# Early Exposure to Low Concentrations of Bisphenol-A can Decrease Zebrafish Social Behavior

by

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ABSTRACT

Bisphenol-A or BPA is a common chemical pollutant that contaminates the environment, specifically water systems, due its mass production in human-made plastic items and subsequent improper disposal. BPA is also an endocrine disruptor that has negative health impacts on organisms exposed to them, ranging from changes in reproduction to neural activity. In this study I researched the impact of early exposure to weak levels of BPA on adult zebrafish (*Danio rerio*) social behavior. Zebrafish are highly social creatures that rely on group living for protection and resource attainment in the wild, meaning any alteration to how they interact with their conspecifics can be detrimental to their survival. For one-week postfertilization, I exposed baby zebrafish to either 0.01 mg/l BPA, 0.001 mg/l BPA, 0.1% DMSO, or water. I raised the fish to adulthood and tested their reaction to a social stimulus. I found that early exposure to low doses of Bisphenol-A led to an increase in zebrafish activity levels (increased distance and time spent traveling) and a decrease in preference towards the social stimulus (more time away from the social stimulus). Increases in activity suggest that the long-term effects of early BPA exposure may be linked to chronic stress. However, all treatment and control groups spent most of their time near the social stimulus when they had visual access to it, implying a natural social drive that was not completely blocked by the exposure to BPA. This also verifies that visual signals are highly important to social behavior, since fish given olfactory access alone did not spend as much time in proximity to the social stimulus. Although even short-term exposure to weak BPA has a lasting impact on zebrafish social behavior, future studies are needed to confirm that these persistent effects are related to stress.

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# CHAPTER 1

## INTRODUCTION

Water pollution is a growing environmental issue that can be a challenge for organisms even when encountered for short periods at low doses, and can be especially deleterious during early developmental stages (Frye et al., 2012). Physical, chemical, and biological hazards detrimentally affect the organisms that consume them, with chemical factors being the most prevalent and harmful (Goel, 2006). Bisphenol-A, or BPA, is a leading, inorganic, chemical pollutant that contaminates multiple water supplies due to its heavy use in the production of plastic food containers and other mass-produced items, such as plastic water bottles and the plastic resin that lines metal cans (vom Saal et al., 2012). This mass production of plastic subsequently leads to high levels of plastic pollution in the environment (specifically in water systems) due to improper industrial maintenance, disposal, and waste. BPA therefore has a profound impact on aquatic life since lakes and rivers are such hot spots for this chemical (Canesi & Fabbri, 2015), meaning aquatic life is exposed constantly to BPA at a variety of concentrations. Although fish such as rainbow trout and zebrafish can process and expel BPA from bodily tissues (Lindholst et al., 2003), even short-term exposures can have major impacts. BPA accumulates in fish brains, gills, livers, kidneys, and muscles (Chen et al., 2017), and leads to genotoxicity and oxidative stress in several species of fish (Afzal et al., 2022). Our goal in this study was to observe the effect of early-life exposure to BPA on adult zebrafish social behavior at low, but environmentally relevant, concentrations.

BPA is a well-known endocrine-disrupting chemical, with important impacts on both the environment and human health. The modern prevalence of BPA in our environment has led to increased public concern, and epidemiologic research to limit exposure (Ho et al., 2022). Organisms are especially vulnerable to endocrine disruptors in their early developmental stages because the brain is highly sensitive to endogenous hormones at that time (Gore et al., 2019). Thus, time of exposure may change the intensity of impact. Endocrine disruptors interfere with not only the endocrine system, but also the synthesis, distribution, and reception of chemical signals and cell function (vom Saal et al., 2012). The endocrine system is extremely important for all animals because it regulates biological processes in the body from conception until death. Because endocrine disruptors obstruct the function and metabolism of endogenous hormones, they disrupt homeostasis, neurological development, reproduction, and behavior in the organisms inflicted by these chemicals (Huang et al., 2015). Exposure to endocrine disruptors has been linked to changes in locomotion, cognitive abilities such as learning and memory, both sexual and social behavior, reproduction, neurological development, anxiety level, and sex differences (Masuo & Ishido, 2011).

