INVESTIGATION OF THE PRESENCE AND IMPACT OF HEAVY METALS IN THE TRINITY RIVER

by

Drew Carlton

Submitted in partial fulfillment of the
requirements for Departmental Honors in
the Department of Biology
Texas Christian University
Fort Worth, Texas

May 5, 2025

INVESTIGATION OF THE PRESENCE AND IMPACT OF HEAVY METALS IN THE TRINITY RIVER

Project Approved:

Supervising Professor: Marlo Jeffries, Ph.D.

Department of Biology

Meredith Curtis, Ph.D.

Department of Biology

ABSTRACT

The Trinity River is an important body of water that serves as a source of drinking water, a location for recreational activities, and an ecologically significant habitat for a variety of organisms. The Trinity River is located in the densely populated and urbanized Dallas-Fort Worth Metroplex, creating an increased risk of pollution from contaminated runoff, wastewater treatment plant discharge, and industrial plant effluent. Heavy metals were of particular concern as a class of contaminants that are relevant in urban environments and can induce long-term negative effects on aquatic organisms. This project aimed to evaluate the presence and biological impact of heavy metals in sediment and surface water samples collected from the Trinity River. Larval fathead minnows were exposed to sediment and surface water samples collected from the Trinity River, and gene expression levels of four biomarkers were measured. Metallothionein was used as a biomarker of exposure to heavy metals, whereas catalase, superoxide dismutase, and heat shock protein 70 were used as biomarkers of adverse effects. All biomarkers showed significant upregulation in specific groups, providing evidence of heavy metal contamination and impact in the Trinity River.

INTRODUCTION

The Trinity River is a vital body of water in the state of Texas, as it serves as a water source for both the Dallas-Fort Worth and Houston Metropolitan areas (Texas Water Development Board, ND). Over 1.4 million Texas residents get their drinking water directly or indirectly from a group of water sources in Tarrant County that include the Trinity River and lakes fed by the Trinity River (City of Fort Worth, 2024). The Trinity River is a popular location for paddlers, hikers, and mountain bikers, as there are over 130 continuous miles of paddling trails and over 100 miles of walking and biking trails in the surrounding area (Tarrant Regional Water District, 2024). In addition to serving as a source of drinking water and recreation, the Trinity River, like many watersheds, also provides numerous ecosystem services, including erosion control, waste treatment, nutrient cycling, climate regulation, and pest control (Cathey et al., 2007). The Trinity River also provides habitat to numerous species, including game fish such as freshwater drum, striped bass, and three different species of catfish (Texas Parks and Wildlife Department, 2002). Given the numerous resources provided by the Trinity River, there is a need to monitor and maintain its health.

As an urban watershed, one of the primary factors threatening the health of the Trinity River is pollution, given the risk of contamination from a variety of sources, including effluent from wastewater treatment plants (WWTPs), industrial plants, and contaminants in runoff. Exposure to effluent from WWTPs has been linked with changes in both gene expression and behavior of aquatic organisms (Bjorkblum et al., 2009; Garcia-Reyero et al., 2011). As population sizes increase, the volume of WWTP effluent has increased, thereby enhancing the likelihood of environmental contamination (Simmons et al., 2017). Thus, the Trinity River is at a heightened risk of contamination from WWTP effluent, given that the Dallas-Fort Worth (DFW)

Metroplex is rapidly growing in population (Dearman, 2023). Further, the Trinity River is effluent-driven, with as much as 95% of the flow coming from effluent in certain locations in the river (Trinity River Authority of Texas, 2012). In addition to WWTP effluent, contaminated runoff serves as a potential source of contamination in urban watersheds (Baker et al., 2014). Residential lawns, roofing materials, and roads are all potential sources of runoff that can eventually lead to contamination of the Trinity River.

One group of compounds often present in each of the aforementioned sources of contamination is heavy metals (Qasem et al., 2021; Klotter, 2003). Heavy metals are of particular concern because of the negative impact they can have on aquatic species and their ability to cause longer-lasting impacts than other chemicals, as heavy metals are not biodegradable and thus persistent in aquatic environments. The changes heavy metals can induce in aquatic species include generalized and oxidative stress (Kadim & Risjani, 2022; Zelikoff, 1993). Generalized stress refers to the denaturation of proteins, the infliction of DNA damage, the inhibition of DNA repair mechanisms, and the disruption of ion concentrations in the cell, all of which can be carried out by heavy metals. Heavy metals are also strong reducing agents that can participate in redox reactions that lead to the production of reactive oxygen species. Those reactive oxygen species then cause oxidative stress with impacts that can include DNA damage, protein denaturation, and lipid peroxidation. Moreover, extended exposure to heavy metals has been linked with longer-term effects such as mortality, immunosuppression, and reproductive impairment (Zelikoff, 1993; Sellin & Kolok, 2006).

