

ANIMAL BEHAVIOUR

LAB PRACTICAL












The effect of ambient temperature on the activity levels of fruit flies (*Drosophila melanogaster*)

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The objective of this lab practical is to examine changes in locomotor activity of fruit flies, *Drosophila melanogaster*, in relation to ambient temperature variations. Both manual and automated data collection methods are implemented.

TABLE OF CONTENTS

INTRODUCTION	3
EXPERIMENT OVERVIEW	4
EXPERIMENTAL PROCEDURE	5
 Step 1 Lab introduction & preparation.....	5
 Step 2 DISCUSSION: Hypothesis and predictions (10-20 minutes)	5
 Step 3 Sample identification.....	6
 Step 4 Locate the fly	7
 Step 5 Video decoding.....	7
 Step 6 Data amalgamation.....	9
 Step 7 Calculations	10
 Step 8 Data exploration.....	11
RESULTS AND CONCLUSION	15
 Step 9 DISCUSSION: Data interpretation (~10 min)	15
 Step 10 DISCUSSION: Evaluate the methodology (~10 min)	15
 Step 11 Reporting your findings.....	16
REFERENCES	17

 Group discussion
 Individual work

INTRODUCTION

Animals are sensitive to fluctuations in their environment and adjust their behaviours accordingly (Traill et al., 2010). Altered environmental parameters, such as variations in the thermal ecosystem may impact breeding activities (Heath et al., 2001), developmental trajectories (Burger, 1998), and foraging and resting patterns (Armbruster & Berg, 1994).

Understanding the effect of different environmental drivers on animal behaviour is particularly relevant in light of climate change and how it is transforming animal habitats.

Behavioural activity is a transition between active and inactive states. This may involve gradual and steady behavioural changes as well as sudden reactions to environmental factors (Cole, 1995). Many of such behaviours are composed of movements that occur in specific sequences and patterns that may be highly species-specific (Katsov et al., 2017). In order to understand the effect of a particular setting on animal behaviour, many researchers examine locomotor activity.

Like many animals, *Drosophila* fruit flies are influenced by the circadian clock (Foster & Helfrich-Forster, 2001) and exhibit sensitivity to photoperiods, regulated by the change in the quantity and quality of light throughout the day (Rieger et al., 2012). This becomes evident when examining their locomotor activity which reveals increased activity in the presence of light (Martin et al., 1999).

Ectothermic animals, such as fruit flies, are highly influenced by temperature where extreme temperatures negatively affect their physiology and biological functions (Garrity et al., 2010). For this reason, it is crucial for these animals to be able to reliably predict regular daily temperature fluctuations and align their activity peaks with optimal temperatures. Sexually mature fruit flies show a temperature preference between 24°C and 26°C which is associated with a peak activity level (Bywalez et al., 2012; Rieger et al., 2003). This is further related to the time of day, where the morning peak of locomotor activity was associated with the morning rise in temperature, and the evening peak relates to the fall in the afternoon temperatures (Bywalez et al., 2012). This suggests that activity patterns of *Drosophila* are influenced by multiple factors, including ambient temperature, light intensity, and the circadian clock.

LEARNING OBJECTIVES

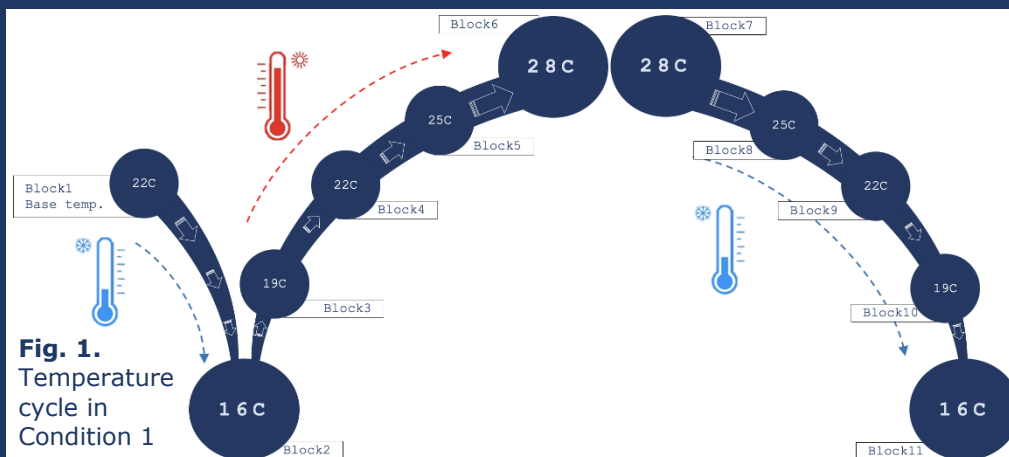
- To gain practical experience decoding animal behaviour from video recordings
- To consider how temperature may affect physiological processes and animal behaviour
- To compare and contrast data from manually versus automatically generated behavioural data and discuss their pros and cons
- To practise hypothesis testing

