Summary:

Mixtures of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), mercury (Hg), and many other toxic chemicals pervade many waterways around the Newark, NJ, area including the Passaic River, Hackensack River, Newark Bay, and the Hudson River. While many studies have investigated the toxicities and gene expression profiles in organisms following exposure to TCDD or Hg singly, few, if any studies have explored these effects from exposure to their binary mixture. Atlantic tomcod is a common bottom-dwelling finfish in these waters that has been shown to bioaccumulate high levels of these toxicants. We examined tomcod for differentially expressed mRNA using RNA-Seq following exposure to graded doses of TCDD or Hg alone and their binary combination. Water and acetone were used as solvent controls. All analyses were performed in R using "edgeR". Of the 146 genes in total differentially expressed in the low dose exposure experiment, 87 (59.6%) genes were differentially expressed only by the combination exposure. Similarly, in the high dose exposure experiment, of the 585 differentially expressed genes in total, 373 genes (67.3%) were significantly differentially expressed by only the high dose combination treatment and not by either the high dose of Hg alone or high dose of TCDD alone. Thus, the combination treatments seemed to have greater and unique effects relative to treatments with either chemical alone. Further study of the effects of TCDD and Hg alone and in combination is warranted to investigate the toxicities of mixtures of these chemicals at higher levels of biological organization.

Introduction:

Bioaccumulation is the process by which certain chemicals amass in an organism or populations of organisms over time. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and mercury (Hg) can be found in the bodies of fishes from contaminated urban and industrial sites because of their bioaccumulation of these toxins. In particular, bioaccumulation may be seen in Atlantic tomcod *Microgadus tomcod*, a bottom-dweller found in Atlantic coast estuaries in the northeastern United States and Canada. Tomcod can be found in areas where they have been highly exposed to these chemicals and areas where they have been relatively unexposed.

Tomcod are particularly good model to study for a number of reasons. They can be matched with a control population of tomcod that haven't been exposed to these chemicals (Brown et al. 2017). They are benthic fish with livers that are relatively high in fat content, making them particularly vulnerable to deposition of TCDD. Further, as these fish do not migrate far from the places in which they are born, they can be seen as a good model for the effect of contaminants in the area on local fish (Rom and Markowitz 2007).

Remnants of tomcod have been found in some of the carnivorous fish of the Hudson River (Wirgin and Waldman, 2004), suggesting that the tomcod provide a vital food source in this, and other waterways in the area. This means that the chemicals of concern of this study, TCDD and Hg can be passed from tomcod to other organisms up the food chain, including to fish eaten by humans. Therefore, understanding the effects of these chemicals in tomcod may promote our understanding of potential effects in multiple

organisms within the food web.

TCDD, a man-made chemical (Schecter et. al., 2018), was not found in nature prior to being produced by humans. It now pervades many waterways around Newark, NJ, and other places. Its toxicity is mediated through the aryl hydrocarbon receptor (AHR) pathway which leads to the upregulation or downregulation of transcription of a number of genes in the "AHR battery" (Mimura and Fujii-Kuriyama, 2003). This can be seen in the number of the mRNAs transcribed per gene. Organisms bred with mutated AHR pathways do not seem to be affected by the toxicities of TCDD (Fernandez-Salguero et al., 1996). All vertebrates including humans, other mammals, birds, and fishes have this pathway. In fact, fishes and some bird species have two forms of AHR: AHR1 and AHR2, with AHR1 being more similar to mammalian AHR and AHR2 being more functional in fishes. But, tomcod and Atlantic killifish *Fundulus heteroclitus* from some populations that have been chronically exposed to TCDD and PCBs seem to be less affected by chemicals that affect the AHR pathway (Wirgin and Waldman, 2004).

In humans, a study conducted in Serveso, Italy found that many people had cancer and diabetes, possibly connected to exposure to TCDD (Bertazzi et al., 1998). In a study of a group of chemicals structurally similar to TCDD, polychlorinated biphenyls (PCBs), there was a potential link between exposure to these chemicals and diabetes (Longnecker et al., 2001). Studies have also found a potential connection between Hg exposure and diabetes in humans (Chang et al. 2011, He et al., 2013; Jeppesen et al., 2015).

Furthermore, many waterways in northern New Jersey have been contaminated with both TCDD and Hg. Therefore, it is important for studies to consider the effects of TCDD and Hg alone and in conjunction with each other.

Hg, like TCDD, has permeated waterways and found its way into several organisms. It has been found not only in the United States but also in Europe where the concentration of Hg in snakes was found to be dependent on what the snakes consumed (Lemaire et al*.,* 2018). Higher Hg levels were associated with the snakes that ate fish. As these organisms are affected by Hg just as humans are, it seems prudent to look at fish which can be contaminated by Hg.

Both TCDD and Hg have been found to individually affect tomcod, however, no field and little laboratory-controlled research has focused on their combined effects. The combination of TCDD and Hg has been found to affect genes in human cells differentially from either chemical alone (Jagannathan et al., 2017), suggesting that this might be the case in tomcod. In this study we focused on the effects of TCDD and Hg on tomcod as a model organism for how fish may be affected by TCDD and Hg in polluted waterways.

The aim of this study was to explore the effects of TCDD and Hg both alone and in combination with each other on global gene expression in tomcod. We checked for differentially expressed mRNAs in tomcod using acetone and water as controls, and varying doses of TCDD and Hg alone, and in combination, in the experimental groups of tomcod.

