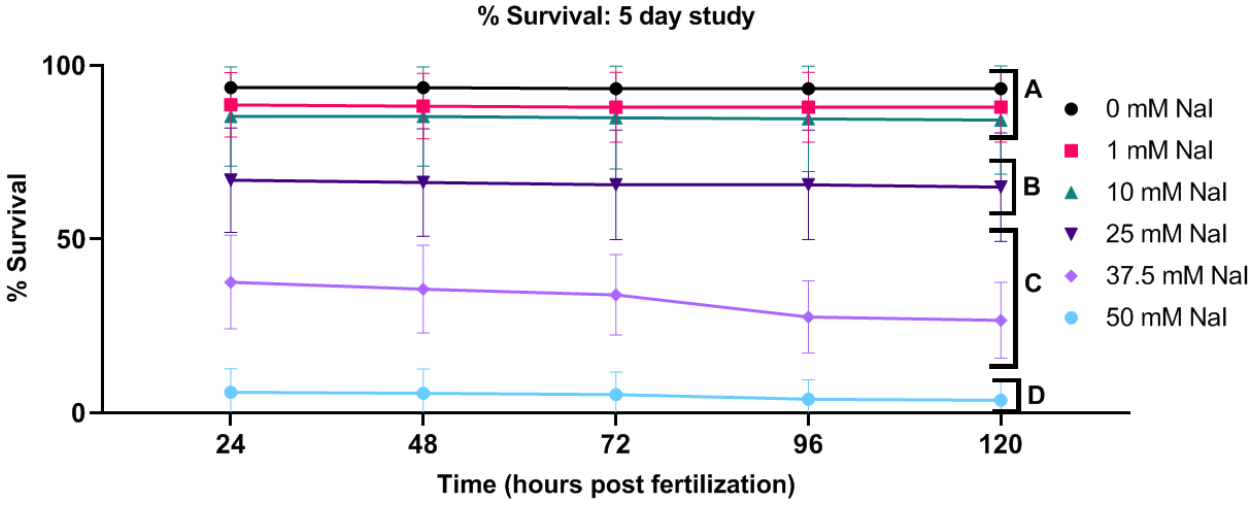


Figure 5: Survival rates following NaI exposure from 1-120 hpf: Zebrafish exposed to NaI had statistically significant mortality at 25, 37.5, and 50 mM starting at 24 hpf and continuing at each time point with the same statistical grouping. N=6 with 50 subsamples per treatment per replicate to total 300 fish in each treatment. Error bars represent standard deviation. Different letters indicate different significant groups at $p<0.05$.

3.3 Morphological Assessment

Assessing development rate and quality can be accomplished with standardized measurements (Singleman and Holtzman, 2014; Kimmel *et al*., 1995). Morphological assessment was performed on 0, 1, 10, and 25 mM dosing groups. Despite having statistically significant mortality (with LC50 at 26.6 mM) and not relevant to dietary concerns, 25 mM was included for the purpose of reflecting environmental exposures and to demonstrate robust clinical symptoms.

NaI caused a statistically significant increase in both head length and brain length at 10 mM ($p<0.0001$), but a statistically significant decrease at 25 mM ($p<0.0001$) (Figure 6A-B). The head width, which included the eyes, had a statistically significant decrease at 1 mM and a statistically significant increase at 10 mM ($p<0.0001$), but no statistically significant change at 25 mM (Figure 6C). Similar to head and brain length, there was a statistically significant increase for total body length at 10 mM and decrease at 25 mM ($p<0.0001$) (Figure 6D).

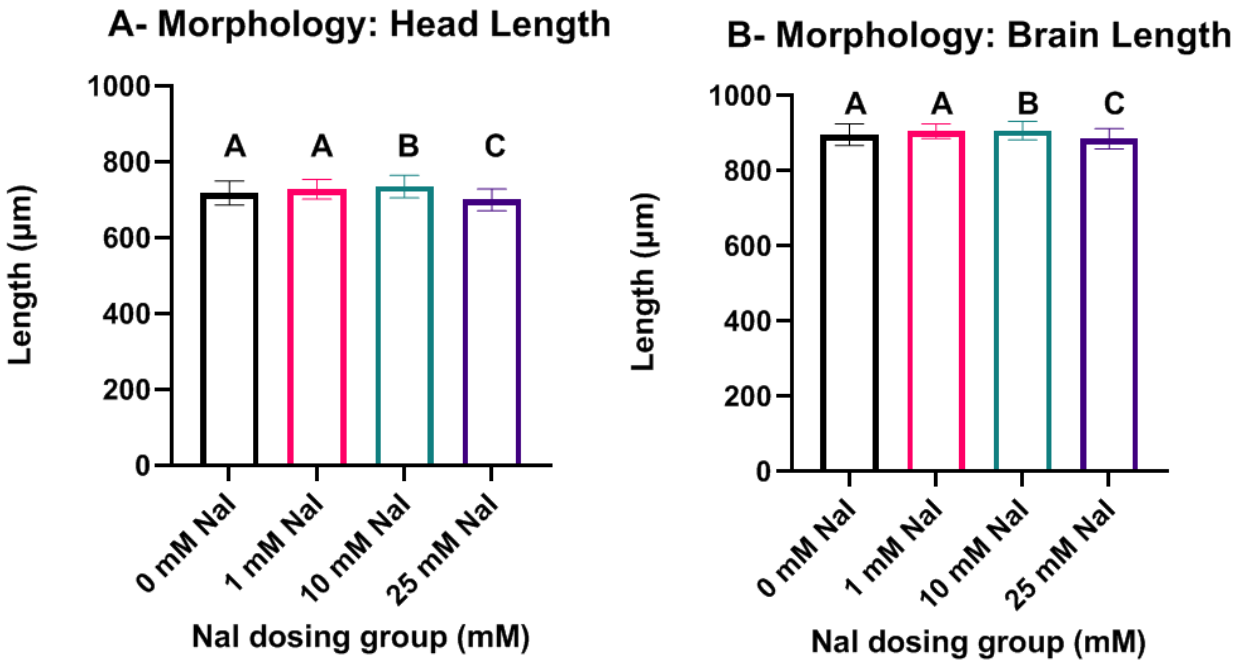
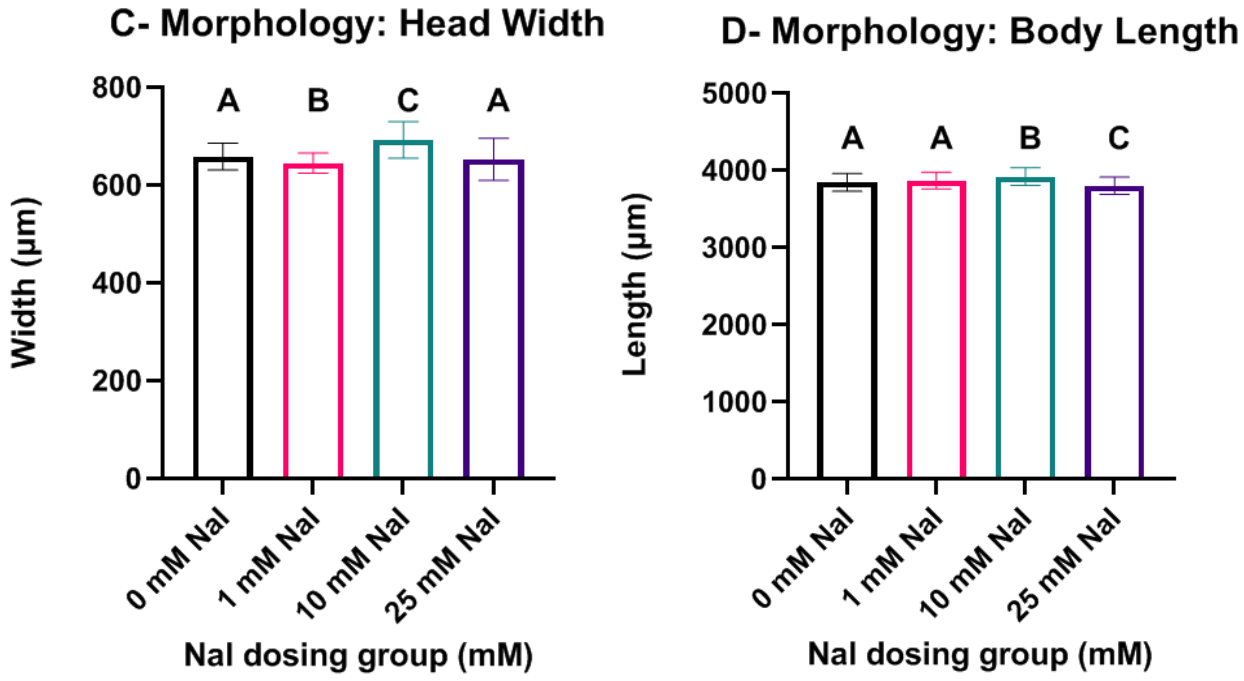


