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# **Molecular Measurement of Toxicity in Fish; Case Examples and Policy Implications**

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## **Abstract**

Stormwater and oil are common urban contaminants that can be harmful to fish species. One way of recognizing exposed and impaired fish is by monitoring gene expression and gene induction. This study focused on the identification and validation of reference genes for measuring contaminant-induced changes in gene expression due to urban influence. In this study, reference genes (which are genes used to normalize data and remain consistent in varying exposures regardless of organism and tissue type) were established. Six genes were identified as reference genes (*ef1a*, *watc1*, *mtm1*, *spop*, *rxrba* and *tubal*) from a longer list of potential reference genes. These genes were stable across development, highly conserved, and were stable among variations of contaminants. The reference genes were tested in the zebrafish-model using embryos exposed to multiple sources of stormwater and oil. RNA was extracted from developing zebrafish and qPCR was performed using past stormwater and oil exposure experiments. These established reference genes are proficient to use as molecular indicators to identify target genes induced by pollution such as stormwater and oil exposures. Information on the toxic effects of stormwater in Seattle, Washington can impact local, state, and nationwide pollution policy and mitigation efforts. Stormwater policy analysis was mainly examined at the state level and was compared to stormwater policy in the state of Rhode Island. While many local initiatives are in place, the ongoing molecular research is an indicator that wastewater needs stronger regulatory measures in order to better mitigate its known impacts.

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**Keywords:** Zebrafish; salmon; reference genes; PCR normalization; qPCR; pollution policy; genetics; toxicology; Bestkeeper; Green Stormwater Infrastructure