One approach for evaluating heavy metal presence and effect relies upon assessing the biomarker responses of exposed fish, like the fathead minnow (*Pimephales promelas*), which has been used extensively in aquatic toxicology testing, including heavy metal exposure testing

(Ankley & Villenueve, 2006; Kadim & Risjani, 2022). A common biomarker indicative of heavy metal exposure is metallothionein, a protein involved in the sequestering of heavy metals in the cell to prevent cellular damage (Kadim & Risjani, 2022; Yang et al., 2024). Biomarkers indicative of adverse effects associated with exposure to heavy metals include catalase, superoxide dismutase, and heat shock protein 70. Catalase and superoxide dismutase both combat oxidative stress through the breakdown of reactive oxygen species, whereas heat shock protein 70 is a chaperone protein that combats the negative effects of generalized stress (Kadim & Risjani, 2022).

The goal of this study was to evaluate the presence and biological impact of heavy metals in sediment and surface water collected from the Trinity River. To accomplish this goal, larval fathead minnows (*Pimephales promelas*) were exposed to sediment and surface water samples collected from three sites along the Trinity River on four occasions from July 26th, 2024, to October 16th, 2024. After seven day exposures, fish health was evaluated by comparing the expression of metallotionein (*mtt*), catalase (*cat*), superoxide dismutase (*sod*), and heat shock protein 70 (*hsp70*) measured in larvae exposed to Trinity River water and sediments to that of control larvae exposed to 'clean' laboratory water.

METHODS

Site Selection and Sampling

Sediment and surface water samples were collected from three sites on the Trinity River. The three sites were selected based on their location downstream of WWTPs and other potential sources of heavy metal contaminants, as shown in Table 1. Each site was also located near a major highway, with the Scrap Metal Recycling Site being located near Interstate Highway 45, the Biosolids Processing Site being located near Interstate Highway 30, and the Naval Air

Station Site being located near Interstate Highway 30. Samples were collected on four dates for four different exposures with collections on July 26th, 2024 (late July), September 2nd, 2024 (early September), September 25th, 2024 (late September), and October 16th, 2024 (mid-October).

Table 1. Sampling site information with coordinates and potential point sources for each site.

Site	Coordinates	Potential Point Sources
Scrap Metal Recycling Site	32°43’45.3”N 96°45’47.4”W	Central Wastewater Treatment Plant Okon Scrap Metal Recycling Plant
Biosolids Processing Site	32°47’17.9”N 97°09’23.9”W	Village Creek Wastewater Treatment Plant SYNAGRO Biosolids Dewatering and Processing Facility
Naval Air Station Site	32°46’51.5”N 97°24’36.3”W	River Oaks Filter Plant Naval Air Station Joint Reserve Base, and Lockheed Martin Cooperation

Figure 1. Map of sampling sites along the Trinity River.

Exposure Setup

Eggs were collected from a stock of lab-grown fathead minnows housed at Texas Christian University six days before each collection date. The eggs were obtained by placement of 10 cm long pieces of 10.2 cm diameter polyvinyl chloride schedule 40 pipe into tanks containing adult fathead minnows to serve as breeding structures. Structures containing eggs were then placed into 1 L beakers filled with dechlorinated municipal water. The beakers were placed in an incubator with a controlled temperature of 27°C and outfitted with an airline. The beakers were cleaned, and 67% of the water was changed every 24 hours. Only fish that hatched on the start date of the exposure period were used in the exposure.