Figure 6: Morphological measurements of head width, and head, body, and brain lengths: Head length (A), brain length (B), head width (C) and body length (D) all had significant increases at 10 mM NaI. Significant decreases were seen at 25 mM NaI for head, brain, and body length. Head width had a significant decrease at 1 mM NaI. Error bars represent standard deviation. N=3 with ~20 subsamples per treatment per replicate to total 56-60 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$.

Figure 6 continued



Jaw length was also included as a supplemental measurement to identify or rule out potential outcomes from other endocrine axes. Jaw length had a similar statistically significant increase at 10 mM, and significant decrease at 25 mM ($p<0.0001$) (Figure 7).

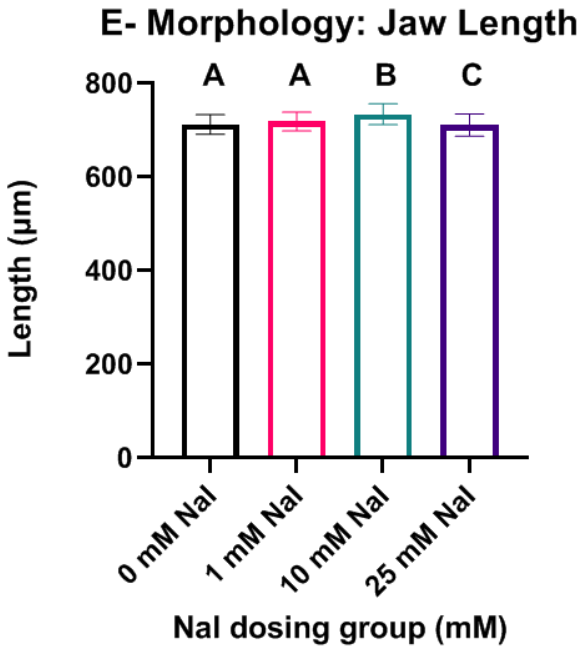


Figure 7: Morphological measurements of jaw length: Jaw length had a significant increase at 10 mM NaI and significant decrease at 25 mM NaI. Error bars represent standard deviation. N=3 with ~20 subsamples per treatment per replicate to total 56-60 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$.

The swim bladder is homologous to human lung tissue, and is thus an important developmental endpoint (Zaccone *et al.*, 2015). Swim bladder volume was calculated as a spheroid using posteroanterior and lateral measurements and had a statistically significant decrease at 10 mM and an extreme decrease at 25 mM ($p<0.0001$) (Figure 8). It should be noted that these extreme decreases were largely influenced by many larvae having not developed a swim bladder, which was measured as zero. This also influenced the 10 mM measurements, which were less frequent. Swim bladder absence did appear in one 1 mM sample, but all control fish had a swim bladder present.

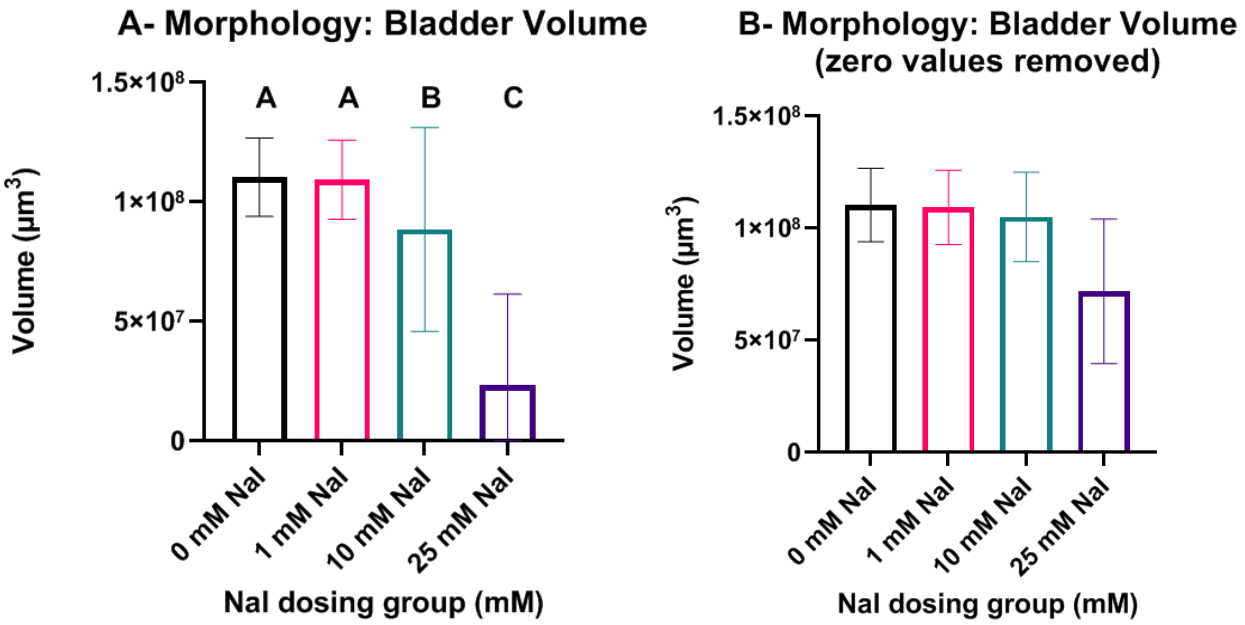


Figure 8: Morphological measurements of bladder volume: Bladder volume significantly decreased at 10 mM NaI and had radical decrease at 25 mM NaI (A). Larvae that had had no visible swim bladder development were measured as zero. The right graph (B) demonstrates values with all zero values removed. Due to low sample size with the 25 mM group under this restriction, no statistical analysis was performed on this scenario. Error bars represent standard deviation. N=3 with ~20 subsamples per treatment per replicate to total 56-60 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$.