Methods:

Atlantic tomcod spawning adults were collected from the Hudson River at West Point, New York, with unbaited box traps set off bulkheads in early January 2018. Adults were immediately transported to NOAA's Northeast Fisheries Center laboratory in Sandy Hook, New Jersey, where they were spawned following the procedures described in Wirgin and Chambers (2006). Embryos were maintained at 6^0 C in a 12h light:12 dark regime until hatch at approximately 30 d post fertilization. Within 3 days of hatch, larval tomcod were placed in an environment in which they were waterborne exposed in 50 ml volumes of 5% ppt artificial seawater in 100 ml glass beakers to TCDD in acetone solvent at 0.01 ppb or 1.0 ppb singly and $HgCl_2$ at 0.01 ppm, 0.1 ppm or 1.0 ppm singly. They were exposed to acetone as a vehicle control since the chemicals (TCDD alone or TCDD and Hg in binary combination) were dissolved in acetone. They were also exposed to water as a vehicle control for the treatments with $HgCl_2$ alone. Combination treatments with Hg and TCDD in which all possible combinations of $HgCl_2$ at 0.1 ppm or 1.0 ppm and TCDD at 0.1 ppb or 1.0 ppb were used in exposure groups. All exposure beakers contained 15 individual larvae. All treatments were replicated 3 times in separate glass beakers as described above. All exposures were terminated at 7 days after which 2 larvae from each of the three beakers were pooled into single 1.5 ml microcentrifuge tubes, snap frozen on dry ice, and immediately transferred to a -80^0 freezer until processing.

Total RNAs were isolated from each of the three replicate pools of larvae for each of the doses of TCDD, Hg, their binary combinations, and acetone and water controls using Ultraspec reagent (Biotexc, Houston, TX) as described in Yuan et al. (2006). Because each of the three pools of larvae for each dose of TCDD or acetone control were from different replicate beakers, they represented true biological replicates. RNA concentrations and purities were evaluated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technol. Inc., Wilmington, DE). The integrity of the RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). RNA-Seq libraries were prepared using an Illumina TruSeq mRNA v2 kit or the TruSeq stranded mRNA kit, with 12 cycles of PCR amplification, starting from 500 ng of total RNA. Barcoded libraries were sequenced as 101 base paired-end reads on an Illumina HiSeq 2500 (v4 chemistry).

We built *de novo* transcript assemblies from the RNA-Seq reads using SOAPdenovaTrans, then annotated them against UniRef IDs and against the zebrafish proteome (Carlson, 2018). Gene expression per transcript was counted using Salmon for each sample. Differential expression was computed using edgeR (Chen et al., 2014; Robinson et al. 2010).

For the analysis, the RNA counts were then converted to UniProtKB IDs and finally to Ensembl IDs. An MDS plot was made. Then an ANOVA test was performed comparing each dose with acetone control and a table of values for the log of the fold change (logFC), log of the counts per million (logCPM), F statistic, p-values and false

discovery rates (FDR) was created. Genes were compared to those in zebrafish in order to attain the function of the protein they would have made using "bioMart" in R and the David Bioinformatics Database (R Core Team 2018; Dennis et. al. 2003; https://david.ncifcrf.gov/home.jsp).

After this, Venn diagrams were created using Eulerr (http://eulerr.co/) in order to visualize the difference between the effects of each of the doses of each chemical or their mixtures on mRNA expression while using Ensembl IDs. Subsequently, pie charts of the significantly expressed mRNAs (p-value ≤ 0.01 and an FDR ≤ 0.05) were made using the ggplot2 library in R (Kahle and Wickham, 2013, Wickham, 2016). The set of significantly expressed mRNAs in each high dose condition were then examined.

Results:

The varying doses of TCDD and Hg and their mixtures as compared to the water and acetone controls were examined in various ways.

