

## Protocol C: Video recording zebrafish behaviour

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### Introduction

The provision of [enrichment for zebrafish](#) is not as common as it is for terrestrial research animals, with the most common form of zebrafish enrichment being [live food, such as rotifer](#). Reasons for this include issues with the practicality of including structural enrichment within tanks and concerns about contamination of tank (and system) water by enrichment items.



There is an evidence base suggesting that the provision of physical enrichment can reduce certain negative welfare indicators in zebrafish (e.g. a reduction in the 'stress hormone' cortisol; see [Stevens et al. 2021](#) for a review of the literature). However, it is perhaps more difficult for us to see the benefits of enrichment in this species and to recognise when zebrafish are experiencing a positive welfare state, compared to mammalian species commonly used in research.

All of these reasons make it especially important to properly evaluate environmental enrichment for zebrafish and, if possible, to [share your findings](#) to advance our understanding of this area.

### About the protocol

This example protocol is a step-by-step guide on how you might prepare for and carry out basic behavioural observations, using video recording equipment, to answer the questions:

- Is an enrichment item used by fish?
- Does the presence of enrichment affect the behaviour of the fish?

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More complex evaluations of enrichment can be found in the published literature.

Within the protocol \*Rand indicates an opportunity to incorporate >randomisation< into your study, and \*Flex indicates a part of the protocol that is flexible depending on your circumstances (e.g. how many animals/tanks are available to study).

General advice is given on how to look at the data generated by this protocol in order to draw conclusions about the questions of interest. More detailed instructions on using MS Excel to look at data and insert charts can be found in Protocols A, B and D. If you would like to carry out statistical tests on your data, we recommend consulting someone with statistical expertise before you begin data collection.

[More information on behaviour observations](#) can be found in the 'Evaluating environmental enrichment' resource. [Published studies that include behaviour observations](#) can be found on the 'Example enrichment study protocols' page.

## Protocol steps

1. Define your behaviours of interest
2. Select the tanks to observe
3. Assign the tanks to a treatment
4. Create a schedule of observations
5. Add the enrichment to the tanks
6. Allow the fish to acclimatise to the enrichment
7. Have a dry run with your equipment
8. Make your recordings
9. Back up your recordings
10. Watch the recordings back
11. Input your data onto a spreadsheet

### 1. Define your behaviours of interest

In this example two measures will be used to evaluate the enrichment:

- The number of interactions with the enrichment items.
- The number of aggressive interactions observed.

More measures can be added but remember that the number and type of behaviours of interest will need to be manageable for you to observe and record.

As you will be video recording behaviour, it will be possible to re-watch the videos if you decide to record the occurrence of another behaviour at a later date.

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In order to collect data consistently, the behaviours must be well defined; similar to when [creating an ethogram](#). Define which behaviours will be counted as aggressive interactions and which will not. The examples shown in Table 1.1 were created using the [general zebrafish ethogram](#).

Behaviour of interest	Definition
Interact with enrichment	Passing through the enrichment item, making contact with it, or resting underneath it.
Aggressive interactions	<p>One fish moves towards a second fish, increasing acceleration, while the second fish avoids the first. Mouth opening and biting may occur. Erratic swimming patterns may be observed, such as the pursued fish making sharp (as opposed to smooth) turns in an attempt to flee from the aggressor.</p> <p><b>Do not</b> count non-aggressive following behaviour, i.e. no increased acceleration, no lunging or striking towards others observed. The other fish does not appear agitated by the follower and does not seek to escape from the follower by swimming erratically; turns are smooth.</p> <p><b>Do not</b> count nose-tail touching. This is a non-aggressive social interaction, commonly seen during courtship. A fish touches the side or tail of another fish with the nose or head.</p>

**Table 1.1.** Examples of how to clearly define which behaviours you will and will not record.

## 2. Select the tanks to observe

Select <sup>\*Rand</sup> eight <sup>\*Flex</sup> tanks to observe. Four tanks will have enrichment, and four will act as controls to gauge the level of aggression in the absence of enrichment.

## 3. Assign the tanks to a treatment

Assign <sup>\*Rand</sup> the tanks you have selected to the enriched or control category. For example, write down the tank numbers on different scraps of paper, shuffle them and then pick them out of a container. The first four you select can be assigned to the 'test tank' treatment, which will contain the new enrichment. The remaining four will be control tanks.