A unique aspect of this experiment is that we exposed the zebrafish when they were embryos, but then measured behavior when they were fully developed. This allowed us to investigate the long-term effects BPA may have on the fish, as opposed to the more frequently studied short-term effects. Due to their undeveloped organs and fluctuating hormones, larval fish are much more vulnerable to any invasive chemicals or pollutants than are adult fish. Exposure to endocrine disruptors can be much more harmful if it occurs during developmental periods of life, such as the perinatal, juvenile and puberty periods, when organisms are more sensitive to hormonal disruption (Frye et al., 2012). In both human and animal models, early life exposure to endocrine disruptors has been directly related to later in life diseases, such as endocrine-related cancer and neurological disorders (Kim et al., 2022). This early exposure to BPA and other endocrine disruptors affects mammals primarily through its impacts on the brain including changes in hypothalamus anatomy and nonapeptide production (Gore et al., 2019, Repouskou et al., 2020). Effects of early BPA exposure on adult behavior are mixed, with some studies finding that BPA increases, and others finding decreases in adult mouse social and sexual behavior (Gore et al., 2019). Studies of the impact of early BPA exposure on adult social behavior of other animals may provide important insights.

Zebrafish or *Danio rerio* have been widely used as a model system for biomedical research to study biological processes and analyze developmental and genetic changes (Rouf et al., 2022). These fish are highly social creatures, similar to humans, that form shoals, which are small groups of fish that interact loosely with each other (see review by Facciol and Gerlai, 2020). Group living provides these fish with several benefits including defense against predators by increasing predator detection, decreasing the risk of capture, and confusing predators; it also increases foraging success and ability to find prey (Peichel, 2004). This forming of shoals is often influenced by early experience, meaning these early times for zebrafish are critical for them to develop proper social and mating skills as adults (Moretz et al., 2007). Any type of disruption can have negative impacts on sociosexual behavior, whether this be a subtle alteration or significant one (Gore et al., 2019). In particular, Saszik and Smith (2018) showed that increased stress leads to a change in dopamine activity ultimately decreasing social behavior in zebrafish. Since BPA causes augmented oxidative stress in zebrafish (Sahoo et al., 2020), we predicted that early BPA exposure would lead to chronic stress in adult zebrafish, increasing overall activity levels and subsequently lowering social behavior. In addition, olfactory and visual signals play a large role in fish shoaling and social behavior. Evolutionary adaptations of cooperative behavior require mechanisms that enhance shoal cohesion subsequently enhancing fish survival, with visual and olfactory signals adapting over time to maintain advantageous shoaling techniques (Engeszer et al., 2004). Although zebrafish use both visual and olfactory cues to form cohesive shoals, visual signals are more important in mediating social behavior in zebrafish (Engeszer et al., 2004). When separately testing these cues, Faustino et al (2017) also found visual signals to be more important for zebrafish when facing an aversive stimulus. In this study, we tested whether visual cues remain the predominant signal after BPA treatment, by looking into both olfactory and visual responses of fish exposed to a social stimulus.

The Environmental Protection Agency deems 0.1 mg/ml of BPA as a “safe” consumable amount for humans (Willhite et al., 2008). We set treatment concentrations well below that, and asked whether early-life exposure to low levels of BPA impact later behavior of adult zebrafish. Specifically, we predicted that (1) early BPA exposure would lead to chronic stress, elevating activity and decreasing social behavior. In addition, since zebrafish sensory preferences are set during early development, we asked (2) whether BPA impacted later use of olfactory and visual signals in social behavior.

# CHAPTER 2

# METHODS

## Subjects and Maintenance

The fish in this study were measured also as part of an experiment testing the impacts of BPA on larval sensory behavior (Suriyampola et al., 2021). We purchased adult wild-type zebrafish from a commercial supplier (Rawlins Tropical Fish Farm in Lithia, Florida, United States), and bred larval zebrafish. We collected only viable eggs from these fish after mating, and then kept these eggs in separate, small containers based on which treatment they were given. We set the water at an ambient temperature of 26 ± 1◦C in static conditions. Each day, we carried out a 50% water exchange and removed any debris and dead larvae from the tanks using a dropper. At 5 days postfertilization, we fed the larvae daily with 5 mL of s-type rotifers (*Brachionus rotundiformis*) from Reed Mariculture®, Campbell, CA, USA (Suriyampola et al., 2021). Once they were large enough, we fed them brine shrimp (3 mL to each container), and then slowly started incorporating beginner flake food (First Bites). Eventually, we moved the fish to a Tecniplast Zebtec Active Blue® standalone system set to standard conditions (28 ± $1^{0}$C, 400 µS electrical conductivity, pH 7.4, 10% daily water exchange, and 14:10 light: dark photoperiod). We fed adult fish with commercial flake food (Tetramin Tropical®) daily each morning. We began the behavioral assays with 66 5-month-old fish. Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Arizona State University (Protocol 20-1742R).