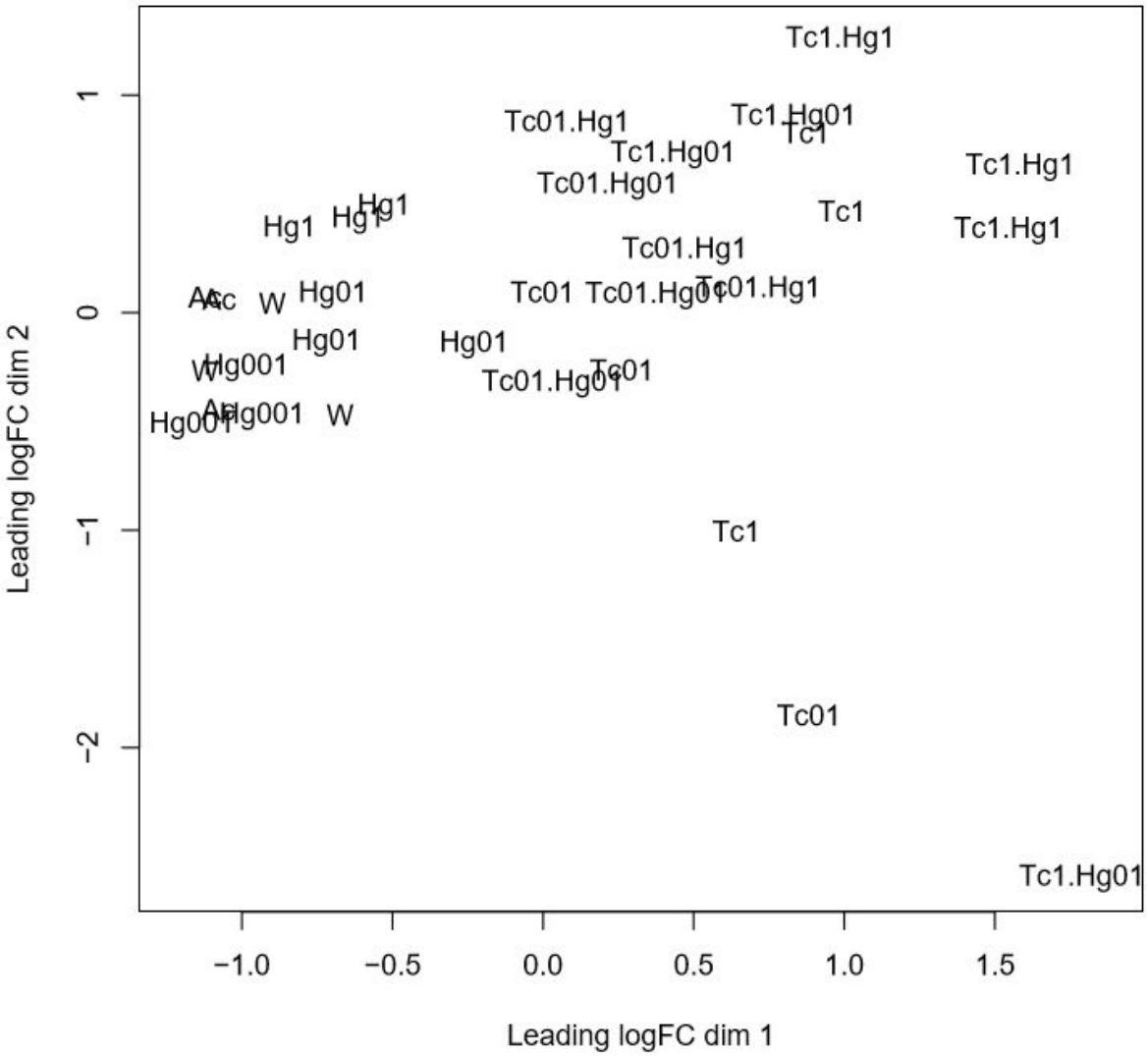


Figure 1: This is an MDS plot. The acetone treatment is abbreviated as Ac. The water control is abbreviated as W. Each treatment of Hg has Hg and then the dosage next to it. Each treatment of TCDD is abbreviated as Tc and has the dosage next to it. A period between the Tc, its dose and an Hg and its dose indicates that it is a combination treatment.

The MDS plot was used to visualize the differences among each of the treatments. The acetone control treatment clustered closely with the water control and lowest dose of Hg treatment (0.01 ppm) (Figure 1). The high dose TCDD (1.0 ppb) and Hg (1.0 ppm) combination treatment clustered closely on the top right with one noticeable outlier that was on the bottom right of the figure (Figure 1). All Hg doses singly clustered closely with the acetone and water control treatments. TCDD doses seem to cluster more closely with the combination treatments. This suggests that the TCDD alone treatments had more similarity with the combination treatments than Hg.

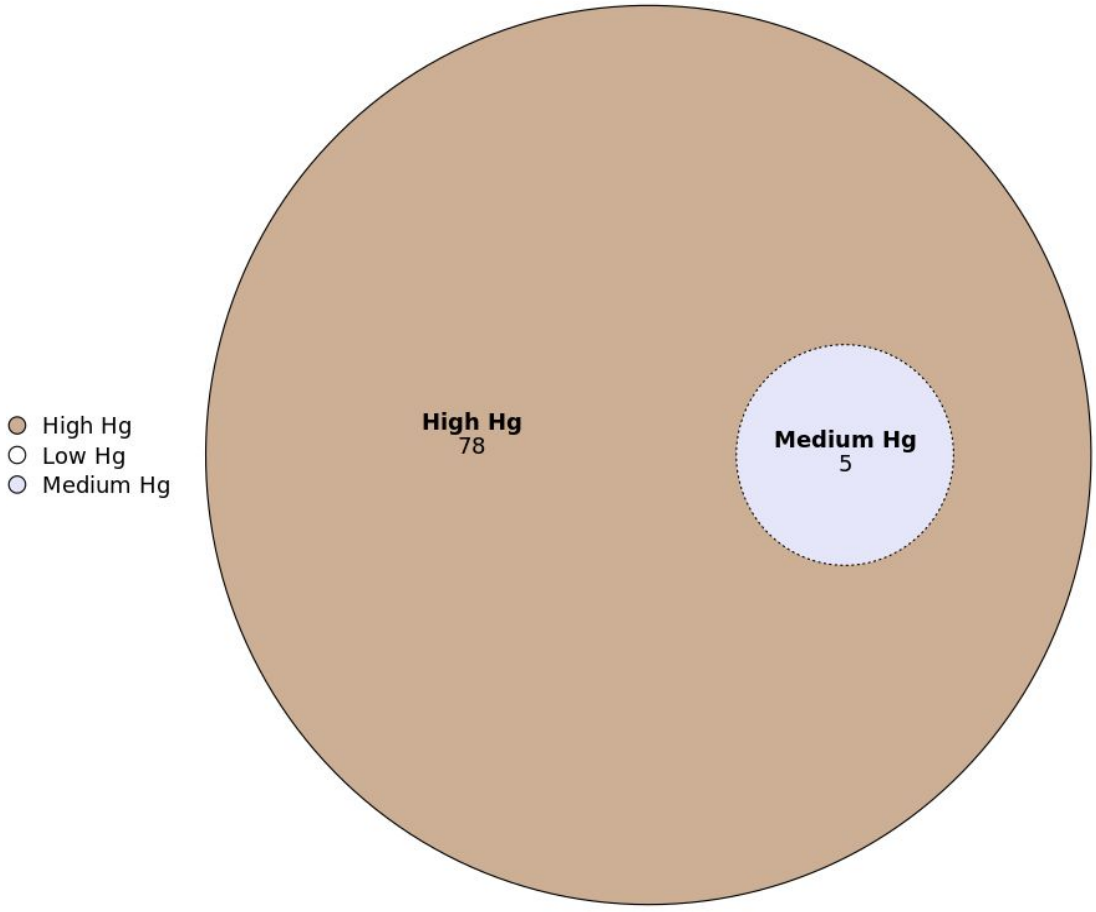


Figure 2: Venn diagram of the number of differentially expressed mRNAs that overlap between varying doses of $HgCl_2$ (p-value ≤ 0.01 and FDR ≤ 0.05) as compared to acetone control. No genes were significantly differentially expressed at the lowest (0.01 ppm) $HgCl_2$ treatment.

