Retrospective temporal trend analysis of gene expression changes in bream livers from river Rhine (Koblenz)

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Anthropogenic substances can enter the aquatic environment with emissions from urban and industrial wastewater treatment plants, where they can cause both short-term and long-term environmental effects. The German Environmental Specimen Bank (ESB) [1] operates 17sampling sites at major German waterways where among other samples blood, liver and fillet samples from Abramis brama (bream) has been collected, analysed and archived at ultra-low temperatures annually since the 1990s. This study represents a pilot project in which toxicogenomic methods were applied to detect not the substances themselves in ESB fish samples, but the effects triggered by them. To test this approach, gene expression patterns were evaluated toxicogenomically in bream samples from the last three decades sampled at the river Rhine and compared with those from the comparatively unpolluted Lake Belau, and. Overrepresentation analyses of differentially regulated gene expression patterns were used to retrospectively identify impaired biological functions in the bream and to correlate these with known pollutant loads.

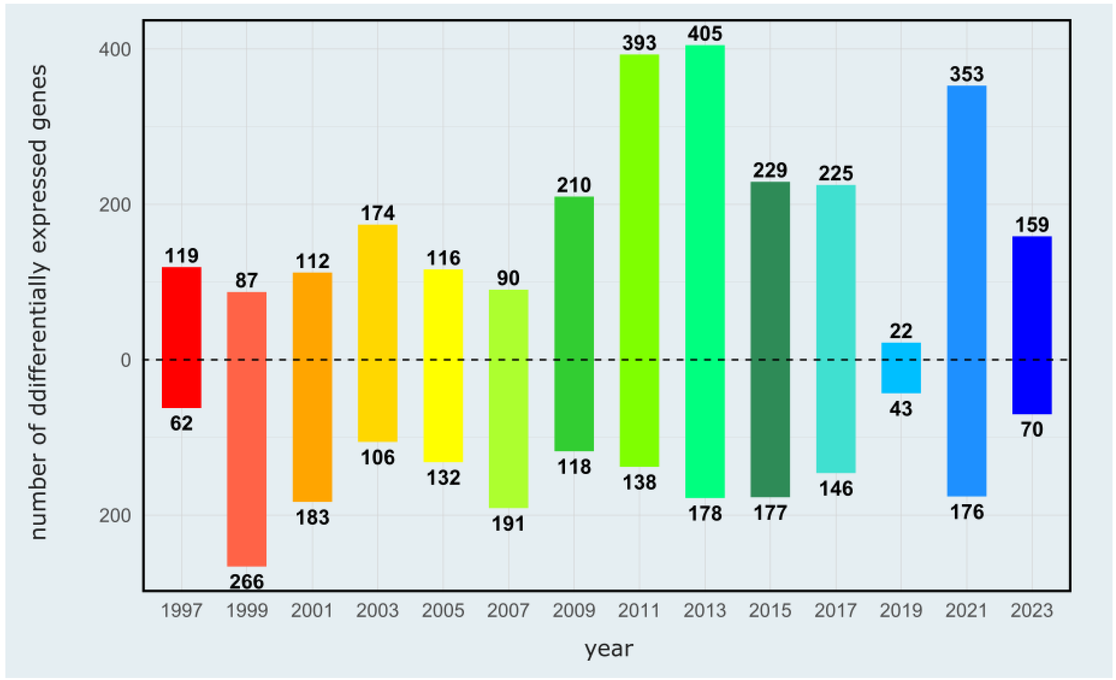

Figure 2: Numbers of differentially expressed genes per year as compared to Belauer See.

Materials and Methods

In the project, liver samples from bream in the German ESB (1997–2023) at the Koblenz (Rhine) and Lake Belau sites were analyzed every two years. Total RNA was stored at <–150 °C, extracted, quality-checked, and sequenced on an Illumina NovaSeq 6000. Reads were mapped to the bream genome using StringTie and STAR [2,3]. Gene expression differences between Koblenz and Lake Belau were assessed, and biologically relevant changes identified. Functional annotation with EnTAP and overrepresentation analysis (ORA) using ClusterProfiler [4] revealed biological functions linked to known chemical pollution (Figure 1).