Ventral distension was an unexpected but prominent outcome of high dosing groups, and the measurement of such was subsequently added during image analysis. Ventral distension was only present in the 25 mM group ($p<0.0001$), and had a statistically significant increase with significant variability, due the majority of affected larvae demonstrating an "all or nothing" response (Figure 9). A total of 31 out of 57 fish had no distension.

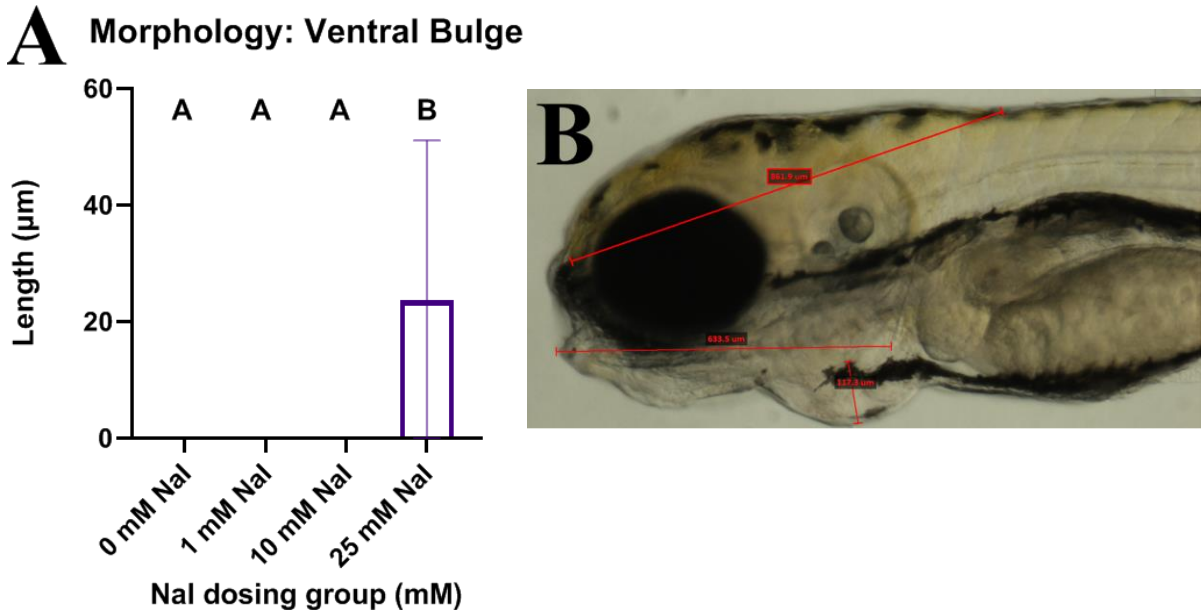


Figure 9: Morphological assessment of ventral distension: (A) Ventral distension only occurred at 25 mM. Variance was high, and many 25 mM larvae had no distension at all. (B) Example larva with ventral distension with measurement approach noted. Error bars represent standard deviation. N=3 with ~20 subsamples per treatment per replicate to total 56-60 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$.

Morphological ratios can determine if there are any developmental asymmetries in treatment groups. The ratio of head length to body length was consistent in all groups except for the 25 mM group, which had a significant decrease ($p<0.001$) despite moderate mouth swelling evident at 25 mM, which was included in the head measurements (Figure 10A). There was a significant increase in the head width to body length ratio at 10 mM ($p<0.001$), which could be affected by exophthalmos occurring in that group (Figure 10B). However, there was a significant decrease in head width to body ratio in the 25 mM group ($p<0.001$), despite the increased prevalence of exophthalmos. There was no significant difference in brain length to body length ratios in all groups ($p>0.05$) (Figure 10C).

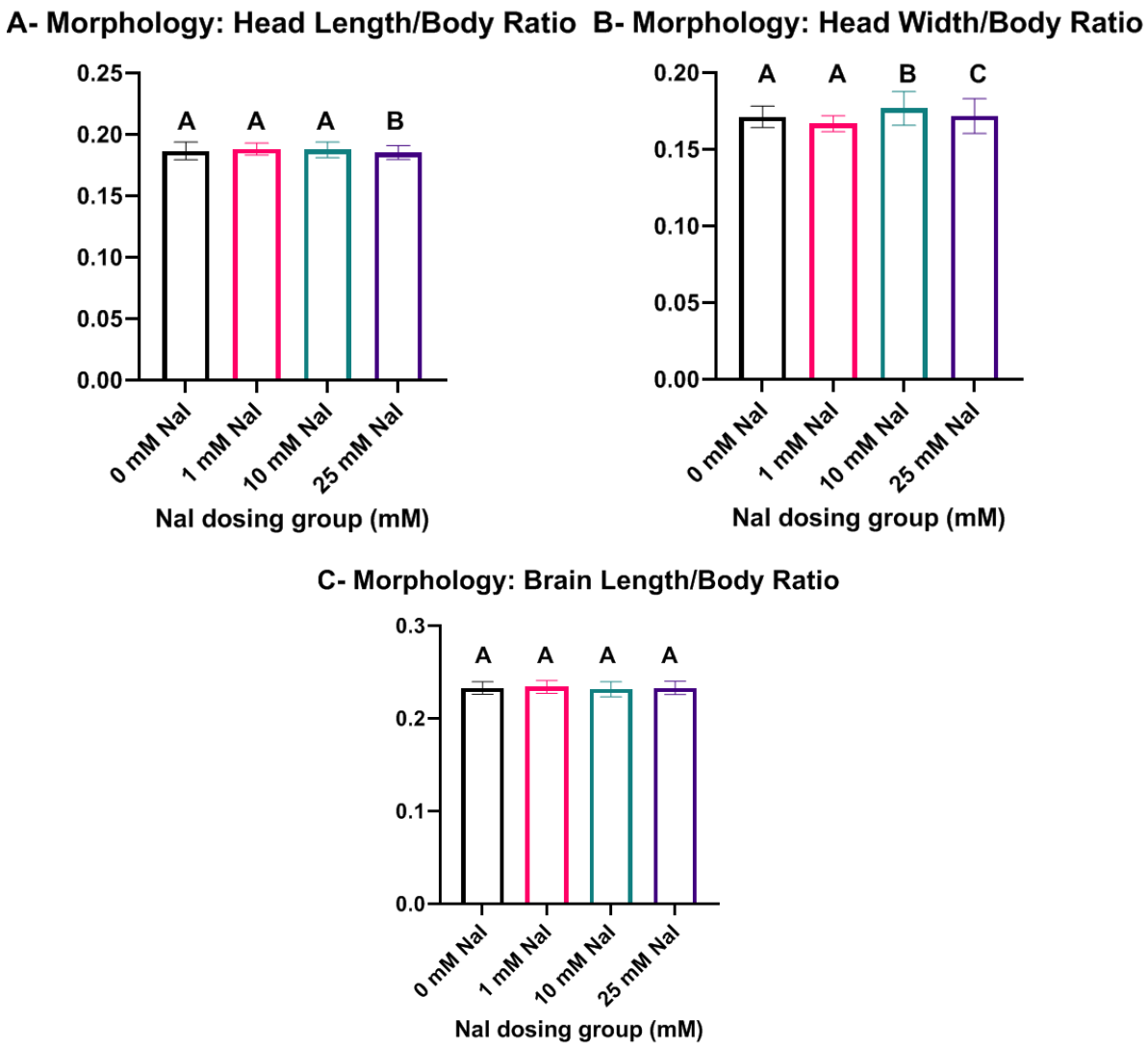


Figure 10: Morphological mean ratios: Significant decrease in head length to body occurred at 25 mM NaI (A). Significant increase occurred in the head width to body length ratio at 25 mM NaI, but had a decrease at 25 mM (B). No significant changes were observed in the brain length to body length ratio (C). Error bars represent standard deviation. N=3 with ~20 subsamples per treatment per replicate to total 56-60 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$.