EXPERIMENT OVERVIEW

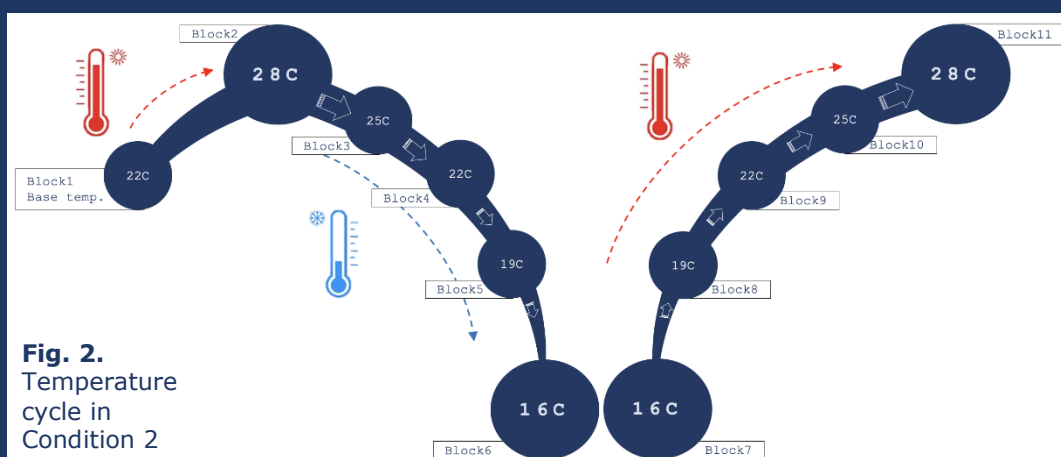
In this experiment, you will be introduced to the concept of behavioural decoding of locomotor activity in fruit flies and examine activity patterns in relation to temperature variation.

- ✦ Fruit flies experienced one of two different conditions in this experiment: 1) Increase-Decrease and 2) Decrease-Increase.
- ✦ Fruit flies in each condition all started at the base temperature of 22°C before experiencing their respective temperature treatment.
- ✦ Each condition had 10 temperature-steps creating 11 “blocks” of ambient temperatures experienced by the flies (block 1 always being 22°C).
- ✦ In each block, flies were allowed 20 min to acclimatise.
- ✦ Towards the end of the acclimatisation period, a 30 sec video was recorded of their activity. These videos can be used to manually decode the total period of fly movement (versus inactivity) during the 30 sec. Furthermore, software automatically measured the total distance moved by each fly during the 30 sec.

Condition A (abbreviation: CA): Increase-Decrease. A plate of 24 individually housed fruit flies experienced a drop in temperature to 16°C (block 2). The temperature was then increased at 3°C increments until reaching 28°C (block 6) where it was kept for one more step (block 7) before incrementally returning to 16°C (Fig. 1).



Condition B (abbreviation: CB): Decrease-Increase. A plate of 24 individually housed fruit flies experienced an increase in temperature to 28°C (block 2). The temperature then decreased at 3°C increments until reaching 16°C (block 6) where it was kept for one more step (block 7) before incrementally returning to 28°C (Fig. 2).



EXPERIMENTAL PROCEDURE | Instructions

Step 1 | Lab introduction & preparation

To begin with, please watch [this](#) video to familiarise yourself with the data collection process undertaken for this experiment. While you are welcome to watch the entire clip (9min 20sec), the first 5min 45sec are the most important.

Before you begin, please ensure that you have the following available:

- ✦ Laptop or computer to watch the video (no sound required)
- ✦ Microsoft Excel
- ✦ Access to the internet
- ✦ Your unique Lab Student ID (a number from 1 to 10) and your Student Set ID (set X, Y or Z) (provided by your lecturer/instructor)
- ✦ Access to *Drosophila* data files (provided by your lecturer/instructor)

Step 2 | DISCUSSION: Hypothesis and predictions (10-20 minutes)

In small groups (either face to face or using virtual break rooms) please discuss with your peers how you think ambient temperature might affect fruit fly behaviour. Use the below questions as inspiration for your discussion, and then form a hypothesis and one or more associated predictions derived from your hypothesis.

