TEACHER'S MANUAL

ANIMAL BEHAVIOUR

LAB PRACTICAL

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

V. GIBSON & DR L. GRINSTED
✉ *Lena.Grinsted@port.ac.uk*

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 automatic data collection methods are implemented.

Number of students:
30 students (can easily be amended for fewer or more students)

Duration of practical:
3 hours (recommended)

Delivery:
Synchronous, virtual delivery using your favourite video conferencing platform OR classroom delivery

UNIVERSITY OF PORTSMOUTH
The Association for the Study of Animal Behaviour
MARCH 2021

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WELCOME LETTER

March 2021

Dear Colleague,

This lab practical has been developed as support for peers who are delivering virtual lab practicals within the field of animal behaviour, physiology and experimental biology. This practical can be held online or in a classroom where each student has access to a computer and internet.

This practical has a strong focus on gaining experience with and understanding the scientific process: hypothesis testing, data exploration and analysis, and a critical examination of the experimental procedure. This practical gives students an appreciation of the difficulties and limitations of both manual and automated decoding of animal behaviour.

We encourage you to emphasise the value of student discussions both before and after the data decoding part, and to direct their discussions towards critical thinking. The results from this lab practical are likely to be different to the hypotheses formulated by the students, and so offer a great opportunity for them to try to identify additional explanatory factors, and think of creative ways to improve the experimental design.

I hope you and your students will find this lab practical rewarding, and I am happy to receive comments and questions at any time.

Best wishes,

Lena Grinsted

Senior Lecturer in Zoology
University of Portsmouth
lena.grinsted@port.ac.uk

LAB PRACTICAL SUMMARY

In the first portion of the lab, the students are instructed to familiarise themselves with the lab requirements, develop a hypothesis, and discuss their predictions with their peers. This is followed by decoding the locomotor activity of fruit flies from 30sec video recordings and a preliminary data exploration. Students are then asked to discuss their findings and reflect on the experimental protocol with their peers, consider how lab results are typically presented, and finally engage in an assessed activity. You, as the teacher, can decide on the format of the resulting assignment and whether it should be followed by a formative peer-assessment, or a summative teacher-assessment. This lab is designed in a way to promote independent, as well as group work.

LAB INTRODUCTION AND PREPARATION | Intro and Step 1

Equipment / software requirements

There is no requirement for specialised lab equipment to be used during this practical. Video recordings of the flies will be streamed via the ASAB website and so no playback software is needed.

Video recordings and file sharing

Please ensure that each student has access to the lab manual and the two spreadsheets: The Decoding File and the Master File.
Students will be required to download the Decoding File into which they will record the movement for each fly. They will then need to copy-paste their data in the shared, live, online Master File.
Once fully completed, each student then downloads the Master File for individual data exploration and analyses.

STUDENT LAB FILES

1) **Lab Manual** - with detailed instructions for the students

2) **Decoding file** - an Excel spreadsheet where students individually report the locomotor activity of their sample flies

2) **Master file** - an Excel spreadsheet used to combine data from all students

Sample allocation

Each student will be instructed to decode the movement of one sample group composed of 16 fruit flies. In order to ensure that all students receive roughly comparable samples to decode, the flies that expressed the most activity were evenly distributed across the student IDs.

Many flies in this experiment did not move at all during the 30sec video recordings. In order for students not to get stuck with lots of videos of nothing happening, many of the videos of inactive flies have been removed from the student data file. In other words, please keep in mind that this dataset does not reflect the entire,

actual experimental dataset, and that sample sizes are therefore different for each temperature treatment. This should not affect the valuable lessons that the students will learn from this lab practical, though.

A total of 160 30sec recordings of individual flies have been allocated to **10 student IDs** so that each student will decode data from 16 flies. This lab practical has been designed for a group of 30 students, and the idea is that the students will be grouped up into **three student sets: X, Y and Z.** Student 1 from each of set X, Y and Z will decode data from the same set of flies, and so three separate recordings are done for all flies. The idea is for students to gain an appreciation of how subjective animal behaviour observations can be, and they will understand this by examining the variation among individual observations of the same video.

Hence, in the beginning of the lab, please assign each student a student ID between 1 and 10, and split students up into three sets X, Y and Z. If you have fewer than 30 students, e.g. 20 students, you could potentially split them into just two sets, X and Y. Alternatively, allocate less than 10 student IDs to your students, e.g. numbers 1-8 for a group of 24. If you have more than 30 students, just duplicate the entire protocol so that two (or more) Master files exist, and students are asked to work with just one of those Master files (no more than 30 students should enter data into the same Master file).