Exposure Protocol

Each exposure period began on the corresponding sample collection date. Four 1L crystalizing dishes were used for the exposure, one for each site and one for a control group. The control group dish was filled with 750 mL of warm dechlorinated municipal water and no sediment. The experimental dishes each received sediment that filled the bottom 1.25 cm in the dish from one sampling site, and 750 mL of surface water collected from the same sampling site. The crystallizing dishes were outfitted with an air line and placed in an incubator with a temperature maintained at 26°C. Water temperature, pH, conductivity, and dissolved oxygen levels were measured using portable electrodes, and a 67% water change was performed every day for each crystallizing dish. Each exposure period lasted seven days. Following the exposure period, fish were sacrificed by immersion in a lethal concentration of buffered tricaine mesylate (MS-222). Body tissues were stored in individual tubes and preserved in a freezer at -80°C.

Gene Expression Analysis

Gene expression analysis on the body tissues collected was performed via qPCR as outlined in Jeffries et al. (2014). Total RNA was extracted from the tissues using a Maxwell Research System and then converted to cDNA through the use of qScript cDNA synthesis. Real-time qPCR of the cDNA with SYBR Green was performed with a CFX Connect real-time PCR detection system. The expression of the target genes was measured by the standard curve method using ribosomal L8 as a reference gene. Gene expression levels of biomarkers were normalized to a housekeeping gene by date, with the geometric mean of ribosomal L8 and ARP used for late July samples, the geometric mean of ribosomal L8 and ARP for early September samples, the geometric mean of ribosomal L8 and ARP for late September samples, and ribosomal L8 for mid-October samples. Gene expression was further normalized to the control group of the late July exposure for each respective biomarker.

Statistical Analysis

A one-way ANOVA test for variance was conducted to determine the statistical significance of differences between experimental groups and the control for each respective date. A Levene's test was performed to evaluate variance. If variances were unequal, a nonparametric Wilcoxon test was run to determine statistical significance. If variances were equal, ANOVA was used to determine statistical significance. For visualization purposes, all targeted gene expression values were standardized to the late July control group. The alpha value for all tests was set to 0.05. All statistical analyses were conducted using JMP stats software.