## Exposure

To prepare an initial BPA stock solution, we dissolved 100 mg of BPA (99% purity, Sigma-Aldrich) in 1 L of dimethyl sulfoxide (DMSO, >99.9% purity, Sigma-Aldrich) to obtain a 0.01 mg/l BPA solution (Suriyampola et al., 2021), and then diluting with more DMSO to obtain also a 0.001 mg/l BPA treatment solution. For one-week postfertilization, we exposed the larvae to 0.01 mg/l BPA, 0.001 mg/l BPA, 0.1% DMSO, or water, refreshing the treatment daily. The DMSO treatment was a second control to measure the impact of the solvent separately from that of the BPA. On the seventh day, we transferred the larvae to 3.5 L Tecniplast tanks, rinsing them twice for 10 minutes each in system water. We began the social assays at 5 months post-fertilization with 66 adult fish from the early-life treatments, distributed as follows:18 fish in water, 9 fish treated with 0.1% DMSO, 19 fish treated with 0.001 mg/l BPA (BPA-Low), and 20 fish treated with 0.01 mg/l BPA (BPA-High).

## Sociality Assay

To measure activity and social behavior, we tested each subject fish twice in a 3-part arena (Fig. 1). We placed one focal fish in the small, central compartment (15 cm x 15 cm) with opaque, perforated sides, giving the subject chemical access to a social stimulus on one side and an empty box on the other (Fig. 1). The social stimulus consisted of a mixed-sex group of four adult fish (2 females and 2 males). We placed the focal fish and stimulus fish in their respective boxes at night, leaving them to acclimate until the next morning with olfactory, but not visual, access to each other. We turned the lights on at 7:30 am the next day, and left the fish undisturbed for 15 min to acclimate.

We then video-recorded the subject fish for 5 min from above with Logitech® C270 HD webcams connected to MultiCam Capture software. After this, we removed the opaque barriers on both sides of the central arena, now allowing the subject both visual and chemical access to the stimulus fish. After 15 min of acclimation, we recorded the subject fish again for 5 min. The side of the social stimulus was alternated intermittently to ensure results are independent of side fidelity.

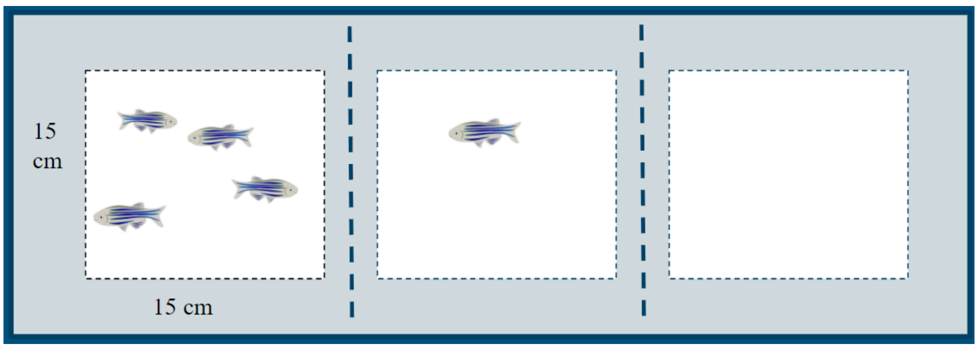


**Figure 1.** Arena for behavioral assays. The left box held the social stimulus, the middle box held the focal fish, and the right box was an empty box. The location of the social stimulus was swapped intermittently between the left and right side for different trials. Each box was 15 cm x 15 cm with transparent, perforated sides. The two vertical, dashed lines represent the opaque, perforated, barriers that we used during the first assay period. The outer rectangle represents the tank that held the three individual boxes inside allowing water to flow between boxes.

## Data Scoring

We used Ethovision XT10® software to track the behavior of the subject fish automatically, recording total time spent moving, total distance traveled, and time spent in each zone of the arena (Fig. 2). We divided the central box of the arena (with the subject fish) into three separate zones: far, near, and very-near zones (Fig. 2). The "far" and "near" zones divided the central arena in half, with the "near" zone corresponding to the half nearest the social stimulus. The "very-near" zone was the area within 3 cm of the social stimulus (roughly the length of an adult zebrafish) and overlapped with the "near" zone.