- ✦ *What do you think happens in the physiology of the flies at different temperatures?*
- ✦ *How might temperature variations impact their metabolic rate?*
- ✦ *What kind of behavioural change (if any) would you expect to see in the locomotor activity (distance moved, time spent moving, and speed of movement) of fruit flies in relation to ambient temperature?*

Note: *The hypothesis could state a relationship between variables that is either positive, negative or neutral; there is no right or wrong option at this stage.*

1 HYPOTHESIS

A hypothesis is an educated guess: a statement of how you think ambient temperature might affect *Drosophila* locomotor activity and/or physiology.

A hypothesis should be based on a strong rationale using previous research findings and your own knowledge.

1 PREDICTION(S)

Predictions should identify your specific dependent variable(s), e.g. 'time spent moving', and your independent variable(s), e.g. 'ambient temperature', and specify which relationship you would expect between these two variables (positive, negative, or neutral).

Step 3 | Sample identification

Each student ID (1-10) has been randomly allocated a set of 16 individual fruit flies at various temperatures to observe and from which to decode activity data. It is important that you correctly identify your allocated flies (corresponding to your student ID).

All videos have a unique ID indicating condition (CA or CB) and block (video 1-11). Each video is a recording of the entire plate of 24 flies. You will need **both the video ID and the fly ID** in order to find the right fly to observe and decode behavioural data from.

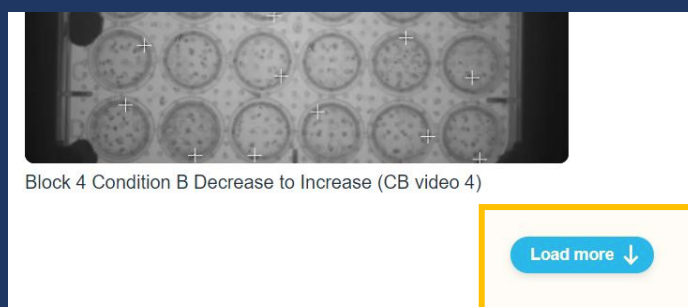
Later on in the practical, after you've decoded your data, your data will be merged with that of your fellow classmates so that you will have a larger dataset to play with.

Please follow the steps below:

- ✦ Create a new folder on your computer and open up this new, empty folder
- ✦ Download the Decoding File and save it in your new, empty folder
- ✦ Open up the Master File (no need to download it at this stage)
- ✦ Locate your Student ID in the Master File (in Column C)
- ✦ You can find your allocated flies by looking at both the video ID (Column D) and fly ID (Column E)
- ✦ Now copy-paste the 16 Video IDs and Fly IDs (corresponding to your student ID) from the Master file into the Decoding file you have saved on your device (into columns B and C)

A	B	C	D	E	F	G	H	I
sort #	STUDENT ID	VIDEO ID	FLY ID	COND	BLOCK	TEMP	MODE	
1	1	1	CA video 11	A2	CA	11	16	dec
2	2	1	CA video 2	B5	CA	2	16	inc
3	3	1	CA video 3	A3	CA	3	19	inc
4	4	1	CA video 5	C3	CA	5	25	inc
5	5	1	CA video 5	D4	CA	5	25	inc
6	6	1	CA video 8	B5	CA	8	25	dec

- ✦ Identify the first of your video IDs in your Decoding file, and locate the specific video at the [ASAB](#) website (you may need to scroll down quite a way, and perhaps click the 'load more' button)



Step 4 | Locate the fly

Once you open and play your first video you will notice a black and white plate with 24 arenas distributed across 4 rows (A-D) and 6 columns (1-6) (*you can see the letters and numbers very faintly on the video and are here depicted more clearly*).

- Each circle represents a separate arena with a fly.
- In order to find a specific fly, please use the Fly ID in your Decoding file to locate the corresponding arena ID. For instance, the D3 fly will be located in row D, column 3.

