# LINKING POLLUTANTS AND PHYSIOLOGICAL PROCESSES IN FISH EXPOSED TO URBAN WATERS

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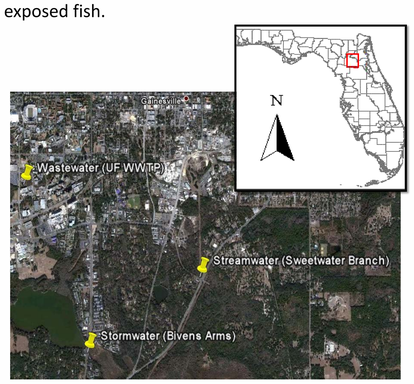
## Introduction

The fact that toxicity is preceded by gene expression alteration allows the use of genomics approaches like ***microarrays*** for early detailed characterization of thousands of genes to understand the perturbation of biological pathways in organisms exposed to toxicants. Here, we show the gene expression profiles of male ***fathead minnow livers*** exposed to three types of urban waters (stormwater, wastewater, streamwater) from Gainesville, Florida. We hypothesized that the patterns of gene expression changes in fish exposed to urban waters represent physiological outcomes to the class of toxicants present in water.

## Materials and Methods

### Water Chemistry

Historic water chemistry analysis of organic contaminants present in wastewater and streamwater was available from ACEPD (2009) and was used to make linkages with observed effects in exposed fish.




### Fish exposure

Water was collected from three sources in Gainesville, Florida: (1) surface water from *Sweetwater branch* downstream of a wastewater treatment plant (referred to as **streamwater**), (2) wastewater from a wastewater treatment plant (referred to as **wastewater**), and (3) lake Bivens Arm that receives stormwater runoff (referred to as **stormwater** ). Chemfluor tubing and a 120 liters steel barrel coated with polyester resin was used to avoid cross-contamination. Water was pumped into four fiberglass cylinders and then into four aquariums **(Figure 1).** Four male fathead minnow fish were transferred to each replicate aquarium and kept for 48 hours of exposure (total fish= 64). Four fish per treatment were selected for gene expression determination (microarray).

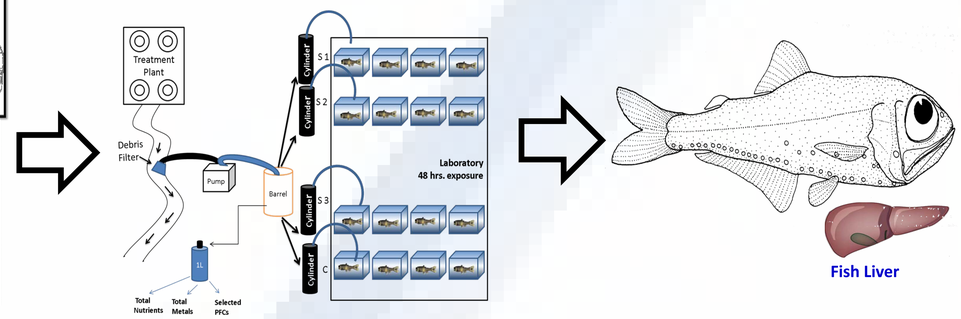
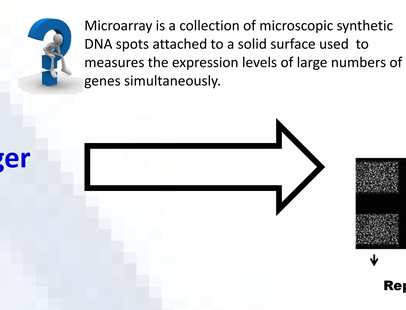



**Figure 1 .** Water collection and Fish exposure design.

### cDNA Microarrays

After 48 hours of exposure, fish were anesthetized and euthanized. All procedures involving live fish were approved by the University of Florida IACUC. Liver RNA was isolated from fish liver randomly collected from each treatment. RNA samples were prepared and hybridized to a **16,000 gene cDNA microarray (Figure 2) .** Microarray light intensity was **normalized by LOESS**. Differentially regulated genes were identified using ANOVA ($p < 0.05$) compared to controls. Fisher's exact test was used to determined the altered biological processes **(Table 1).**




Microarray is a collection of microscopic synthetic DNA spots attached to a solid surface used to measures the expression levels of large numbers of genes simultaneously.

