Robots vs. Researchers: A Comparison of Larval Zebrafish Assessment Methods

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Research Goal and Approach

The U.S. Environmental Protection Agency (EPA) is using the Vertebrate Automated Screening Technology (VAST) robotic system for automated morphological analysis of zebrafish larvae.

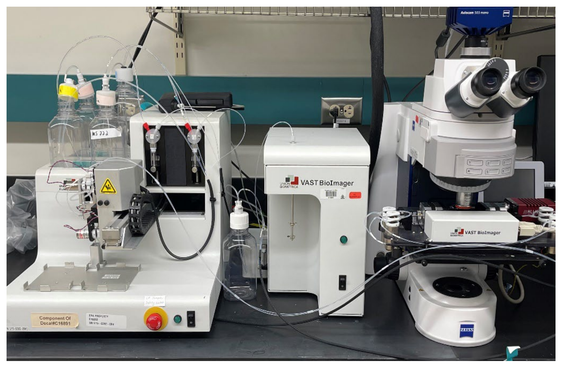
We compared human visual analysis to the automated analysis, by testing for statistically significant differences in various morphological endpoints between larvae rated as abnormal vs. larvae rated as normal. Our goal was to quantify morphological endpoints in addition to our traditional qualitative visual assessment.

Methods

Embryonic zebrafish were exposed to a chemical known to cause malformations during development.

Usual Field of View for the Human Assessors

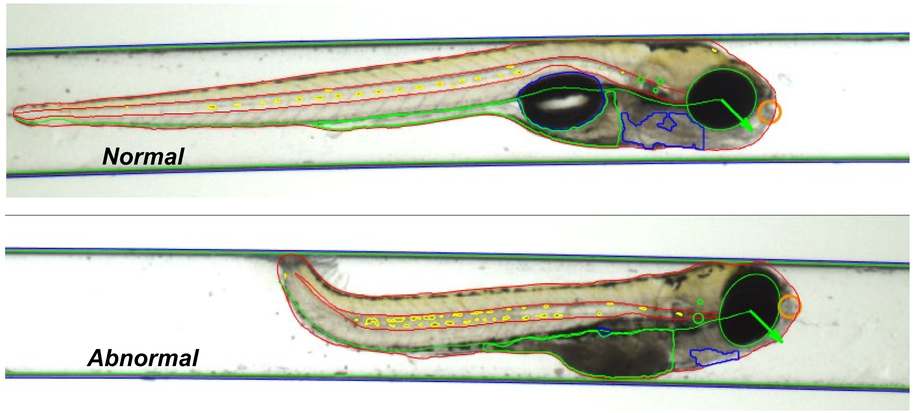
Exposure to the chemical ended at 6 days post fertilization (dpf), the larvae were visually assessed by trained humans, and classified as normal or abnormal.



VAST system

The larvae were imaged laterally by the VAST robot. From VAST images, FishInspector software measured and quantified morphological features. These measurements were organized in a spreadsheet generated by KNIME workflow.

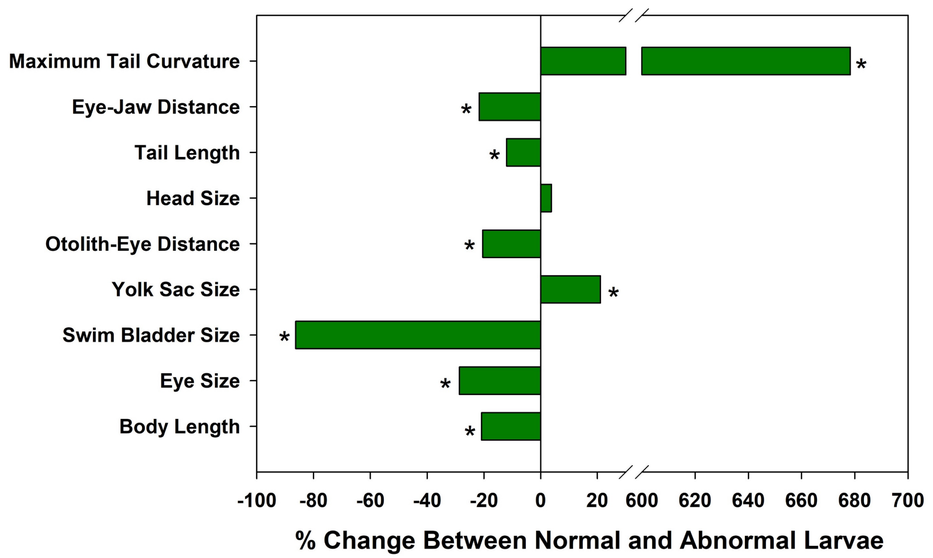
FishInspector Image Analysis of VAST images



FishInspector software generates tracings of morphological endpoints.

Results: Comparison of Human and Robotic Analyses

The larvae that were classified as "abnormal" by human observers showed many significant differences in their morphological structure compared to the larvae that were classified as "normal."



Results (continued)

- **The largest differences were noted in the swim bladder size (86% decrease) and the degree of tail curvature.**
- **In general, the abnormal larvae were smaller, had smaller eyes, more yolk, and had an uninflated swim bladder.**
- **As a zebrafish larva grows, the eye-jaw and the otolith-eye distances increase. The abnormal animals showed retardation of these normal increases.**

Conclusions

Our results support and extend our visual analysis. The VAST system and Software-generated measurements further expand the level of precision beyond normal/abnormal classifications.

Acknowledgements

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