3.4 Behavior Assessment

Behavior tests, particularly studies that compare behavior in light and dark environments, are used to quantify locomotion, movement patterns, and anxiety-like behavior in zebrafish (Stewart *et al.*, 2011). The behavior of treated larvae was measured using an alternating dark/light environment at 120 hpf using the Noldus Daniovision system. All measurements taken were

measured separately at each 10 minute interval. The first measurement interval is a dark period, followed by light, etc.

For distance moved, treatment and treatment-phase interaction was significant ($p<0.0001$, $F=33.90$; $p<0.0001$, $F=7.61$, respectively). In the first dark phase, larvae in the 10 mM group and 25 mM group displayed a significant increase in total distance moved in comparison to the control group ($p<0.001$) with the 25 mM group having a significantly higher increase than the 10 mM group ($p=0.0001$) (Figure 11). In the first light phase the 10 mM group had a significant decrease ($p=0.0140$). Conversely, the 25 mM group had a significant increase ($p<0.0001$). In the second dark phase, a significant increase for the 10 mM group ($p=0.0023$) and a drastic increase in the 25 mM group ($p<0.0001$) was observed. The 25 mM group had a significantly higher increase than the 10 mM group ($p=0.0120$). In the second light phase, a significant increase in the 25 mM group ($p<0.0001$) was only seen. Lastly, the third dark phase showed an increase in both the 10 ($p=0.0041$) and 25 ($p<0.0001$) mM groups.

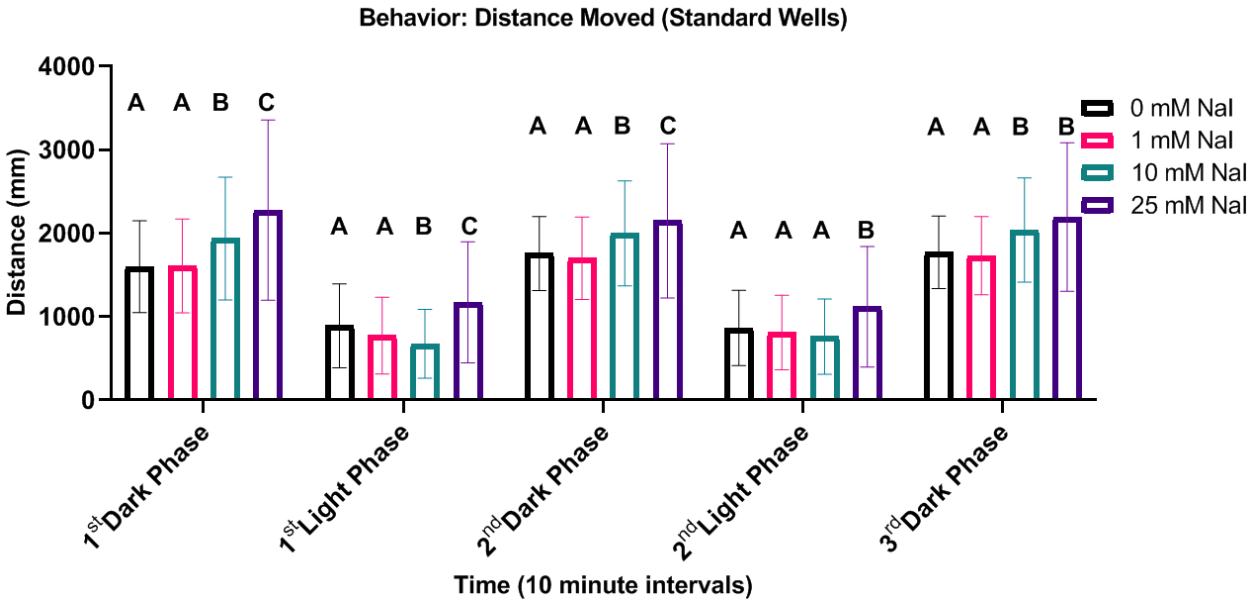


Figure 11: Larvae distance moved: The 10 mM group tended to alternate between periods of comparative activity and inactivity with increased distance moved in the dark phases and decreased distance moved in the light phases. The 25 mM group consistently remained more active throughout the study in all phases. Error bars represent standard deviation. N=9 with 24 subsamples per treatment per replicate to total 216 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$ within phases.

Time spent moving was also measured by phase (Figure 12). For time spent moving, treatment was not significant (p=0.0903, F=2.17), but treatment-phase interaction was significant (p<0.0001, F=13.56). In the first dark phase, there was a significant increase in time spent moving by the 10 mM group (p=0.0044), but not by any others. The first light phase showed a significant decrease for the 1 mM (p=0.0278) and 10 mM (p=0.0001) treatment groups and a significant increase was shown in the 25 mM group (p=0.0058). The second light phase showed a small but significant increase in the 10 mM group (p=0.0451), but all other groups remained similar to the control. In the second light phase, time spent moving was significantly lower in the 10 mM group (p=0.0024) and increased in the 25 mM group (p=0.0511). The third dark phase had no significant differences in any of the groups compared to the control.