## 4. Create a schedule of observations

Factor in time for fish to acclimatise to the presence of the new enrichment (one to two weeks), and for fish to settle after setting up the recording equipment. Keep these periods the same for all tanks.

You may wish to look at behaviour during feeding time, or you may wish to avoid this period of excitement. In any case, it is important to be aware of factors that may cause a disturbance and to record this information for reference when you are interpreting your findings.

For the purpose of this example, there is only access to one camera, so recordings cannot take place simultaneously. If it is possible to carry out simultaneous recordings, prioritise recording from a test and control tank at the same time.

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Your schedule will depend on how many tanks you have chosen to observe and your other commitments. You may wish to make multiple recordings in one day or spread them out over a longer period.

Table 1.2 shows a recording schedule for eight tanks (with a single camera) spread over two weeks.

<b>Week 1</b>	
<b>Tank</b>	<b>Recording slot</b>
Test 3	Tues 10 AM
Test 2	Tues 3 PM
Control 1	Weds 10 AM
Control 2	Weds 3 PM
<b>Week 2</b>	
<b>Tank</b>	<b>Recording slot</b>
Control 4	Mon 11 AM
Test 1	Mon 4 PM
Test 4	Tue 11 AM
Control 3	Tue 4 PM

**Table 1.2.** Schedule for recording fish behaviour.

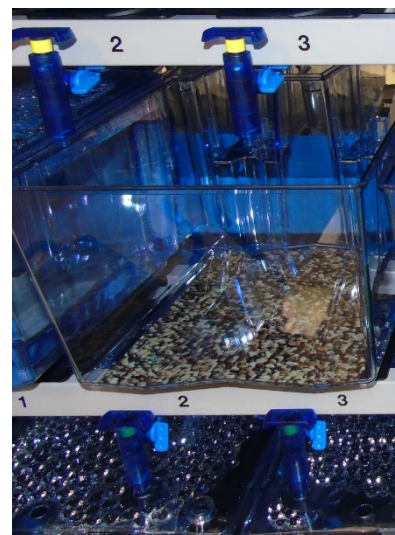
Note that two control tanks and two test tanks have been assigned to the recording slots, but otherwise the allocation was random. The observations will be spread over the day, to attempt to account for other factors that may affect behaviour (e.g. differences in activity levels between the morning and afternoon).

### 5. Add the enrichment to the tanks

The control tanks should be your current, standard tank set up, before the enrichment you are evaluating is introduced. For this example, the control tanks are barren, with an image of gravel placed underneath them.

To create the test tanks, add any standard items included in the control tanks (in this case, the image of gravel) and then add the enrichment item(s) you want to trial. For the purpose of this example, physical enrichment (shelters) will be trialled. Note the date that the enrichment was added to the tanks, this may be useful information (e.g. if you decide to observe longer term responses to enrichment over time).

Two shelters will be added to each test tank in an attempt to provide sufficient physical enrichment for the number of fish within the tank, and in anticipation of competition over the resource.



*Image credit: Dr Will Norton, University of Leicester*

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Different combinations of enrichment items can have different outcomes, so make sure you have made a note of exactly what each tank set up contains (e.g. control: gravel sticker only and test: gravel sticker with two identical shelters) so that you have this information when you come to report your results.

## **6. Allow the fish to acclimatise to the enrichment**

Studies where fish have indicated a preference for enrichment typically allow an acclimation period of several days ([Stevens et al. 2001](#)).

This suggests that for an accurate evaluation of enrichment items the fish will require time to settle before you begin your observations.

Allow the fish one to two weeks between introducing the enrichment and recording data. During this period, monitor the test tanks closely to ensure that no harm comes to the animals.

## **7. Have a dry run with your equipment**

You will need to find the best position for the camera so that the whole tank and all the fish are visible on the recording. This may be filming through the front of the tank or an aerial view.

Make a recording and check the video. Ensure that there is enough device memory to record for the intended period.

## **8. Make your recordings**

Following your schedule, carefully set up the recording equipment and begin the recording.

Leave the camera filming for one hour <sup>\*Flex</sup>.

## **9. Back up your recordings**

Create a backup (or two!) of your experimental videos as soon as possible (e.g. save them onto a PC and upload them onto Cloud storage). If your device has limited space to store recordings, delete them once they are backed up. This will prevent your next recording getting cut short due to lack of device memory.