RESULTS

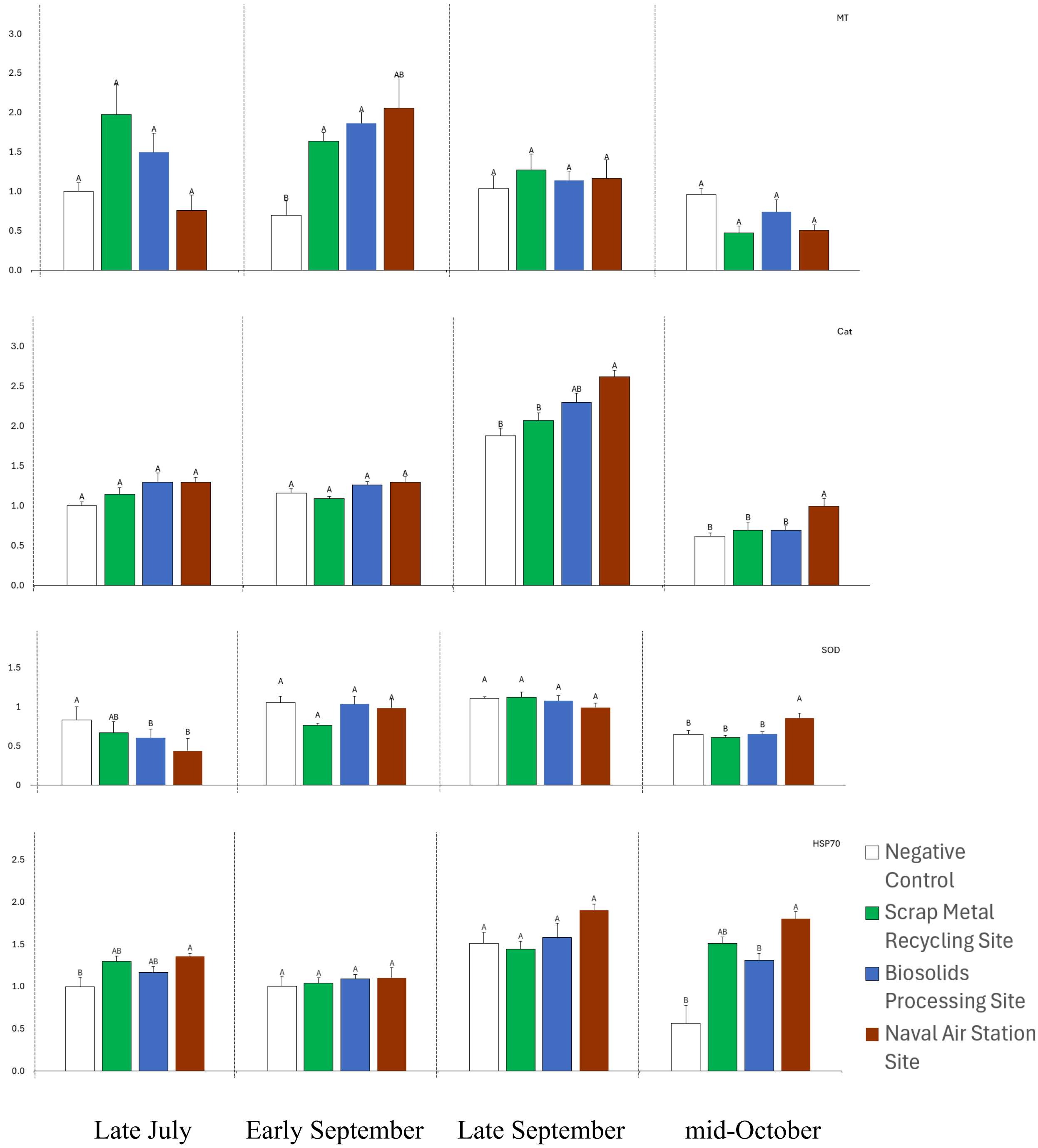

Figure 2. Graphs showing differential expression of three biomarkers of heavy metals (mtt=metallothionein, cat=catalase, sod=superoxide dismutase, hsp70=heat shock protein 70) across four 2024 sampling dates (July 26th, September 2nd, September 25th, October 16th). For visualization, all gene expression data are normalized to that of the late July control group.

Late July Biomarker Responses

Metallothionein expression was found to be significantly different between the fish exposed to the sediment and surface water samples collected from the sites (ANOVA, p=.0192, Figure 2), but no significant differences were found between any two groups. No significant differences in catalase expression were found in this exposure period. Superoxide dismutase was found to be significantly down-regulated in the fish exposed to samples collected from the Biosolids Processing Site (Tukey-Kramer, p=.0028, Figure 2) and the Naval Air Station site (Tukey-Kramer, p=.0353, Figure 2) when compared to the control group. Heat shock protein 70 was significantly upregulated in the fish exposed to samples collected from the Naval Air Station Site when compared to the control group (Tukey-Kramer, p=.0237, Figure 2).

Early September Biomarker Responses

Metallothionein was significantly upregulated in the fish exposed to surface water and sediment samples collected from the Scrap Metal Recycling Site (Steel-Dwass, p=.0405, Figure 2) and the Biosolids Processing site (Steel-Dwass, p=.0405, Figure 2) when compared to the control group. No significant differences were found in the expression of catalase, superoxide dismutase, or heat shock protein 70.

Late September Biomarker Responses

No significant differences were found in the expression of metallothionein in this exposure period. Catalase was found to be significantly upregulated in the fish exposed to samples collected from the Naval Air Station Site when compared to the control group (Tukey-Kramer, p=.0461, Figure 2) and to the fish exposed to samples collected from the Scrap Metal Recycling Site (Tukey-Kramer, p=.0284, Figure 2). No significant differences in expression of superoxide dismutase or heat shock protein 70 were detected in this exposure period.

Mid-October Biomarker Responses

No significant differences were found in the expression of metallothionein in this exposure period. Catalase was significantly upregulated in the fish exposed to samples collected from the Naval Air Station site when compared to the control group (Tukey-Kramer, p=.0030, Figure 2) and to fish exposed to samples collected from the Scrap Metal Recycling site (Tukey-Kramer, p=.0039, Figure 2) and the Biosolids Processing Site (Tukey-Kramer, p=.0039, Figure 2). Heat shock protein 70 was significantly upregulated in fish exposed to surface water and sediment samples collected from the Naval Air Station site when compared to the control group (Tukey-Kramer, p=.0005, Figure 2) and compared to fish exposed to samples collected from the Biosolids Processing site (Tukey-Kramer, p=.0022, Figure 2). Superoxide dismutase was upregulated in the fish exposed to surface water and sediment samples collected from the Naval Air Station site when compared to the control group (Tukey-Kramer, p=.0205, Figure 2) and when compared to fish exposed to samples collected from the Scrap Metal Recycling site (Tukey-Kramer, p=.0052, Figure 2) and the Biosolids Processing site (Tukey-Kramer, p=.0232, Figure 2).