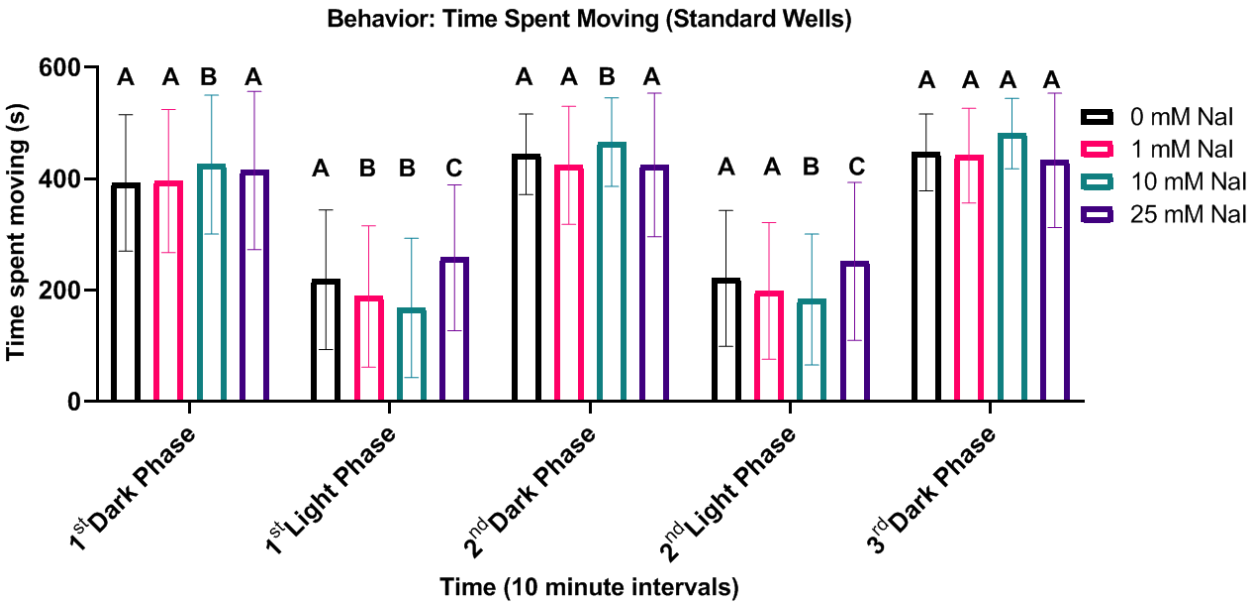


Figure 12: Larvae time spent moving: The 10 mM group tended to alternate between periods of comparative activity and inactivity with increased distance moved in the dark phases and decreased distance moved in the light phases. The 25 mM group was more active only in the light phases, while the 1 mM group had a decrease in time spent moving only in the first light phase. Error bars represent standard deviation. N=9 with 24 subsamples per treatment per replicate to total 216 total fish in each treatment group. Different letters indicate different significant groups at p<0.05 within phases.

Velocity is considered to be the total distance moved divided by the time spent moving. For velocity, treatment and treatment-phase interaction was significant (p<0.0001, F=34.86; p<0.0001, F=8.44, respectively). In the first dark phase, the 1 mM group had no significant

difference compared to the control, but the 10 mM group ($p<0.0001$) and 25 mM group ($p<0.0001$) had a dramatic increase, with the 25 mM group being significantly higher than the 10 mM ($p<0.0001$) (Figure 13). In the first light phase, a significant decrease in the 10 mM group ($p=0.0140$) and a dramatic increase in the 25 mM group ($p<0.0001$) was observed. In the second dark phase, the 10 mM group was significantly higher ($p=0.0025$) and the 25 mM group dramatically higher ($p<0.0001$) with 25 mM being significantly higher than 10 mM ($p=0.0075$). The second light phase was less robust, with only the 25 mM group being significantly higher ($p<0.0001$). However, the increase resumed in the third dark phase, with 10 mM being significantly higher ($p<0.0001$). The 25 mM was more so, ($p<0.0001$) and was still significantly higher than the 10 mM group ($p=0.0255$).

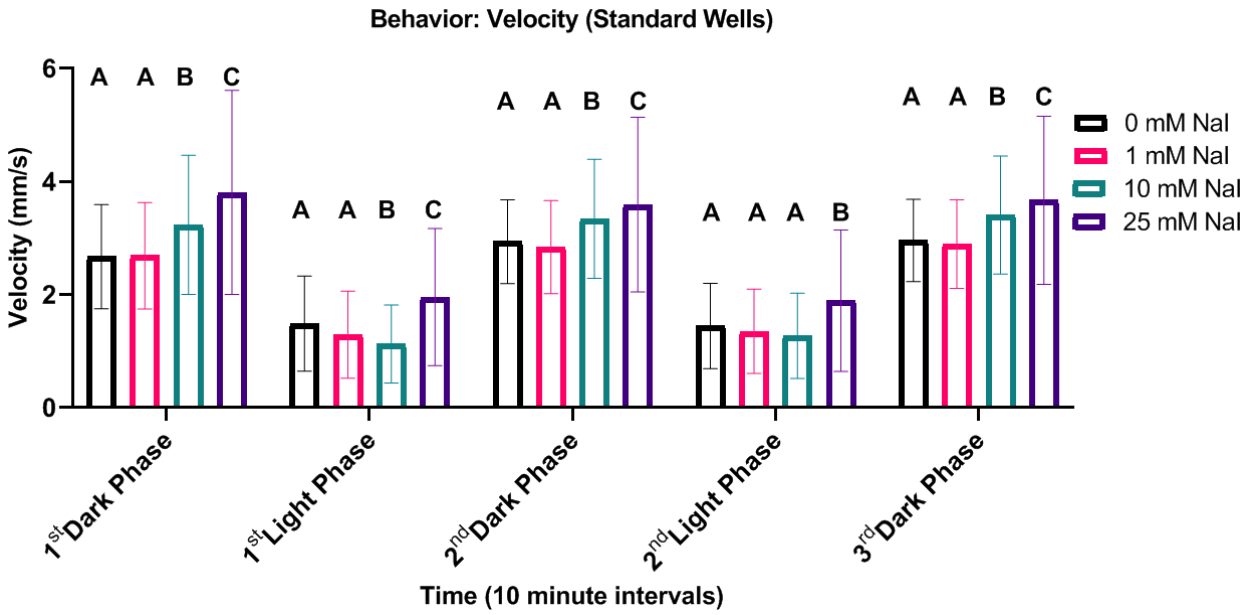


Figure 13: Larvae velocity: Velocity, in this case, can be summarized as the average speed of which a larvae swims when it chooses to do so. The 10 mM group tended to alternate between periods of comparative activity in dark phases and inactivity in the light phases, while the 25 mM group consistently remained more active throughout the study. Error bars represent standard deviation. N=9 with 24 subsamples per treatment per replicate to total 216 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$ within phases.

Measuring distance in large wells yielded similar results. For distance moved in larger wells, treatment and treatment-phase interaction was significant ($p<0.0001$, $F=34.23$; $p<0.0001$,

F=6.71, respectively). The first light phase had significantly higher distance in the 10 mM group (p=0.0044) and the 25 mM group (p=0.0023). The first light phase had no significant increases or decreases in distance moved compared to the control. The second dark phase had significantly higher distance moved in the 10 mM group (p=0.0018) and the 25 mM group (p=0.0175). The 10 mM group had a significant increase in both the second light phase (p=0.0050) and the third dark phase (p=0.0444), but the 25 mM group had no appreciable difference on either of the last two phases.