Figure 2 shows the number of differentially expressed mRNAs of pooled tomcod larvae that were exposed to different doses of $HgCl_2$ alone compared to acetone control (p-value ≤ 0.01 and FDR ≤ 0.1). This visual suggests that the high dose (1.0 ppm) Hg clearly has a larger effect than the lower (0.01 and 0.1 ppm) doses of Hg. It seems that the lowest dose of Hg has no significant effect when compared to acetone. The medium dose seems to have some effect (5 differentially expressed genes) but it is not as pronounced as that of the highest dose of Hg which has 78 genes significantly expressed alone and also has the 5 differentially expressed genes at the medium dose of Hg. A comparison of the number of differentially expressed genes by TCDD doses is warranted to see how much overlap there is between the doses.

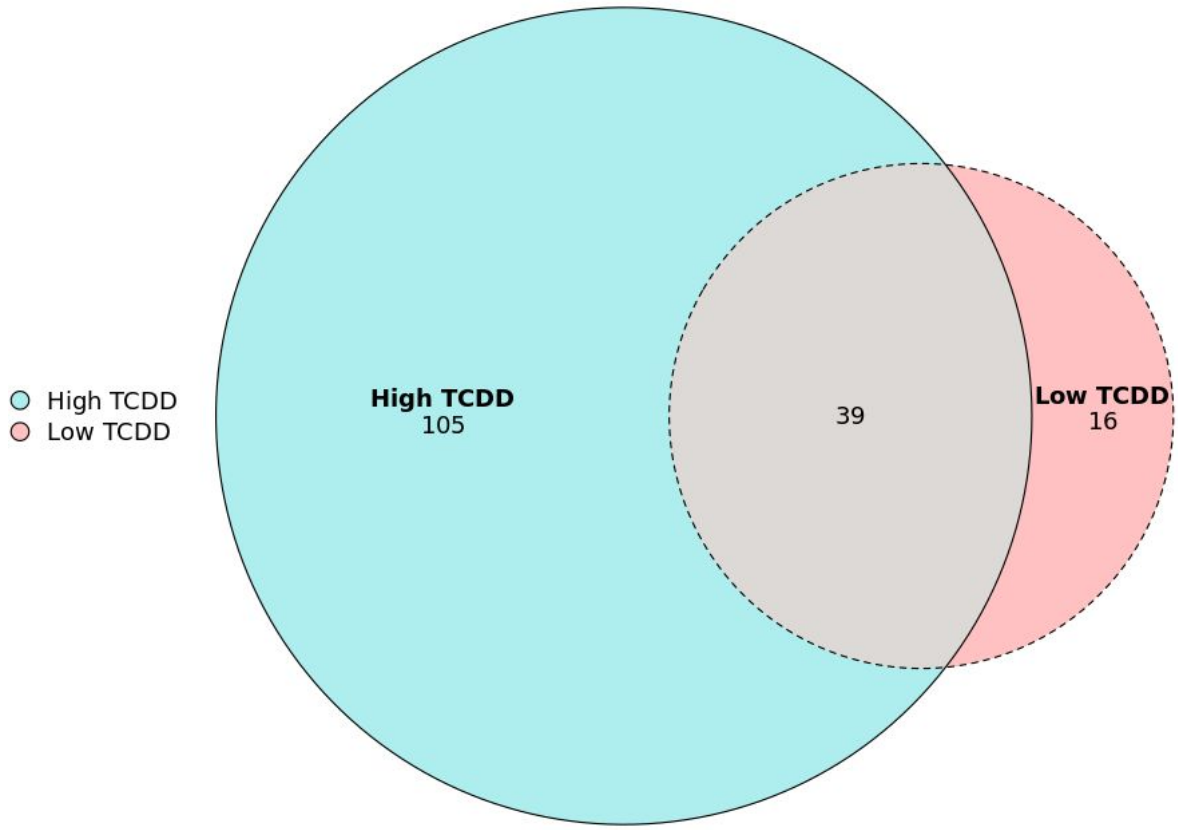


Figure 3: Venn diagram of the number of differentially expressed mRNAs in pooled tomcod larvae that were treated with two levels of TCDD (p-value ≤ 0.01 and FDR ≤ 0.05) as compared to acetone vehicle control.

Figure 3 shows the number of differentially expressed mRNA levels in pooled tomcod larvae exposed to two doses of TCDD after they were compared to the acetone control. The p-value and FDR were restricted in each case (p-value ≤ 0.01 and FDR ≤ 0.05). The high (1.0 ppb) dose of TCDD resulted in 144 differentially expressed genes while the low (0.01 ppb) dose of TCDD resulted in only 55 differentially expressed

genes. There was overlap of 39 genes that were differentially expressed at both the low and high doses of TCDD indicating that some genes were differentially expressed in both treatment groups. As expected, the high dose in this case significantly affected the RNA expression of more genes overall than the low dose of TCDD.

