A Benchmark Concentration Analysis Method for Larval Zebrafish Locomotor Response Data Using ToxCast Pipeline Software

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Issues Addressed by this Work

This work produces a reproducible analysis procedure for larval Zebrafish (*Danio rerio*) locomotor response (LMR) data applicable to high-throughput research by applying ToxCast curve-fitting software (tcplfit2).

Applying tcpl to Zebrafish Behavioral Data

Acclimation = A = $\{t_1, t_2, \ldots, t_{10}\}$
Light = L = $\{t_{11}, t_{12}, \ldots, t_{30}\}$
Dark = D = $\{t_{31}, t_{12}, \ldots, t_{50}\}$
Total = T = $\{t_{11}, t_{12}, \ldots, t_{50}\}$

Startle vs Average Speed in Light
$= strtlAavg = s_{31} - avgS_L$

Area Under Curve = $AUC = \frac{1}{2}(s_k + s_j) + \sum_{i=j+1}^{k-1} s_i$

Startle Fold-Change
$= strtlF = \frac{s_{31}}{s_{30}}$

AUC Ratio
$= AUC_r = \frac{AUC_D}{AUC_L}$

Habituation = $hbt = \frac{s_k}{s_j}$

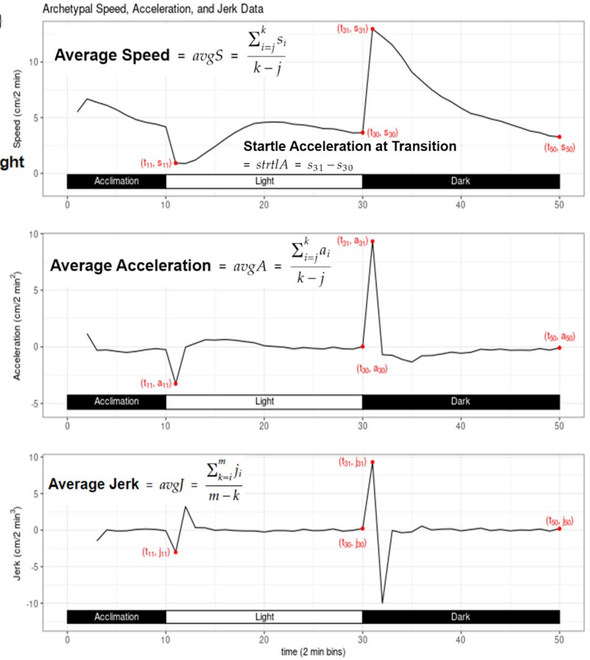

Fig 1: *Endpoints for LMR. Most endpoints are calculated for Light, Dark, and Total separately. Acclimation phase is not used in analyses.*

Box-Cox power transform

$$Y_i^{(\lambda)} = \begin{cases} \frac{(Y_i + c)^{\lambda} - 1}{\lambda} & (\lambda \neq 0) \\ \log(Y_i + c) & (\lambda = 0) \end{cases}$$

Equation for cutoff:

$$Var(\overline{x_t} - \overline{x_0}) = \widehat{SE_{diff}}^2 = \frac{{s_0}^2}{n_t} + \frac{{s_0}^2}{n_0} \quad ; \quad cutoff = 3 \cdot \widehat{SE_{diff}}$$

Results

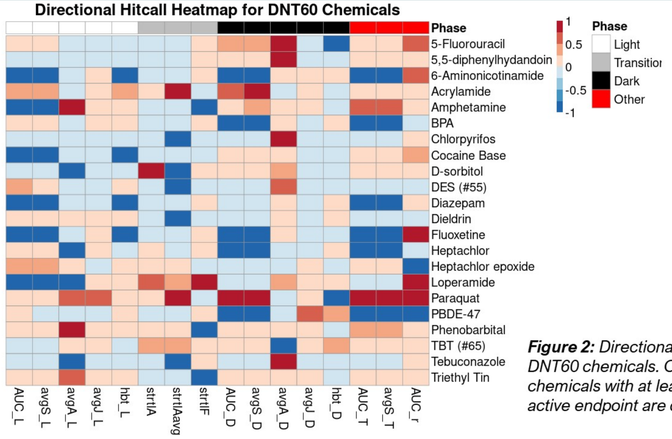

Figure 2: *Directional hitcalls for DNT60 chemicals. Only hitcalls chemicals with at least one active endpoint are displayed.*