NOTE: *This step is extremely important to get right! Some of your classmates will be decoding data from the same flies as you, and so matching the correct data with the correct fly ID is crucial.*



Step 5 | Video decoding

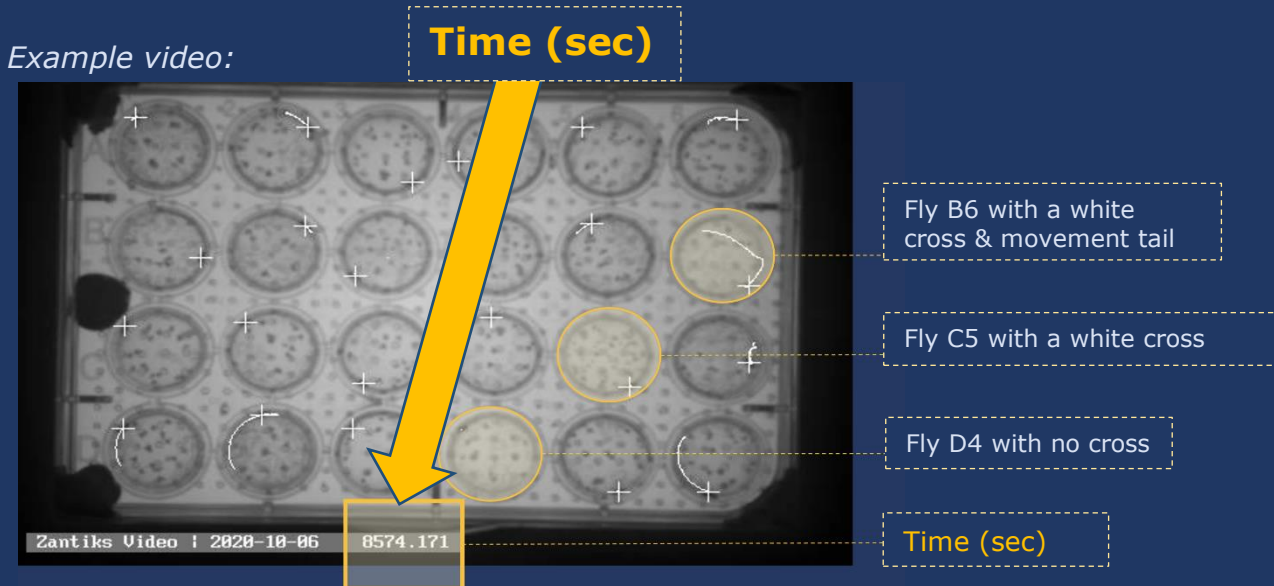
Having located your first fly, the next step is to decode the movement of this fly in the Decoding File by following the steps on the next page. (*You will repeat these steps for each of your 16 flies, one at a time*).

- The variable you will be recording is the *time spent moving* for each fly.
- This will be recorded by noting down the *start time* and the *stop (end) time* for each single move that the fly does within the 30sec video. Excel then automatically calculates the duration of movement for each single move, as well as the total duration of movement during the whole video.
- The Excel sheet allows for recording multiple movements per fly, but some flies will not move at all, some will move only once, and some will move several times.

	G	H	I	J	K	
		MOVE 1			MOVE 2	
Start		End	Duration	Start	End	Dur
	8574.2	8576.8	2.6	8577.0	8583.9	
	0.0	0.0	0.0	0.0	0.0	
	0.0	0.0	0.0	0.0	0.0	

For each of your 16 flies:

- Locate the position of your fly within the arena. Most flies can be identified by a white cross over them.
- Play the video from the beginning and record the **start** and **end** time of each movement made by the fly during the 30 sec video.
- To do so, pause the video each time the fly starts to move and when it stops, and then use the time format found at the bottom of the video to enter into the Video decoding file (identified in yellow in the screenshot below). (*You will have to move the cursor AWAY from the video recording in order to see the time format on the recording!*)



- If the fly moves more than once in the 30 sec video, all individual periods of movement starts and stops should be included as a separate movement, e.g., if your fly moved twice during the video, please provide start and end times for both events.
- If the fly does not move at all, simply write a '0' for both Start and End of Move 1. This then populates the total duration cell with '0'.
- If the fly is already moving at the start of a video, then use the time of the start of the video as the start of its first movement.
- Please watch each video multiple times to ensure the accuracy of your recording.

NOTE: All columns colour coded in a blue shade contain formulae that automatically populate the cells based on your input into other cells. For example: the duration columns will be populated with values (= End time – Start time) once you enter the start and end time. The Total duration (column Y) and the unique Fly/VideoIDs (column Z) also contain formulae.

IT IS IMPORTANT THAT YOU DO NOT AMEND CELLS IN THESE BLUE COLUMNS AS IT COULD LEAD TO INACCURATE DATA!