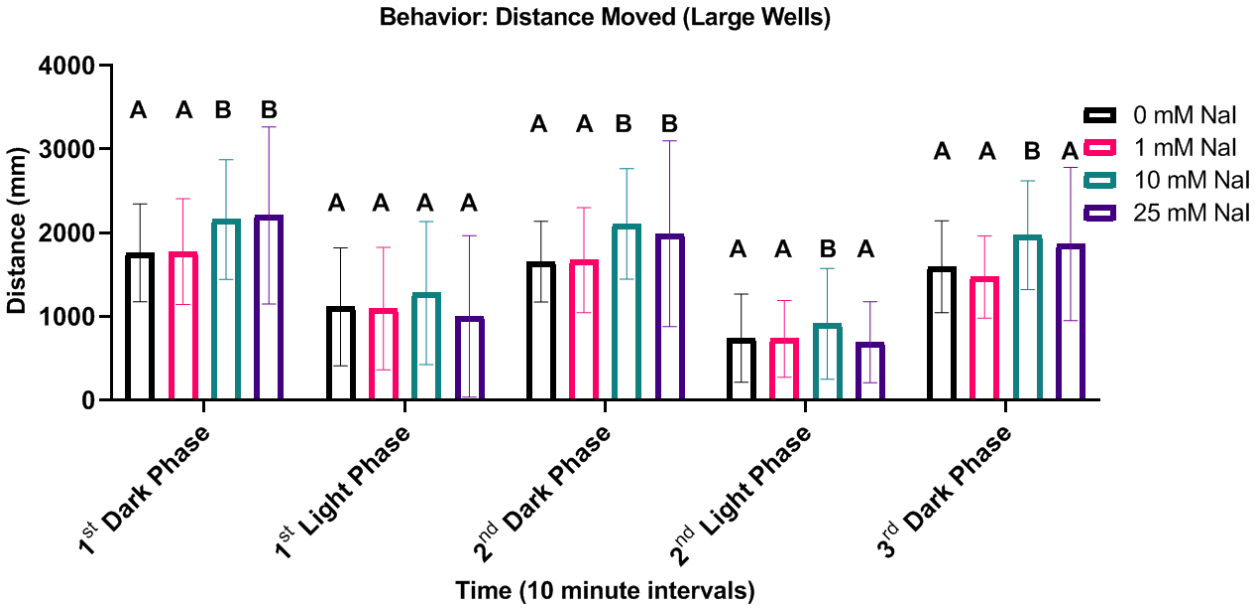


Figure 14: Large well larvae distance moved: The behavior closely resembles distance moved in the dark phases in standard wells with increased distance moved in the 10 mM and 25 mM groups, but in the light phases the 10 mM group was increased instead of decreased when significance was observed. Error bars represent standard deviation. N=13 with 4 subsamples per treatment per replicate to total 52 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$ within phases.

Total time spent moving was also recorded for the large well study and mostly demonstrated a wider variance in the dark phases. For time spent moving in larger wells, treatment and treatment-phase interaction was significant ($p<0.0001$, $F=49.18$; $p<0.0001$, $F=11.63$, respectively). The first light phase showed a decrease in time spent moving for the 25 mM group (p=0.0054), while the second dark phase included a significant increase in time spent moving in the 10 mM group (p=0.0058). The first and third dark phases and second light phase had no significant changes.

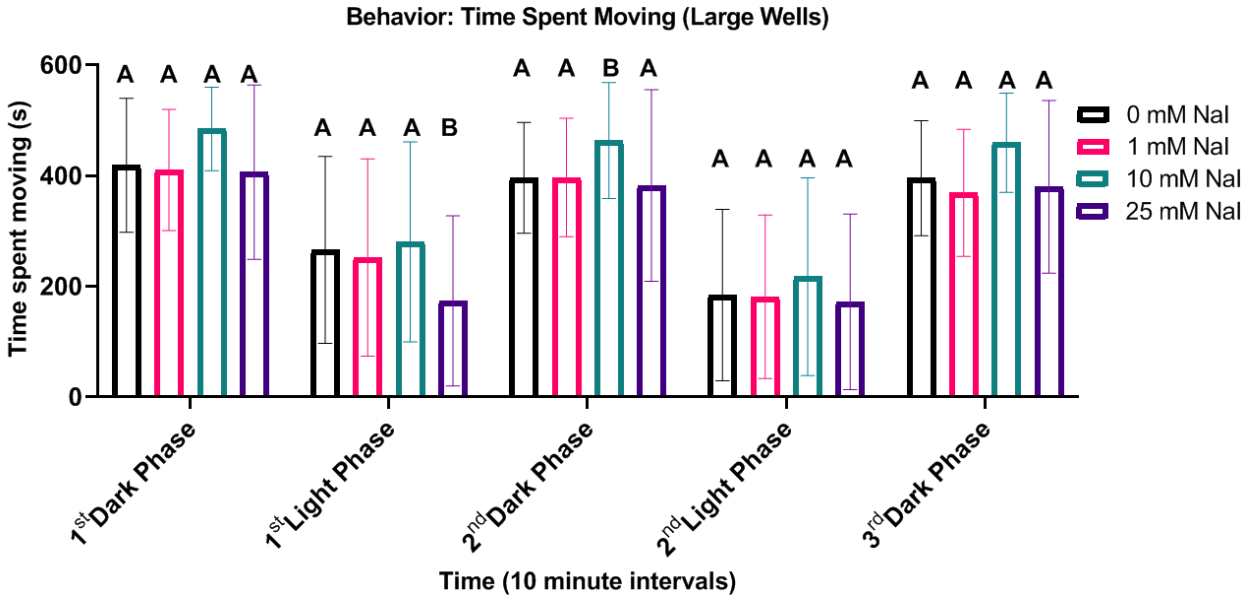


Figure 15: Large well larvae time spent moving: Overall, less significant differences were observed in the larger well study compared to the standard wells with an overall increase in variance in the light phases. In addition, time spent moving was decreased in the 25 mM group in the first light phase in the larger well study compared to the opposite observation of increased time spent moving in the standard wells. Error bars represent standard deviation. N=13 with 4 subsamples per treatment per replicate to total 52 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$ within phases.

Velocity for the large well study loosely mirrored the standard well results, but with less significant differences observed. For velocity in larger wells, treatment and treatment-phase interaction was significant ($p=0.0006$, $F=5.11$; $p<0.0001$, $F=3.31$, respectively). The first dark phase had significant increases in the 10 mM ($p=0.0028$) and 25 mM groups ($p=0.0021$). The first light phase had no significant differences in any group. The second dark phase had a significant increase in the 10 mM group ($p=0.0005$).There were no significant differences in the second light or third dark phases.