- 22/61 (36%) chemicals were found to have a *hitcall* greater than the significance level of 0.8 in at least one of the 16 endpoints.
- Conservative detection of activity for chemical set.
- Average jerk endpoints (avgJ) for both Light and Dark were only two endpoints where chemical activity was not detected.

Endpoint	Number of Chemicals Found Active	Endpoint	Number of Chemicals Found Active
Area Under the Curve in Light	6	Area Under the Curve in Dark	6
Avg Speed in Light	6	Avg Speed in Dark	7
Avg Acceleration in Light	5	Avg Acceleration in Dark	4
Avg Jerk in Light	0	Avg Jerk in Dark	0
Habituation in Light	4	Habituation in Dark	2
Startle Acceleration	1	Area Under the Curve Over Total Experiment	7
Startle vs. Average Speed in Light	7	Avg Speed over Total Experiment	7
Startle Factor	4	Ratio of AUC_D to AUC_L	5

Figure 3: *Number of chemicals found active in each of the 16 constructed endpoints.*

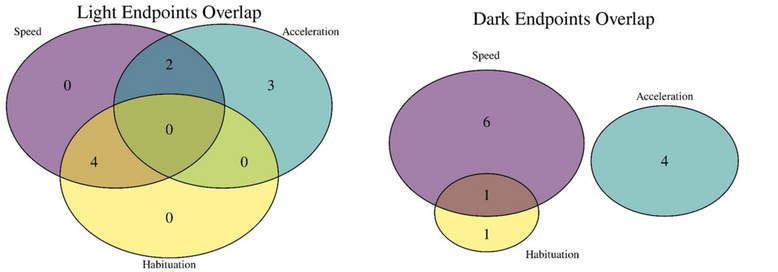

Figure 4: Left: *Overlap of chemicals found active by endpoints describing Light phase.* ***Center:*** *Overlap of chemicals found active by endpoints describing transition from Light to Dark.* ***Right:*** *Overlap of chemicals found active by endpoints describing Dark phase.*

Conclusions

- The ability of this battery of endpoints to multidimensionally describe zebrafish behavior and identify unique chemical effects in a high-throughput format has been displayed.
  - *hitcalls* and BMCs were produced for 976 chemical endpoint pairs.
  - Endpoints identified unique sets of chemicals as active indicating the utility of this set of endpoints in detecting behavioral changes.

Issues to Address

- Possible inverted U-shaped concentration response behavior frequently seen in Zebrafish LMR assays is inaccurately modelled by tcplfit2 curve fitting functions.
  - A new curve-fitting function may be added to supplement set of 9 included in tcplfit2.

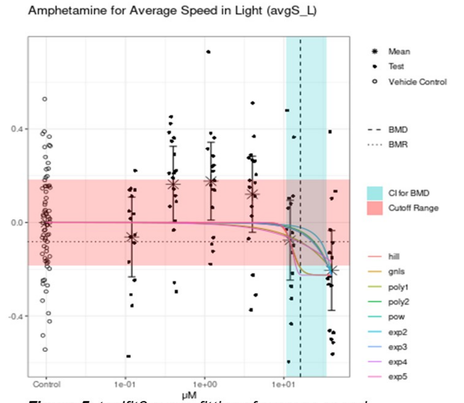

Figure 5: *tcplfit2 curve-fitting of average speed in Light data for Amphetamine exposure*

Disclaimers

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