FINISHED QUICKLY?

If you finished well before your other classmates, please continue to decode data from the videos specified as "extra" in the Student ID column at the bottom of the Master file.

Step 6 | Data amalgamation

After you have recorded the activity of all (or most) of your 16 flies, the next step is to amalgamate your data with the data of other students. This way you will gain access to the locomotor behaviour of the remaining flies and will be able to carry out statistical analysis with a larger sample size.

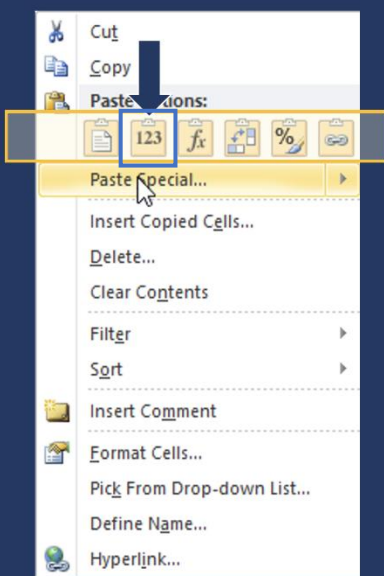
Before you begin, please ensure that your decoding file is complete and that there are no omissions in columns Y and Z. It is always important to double check your work for any potential errors. Once you are satisfied with the outcome, open the live, online Master file and find your lab student ID (*do not download the Master file at this stage*).

Follow these steps:

- You will need to copy and paste the 16 'Total duration' values from your decoding file into one of the 'Duration' columns (columns J, K or L) according to your student set X, Y or Z in the Master file in the rows that correspond to your lab student ID.
- Since the Total duration (column Y) in the decoding file was populated using formulas, you will need to paste the values as plain values/plain text (see instructions for Windows and Mac users below).
- If you are having trouble pasting the data as plain values, then please type in your data manually.

Procedure for pasting plain values/plain text in excel:

Windows



Mac



Step 7 | Calculations

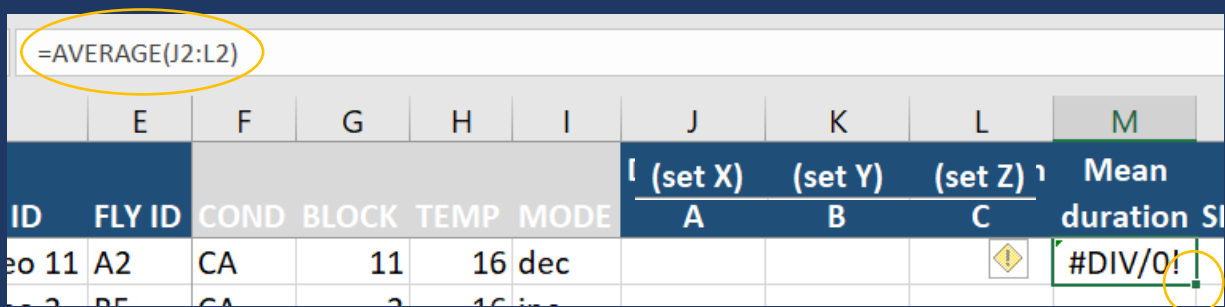
After you and all of your classmates have pasted your data into the live, online version of the Master file, you will need to download the Master file and save it on your computer. This will allow you to work with the data independently.

- ✦ Download the Master file into your new folder on your computer.

You can see that several students (from student sets X, Y and Z) have decoded data from the same flies. This will allow you to calculate a mean of the multiple observations for each fly, and also examine that variability and subjectivity in manual data decoding.

Calculate the mean duration as follows:

- ✦ In cell M2 write the following: =average(J2:L2)
- ✦ Now drag that formula down to cover all flies: Click on cell M2. Now either double click on the little green square at the bottom right hand corner, or click the green square and hold and drag it all the way down to cover all rows.
- ✦ If some rows do not contain any values in column J, K or L, then an error message shows up in column M. This is OK for now.