## **10. Watch the recordings back**

Discount the first 30 <sup>\*Flex</sup> minutes of the video as an acclimatisation period, to account for the disturbance of setting up the camera. Keep this period the same for all the videos.

Observe behaviour starting just after 30 minutes <sup>\*Flex</sup>, for a period of ten minutes. Record all incidents of any fish within the group performing [the behaviours that you previously defined](#).

Pause the video when you notice a behaviour of interest and record it on a [data collection sheet](#) or straight onto an Excel spreadsheet. You may need to rewind the video to note all the behaviours, especially if more than one fish is near to or interacting with the enrichment at once. You may want to count the interactions with enrichment first and then play the same section of the video back again to record incidents of aggression.

Because interactions with the enrichment should be obvious, increasing the playback speed of the videos may be possible to collect the data more quickly. If you have more than one enrichment item, it may help to watch the video with a colleague, with each of you focusing on a different item.

Recording incidents of aggression within a group may be more challenging. If you enlist the help of colleagues, ensure that they are clear on what should be recorded as aggression.

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Your notes might look something like Figure 1.1.

Tank #	Day since enrichment added	Control/Test	Interactions with enrichment	Incidents of aggression	Notes
3	9	Test	III - 3	II - 2	Tues 10 AM
2	9	Test	IIII - 9	IIII - 4	Tues 3 PM
1	10	Control	NA	II - 2	Weds 10 AM
2	10	Control	NA	III - 3	Weds 3 PM
4	15	Control	NA	II - 2	Mon 11 AM
1	15	Test	IIII - 8	II - 2	Mon 4 PM
4	16	Test	IIII - 4	0	Tue 11 AM
3	16	Control	NA	II - 2	Tues 4 PM

**Figure 1.1.** An example of a completed data collection sheet.

Note that this stage of data collection is at risk of [bias](#) due to preconceptions about how enrichment may affect aggression.

To avoid this, enlist the help of colleagues. Ideally these colleagues should not be aware of the purpose of the study, but this may not be possible.

Multiple people can watch and score the same videos to help maintain [observer consistency](#).

### 11. Input your data onto a spreadsheet

At this stage it will be possible to [blind](#) the data to minimise the risk of bias during data analysis.

### Looking at the data

General advice is given how to look at the data generated by this protocol to answer the draw conclusions about the questions of interest. If you would like to carry out statistical tests on your data, we recommend consulting someone with statistical expertise before you begin data collection.

Remind yourself of the aims of the study. The questions of interest are shown below with suggestions on how to approach them.

#### Is an enrichment item used by fish?

Following Protocol C will have generated count data on how frequently the fish interact with the enrichment.

This will give you a general idea of whether the fish find the enrichment useful, but it will be up to you to interpret if the number of interactions observed indicates interest.

*Detailed guidance on using the COUNTIF and SUM functions is included in 'Looking at your data' for [Protocol A](#) and [Protocol D](#).*

*More information on using different types of charts can be found in the 'Looking at your data' section of [Protocol B](#).*

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Collecting this data at different time points will allow you to investigate whether use of the enrichment increases, decreases or generally stays the same at different times of the day, or over longer periods (e.g. by comparing the number of interactions with the enrichment counted on week one vs. week six).

A change in enrichment use over time can be visualised using a figure such as a line graph.

### **Does the presence of enrichment affect the behaviour of the fish?**

Following Protocol C will have generated count data on how frequently aggressive interactions occurred.

Find the total number of aggressive interactions observed for the Control tanks and the Test tanks and compare these numbers.

You can visualise this data as a bar chart, or a scatterplot (with individual tanks plotted as individual data points).

### **General advice on summarising your data**

Use the =SUM function in Excel to give you the total number of interactions.

Use the =MEAN function in Excel to give you the mean (average) number of interactions.

SORT and FILTER the data to break down your data summary by tank.

Use Insert Charts in Excel to visualise your data. Tutorials are available online (e.g. on YouTube) if you require extra help with this.

The basic steps for creating a chart in Excel are:

- Arrange the data you want to visualise into a table.
- Highlight the table using the cursor.
- Select the 'Insert' menu.
- Choose the chart you wish to plot.
- Amend the chart as necessary (e.g. adding axis labels, changing the formatting or changing how the data is presented).

### **What next?**

Carefully consider what you have found and whether any further investigation would be useful.

Refer to the Evaluating Enrichment Resource for advice on [implementing](#) and [sharing your findings](#).

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