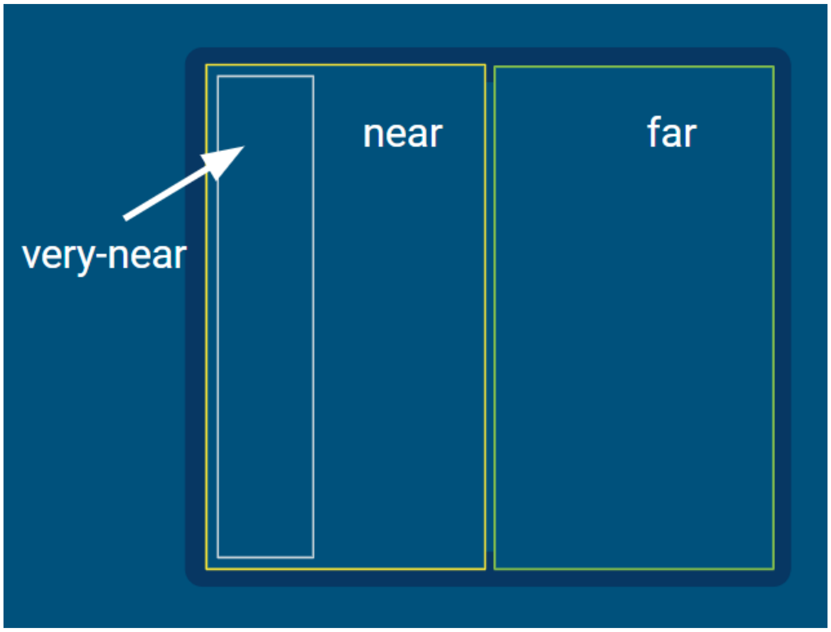


**Figure 2**. Schematic of the central arena. We scored subject location by splitting the arena into three zones (very-near, near, and far) with respect to the social stimulus, with the near and very-near zone overlapping. We used Ethovision® to track the subject during a 5-min trial, scoring the total amount of time each fish spent in each zone.

## Data Analysis

We extracted all data collected by Ethovision®, and analyzed it using R (R Core Team 2020). We then used the core functions of R, and packages "nlme" (Pinheiro et al., 2013) and "multcomp" (Hothorn et al., 2008) to conduct a repeated-measures

ANOVA with Tukey posthoc analyses to test for differences between treatments. We used a repeated-measures model since each subject fish was measured twice: once with visual and olfactory access to the social stimulus, and once with olfactory access only. This allowed us to measure any significant differences between fish in BPA-High, BPA-Low, DMSO and water groups, while measuring the impact of visual and olfactory signals. By testing if BPA-treated fish showed varying results compared to control groups, we can surmise whether or not BPA has long-term effects on zebrafish social behavior. In addition, we included a factor indicating whether the subject fish had visual, as well as olfactory, access to the social stimulus in order to test also for differences in sensory behavior. Specifically, we tested the impact of BPA on activity levels by measuring "Distance Moved (cm)" and "Time Spent Moving (s)", and the impact of BPA on social behavior by measuring "Time in the Very-Near zone (s)" and "Time Spent in the Far Zone (s)".

# CHAPTER 3

# RESULTS

## Activity Levels:

### Zebrafish treated with BPA during early life were more active as adults

Zebrafish that experienced the low concentration of BPA during early life were significantly more active than were those that had experienced a high concentration of BPA or the two control conditions. Zebrafish in the BPA-Low group traveled a mean distance of 1824 cm (SE = 78.9) in 5 min, as compared to 1602 cm (SE = 60.1) for fish in the BPA-High group, 1621 cm (SE = 73.6) for fish in the DMSO group, and 1311 cm

(SE = 71.5) for fish in the water group (Fig. 3). Fish treated with BPA moved significantly farther distances (measured in centimeters) than did fish in the DMSO or water control groups, leading to a significant effect of BPA treatment in the ANOVA ($F = 9.1$; $df = 3, 123$; $P < 0.01$), supported also by significant differences in Tukey posthoc tests between fish exposed to water and both BPA treatment conditions ($P < 0.05$), but not between fish in the water and DMSO control groups ($P > 0.05$). Visual access to the social stimulus and the interaction between treatment and visual access were not statistically significant predictors of distance moved ($F = 0.0$; $df = 2, 123$; $P = 0.98$ and $F = 0.6$; $df = 3, 123$; $P = 0.62$, respectively).