DISCUSSION

Biomarker Responses In Late July

The upregulation of heat shock protein 70 in the fish exposed to sediment and surface water samples collected from the Naval Air Station site indicates the induction of generalized stress (Kadim & Risjani, 2022). Since there was no corresponding significant increase in metallothionein for that site on the same sampling date when compared to the control group, no definitive conclusions can be drawn about the presence of heavy metals in the Naval Air Station site on that date. Generalized stress and heat shock protein 70 expression can result from a variety of factors other than heavy metal exposure including increased temperature, changes in salinity, exposure to ultraviolet radiation, infection by pathogenic species, and exposure to microplastics (Moreira-De-Sousa et al., 2018; Kuats et al., 2024).

Biomarker Responses in Early September

The upregulation of metallothionein in the fish exposed to sediment and surface water samples collected from both the Scrap Metal Recycling site and the Biosolids Processing site, when compared to the control group, provides evidence of heavy metal presence in the Trinity River at those sites on the sampling date (Kadim & Risjani, 2022). Since there were no corresponding significant changes in biomarkers of effect, no definitive conclusions can be drawn about the impact of heavy metals on the early September sampling date.

Biomarker Responses in Late September

The significant upregulation of catalase in the fish exposed to sediment and surface water samples collected from the Naval Air Station site indicates the induction of oxidative stress in those fish (Kadim & Risjani, 2022). Since there was no corresponding increase in metallothionein at that site on the late September sampling date, no conclusions can be drawn about the presence of heavy metals in the samples or that heavy metals were responsible for the induction of oxidative stress. Oxidative stress can result from a variety of factors outside of heavy metals including exposure to microplastics which are commonly found in urban watersheds like the Trinity River (Suman et al., 2021).

Biomarker Responses in Mid-October

The significant upregulation of catalase, superoxide dismutase, and heat shock protein 70 in the fish exposed to samples collected from the Naval Air Station Site indicates the induction of oxidative and generalized stress in those fish (Kadim & Risjani, 2022). Because there was no corresponding increase in the expression of metallothionein, it cannot be concluded that heavy metals were responsible for these impacts on aquatic organisms. As highlighted previously, both generalized and oxidative stress can be induced by other environmental factors including microplastics which are commonly found in urban watersheds and have been associated with the induction of both generalized and oxidative stress (Suman et al., 2021; Kuats et al., 2024).

Temporal Variation in Biomarker Responses

Across the four sampling dates, there was no consistent trend in differential expression of specific genes, suggesting temporal variation in the presence of heavy metals and other environmental factors capable of inducing generalized and oxidative stress. One potential explanation for this is the seasonal variation of contamination associated with runoff events, as well as seasonal variation in chemical use. For example, residential fertilizer use is most common from late summer to early fall (Janssens & Stocks, 2012; Lee et al., 2014). Because fertilizers can contain heavy metals, including Pb, As, and Cd, runoff following rainfall events occurring in the late summer and early fall may explain why the expression of metallothionein was significantly different between groups in our earlier exposure periods (late July and early September). Likewise, weather patterns and subsequent fluctuations in water flow also may explain the temporal variation in metallothionein expression, as discharge rates and precipitation amounts are each subject to seasonal variation. Discharge rate refers to the amount of water passing through a certain point in the river in a specific amount of time. Higher flow rates result in increased sediment disturbance, which can lead to an increased likelihood that chemicals associated with sediments, including metals, enter the aqueous phase and become bioavailable to aquatic organisms. Precipitation events result in variations in the amount of runoff from residential lawns and roads, which leads to variations in subsequent contaminant loads.

Another factor in the variation in the biomarker responses of fish exposed to heavy metals is the variance in effluent released by both WWTPs and industrial plants into the Trinity River. Effluent from WWTPs and industrial facilities was identified as an important potential source of heavy metal contamination, but the release of effluent into the Trinity River is not guaranteed to be consistent throughout sampling dates.

