

The Fish Behaviour Index (FBI)

- **Use of the FBI is conditional upon citing:** Anthony G. Deakin, Jonathan Buckley, Hamzah S. AlZu'bi, Andrew R. Cossins, Joseph W. Spencer, Waleed Al'Nuaimy, Iain S. Young, Jack S. Thomson and Lynne U. Sneddon (2019) Automated monitoring of behaviour in zebrafish after invasive procedures. *Scientific Reports*, In press
- The FBI runs in Excel and is unsupported but informal enquiries can be made to Anthony Deakin (anthonyd@liverpool.ac.uk) or to Lynne Sneddon (lsneddon@liverpool.ac.uk)
- **This work was funded by NC3Rs UK**
(<https://www.nc3rs.org.uk/detection-assessment-and-alleviation-pain-laboratory-zebrafish>)

The current fish behaviour index, FBI, analysis module is self-contained within a Microsoft Excel spreadsheet with the following attributes:

Current version:	FBI 1.xlsm
Requires:	Microsoft Excel
Location:	may be run from any location
Modes:	2 usage modes: A. post-hoc analysis of zebrafish behaviour B. live analysis of zebrafish behaviour
Available:	Figshare DOI: 10.6084/m9.figshare.7991600

Opening the FBI 1 spreadsheet shows the screen displayed on the following page. The different areas of the screen are labelled as **Data Area**, **Output Area** and **Input Area**.

There are also two macro buttons “Run” and “Stop” that are used in live mode B.

AutoSave Off

FBI 1.xlsm - Excel

Anthony Deakin

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A1. Post-hoc Analysis Of Zebrafish Behaviour

The post-hoc analysis mode uses data on fish movement generated by tracking software.

The data generated by the University of Liverpool tracking software, which is illustrated on SI videos 1 and 2, is output to an intermediate file named “Data.txt” in a directory “C:\FishData” as well as to a video specific output file, for example “Video 1 10% acid lip.data”. In this example, the subject zebra fish lip was treated with 10% acetic acid *after* Video 1.

Data Area

The data file is in text format and has the following comma-delimited structure:

0.21629,142.11,108.46,98.39,110.41 where the first data item is an internal program stamp to be ignored here and the following four data items are the pairs of x and y coordinates for the fish from the front tank view and the overhead view.

The coordinates are relative to the image size. If the image size changes between experiments, by for example moving camera location or zoom, the dimensions of the image require to be updated in the Input Area.

Note, if the frame rate changes from 8 frames per second, this requires to be updated in the Input Area and other changes are required.

A2. Post-hoc Analysis Of Zebrafish Behaviour

Input Area

With the “Video 1 10% acid lip.data” example, the frame rate is 8 and the video dimensions are 514 x 268 pixels for the front view of the tank and 372 x 244 pixels for the overhead view.

(Note: for technical reasons the tracking software records coordinates at 50% of the video dimensions).

Output Area

When data is present in the Input Area, the analysis is shown in the Output Area.