When looking at time spent moving measured in seconds (Fig. 4), the BPA-treated fish also spent significantly more time moving than did fish in the DMSO or water control groups, again leading to a significant effect of BPA in the ANOVA ($F = 6.5$, $df = 3, 123$, $P < 0.01$), with the Tukey posthoc tests showing a significant difference between fish in the water and the BPA-Low treatment groups ($P < 0.01$). Visual access to the social stimulus was a statistically significant predictor: fish with only olfactory access to the social stimulus spent, on average, more time moving (54 s, SE = 4.25) than fish with both visual and olfactory access (11 s, SE = 3.20) ($F = 36.1$; $df = 2, 123$; $P < 0.01$). However, the interaction between BPA treatment and visual access to the social stimulus was not statistically significant ($F = 0.5$, $df = 3, 123$, $P = 0.68$).

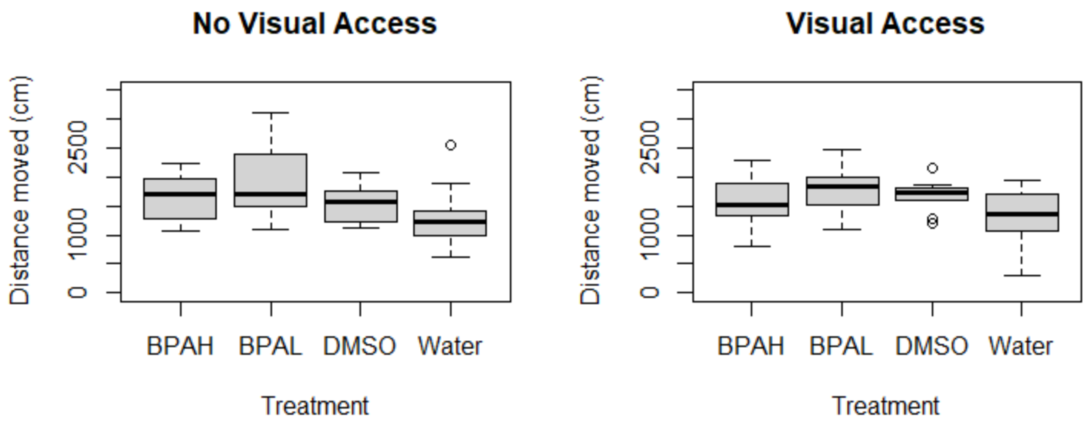


**Figure 3: Distance Moved (cm).** Zebrafish that experienced both low and high levels of BPA (BPAL and BPAH) during early development were more active than the control groups, regardless of visual access to the social stimulus. The panel on the left depicts results for trials in which the subject had only olfactory access to the social stimulus. The heavy horizontal black bar in each box represents the median distance moved in centimeters for each treatment, the box edges mark the upper and lower quartiles, whiskers reflect minimum and maximum values, and circles identify outlying points.

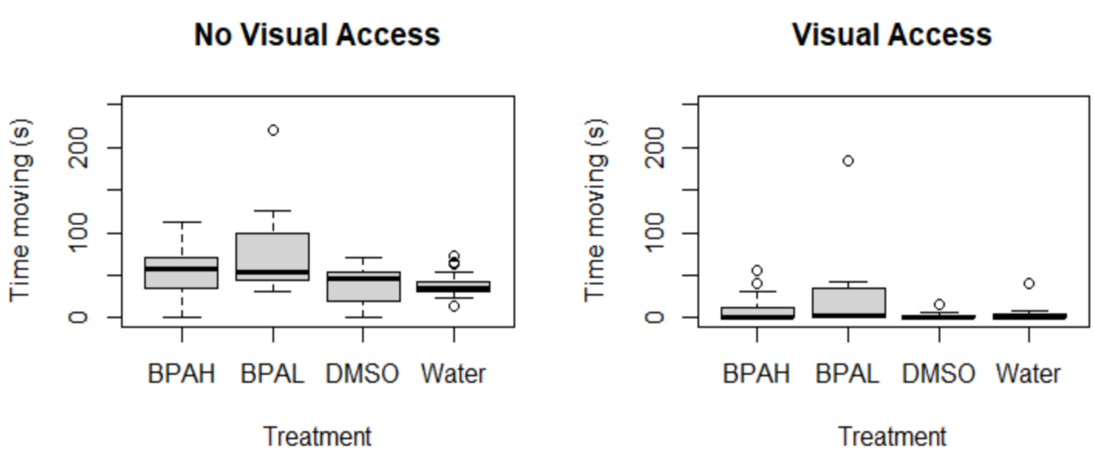

**Figure 4: Time Spent Moving (s)**. Fish with olfactory, but not visual access, to the social stimulus (left panel) spent more time moving than did fish with both visual and olfactory access to the social stimulus (right panel), with BPA-treated fish spending more time moving than control groups. Boxplot elements are as in Fig. 3.