### How microarrays works?

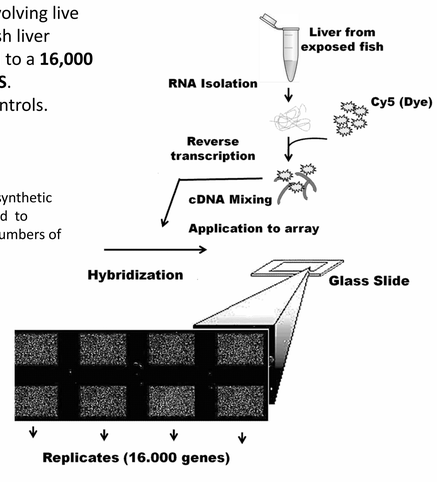



**Figure 2.** Diagram of microarray procedure.

## Results

### Water Chemistry

Within the Organic Wastewaters Contaminants detected in the waters analyzed here, Carbamazepine, Pefluorochemicals (PFCs) and DEET were above 100 ng/l concentration. Particularly relevant because the mode of action observed in fish here, the total PFCs concentration were 278 and 213 ng/l for streamwater and wastewater respectively.


### Cellular Pathways

In stormwater and streamwater exposed fish, the main effects were down regulation of HMGCR enzyme which is a key enzyme in cholesterol biosynthesis (mevalonate pathway). While in wastewater exposed fish, apoptotic related genes were differentially regulated, as shown in **Figure 3**.

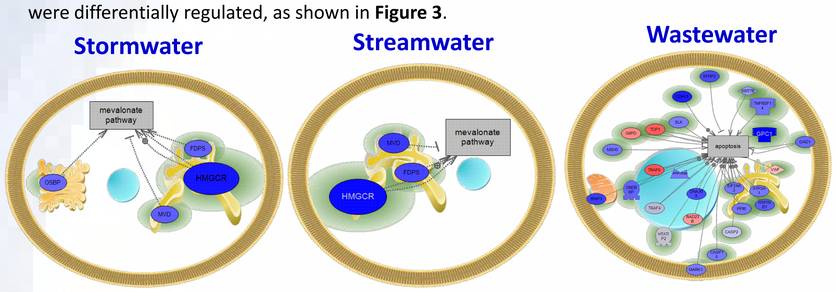



**Figure 3.** Selected cell processes altered in fish after exposure to different waters. Blue indicates down regulated genes. Red indicates up regulated genes.

### Gene Expression

**Figure 4** shows highest number of genes alterations in **A) stormwater** (1028), followed by **B) streamwater** (787) and **C) wastewater** (575 genes) as compared to controls (ANOVA, $p< 0.05$) .

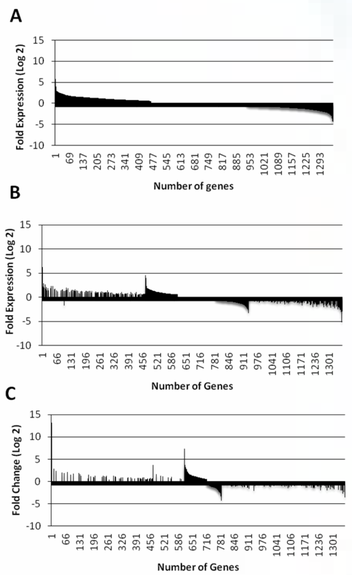



**Figure 4.** Genes up and down regulated after 48 hours fish exposure to different waters.

### Physiological Effects

A total number of 18 biological processes were altered in stormmwater, 19 in streamwater, and 12 in wastewater exposed fish. **Table 1** summarize the main altered processes.