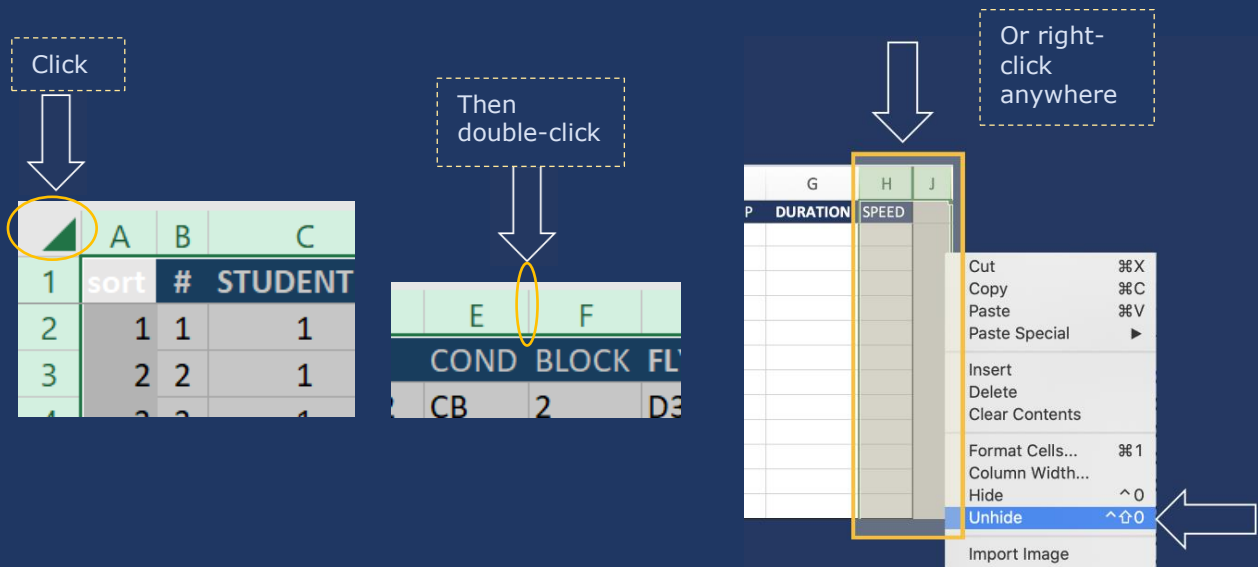


	E	F	G	H	I	J	K	L	M
ID	FLY ID	COND	BLOCK	TEMP	MODE	(set X) A	(set Y) B	(set Z) C	Mean duration S
11	A2	CA	11	16	dec				#DIV/0!
2	BF	CA	2	16	imp				

We now have the manually decoded data for duration of movement, and we also have the automatically recorded distance moved by each fly (estimated using software). This means we can calculate the average speed of movement (during periods of movement) using the following formula:

$$\text{average speed of movement} = \frac{\text{distance moved}}{\text{duration of movement}}$$

- ✦ The distance data can be obtained from the Master file by revealing the hidden column K. To unhide all currently hidden columns, click on the top left hand corner in excel (above row 1 and to the left of column A) in order to select the entire sheet (see screenshots on the next page).
- ✦ When the entire sheet turns a shade of grey it is selected and you can then double click on any line between column letters OR right-click anywhere in the sheet and select "unhide" from the drop-down menu. This will reveal two new columns: one containing distances moved (measured in mm) for each fly and one for calculating average speed of movement.



Now you can calculate the average speed:

- ✦ In cell N2, write '=' and then click on the associated distance (cell O2), then write '/' and click on the associated mean duration (cell M2) and press enter (the cell should now contain the formula =O2/M2).
- ✦ If the duration is zero, the N2 cell will come up with an error, but ignore that for now.
- ✦ Extend the formula to all rows by double clicking on the bottom right-hand corner of cell N2 where you see a little green square (as explained above).
- ✦ Speeds should now have been calculated for all flies in the dataset, albeit with some error messages.