Spatial Variation in Biomarker Responses

Different sites along the Trinity River do not experience equivalent changes in weather patterns, meaning our different sampling sites could experience different effects of weather on a singular sampling date. Additionally, our sampling sites were not equidistant from the identified point sources, creating another potential source of variance between exposure groups. Further, each of the sites is subjected to contamination from specific sources such as the scrap metal recycling facility, the biosolids processing plant, and the naval air station joint base. These different facilities release effluent with varying concentrations of compounds, including heavy metals. Varying distances of sampling sites from effluent release locations, varying chemical makeup of the effluent, and varying weather events could help explain the regulation of biomarkers of heavy metal exposure and impact.

Alignment of Biomarker Responses

The biomarkers selected for inclusion in the present study have shown sensitivity in response to heavy metals exposure in larval fathead minnows; however, previous studies have reported some inconsistency in the responsiveness of these biomarkers in aquatic organisms exposed to various concentrations of heavy metals. It is not uncommon to see all biomarkers upregulated in response to metals as the upregulation of metallothionein does not always prevent oxidative stress and generalized stress meaning biomarkers of adverse impacts can experience upregulation along with metallothionein (Sibiya et al., 2022). Research has shown that this is not always the case as in a study that involved the exposure of *Astyanax serratus* to Ti and Pb, metallothionein was significantly upregulated in the groups coexposed to both, but not significantly changed in groups exposed to only Pb (Delmond et al., 2019). In another study that involved the exposure of adult zebrafish to concentrations of Ag, catalase and superoxide dismutase were significantly down-regulated in certain experimental groups compared to control (Renuka et al., 2020). However, this finding contrasts with that of another study that involved the exposure of *Ruditapes decussatus* to concentrations of Ti that resulted in significant increases in catalase and superoxide dismutase compared to control (Saidani et al., 2019). The lack of significant change in expression of biomarkers of effect, such as superoxide dismutase and catalase, can in part be attributed to the efficacy of metallothionein. Metallothionein is involved in the sequestering of heavy metals for the prevention of negative impacts such as oxidative stress (Yang et al., 2024). Therefore, metallothionein expression may have sequestered heavy metals, thereby, limiting their ability to induce oxidate stress, which in turn limits the need to induce the expression of superoxide dismutase and catalase.

Other Contaminants

The biomarker responses of fish exposed to water and sediments collected in late September and mid-October exposure periods indicated the occurrence of generalized and oxidative stress, however, there was no evidence of heavy metal exposure. Specifically, catalase was significantly upregulated in the Naval Air Station Site in the late September experiment, indicating oxidative stress was induced in the fish. Similarly, the Naval Air Station group in the mid-October exposure period had significantly upregulated catalase and superoxide dismutase, and heat shock protein 70 compared to the control. If heavy metals were the reason for the significant differences seen in the biomarkers of effect, we would expect metallothionein to be upregulated. This suggests that a different contaminant or suite of contaminant may have been present. For example, it is known that microplastics, which are likely to be found in urban systems are capable of inducing generalized and oxidative stress (Kuats et al., 2024; Suman et al., 2021). As such, future studies aimed at identifying chemical contaminants, beyond heavy metals, in the Trinity River are warranted.

CONCLUSIONS

The differential expression of the biomarkers of heavy metals allows for a few conclusions to be drawn. Upregulation of metallothionein in early sampling dates indicates the presence of heavy metals in the surface water and sediment of the Trinity River. The lack of significant increases in the expression of biomarkers of effect indicates that heavy metals are not necessarily having a significant adverse impact on aquatic species. A topic for a future study could include chemical testing of surface water and sediment samples collected from the Trinity River for heavy metals to help explain the metallothionein results found in this project. Secondly, upregulation of biomarkers indicative of oxidative and generalized stress, with no change in metallothionein in later sampling dates, provides evidence of a different chemical in the Trinity River causing negative impact on aquatic species. A future study could target the possibility of another chemical in the Trinity River that is responsible for the changes in biomarker expression observed in this project.