Location Fish Spent Most Time in:

Zebrafish treated with low levels of BPA spent more time at a distance from the social stimulus than did fish in other treatment groups, but all fish with visual access to the social stimulus spent nearly all of the time in the very-near zone

Zebrafish are highly social: regardless of treatment group or visual access, fish spent most of their time adjacent to the stimulus social group in the near and very-near zones (Fig 2). The average time spent in the near zone was 204.3 s (SE = 7.07), with 136 of these seconds (SE = 10.15) in the very-near zone. Fish spent an average of 84.3 s (SE = 6.85) in the far zone. Fish treated with BPA in early life spent roughly the same amount of time in the very-near zone than did fish in the DMSO or water control groups, signifying no significant effect of BPA on location as tested by ANOVA ($F = 1.1$, $df = 3, 123$, $P = 0.33$, Fig. 5). Visual access to the social stimulus was a significant predictor of time spent in the very-near zone ($F = 104.6$, $df = 2, 123$, $P < 0.01$; Fig. 5), however the Tukey posthoc test shows no significant differences in time spent in the very-near zone between fish that had experienced different BPA treatments ($P > 0.05$ for all comparisons). Zebrafish with both visual and olfactory access to a social stimulus spent, on average, much more time in the very-near zone (230 s, SE = 11.44), than did fish that had olfactory access only (67 s, SE = 5.43, Fig. 5). There was no significant interaction found between treatment and visual access to the social stimulus (F = 0.9, DF = 3, 123, P = 0.44).

When looking at their time spent in the far zone, BPA-treated fish did spend significantly more time in the far zone than did DMSO or water treated fish, yielding a significant effect of BPA in the ANOVA (F = 5.0, DF = 3, 123, P < 0.01, Fig. 6). The Tukey posthoc tests also support this, finding significant differences between time spent in the far zone by fish in water and BPA-Low treatment conditions (P < 0.01). Visual access to the social stimulus and the interaction between treatment and visual access were statistically significant predictors of time spent in the far zone (F = 50.9, DF = 2, 123 P < 0.01 and F = 3.3, DF = 3, 123, P = 0.02, respectively).

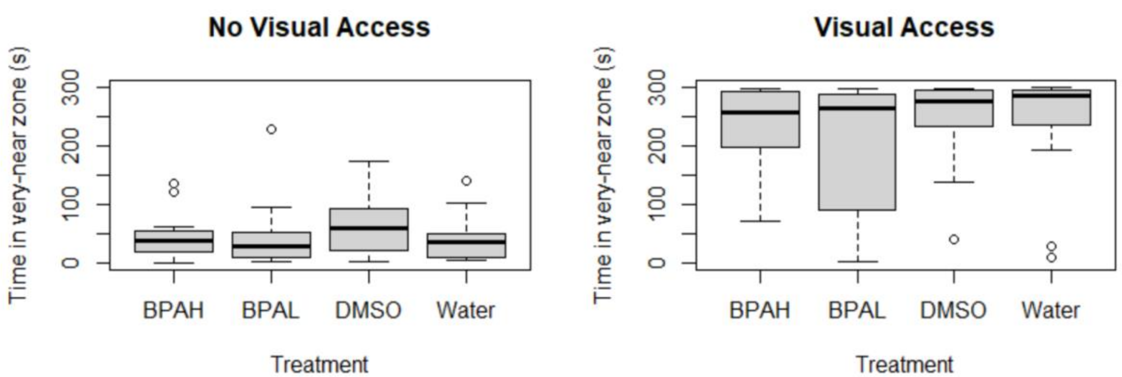


**Figure 5: Time Spent in the Very-Near Zone (s)**. Fish with visual and olfactory access (right panel), regardless of BPA treatment, spent significantly more time in the very-near zone, than did fish with only olfactory access (left panel). The very-near zone was the small area immediately adjacent to the social stimulus. Boxplot elements are as in Fig. 3.