i UNITS OF MEASUREMENT

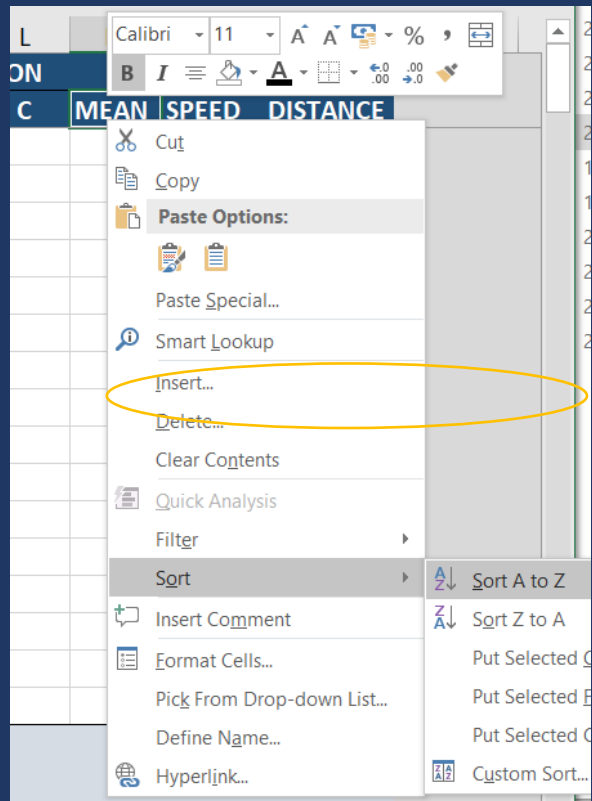
Since the distance is measured in mm and time is in seconds (sec), the unit of speed will be in mm per second (mm/sec).

Step 8 | Data exploration

Now it is time to quality check and explore our data. This is an extremely important part of any scientific study. Most datasets will contain some errors, typos, and mistakes that need correcting before we can analyse the data and correctly interpret the results. First we will sort the data in various ways to look for mistakes and errors.

First sort your data according to duration of movement:

- ✦ Right-Click on the Mean duration cell (M1) and choose Sort and Sort A-Z.
- ✦ (You could also choose Custom sort and sort according to other factors.)
- ✦ Scroll up and down in the file, having a look at the data in the Duration, Speed and Distance columns, familiarising yourself with the data.
- ✦ If there are any rows in the bottom of the dataset containing no values in the Mean duration column (column M), then go ahead and delete those empty rows by clicking on the row numbers (on the left hand side of the screen), right-click and choose 'Delete'. This should get rid of any cells with error messages in column M.
- ✦ Pay particular notice to anything at the top or at the bottom of the Mean duration column (column M) that looks like it is not a number or perhaps a number which is unreasonably high, i.e. >30sec which is the maximum length of each video, and so is likely a typo.
- ✦ Correct any mistakes you have identified in the Mean duration column.



NOTE: You may have to go back and check the duration of movement of a few videos in order to correct all mistakes!

Dealing with remaining error messages:

- ✦ Now focus on the Speed column (column N). It is not possible to divide a number with zero, and so any rows that contain zero in the Mean Duration column will show an error in the Speed column.
- ✦ In this case, we know that speed is zero when the fly has not moved at all, so we can replace error messages in Speed cells with zero.
- ✦ Click on the topmost of the cells with an error message in column N and type '0' and enter.
- ✦ Click the cell again. Now click on the small green square and this time draw it downwards to cover all cells with errors in them.

NOTE: Do not double click on the green square in this case, as it would then overwrite every single cell within that column with zero.

Now consider the discrepancies between automated and manually decoded data.

- Focus on the rows where Distance (and Speed) = 0, but where we have manually decoded Duration of movement >0.
- Consider going back to each of these videos and quality check those data points, using your own judgement to decide whether the manual or automated data is correct in each case.

Now explore how your three variables relate to temperature. Plot 3 different scatter plots in Excel displaying:

- Distance over temperature
- Duration travelled over temperature
- Speed travelled over temperature

For instance, to plot speed over temperature follow these instructions:

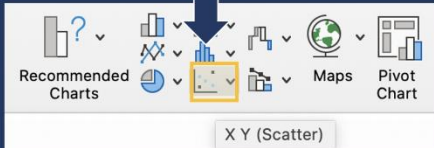
- Select the two columns you want to plot by first clicking on the letter of one column (to select the entire column) and then hold the Ctrl key (on a PC) or Cmd (on a Mac) while selecting the other relevant column
- Insert a scatterplot via the 'Insert' tab

Select the columns you want to plot e.g., temperature and speed


In the Charts group, click the Scatter symbol

Under Scatter, select the first option - Scatter

F	G	H
Temp	DURATION	SPEED
28	1.04	10
28	2.41	0
19	0.67	15.5
16	1.53	3.8
19	2.83	2.26
19	2.13	1.21
25	0.76	2.62
19	1.56	6.64
22	3.02	1.46
22	2.80	0
25	2.68	0
25	3.25	0.89