REFERENCES

Ankley, G. T., & Villeneuve, D. L. (2006). The fathead minnow in aquatic toxicology: past, present and future. Aquatic toxicology (Amsterdam, Netherlands), 78(1), 91–102. https://doi.org/10.1016/j.aquatox.2006.01.018

Baker, B. H., Martinovic-Weigelt, D., Ferrey, M., Barber, L. B., Writer, J. H., Rosenberry, D. O., Kiesling, R. L., Lundy, J. R., Schoenfuss, H. L. 2014. Identifying Non-point Sources of Endocrine Active Compounds and Their Biological Impacts in Freshwater Lakes. https://doi.org/10.1007/s00244-014-0052-4

Björkblom, C., Högfors, E., Salste, L., Bergelin, E., Olsson, P., Katsiadaki, I., Wiklund, T. 2009. Estrogenic and androgenic effects of municipal wastewater effluent on reproductive endpoint biomarkers in three-spined stickleback (Gasterosteus aculeatus), Environmental Toxicology and Chemistry, Volume 28, Issue 5, Pages 1063–1071. https://doi-org.ezproxy.tcu.edu/10.1897/08-337.1

Cathey, J.C., Locke, S.L., Feldpausch, A.M., Parker, I.D., Frentress, C., Whiteside, J., Mason, C., Wagner, M.W., 2007. Linking water conservation and natural resource stewardship in the Trinity River Basin. Texas Parks and Wildlife Department, Austin, TX. https://tpwd.texas.gov/huntwild/wild/research/highlights/taxa/publications/Cathey_etal_2007_TrinityRiver.pdf

City of Fort Worth. 2024. 2024 Water Conservation Plan. https://www.fortworthtexas.gov/files/assets/public/v/4/water/documents/save-water/fort-worth-2024-water-conservation-plan.pdf

Dearman, E. 2023. Dallas-Fort Worth no longer leads nation in population gain. Here's where we rank, Fort Worth Star-Telegram. https://www.star-telegram.com/news/local/article301928999.html

Delmond, K. A., Vicari, T., Guiloski, I. C., Dagostim, A. C., Voigt, C. L., de Assis, H. C. S., Ramsdorf, W. A., Cestari, M. M. 2019. Antioxidant imbalance and genotoxicity detected in fish induced by titanium dioxide nanoparticles (NpTiO2) and inorganic lead (PbII) Environ. Toxicol. Pharmacol., 67 (2019), pp. 42-52.

Garcia-Reyero, N., Lavelle, C., Escalon, B., Martinovi, D., Kroll, K., Sorensen, P., Denslow, N. 2011. Behavioral and genomic impacts of a wastewater effluent on the fathead minnow, Aquatic Toxicology 101 (2011), Pages 38-48. https://doi.org/10.1016/j.aquatox.2010.08.014

Janssens, L., Stoks, R. 2012. How does a pesticide pulse increase vulnerability to predation? Combined effects on behavioral antipredator traits and escape swimming, Aquatic Toxicology Volume 110-111, Pages 91-98. https://doi.org/10.1016/j.aquatox.2011.12.019

Jeffries, M.K.S., Kiss, A.J., Smith, A.W., Oris, J.T., 2014. A comparison of commercially available automated and manual extraction kits for the isolation of total RNA from small tissue samples. BMC Biotechnol. 14 (1), 94.

Kadim, M. K., Risjani, Y. 2022. Biomarker for monitoring heavy metal pollution in aquatic environment: An overview toward molecular perspectives, Emerging Contaminants, Volume 8, Pages 195-205. https://doi.org/10.1016/j.emcon.2022.02.003

Klotter, J. (2003). Heavy metals in fertilizer. (Shorts). Townsend Letter for Doctors and Patients, (234), 27+. https://link.gale.com/apps/doc/A95676493/HRCA?u=txshracd2573&sid=bookmark-HRCA&xid=4127525d

Kauts, S., Mishra, Y., & Singh, M. P. (2024). Impact of Polyethylene Terephthalate Microplastics on Drosophila melanogaster Biological Profiles and Heat Shock Protein Levels. Biology, 13(5), 293. https://doi-org.ezproxy.tcu.edu/10.3390/biology13050293

Lee, K. E., Barber, L. B., Schoenfuss, H. L. 2014. Spatial and Temporal Patterns of Endocrine Active Chemicals in Small Streams Indicate Differential Exposure to Aquatic Organisms, Journal of the American Water Resources Association, Volume 50, Number 2, Pages 401-419. https://doi.org/10.1111/jawr.12162

Moreira-de-Sousa, C., de Souza, R. B., & Fontanetti, C. S. (2018). HSP70 as a Biomarker: an Excellent Tool in Environmental Contamination Analysis—a Review. Water, Air & Soil Pollution, 229(8), 1. https://doi-org.ezproxy.tcu.edu/10.1007/s11270-018-3920-0