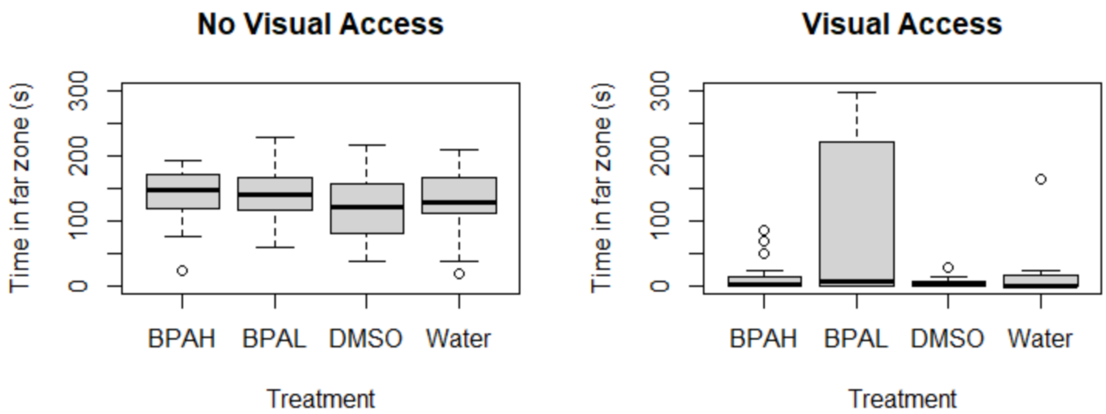


**Figure 6: Time Spent in the Far Zone (s)**. BPA-treated fish spent more time in the far zone than the control groups. Fish with no visual, but olfactory access (left panel) spent more time in the far zone than fish with both visual and olfactory access (right panel). The far zone was the half of the subject box that was furthest from the social stimulus. Boxplot elements are as in Fig. 3.

# CHAPTER 4

# DISCUSSION

As predicted, we found that early exposure to low doses of Bisphenol-A led to an increase in adult zebrafish movements and decrease in proximity to the social stimulus. We did not find evidence of early BPA treatment leading to a shift in sensory preferences, however visual access showed higher receptiveness than olfactory access in a social context. Our results reveal that even low concentrations of BPA can have long-lasting effects on an organism's (specifically zebrafish's) social behavior and movements, affecting their chance of survival in an increasingly polluted environment. These are crucial findings for epidemiology research as they distinguish the importance of eliminating these toxins due to their adverse effects shown in zebrafish which may be relevant to other species as well. Zebrafish share 70% of their genes with humans and have the same major organs involved in the metabolic process as humans, making them great models for studying human diseases and human internal operations. (Tsegay et al 2019). This means the changes BPA has on zebrafish behavior can not only cause a fundamental impact to aquatic life, but also humankind, supporting current understanding of the detrimental quality of endocrine disruptors and need for their eradication.

Although it dissolves rapidly, BPA pseudo-persists in the environment, as it is continually infused into the water (Flint et al., 2012). This continual exposure means that the chemical can never fully escape the organism's system (Lindholst et al., 2003). Here, though, we focused on transient exposure to BPA during a fixed point during early development, since time of exposure may be especially important to the effects of Bisphenol-A. Larval zebrafish show little, if any, social behavior before three weeks of age, when they begin to prefer to be in an arena with visual access to other fish of similar size (Dreosti et al., 2015). Since here, we treated fish with an endocrine disruptor before they developed social behavior, one might expect BPA to have no impacts at all on social behavior. However, BPA can have profound acute effects on larval zebrafish sensory behavior, for example, making them more susceptible to changes in vision as a result of light pollution (Suriyampola et al., 2021). In this study, we found that short-term exposure early in life led to long-term effects for zebrafish social behavior by making them more active and less social. However, the mechanisms that led to this impact are unclear. BPA effects were at highest intensity during the week of exposure and since zebrafish are able to rapidly expel toxins from their bodily tissues (Lindholst et al., 2003), BPA likely did not stay in the fish's system for long after exposure ceased. Why then are we still seeing effects to fish behavior and movements months after the BPA exited their system? What happened during the week of exposure to cause these observed long-term effects? To answer these questions, additional research is necessary to understand the persistence of this endocrine disruptor's impacts.

In this study, BPA-Low treated fish had the most significant differences as opposed to BPA-High groups, when compared to control groups. With a non-monotonic dose response, low levels of BPA may have stronger impacts than higher levels. This nonlinear relationship between dose and effect is not uncommon for studies regarding natural hormones and endocrine disruptors (Vandenberg et al., 2012), including studies of zebrafish behavior (Lopes 2021). Endocrine disruptors challenge traditional toxicology concepts, showing effects at concentrations well below those that are currently being used in toxicological studies (Welshons et al., 2003). This creates challenges for risk assessment since some toxicology studies may grossly underestimate their effects by using only higher doses to study biological impact. Hormones act indirectly by binding to specific protein receptors and the relationship between receptor occupancy and hormone concentration, as well as between receptor occupancy and response can be nonlinear (Welshons et al., 2003). Since endocrine receptors, like BPA, can mimic hormonal mechanisms, other studies have also found the same non-monotonic dose response to organisms exposed to low concentrations of these chemicals (Welshons et al., 2003). These studies agree with our own result that BPA-Low treated fish had the most dramatic results. Further research is needed to understand the mechanisms behind this nonlinear dose response and uncover methods to decrease adverse effects for both small and large doses.