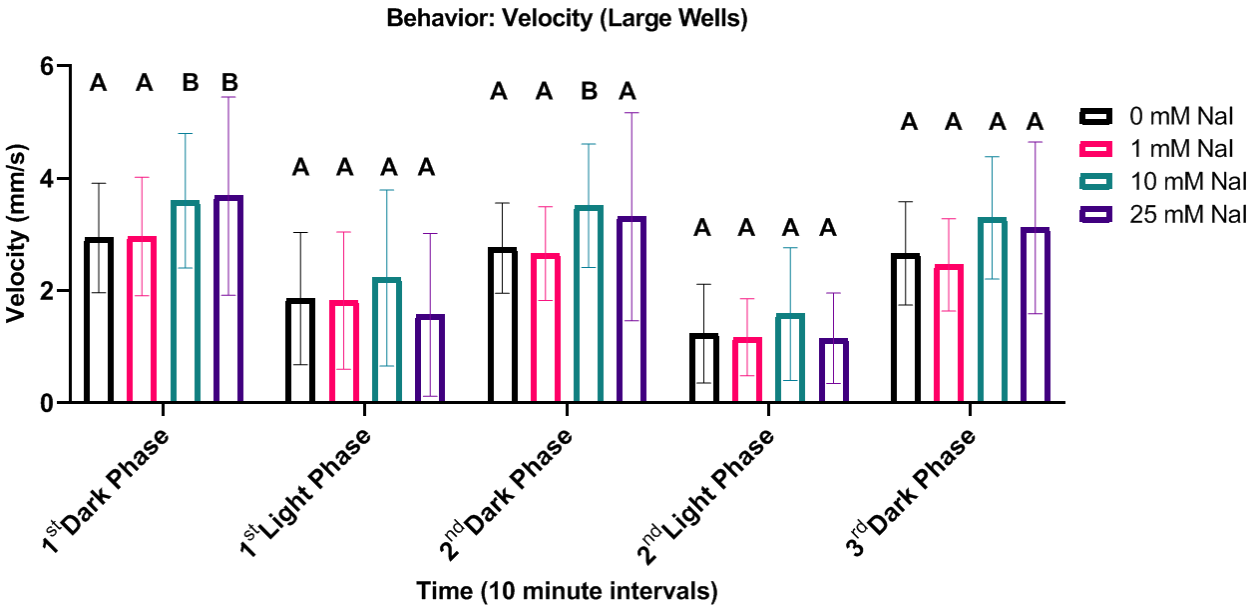


Figure 16: Large well larvae velocity: Increased velocity in the 10 and 25 mM groups in the first dark phase and in the 10 mM group in the second dark phase aligns with observations in the standard well study, but overall less significant observations were seen in the large well study compared to the standard wells. Error bars represent standard deviation. N=13 with 4 subsamples per treatment per replicate to total 52 total fish in each treatment group. Different letters indicate different significant groups at $p<0.05$ within phases.

3.5 Gene Transcript Response Assessment

Gene expression pertaining to the sodium iodine symporter (*slc5a5*), thyroid peroxidase (*tpo*), and a component of thyroid stimulating hormone (*tshba*) was studied to determine any comparative differences in expression. No significant differences in gene expression were observed for all dosing groups (*slc5a5*: p=0.6795; *tpo*: p=0.2589; *tshba*: p=0.8868) (Figure 17).

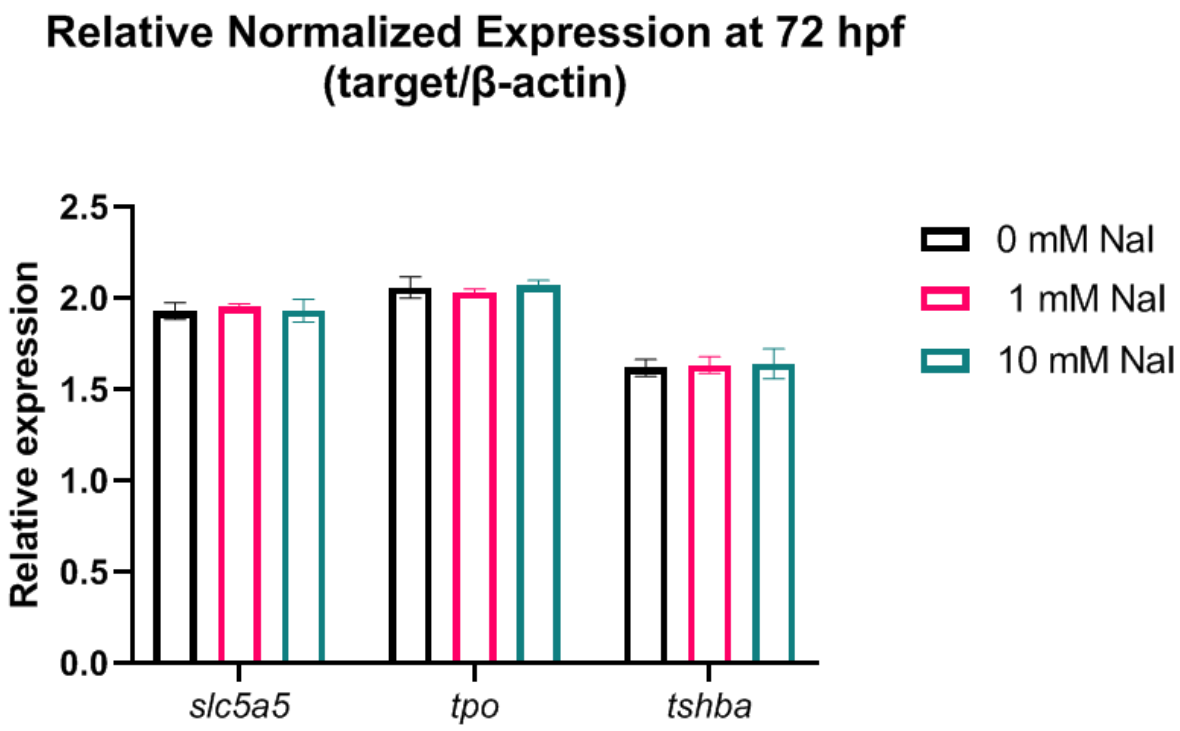


Figure 17: Gene expression for slc5a5, tpo, and tshba: No significant differences in gene expression were observed for all dosing groups (slc5a5: p=0.6795; tpo: p=0.2589; tshba: p=0.8868). Error bars represent standard deviation. N=4 with 50 subsamples per treatment per replicate to total the genetic material of 200 total fish per treatment group.

CHAPTER 4: DISCUSSION

As stated earlier, there are multiple nuanced manifestations of thyrotoxicosis, and many of the relevant mechanisms are still poorly understood (Pramyothin *et al.*, 2011). As a result, the initial evaluation of zebrafish as a relevant model for thyrotoxicosis should focus on equivalent outcomes between zebrafish and humans. Since iodine is an essential nutrient for zebrafish as well as humans (Brown, 1997; Burman and Wartofsky, 2000), we should expect to see similar dose dependent outcomes that are beneficial and adverse, as well as a subclinical dosing range that gives both beneficial and harmful outcomes. To that end, this study investigated the toxicity of excess iodine in the developing zebrafish.

While the survival curve at 24 hpf was a standard and uneventful decline downward as dosing increased, there was a finding of significant interest comparing the hatch rate at 48 hpf. There was a significant increase in the hatch rate of the 37.5 mM group at 48 hpf, which was followed by an equivalent increase in death 48 hours later. It should be noted that the thyroid gland is fully formed by 40 hpf (Rohr and Concha, 2000), but thyroid hormone is present in zebrafish eggs and embryos at 0 hpf (Chang *et al.*, 2012), suggesting that for the majority of the time prior to hatching maternal thyroid hormone is significantly contributing to the thyroid hormone levels prior to thyroid development similar to other fish (Brown *et al.*, 1988). This is equivalent to human development, where the fetus is entirely dependent on maternal thyroid hormone in the first trimester, primarily dependent for the second, and appreciably independent in the third trimester (Rovet and Willoughby, 2010).

