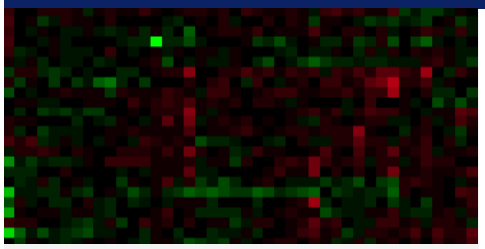
U. S. Geological Survey's Western Fisheries Research Center (WFRC)



A heat map showing relative gene expression as intensity of fluorescence. Up-regulated genes are shown in red and down-regulated in green.

Using DNA Microarray to Investigate the Influence of Environmental Contaminants on Innate Immunity

DNA Microarrays

- DNA microarrays allow scientists to examine the expression of thousands of genes simultaneously.
- Gene expression is measured by quantifying the messenger RNA present in a sample using specialized fluorescent nucleotides
- Used for about 25 years in research on human diseases like cancer, DNA microarrays have only recently been applied to broader studies of environmental health.
- Robotic machines and new options for affordable data management make more sophisticated analysis possible than ever before.
- Changes in gene expression measured by microarray after exposure to contaminants give scientists insights into the effect of contaminants on functions like innate immunity.

Contaminants and Disease

Exposure to environmental contaminants and the emergence of wildlife, zoonotic and other environmental diseases are growing concerns worldwide. Suppressed immunity in individuals exposed to endocrine disrupting compounds (e.g. ethinylestradiol, aka EE2) in the environment may accelerate the emergence of new health threats and increase the prominence of established threats to wildlife and human health. Much science has focused on investigating the impacts of endocrine disruptors on reproduction. However, understanding the influence of contaminants on immunity is also an important piece of predicting and managing wildlife and human health impacts of endocrine disruptors. U.S. Geological Survey (USGS) scientists at Western Fisheries Research Center in Seattle, Washington are using advanced genetic techniques such as DNA microarrays to examine the impacts of contaminants on innate immunity at a molecular level.

US bass populations are susceptible to endocrine disrupting chemicals. Photo by DE Tillitt.

What is innate immunity and why does it matter?

Innate immunity includes generalized responses to infection like inflammation, phagocytosis and the killing of bacteria. These responses depend on pattern recognition receptors (PRRs) that have been highly conserved over evolutionary time, so that PRR genes in a fish are similar to PRR genes in a human. Because innate immunity represents an organism's primary defense to a novel pathogen in the environment, suppressed innate immunity has important implications for the susceptibil-

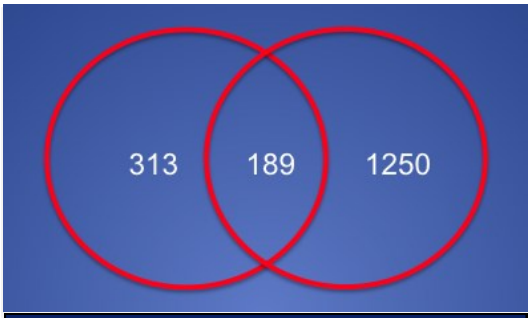
Why study the effects of contaminants in zebrafish?

In many ways zebrafish are perfectly suited to an investigation of the effects of contaminants on immunity. For one, the zebrafish genome has been completely sequenced, meaning that all its genes are known. This allows scientists to determine which specific genes are abnormally turned up or down, after exposure to contaminants. For decades zebrafish have served as an important model organism for studies of development and many transgenic strains are available that will fluoresce in various colors when expressing particular proteins. This provides a valuable tool to look at the activation of specific genes responsible for critical stages of the signal pathway responsible for a pro-inflammatory response. Additionally zebrafish mature rapidly and reproduce reliably in captivity so that researchers can investigate potential transgenerational effects that are passed from parents to offspring through methylation or other permanent alteration of the DNA.

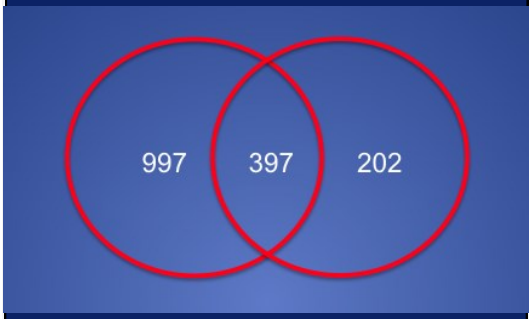
What are DNA microarrays and what can they tell us about the effect of contaminants on immunity?

Microarrays measure the expression of many genes simultaneously. Functions like immunity depend on the coordinated actions of many proteins, enzymes and other biological compounds. To study how immunity is influenced by environmental factors requires examining the expression of many genes at once. In this case microarrays allow scientists to track patterns of expression for specific genes, such as PRR genes and other genes implicated in innate immunity. Expression is quantified after exposing adult zebrafish to contaminants that occur in the environment and then challenging them with a compound representing a pathogen associated molecular pattern (PAMP) from a bacterial pathogen. Characterizing gene expression is one component of a broader approach to examining the effects of contaminants on immunity. Such molecular approaches compliment cellular tests of white blood cell function, tests of transgenerational effects that occur within the epigenome and are passed from parent to offspring, as well as field tests done in bass.

A view of the Patuxent River in Maryland, a river known to have high levels of contamination..



Venn diagram showing the number of zebrafish genes that were significantly up- or down-regulated upon exposure to environmentally relevant levels of EE2 (left-low dose 502; right-high dose 1439 with 189 genes being dysregulated at either concentration).



Venn diagram displaying the number of zebrafish genes that were significantly ($p<0.05$) up- or down-regulated upon stimulation with a specific bacterial (left) or viral (right) PAMP. Approximately 400 genes are found at the intersection. USGS scientists are addressing how these immune-stimulated genes and their associated pathways are co-regulated upon exposure to both EE2 and PAMPs.

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