Zebrafish treated with BPA were more mobile in both distance moved and time spent moving than were zebrafish in the control groups. This is an interesting finding since most previous research on Bisphenol-A impacts on zebrafish movements show a decrease in locomotion. Zebrafish's normal swimming behavior is often regulated by the release of dopamine, and past studies show that endocrine-disrupting chemicals disrupt neurotransmitter levels, therefore altering dopamine regulation (Gu et al., 2022). In this experiment, though, zebrafish displayed opposite results by boosting activity levels. One possible explanation for this increase in movement is due to an increase in their stress or cortisol levels over the several months between the early BPA treatment and adult behavioral assays. BPA is a top inducer of stress, along with several other chemical pollutants that have been linked to raised cortisol levels and stress axis gene expressions in fish (Wei et al., 2020). Chronic stress can induce a change in dopamine levels, ultimately decreasing social behavior in fish (Saszik and Smith., 2018). Anxiety behavior, such as quicker movements, generally parallel cortisol levels. For example, in a study that measured the relationship between novelty stress and behavior, higher stress levels directly correlated with more erratic movements in zebrafish (Cachat et al 2010). Similarly, Wei et al. (2020) found that long term exposure of zebrafish to an endocrine-disruptor led to increased cortisol levels within the whole body, instilling anxiety-like behavior in the fish, including more erratic movement and more time spent in specific zones of the testing tank. In this study, we found similar impacts with short-term BPA exposure early in development, suggesting that chronic stress may persist for long periods of time.

Our results also showed that BPA-treated fish were less motivated by a social stimulus, spending more of their time far from the social stimulus. Salahinejad et al. (2020) found similar results for Bisphenol-S (which is considered to be a safer alternative to Bisphenol-A), with large changes to social behavior and shoaling. Treated fish displayed decreased shoal cohesion and a lower preference to groups, likely as a result of disruption to the isotocinergic and vasotocinergic neuro-endocrine systems (Salahinejad et al., 2020). These shifts may again be linked to stress. Zebrafish isolated at birth also displayed profound disruption to their neural activity, such that social interaction became overwhelming as opposed to rewarding (Tunbak et al., 2020). These isolated or “lonely” fish, as the researchers labeled them, recovered social behavior when treated with Buspirone (which has been shown to reduce anxiety), again suggesting that stress was involved (Tunbak et al., 2020). Here, we found similar long-term effects after short-term exposure to BPA. Additional research is needed to determine how exactly acute stressors have such profound long-term impacts.

Our findings emphasize the importance of vision to social behavior as well. The natural social drive in zebrafish is extremely strong, as visual access to the social stimulus caused most fish, regardless of treatment, to spend the majority of the five minutes in the very-near zone. Zebrafish use both vision (e.g., Engeszer et al., 2004) and olfaction (e.g., Gerlach & Wullimann, 2021) in learning social preferences for particular types of individuals. Although visual cues are more effective than olfactory signals, both cues also can create positive benefits of social behavior on individual fear response (Faustino et al., 2017). Visual circuits have been vastly studied in zebrafish with research finding the visual system in fish to be highly sensitive to local object motion (Bollmann, 2019). This explains why fish in our study were more responsive when given visual access to a group of conspecifics. In this investigation, visual cues were verified as more important, since fish given olfactory access alone did not spend as much time in proximity to the social stimulus.

Zebrafish rely on shoaling, mating, communication, and other behavior with conspecifics to survive and reproduce in the wild. The strength of a shoal is often dependent on environmental and individual factors such as changing habitats, early experience, with most changes coming from stress-inducing situations (Facciol & Gerlai, 2020). Fish in this study were kept in a controlled environment, with BPA exposure being the factor leading to high levels of stress and ultimately weaker shoaling techniques. Our study used very low concentrations of BPA, which was still able to disrupt their normal social behavior. Results also suggest that even short-term exposure to weak concentrations may lead to chronic stress that has profound impacts on adult social behavior. This begs the need for further research and safety precautions to be placed on such chemicals as to avoid any lasting negative effects and understand the full extent of its toxicity.

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