It is well established that morphological measurements are a reliable means of assessing development (Singleman and Holtzman, 2014; Kimmel *et al.*, 1995). Thus, it is reasonable to assume that the universal increases in size for the 10 mM group demonstrated metabolic and developmental benefit, while the decreases in size for the 25 mM group demonstrated a detrimental effect. In addition, significance of some morphology ratios indicate asymmetrical development of structures. Of particular note is the swim bladder volume measurement. Swim bladder development in fish is governed by the thyroid pathway. The swim bladder is homologous to lung tissue in humans and similarly the thyroid pathway also plays an important role in human lung development (Zaccone *et al.*, 2015). Despite the initial apparent universal benefits experienced morphologically by the 10 mM group, both the 10 mM and 25 mM treatment groups demonstrated

a decrease in size or collapse of the swim bladder, a well-established indicator of thyroid disruption in zebrafish (Chen *et al.*, 2020; Godfrey *et al.*, 2017; Hagenaars *et al.*, 2014; Li *et al.*, 2011; Stinckens *et al.*, 2018; Wang *et al.*, 2020). This suggests a potentially alarming scenario if this trend occurs in human development for interference in lung development. While there are some reports that a prenatal iodine deficiency can result in impacts to lung development in rodents (Godbole *et al*., 2012), further research is needed into the connection between iodine excess, lung development, and the thyroid pathway. In this instance, standardized, superficial measurements all suggest developmental benefits, but upon closer inspection, there are already signs of insults to the HPT axis. Human equivalent advanced screening processes come at significant cost, and are unlikely to be widely performed in public health programs. This scenario coincides with previous human studies, hinting at increasing prevalence of adverse outcomes to common iodine exposures that are not easily detected (McConahey *et al.*, 1962; Staii *et al*., 2010).

In addition, ventral distension, an unexpected but promptly measured outcome in morphological measurements, had a significant occurrence in the 25 mM group. This occurred at a location distending from the confirmed location of thyroid in zebrafish (Trubiroha *et al*., 2018). More definitive measurements and imaging techniques such as paraffin fixing or alcian blue staining are required to confirm, but this could possibly be a zebrafish equivalent of goiter. It should be noted that the 10 mM group, and only this group, demonstrated multiple instances of ventral distension with higher severity than the 25 mM group, but was ultimately removed from statistical analysis as outliers via the Grubb's test. Further replicates may be necessary to determine if this subset is a vulnerable subpopulation. The lack of such extreme outliers in the 25 mM group could be due the inability of the possible subpopulation to survive at a higher dose than 10 mM.

In the standard size well behavioral study, overall trends saw significant increases in movement in both the 10 mM and 25 mM groups in dark phases. In light phases, movement in the 25 mM groups was increased, but movement was decreased in the 10 mM groups. These observations indicate the possibility of adverse outcomes for many systems, including vision, neuronal, or muscular problems (Sonnack *et al*., 2015). However, as one of the key symptoms of subclinical thyrotoxicosis is anxiety and restlessness, an additional testing environment was added to compare and contrast (large round wells). The differences in this environment can give insight in helping to distinguish between what behaviors in the standard wells were caused by psychological distress and physiological distress. First, the greater space allows for more space for

larvae to accelerate and decelerate in the instances where fine motor control may be hindered. The circular shape also allows for the ability to swim in a circuit pattern without the need to stop or slow at corners. Lastly, the circular wells only offer a clear view of a maximum of four neighbors at a given time, due to the gap of empty water in between two circles arranged in rows. In this environment, socialization is optional, and if a larvae is unsettled by the behavior of their neighbor, they can easily swim to the opposing side to view other larvae with behavior they find more agreeable. This change in environment produced interesting differences between the 10 mM group and 25 mM group in the light phases. In general, zebrafish are expected to move less in light phases than in dark phases. While darkness offers relative safety, movement in light exposes position to predators, so it is generally minimized (Stewart *et al*., 2011). The 10 mM group had a significant decrease in movement during a light phase in the standard well environment, indicating more anxiety, but the 25 mM group moved significantly more in the light phases in the standard well environments. This is in stark contrast to the 25 mM behavior in light environments in the large well scenarios, where the movement was significantly less. The hyperactive behavior resumed once the light period ended, and the larvae felt safer shrouded in darkness. One possible explanation for this is that the restlessness of the 25 mM group exceeded their fear in standard well environments, whereas in the large well environments, the 25 mM larvae felt more isolated by a lack of ability to see their neighbors (which may have been repulsed by neurotic behavior), which would have resulted in their extreme low movement.

Gene transcript analysis of genes associated with iodine intake and thyroid hormone synthesis showed no change across dosing groups, even when behavioral and morphological results were observed that appeared to be characteristic equivalent symptoms of thyrotoxicosis. While it should be noted that RNA harvesting did begin at 72 hpf, two days before behavioral and morphological studies, other studies have confirmed that that the thyroid gland is fully formed and functional by 40 hpf (Rohr and Concha, 2000; Tanaka *et al.*, 1995), with colloid forms clearly visible in imaging (Trubiroha *et al.,* 2018). Iodine intake is reported to occur at least 72 hpf as well (Brown, 1997). It is unlikely that zebrafish developed strikingly similar equivalents to human thyrotoxicosis from iodine alone without increased thyroid hormones playing a role. An alternative explanation is that, like mammals, zebrafish could be subject to the Wolff-Chaikoff effect. This is effectively an emergency shutdown mechanism which protects the thyroid, and thus the rest of the HPT axis from an excessive production of thyroid hormones. Once thyroid hormone levels have

reached a certain threshold, the binding of iodide and subsequently, its conversion into thyroid hormones is temporarily reduced for a period of one to two days (Wolff and Chaikoff, 1948). The mechanism for this has been confirmed to be diminished sodium-iodine symporter and thyroid peroxidase expression (Eng *et al.*, 1999). This mRNA expression is exactly what was measured in this study, in addition to *tshba*, which is functionally downstream of the sodium-iodine symporter (*slc5a5*) and thyroid peroxidase (*tpo*). While normally an animal model is ideal when it shares mechanisms relevant to humans, this might be a point of caution for both time point selection and relevance in dosing windows. Due to the quick development of zebrafish, 48 hours of Wolff-Chaikoff protection would be more meaningful in zebrafish than in humans, and results may only be relevant for single time point exposures or exposure time significantly longer than 48 hours. While further investigation is needed to determine the exact point in which the Wolff-Chaikoff effect takes place for dosings beginning immediately after fertilization, the increased hatch rate and subsequent mortality of the 37.5 mM group would suggest that the window could begin at 24-48 hpf, and end between 72 hpf and 96 hpf. Thus, there is a need to include additional molecular analysis, thyroid level assessments, and thyroid pathology to further define this outcome.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

In this study, the toxicity of excess NaI was determined by measurement of survival rates, behavioral assays, and morphological measurements at 120 hpf, with an evaluation of gene expression of *slc5a5, tpo,* and *tshba* at 72 hpf with qPCR. There were beneficial and detrimental effects demonstrated at 10 mM, with universally detrimental effects demonstrated at 25 mM. Homologous signs and symptoms of subclinical and overt thyrotoxicosis could be observed in the 10 mM and 25 mM dosing groups, respectively. No changes in transcript response were observed at 72 hpf, possibly due to the Wolff-Chaikoff effect. Overall, the zebrafish model appeared to be a promising model to evaluate the multifaceted and nuanced adverse outcomes of excess iodine, particularly for developmental stages and identifying vulnerable subgroups. Further investigations are necessary to identify internalization, biodistribution, clearance, specific time points of genetic responses, windows of vulnerability, and long-term responses.

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