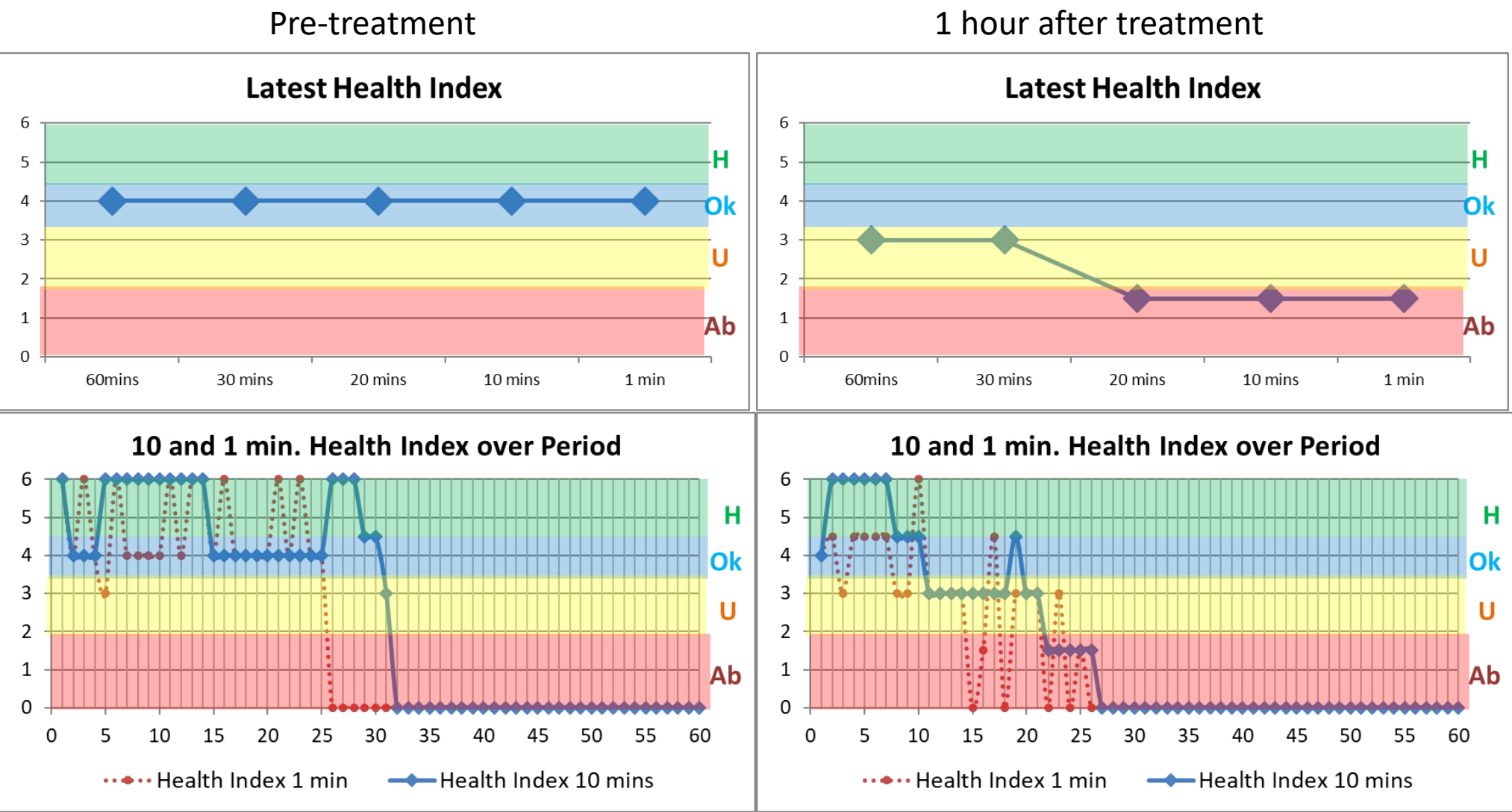
This consists of calculations of Distance and Activity, scatter plots of the fish movement in the tank (front and overhead views), Zone Analysis, Activity View, plus evaluations of the history of the FBI over 10 and 1 minutes as well as the latest 1 minute, 10 minutes, 20 minutes, 30 minutes, and 60 minutes. Data is extrapolated to the longer timescale where required.

New Data

To process another video, existing data is deleted, either manually or using the inbuilt macro “ClearData”. Then new data is copied in from the data file, for example “Video 2 10% acid lip.data”. This file is for the same 10% Acid Lip fish subject at 1 hour *after* treatment. The data is pasted from cell F10 onwards.

(Note: the video data file can be opened as comma delimited format and the data copied and pasted directly to FBI at F10 to J12009 in this example or pasted into the Data.txt file which is linked to FBI).

A3. Post-hoc analysis of zebrafish behaviour, videos 1 and 2 10% acid lip.data example.



B1. Live Analysis Of Zebrafish Behaviour

1. Set up the Zebrafish Tracking Software (Figshare DOI:10.6084/m9.figshare.7971122) and run the software
2. Do not overlap the tracking software and FBI screens
3. The current live FBI analysis mode generates the same results as the post-hoc examples (the accompanying examples on Figshare were recorded live).
4. Live FBI should be started, 1 minute after the tracking software has been started, by clicking on the “Run” macro button. This clears the data area. A time interval in seconds for updating is then entered, currently 73 seconds is used. At the expiry of the interval, it then copies the coordinate data from “C:\FishData\data.txt”, as produced by the tracking software, to “C:\FishMon\data.txt” to which FBI is linked and refreshes the outputs. The previous data will clear out and the status will register as abnormal (this will update as data is refreshed). Do not click data files during the live data acquisition
5. The FBI updates every 60s.
6. The analysis loop is stopped by clicking the “Stop” macro button which enables the current loop to complete. You may need to wait 73s to the FBI stopping. At the end save the spreadsheet.
7. In these examples, the videos were recorded for 25 minutes each. The output graphs would then be truncated at 25 minutes.