**Table** 1. Selected biological processes altered.

| GO_Biological_Processes Category | p>value |
|---|---|
| **Stormwater** | |
| go:0006350; transcription | 0.0004 |
| go:0008299; isoprenoid biosynthetic process | 0.0004 |
| go:0007050; cell cycle arrest | 0.0025 |
| go:0006355; regulation of transcription, dna-dependent | 0.0032 |
| go:0007049; cell cycle | 0.0074 |
| go:0006512; ubiquitin cycle | 0.0211 |
| go:0000226; microtubule cytoskeleton organization and biogenesis | 0.0219 |
| go:0006284; base-excision repair | 0.0388 |
| go:0016310; phosphorylation | 0.0388 |
| go:0008202; steroid metabolic process | 0.0389 |
| go:0009615; response to virus | 0.0389 |
| go:0042742; defense response to bacterium | 0.0494 |
| **Streamwater** | |
| go:0008299; isoprenoid biosynthetic process | 0.0001 |
| go:0006457; protein folding | 0.0012 |
| go:0007050; cell cycle arrest | 0.0025 |
| go:0001889; liver development | 0.0103 |
| go:0007275; multicellular organismal development | 0.0140 |
| go:0006350; transcription | 0.0142 |
| go:0006812; cation transport | 0.0156 |
| go:0016310; phosphorylation | 0.0230 |
| go:0048268; clathrin cage assembly | 0.0230 |
| go:0042127; regulation of cell proliferation | 0.0297 |
| go:0007049; cell cycle | 0.0362 |
| go:0006298; mismatch repair | 0.0369 |
| go:0009058; biosynthetic process | 0.0419 |
| go:0016568; chromatin modification | 0.0419 |
| **Wastewater** | |
| go:0005975; carbohydrate metabolic process | 0.0032 |
| go:0030154; cell differentiation | 0.0052 |
| go:0042981; regulation of apoptosis | 0.0160 |
| go:0006298; mismatch repair | 0.0179 |
| go:0016998; cell wall catabolic process | 0.0179 |
| go:0043065; positive regulation of apoptosis | 0.0189 |
| go:0006289; nucleotide-excision repair | 0.0308 |
| go:0006816; calcium ion transport | 0.0327 |
| go:0007018; microtubule-based movement | 0.0381 |
| go:0008654; phospholipid biosynthetic process | 0.0458 |

## Conclusions

• Fishes exposed to three urban waters showed alteration of genes related with DNA damage. Because of the fundamental role of DNA molecule, we suspect that the urban waters are exerting relevant toxic responses in aquatic biota (e.g., fish).

•Results suggest alteration of genes related with fatty acid metabolism (cholesterol biosynthesis) and cell cycle arrest in **streamwater** and **stormwater** exposed fish and cell wall catabolic process and apoptosis in **wastewater** exposed fish. The presence of PFCs in our sites and their known environmental persistence together with specific gene alterations suggest that fish exposed to these waters had signature effects linked to presence of PFCs. Several studies elsewhere point the liver as a target tissue of PFCs contamination (e.g., Martin et al. 2003a,b; Wei et al. 2008) and the common physiological effects in fish due to PFCs exposure include the disruption of fatty acid metabolism, lipid and cholesterol transport (Wei et al. 2008), cell death (Wei et al. 2009), oxidative stress (Liu et al. 2007), and cell wall catabolic process (Hu et al,2002); all of these effects were observed in our study.

•Due to the important role of cholesterol in animal physiology (reproduction, cell membrane, maintenance, etc) and cell membrane as cellular barrier, we suggest that the PFCs exposure could directly and indirectly exert important toxic effects in fish as others chemicals found in our waters do not have known particular effects like those observed here.

## References

1. ACEPD, Alachua County Environmental Protection Department. (2009).Results of a Screening Analysis for Organic Wastewater Contaminants in Sweetwater Branch and Recommendations for Further Sampling and Analysis 15 p.
2. Haughom and Spydevold, 1992 B. Haughom and O. Spydevold, The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. Biochem. Biophys. Acta, 1128 (1992), pp. 65–72
3. Hu et al., 2003 W.Y. Hu, P.D. Jones, W. De Coen, L. King, P. Fraker, J. Newsted and J.P. Giesy, Alterations in cell membrane properties caused by perfluorinated compounds. Comp. Biochem. Physiol. C, 135 (2003), pp. 77–88
4. Liu C, Yu K, Shi X, Wang J, Lam PKS, Wu RSS, et al. 2007. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (Oreochromis niloticus). Aquatic Toxicology 82(2): 135-143.
5. Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003a. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 22(1): 196-204.
6. Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003b. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 22(1): 189-195.
7. Wei Y, Liu Y, Wang J, Tao Y, Dai J. 2008. Toxicogenomic analysis of the hepatic effects of perfluorooctanoic acid on rare minnows (Gobiocypris rarus). Toxicology and Applied Pharmacology 226(3): 285-297.
8. Wei Y, Shi X, Zhang H, Wang J, Zhou B, Dai J. 2009. Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (Gobiocypris rarus) using toxicogenomic analysis. Aquatic Toxicology 95(1): 27-36.

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