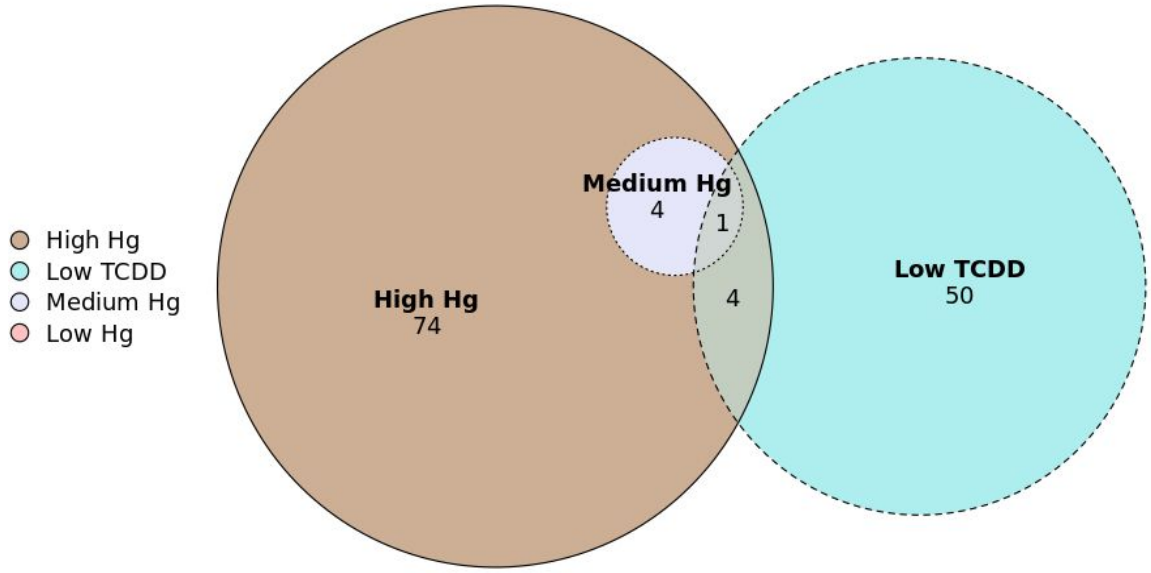


Figure 4: Venn diagram of the number of differentially expressed genes in pooled tomcod larvae treated with a low (0.01 ppb) dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and varying (0.01, 0.1, and 1.0 ppm) doses of $HgCl_2$ (p-value ≤ 0.01 and FDR ≤ 0.05) as compared to acetone vehicle control. Treatment of tomcod with low dose Hg alone had no significant effect on expressed mRNA levels.

There was some overlap in differentially expressed genes in tomcod larvae treated with the low dose TCDD and medium and high Hg doses (Figure 4). The most significant

overlap of TCDD and Hg in this diagram is between the highest dose of Hg and the low dose of TCDD (4 mRNAs). The medium dose of Hg's 4 mRNAs overlaps with the high dose of Hg and the low dose of TCDD. This suggests the high Hg dose has more of an effect on RNA expression than either the medium or low doses of Hg. The low dose of TCDD alone affects more mRNAs overall than the medium dose of Hg alone.

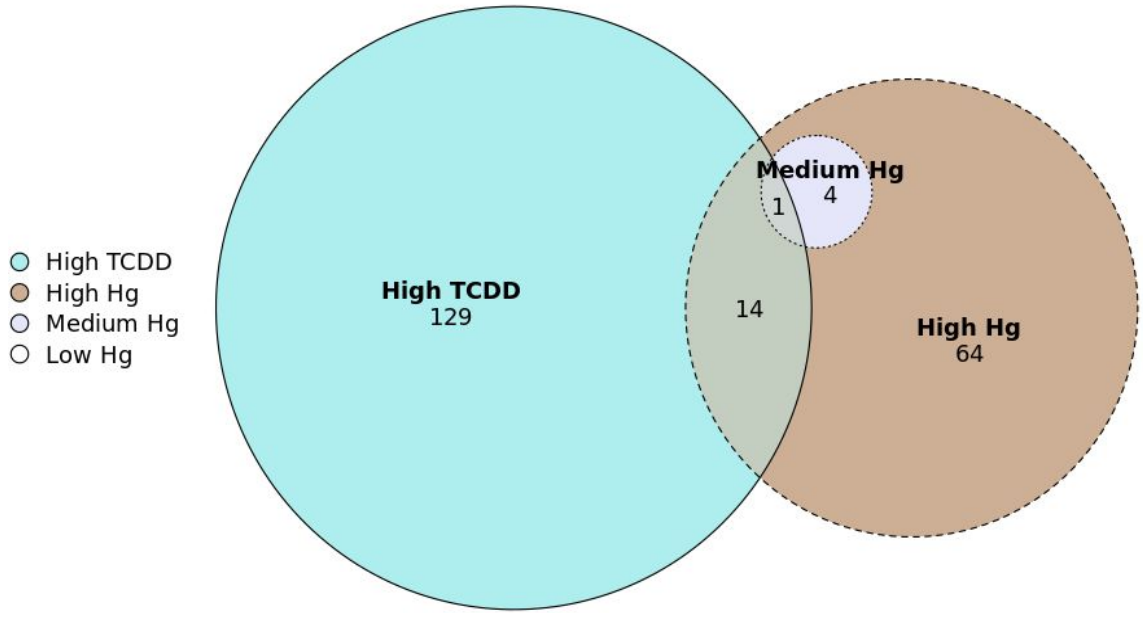


Figure 5: Venn diagram of the number of significant differentially expressed genes in pooled tomcod larvae that were treated with a high (1.0 ppb) dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alone and varying (0.1 and 1.0 ppm) levels of $HgCl_2$ alone (*p*-value ≤ 0.01 and FDR≤ 0.05) as compared to the acetone vehicle control.

The highest dose of TCDD affected more mRNAs overall than any of the three doses of Hg alone (Figure 5). High TCDD (144 genes) also affected more genes overall

than the low TCDD treatment (55 genes) or any of the doses of Hg (Figure 4 & Figure 5). There were 83 genes that were differentially expressed at the high dose of Hg but only 15 of these genes were in common with the high dose of TCDD.

