

## Pathway-Based Analysis of Fish Transcriptomics Data Across Effluent Gradients in Minnesota Rivers

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As part of a larger effort to assess the health of streams and rivers in Minnesota, a series of caged fish experiments were conducted in three locations: Ely, Hutchinson, and Rochester. The experimental design placed caged fish (fathead minnows, *Pimephales promelas*; FHM) across a gradient of municipal effluent (upstream, at effluent, and downstream). While multiple endpoints and time points were evaluated, this study focuses FHM female ovary transcriptomics based on a 4 day field exposure. Overall, the number of differentially expressed genes were higher in Rochester (5847), than Hutchinson (1295) or Ely (867). Hierarchical clustering and PCA showed distinct separation among the upstream, downstream, and effluent sites at each location. Gene Set Enrichment Analysis (GSEA) showed the overall number of gene sets enriched followed the same pattern as the differential expression, with highest enrichment in Rochester and lowest in Ely. Comparing across the effluent gradient, different patterns were observed at each site. However, comparisons of upstream-downstream and downstream-effluent were able to identify several gene clusters that showed the same enrichment pattern across all three sites. In general, results from female FHM ovary Transcriptomics follow a similar pattern to those of the analytical chemistry and studies with male FHM livers (Transcriptomics and metabolomics). This study demonstrates the potential utility of transcriptomics in effects-based monitoring efforts. *The contents of this abstract neither constitute nor reflect official US EPA policy.*

**Key words:** transcriptomics, GSEA, pathway based analysis, effects-based monitoring

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