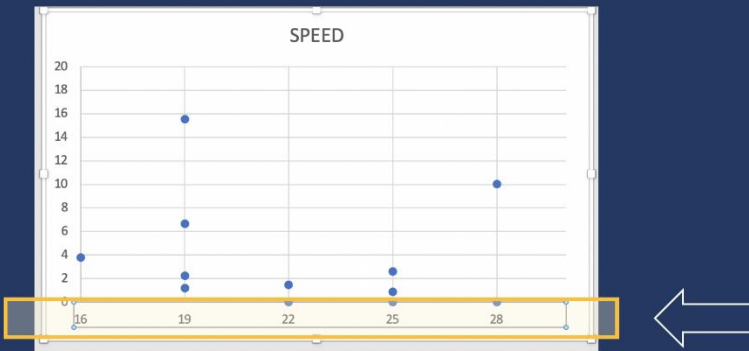


X Y (Scatter)



Once you have generated a scatter plot, you will have the option to modify it, e.g., to add a trend line, or change the minimum and maximum points on the axis, or change the unit increments.

To edit your axes, double click on the axis and a format pane should appear on the right of your screen. Here you can change the minimum and maximum values, and the unit increments.



Format Axis

Axis Options | Text Options

Axis Options

Bounds

- Minimum: 16.0
- Maximum: 30.0

Units

- Major: 3.0
- Minor: 0.6

Vertical axis crosses

- Automatic
- Axis value: 16.0
- Maximum axis value

Display units: None

Show display units label on chart

Logarithmic scale: Base 10

Values in reverse order

▶ Tick Marks

▶ Labels

▶ Number

To add a trendline, click on the graph. Then click on the plus-sign in the top right corner and find Trendline. Choose Linear.

CHART ELEMENTS

- Axes
- Axis Titles
- Chart Title
- Data Labels
- Error Bars
- Gridlines
- Legend
- Trendline

RESULTS AND CONCLUSION

Step 9 | DISCUSSION: Data interpretation (~10 min)

Based on the visual representation of your data, discuss with your peers (using small groups or virtual breakout rooms) the patterns observed. Use the below questions for inspiration in your discussion, but focus mainly on whether or not you have found support of the hypothesis you formulated in the beginning of this practical.

- ✦ *Does temperature appear to affect Drosophila activity and if so, in which way?*
- ✦ *Is there a difference between how duration of movement, speed of movement and distance moved relate to ambient temperature? If so, what could be the reasons for this?*
- ✦ *How much variability is there in your data points at different temperatures and what might influence this variability?*
- ✦ *What are your dependent and your independent variables when analysing the data?*
- ✦ *Which statistical analysis might you use to analyse the data?*
- ✦ *Discuss whether the apparent patterns in the data might or might not provide support for your hypothesis.*
- ✦ *Why do you think your hypothesis is or is not supported?*
- ✦ *Can you think of other variables that might affect fruit fly activity patterns?*

HYPOTHESIS TESTING

Discuss whether or not your hypothesis appear to be supported by the results.

Step 10 | DISCUSSION: Evaluate the methodology (~10 min)

Discuss with your peers the strengths and weaknesses of decoding data manually and automatically. Is there a substantial difference between the two methods?

Things to consider:

- ✦ *Accuracy of the measures taken e.g., human error, variations between researchers, judgement over what constitutes a movement.*
- ✦ *Time constraints of the manual method – what are the benefits of this approach despite being more time consuming?*
- ✦ *In what circumstances/experimental conditions, would one method be more suitable than the other?*

Evaluate the entire experimental procedure and discuss ways to improve the experiment.

- ✦ *Consider the setup and entire experimental procedure.*
- ✦ *Consider sample sizes.*
- ✦ *Consider whether other variables should have been measured and taken into account.*

Step 11 | Reporting your findings

The results from a lab experiment are typically presented in a formal laboratory report. This is your opportunity to demonstrate what you did, how you did it, what you learned, what conclusions can be drawn from your findings, and how future experiments could be improved.

Lab reports can vary in length with slight variations in structure depending on your field conventions. However, a similar basic structure is typically shared across different scientific disciplines:

- ✦ Title
- ✦ Introduction (including background, aims of the study, potential hypotheses and predictions)
- ✦ Materials and methods (including statistical methods used)
- ✦ Discussion (relating back to your aims and hypotheses while putting your findings into context, and including a concluding paragraph at the end)
- ✦ Reference list

i LAB NOTES

You should always take detailed notes during a lab practical so that you remember what and how you did the experiment afterwards. Please note that a lab report (a submittable piece of work) differs from your lab logbook/notebook (a collection of your own notes for you to use when you write your report).

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