Figure 6: Venn diagram of the number of significantly differentially expressed genes in pooled tomcod larvae by exposure to a medium (0.1 ppm) dose of $HgCl_2$ alone, a low (0.01ppb) dose of TCDD alone, and to the binary combination of the medium dose of $HgCl_2$ and the low dose of TCDD. Differentially expressed mRNAs were matched to Ensembl IDs. The *p*-value ≤ 0.01 and the FDR ≤ 0.01. The low dose (0.01 ppm) of Hg

alone treatment resulted in no significantly differentially expressed genes and therefore is not shown on the diagram.

There were 55 genes that were differentially expressed in larvae by treatment with the low dose of TCDD alone. Similarly, 5 genes were differentially expressed in larvae treated with the medium dose of Hg alone. Furthermore, there were 33 differentially expressed genes that overlapped between the low dose TCDD treatment and treatment with the binary combination of TCDD and medium dose of Hg. Most interestingly, there were 87 genes that were differentially expressed in larvae by the binary combination treatment that were not differentially expressed by treatment of larvae by the TCDD or Hg alone. Thus, 59.6% of all that genes (n=146) that were differentially expressed in this experiment were only seen in larvae exposed to the combination of TCDD and Hg.

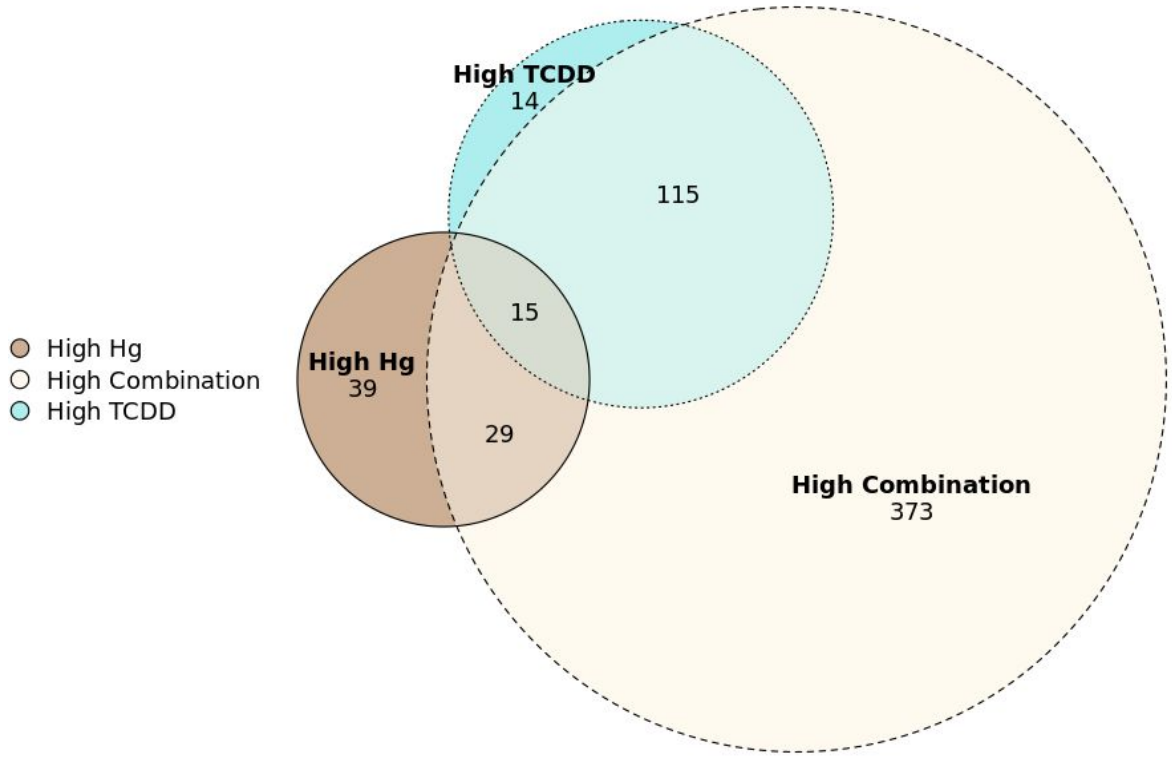


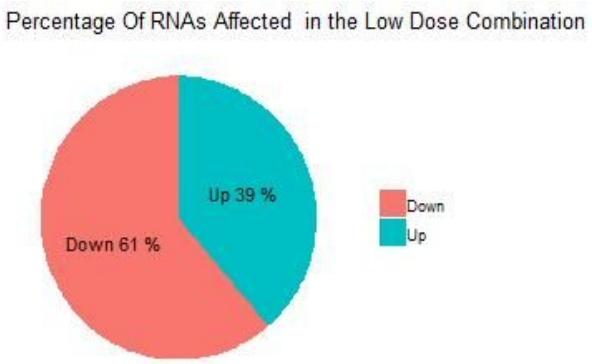
Figure 7: Venn diagram of the number of significantly differentially expressed genes in pooled tomcod larvae treated with the high (1.0 ppm) dose of $HgCl_2$ alone, high (1.0 ppb) dose of TCDD alone, and their binary combination. Differentially expressed mRNAs were matched to UniRef100 IDs. The p-value ≤ 0.01 and FDR ≤ 0.05.

Exposure to the high dose of TCDD alone and Hg alone resulted in the significant differential expression of 144 and 83 genes, respectively. Of these, there were 15 genes that overlapped between the high doses of TCDD and Hg alone. Most significantly, there were 373 genes that were differentially expressed by the binary combination that were not differentially expressed when treated with either TCDD or Hg alone. Thus, of the 585 differentially expressed genes in this experiment, 63.7% were only differentially

expressed by treatment with the binary combination of high doses of TCDD and Hg. In both Figures 6 and 7, it can be seen that the combination treatments seem to differentially express genes in tomcod larvae that neither the TCDD nor Hg treatments alone differentially express. This suggests that the combination treatment have unique effects relative to the treatment with each chemical alone.