Breakout groups

Allocate students to virtual breakout rooms or groups in the classroom. Choose an appropriate group size that suits your students and facilitates good discussion. For a group of 30 students, split students up into 6 groups of 5 by splitting each of set X, Y and Z up into two groups (refraining from mixing students from different sets (X/Y/Z) will ensure that students in the same group are not working on identical videos):

Group 1: X 1-5
Group 2: X 6-10
Group 3: Y 1-5
Group 4: Y 6-10
Group 5: Z 1-5
Group 6: Z 6-10

The students can either remain in these groups throughout the practical (recommended), or you can bring them away from their groups during the steps that require independent work (steps 3-8). If you keep them in their groups throughout, they can easily engage in discussion when a step requires them to (steps 2, 9 and 10 in the Lab Manual), and still work independently when needed while allowing for group chat (steps 3-8).

Lab prep checklist

- ☑ Familiarise yourself with the provided PowerPoint slides to use as an introduction to the lab session, and amend them if you so wish
- ☑ Confirm the number of attendees and amend the student ID allocations according to the number of students
- ☑ Select the delivery method (virtual vs face-to-face)
- ☑ Ensure students have online access to the necessary files: 1) Lab Manual, 2) Decoding File, 3) Master File
- ☑ Allocate to each student their student ID number (1-10), Student set (X/Y/Z), and breakout group
- ☑ Explain to students that they should follow the Student Lab Manual closely for directions and information on how to complete each step of the lab

Suggested (rough) timetable for the 3h lab practical

Teacher presentation ~15min *(use PowerPoint slides)*
Self-directed intro ~10min *(see PowerPoint slides for details)*
Steps 2 group discussion 15-20min
Steps 3-5 data decoding 40-60min *(depending on how they get on)*
Step 6 data amalgamation 10-15min *(can also include a short break)*
Step 8 data exploration 40-60min *(depending on how they get on and how much time is left – you need 30-40min for the last bits)*
Steps 9-10 group discussion 15-20min
Teacher wrap-up 15-20min *(deliver take-home messages)*

INTRO AND PRE-DATA DISCUSSIONS | Steps 1-2

In the introduction, you will explain to the students what the difference between a hypothesis and a prediction is, using the provided PowerPoint slides if you wish. The students will need to formulate a hypothesis about what they think is the general pattern between locomotor activity and ambient temperature. They can further derive specific predictions based on their hypothesis about the three dependent variables in focus in this practical:

1. Time spent moving
2. Distance moved
3. Average speed of movement

You can encourage the students to take a screenshot of the slides that explains hypotheses/predictions, or you can share the slides with them, before they are told to go into their groups and follow step 2.

DATA | Steps 3-8

Data decoding

Make sure to visit the groups and/or individuals, ensuring that everyone progresses nicely with these steps.

Please specify how individuals can approach you to ask clarifying questions. This can be difficult to figure out if students find themselves "stuck" in a virtual break-out room where it is not obvious how to contact the teacher.

Data amalgamation

Students would not have enough data to work with if they only had access to their own data. This is why data from all students are merged into one Master File.

It is important that the students paste their data in the correct place (i.e. relating to the correct fly ID, their own student ID, and the student set X, Y or Z) in a live, online version of the Master file.

It is extremely important that students **paste plain values** rather than simply pasting the content of their Decoding File, as these cells contain formulae.

Once the file is populated with data from all (or at least most) students, the file should then be downloaded by all students before each student sets to work on the file.

NOTE: *Students that finish with their 16 flies before everyone else can be encouraged to continue decoding data from the 'extra' flies at the bottom of the Master file.*

NOTE: *It is OK to amalgamate data and continue with the following steps even if not all students have finished with their 16 flies. The remaining protocol will still work well.*