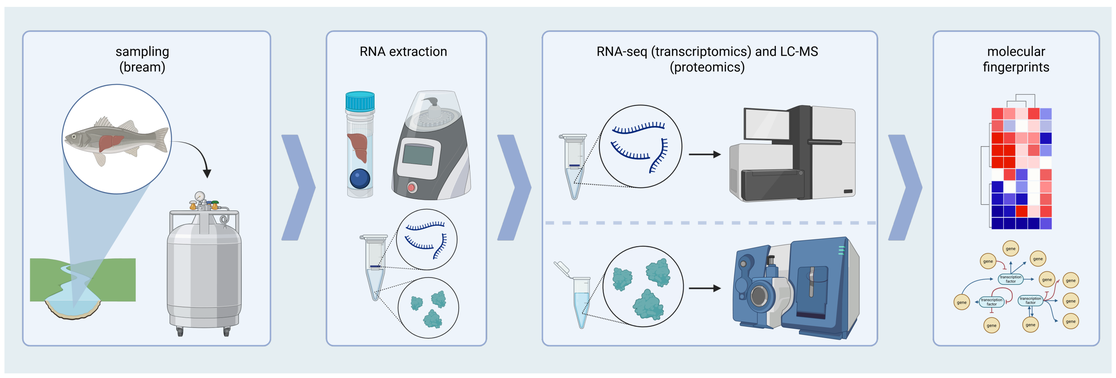

Figure 1: Experimental workflow for temporal trend analysis of gene expression changes in bream livers from river Rhine (Koblenz).

Results

High-quality RNA (RIN > 7) was obtained from all archived samples, enabling successful read mapping to the bream genome. Differentially expressed genes were identified across years using defined expression thresholds (Figure 2). ORA based on functional genome annotation revealed biological functions potentially affected at the Koblenz site compared to Lake Belau. Consistently enriched processes included organic acid, oxoacid, and sugar metabolism, while others, such as fat metabolism, were enriched only in specific years (Figure 3).

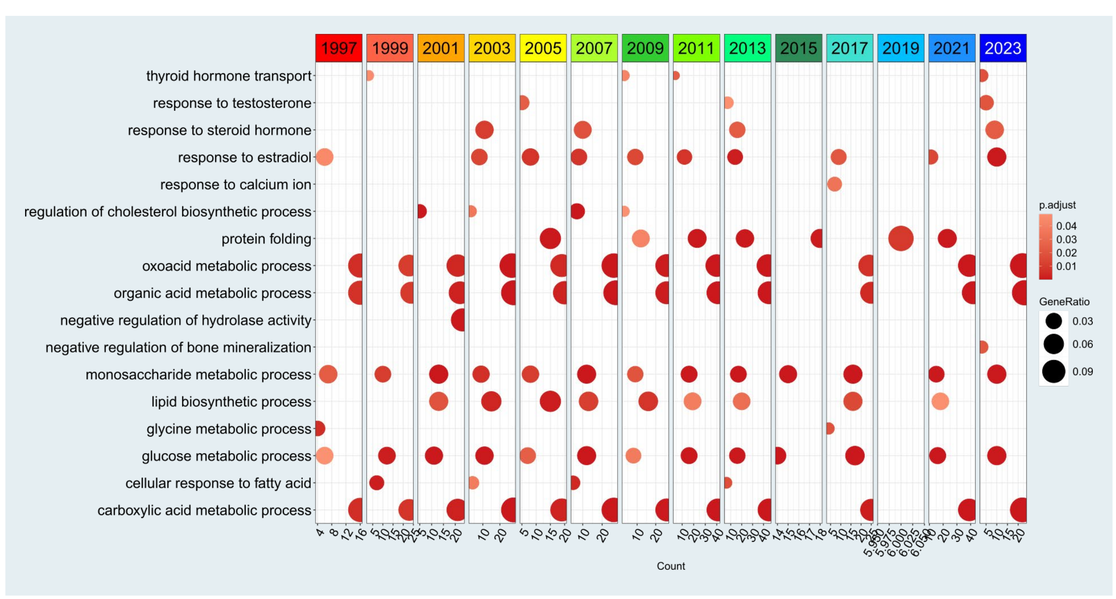

Figure 3: Overrepresentation Analysis of the biologically relevant genes identified to be differentially expressed at each time point when comparing Koblenz and Lake Belau. The colour code of the bubbles represents the Benjamini-Hochberg corrected p-values and the bubble sizes represent the gene count for each biological process.

Conclusion

This pilot study tested the feasibility of using archived German ESB samples for retrospective toxicogenomic analysis. Sample quality supported transcriptomic analysis, and bioinformatic evaluation yielded reliable results. However, identifying the exact drivers remains challenging due to multiple influencing factors beyond chemical pollution. Overall, the study demonstrates the potential and limitations of long-term archived samples for toxicogenomic monitoring.

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