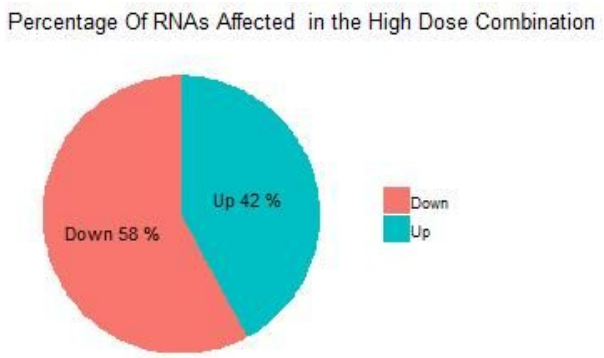
TCDD and Hg Combination Treatment

(A)



	Regulation	The Number Of RNAs
1	Up	48
2	Down	76

(B)



	Regulation	The Number Of RNAs
1	Up	224
2	Down	308

Figure 8: Pie charts depicting the number of significantly differentially expressed mRNAs (*p*-value of less than 0.01 and a false discovery rate of 0.05 or less) that were up-or down-regulated in tomcod larvae that were treated with low (A) or high binary combination (B) doses of TCDD and Hg

The differentially expressed genes for each condition were analyzed in pie charts with accompanying tables of the number of statistically significant mRNAs for each treatment group. In the pie charts above it can be seen that there were more mRNAs significantly differentially expressed in the high dose combination treatment (n=532) than in the low dose combination treatment (n=124) (Figure 8). In both treatments, there were more genes that were down regulated than upregulated. In the case of the low dose

combination treatment, there were 28 more downregulated genes than upregulated genes while in the high dose combination treatment there were 84 more genes that were downregulated than upregulated.

The number of significantly differentially expressed genes across all treatments was then summarized. The high dose Hg treatment alone resulted in 83 significantly differentially expressed genes in tomcod larvae. The high dose TCDD treatment alone resulted in 144 significantly differentially expressed genes. The high dose combination treatment resulted in 585 genes that were significantly differentially expressed. Only 130 significantly differentially expressed genes were shared between treatment with the high dose of TCDD alone and the high dose combination treatment. In total, 44 significantly differentially expressed genes in the high Hg treatment were in common with the combination treatment. Finally, 358 genes (67.3%) were significantly differentially expressed by only the high dose combination treatment and not by either high Hg alone or high TCDD alone.

Discussion:

In this study, the effects of TCDD and Hg treatment alone were different from the effect of their binary combination treatment. For example, 67.3% of mRNAs differentially expressed in the high dose combination treatment were affected by only the high combination treatment and not by either the high dose of Hg alone or the high dose of TCDD alone. Similarly, in the low dose combination treatment experiment, 59.5% of

differentially expressed genes were only seen with the low dose combination treatment and not with the low dose exposures to either Hg or TCDD alone. This suggests that the combination treatment has a greater effect that either Hg alone or TCDD alone have. Further, the combination treatment had approximately 24% of its genes in common with TCDD. This was greater than the percentage of genes it had in common with Hg (8.27%), suggesting that TCDD had a greater influence on the combination treatment.

These results are consistent with an *in vitro* study in human cells (Jagannathan et al., 2017) which tested the effects on global gene expression of TCDD alone, Hg alone, and their combination in immortalized human BEAS-2B lung cells. They found that more than 70% of genes that were significantly differentially expressed by a TCDD treatment were also differentially expressed by a combination treatment of TCDD and Hg (Jagannathan et al*.,* 2017). The relatively lower effect of TCDD exposure seen in our study may be because tomcod from the Hudson River (our source of specimens) have been shown to exhibit resistance to TCDD-induced gene expression and toxicity (Wirgin et al., 2011). However, these tomcod in our current study seem to be more vulnerable to the binary combination of TCDD and Hg.

Given that tomcod seem to have some genetic resistance to TCDD (Wirgin et al., 2011), the less resistant species that are in the same environment may have a different and stronger response to exposure to the same chemicals singly and in binary combination (University of California Museum of Paleontology, 2011). Even though tomcod is not a traditional model organism it can inform our understanding of how

chemical pollution in other water bodies in North America has affected other fishes that live in these impacted environments.

There are a number of strengths to the present study. This experiment was conducted on larvae in a controlled laboratory environment. Therefore, the results are more likely to be reproducible than observations in the field. The analysis for this study used *p*-value cut offs of less than 0.01 and a false discovery rate of less than 0.05. Therefore, it is less likely to have false positives creeping into the results. The "bioMart" package in R was used along with DAVID (Durinck et al. 2005; Huang et al. 2008; Huang et al. 2009) and that allowed the researcher to effectively and precisely figure out the updated function of the genes in question.

This study also had some limitations. It based its inferences on mRNAs measured. While it is clear that DNA is transcribed into RNA which is then translated into protein, the number of mRNAs made does not necessarily correlate with the number of proteins made (Dickson et al., 2007). Therefore, ideally the protein levels need to be measured along with the RNAs to see the effects on the tomcod that TCDD alone, Hg alone and combination treatments have. Given that this study was conducted in a laboratory environment, it may not simulate how organisms may mitigate their exposure in their natural environment.

In the future, a study in a mammalian organism model following the same methodology might be useful in understanding how these chemicals directly affect human beings. Conservation of responses *in vivo* between fishes and *in vitro* in human

cells would strongly support the functional significance of these chemically induced alterations in gene expression. Further, it might be better to see the long-term effects of chronic exposure to TCDD and Hg both alone and in combination throughout the lifespan of aquatic organisms if the focus is on organisms which reside in water bodies. Moreover, it might be good to include treatments in which organisms get sporadic exposure to TCDD or Hg and see how this sporadic exposure affects the organisms' lifespan and quality of life.