Qasem, N.A.A., Mohammed, R.H. & Lawal, D.U. 2021. Removal of heavy metal ions from wastewater: a comprehensive and critical review. npj Clean Water 4, 36 (2021). https://doi.org/10.1038/s41545-021-00127-0

Renuka, R. R., Ravindranath, R. R. S., Raguraman, V., Yoganandham, S. T., Kasivelu, G., Lakshminarayanan, A. 2020. In vivo toxicity assessment of Laminarin based silver nanoparticles from Turbinaria ornata in adult zebrafish (Danio rerio) J. Cluster Sci., 31 (2020), pp. 185-195. https://doi.org/10.1007/s10876-019-01632-6

Saidani, W., Sellami, B., Khazri, A., Mezni, A., Dellali, M., Joubert, O., Sheehan, D., Beyrem, H. 2019. Metal accumulation, biochemical and behavioral responses on the Mediterranean clams Ruditapes decussatus exposed to two photocatalyst nanocomposites (TiO2 NPs and AuTiO(2)NPs)Aquat. Toxicol., 208, pp. 71-79. https://doi.org/10.1016/j.aquatox.2019.01.003

Sellin, M. K., & Kolok, A. S. 2006. Cadmium exposures during early development: do they lead to reproductive impairment in fathead minnows?. Environmental toxicology and chemistry, 25(11), 2957–2963. https://doi.org/10.1897/05-559r1.1

Sibiya, A., Gopi, N., Jeyavani, J., Mahboob, S., Al-Ghanim, K.A., Sultana, S., Mustafa, A., Govindarajan, M., Vaseeharan, B., 2022. Comparative toxicity of silver nanoparticles and silver nitrate in freshwater fish Oreochromis mossambicus: a multi-biomarker approach. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 259.

Simmons, D.B.B., Miller, J., Clarence, S., McCallum, E.S., Balshine, S., Chandramouli, B., Cosgrove, J., Sherry, J.P. 2017. Altered expression of metabolites and proteins in wild and caged fish exposed to wastewater efuents in situ. https://doi.org/10.1038/s41598-017-12473-6

Suman, K. H., Haque, M. N., Uddin, M. J., Begum, M. S., & Sikder, M. H. (2021). Toxicity and biomarkers of micro-plastic in aquatic environment: a review. Biomarkers, 26(1), 13–25. https://doi-org.ezproxy.tcu.edu/10.1080/1354750X.2020.1863470

Tarrant Regional Water District. 2024. Recreation master plan. https://www.trwd.com/wp-content/uploads/2024/12/24_1218-TRWD-Recreation-Master-Plan-compressed.pdf:contentReference[oaicite:0]{index=0}

Tetreault, G. R., Kleywegt, S., Marjan, P., Bragg, L., Arlos, M., Fuzzen, M., Smith, B., Moon, T., Massarsky, A., Metcalfe, C., Oakes, K., McMaster, M. E., Servos, M. R. 2021. Biological responses in fish exposed to municipal wastewater treatment plant effluent in situ. Water Quality Research Journal, Volume 56.2, Pages 83-99. https://doi.org/10.2166/wqrj.2021.031

Texas Parks and Wildlife Department, 2002. Trinity River (Upstream of Lake Livingston). Texas Parks and Wildlife Department, Austin, TX.

https://tpwd.texas.gov/publications/pwdpubs/pwd_rp_t3200_1059c/trinity_river1.phtml

Texas Water Development Board. (n.d.). Trinity River Basin. https://www.twdb.texas.gov/surfacewater/rivers/river_basins/trinity/index.asp

Trinity River Authority of Texas. 2012. Upper Trinity River Flow Discrepancy Study, Clean Rivers Program Project #6012.001.012. Page 6. https://cms9files.revize.com/trinityriverauth/Documents%20Center/Basin%20Planning/Reports/Special%20Studies/Upper_Trinity_River_Flow_Discrepancy_Study_FinalReport.pdf

Yang, R., Roshani, D., Gao, B., Li, P., & Shang, N. (2024). Metallothionein: A Comprehensive Review of Its Classification, Structure, Biological Functions, and Applications. Antioxidants, 13(7), 825. https://doi.org/10.3390/antiox13070825

Zelikoff, J. 1993. Metal Pollution-Induced Immunomodulation in Fish, Annual Review of Fish Diseases, 1993, Pages 305-325.