The present study elucidated the unique effects of the combined treatment versus Hg or TCDD exposure alone on tomcod. These findings indicate the need for a more in-depth and long-term exploration of the impact of the combined exposure to TCDD and Hg on gene expression in other organisms, including humans. Future studies need to explore how these effects on the molecular level translate into toxic alterations at the organismic and population levels.

References:

Bertazzi, P., Bernucci, I., Brambilla, G., Consonni, D., and Pesatori, A. (1998). The Seveso studies on early and long-term effects of dioxin exposure: a review. Environ Health Perspectives *106,* 625–633.

Brown, S., Heguy, A., Zappile, P., Chen, H., Goradia, A., Wang, Y., Hao, Y., Roy, N., Vitale, K., R, C., Chambers, R. C., and Wirgin, I. (2017). A dramatic difference in global gene expression between TCDD-treated Atlantic tomcod larvae from the resistant Hudson River and a nearby sensitive population. Genome Biology and Evolution *9*, 2251–2264.

Carlson, M. (2018). org.Dr.eg.db: Genome wide annotation for Zebrafish.

Chang, J.-W., Chen, H.-L., Su, H.-J., Liao, P.-C., Guo, H.-R., and Lee, C.-C. (2011). Simultaneous exposure of non-diabetics to high levels of dioxins and mercury increases their risk of insulin resistance. Journal of Hazardous Materials *185*, 749–755.

Chen, Y., Lun, A.T.L., and Smyth, G.K. (2014). Differential expression analysis of complex RNA-seq experiments using edgeR. In Statistical analysis of next generation sequencing data, S. Datta, and D. Nettleton, eds. (Cham: Springer International Publishing), pp. 51–74.

Dennis, G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C. and Lempicki, R.A. (2003). DAVID: database for annotation, visualization, and integrated discovery.

Genome Biology, *4*(9), p. P3.

Dickson, B.C., Mulligan, A.M., Zhang, H., Lockwood, G., O'Malley, F.P., Egan, S.E., and Reedijk, M. (2007). High-level JAG1 mRNA and protein predict poor outcome in breast cancer. Modern Pathology *20*, 685-693.

Durinck, S., Moreau, Y., Kasprzyk, A., Davis, S., De Moor, B., Brazma, A., and Huber, W. (2005). BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. Bioinformatics *21*, 3439–3440.

Fernandez-Salguero, P., Hilbert, D., Rudikoff, S., Ward, J., and Gonzalez, F. (1996). Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-Induced toxicity. Toxicology and Applied Pharmacology *140*, 173–179.

He, K., Xun, P., Liu, K., Morris, S., Reis, J., and Guallar, E. (2013). Mercury exposure in young adulthood and incidence of diabetes later in life. Diabetes Care *36*, 1584-1589.

Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2008). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols *4*, 44-57.

Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2009). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic

Acids Research *37*, 1–13.

Jagannathan, L., Jose, C., Tanwar, V., Bhattacharya, S., and Cuddapah, S. (2017). Identification of a unique gene expression signature in mercury and 2,3,7,8-tetrachlorodibenzo-p-dioxin co-exposed cells. Toxicology Research *6*, 312–323.

Jeppesen, C., Valera, B., Nielsen, N.O., Bjerregaard, P., and Jørgensen, M.E. (2015). Association between whole blood mercury and glucose intolerance among adult Inuit in Greenland. Environmental Research *143*, 192–197.

Kahle, D., and Wickham, H (2013). ggmap: Spatial visualization with ggplot2. The R Journal *5*, 144–161.

Law, C.W., Chen, Y., Shi, W., and Smyth, G.K. (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biology *15*, R29.

Lemaire, J., Bustamante, P., Olivier, A., Lourdais, O., Michaud, B., Boissinot, A., Galán, P., and Brischoux, F. (2018). Determinants of mercury contamination in viperine snakes, Natrix maura, in Western Europe. Science of The Total Environment *635*, 20–25.

Longnecker, M.P., Klebanoff, M.A., Brock, J.W., and Zhou, H. (2001). Polychlorinated biphenyl serum levels in pregnant subjects with diabetes. Diabetes Care *24*, 1099-1101.

Mimura, J., and Fuji-Kuriyama, Y. (2003). Functional role of AhR in the expression of toxic effects by TCDD. Biochim Biophys Acta *1619*, 263–268.

R Core Team (2018). R: A language and environment for statistical computing (Vienna, Austria).

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140.

Rom, W.N. and Markowitz, S.B. eds. (2007). *Environmental and Occupational Medicine*. Lippincott Williams & Wilkins.

Schecter, A., Birnbaum, L., Ryan, J., and Constable, J. (2008). Dioxins: An overview. Environmental Research *101*, 419–428.

University of California Museum of Paleontology and National Center for Science Education (2011). Toxic river means rapid evolution for one fish species. *University of California Berkeley*.

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis (New York: Springer-Verlag).

Wirgin, I. and Chambers, R.C. (2006). Atlantic tomcod Microgadus tomcod: A model species for the responses of Hudson River fish to toxicants. In *American Fisheries Society Symposium 51*, 331-364. American Fisheries Society.

Wirgin, I., and Waldman, J. (2004). Resistance to contaminants in North American fish

populations. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis *552*, 73–100.

Yuan, Z., Courtenay, S., Chambers, R. C., & Wirgin, I. (2006). Evidence of spatially extensive resistance to PCBs in an anadromous fish of the Hudson River. Environmental Health Perspectives, *114*, 77-84.

Zhou, X., Lindsay, H., and Robinson, M.D. (2014). Robustly detecting differential expression in RNA sequencing data using observation weights. Nucleic Acids Research *42*, e91–e91.