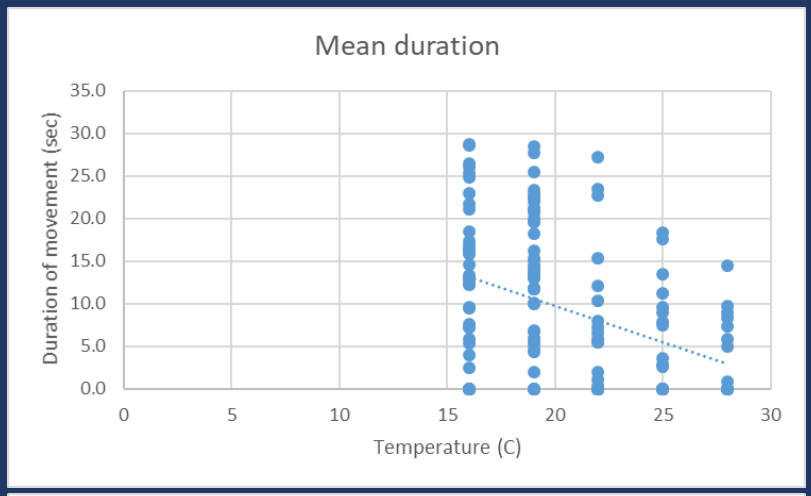
DISCUSSIONS: CRITICAL THINKING | Steps 9-10

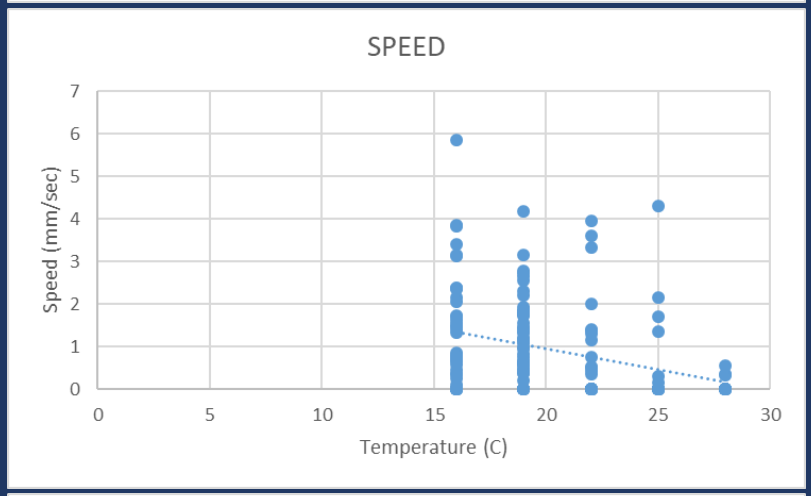
Please encourage your students to discuss whether or not they found support for their hypothesis, and if not, why not. Remind students that it is extremely important for scientists to think critically about their experimental design and consider how the design could be improved for future studies. Scientists also need to carefully consider how broadly (or how narrowly) their conclusions may extend.

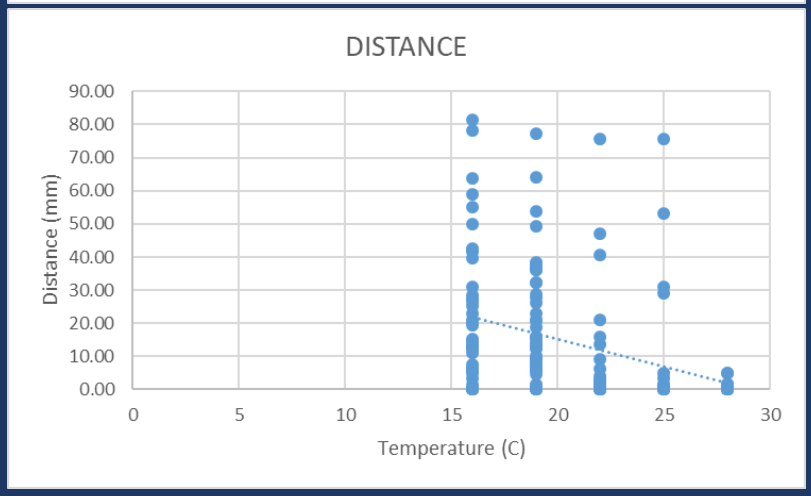
Findings

Your students will find that flies are more active at colder temperatures (Fig. 1 and 2). In other words, there is a negative effect of temperature on activity. Your students are likely to have hypothesised a positive effect of temperature on activity, perhaps due to basal metabolic rate being positively correlated with temperature.

Your students will produce three figures that look like this:







The discrepancy between predictions and findings might have something to do with circadian rhythms: *Drosophila* are most active at dawn and dusk and the associated increase/decrease in ambient temperature is an indicator for them to change their activity to match those time periods (Bywalez et al., 2012; *if you would like to recommend your students a single paper to read, choose this one*). But in lab conditions the experimental protocol of exposing flies to increasing and decreasing temperatures, according to condition A and B, may have influenced their patterns of activity. Indeed, there is an interaction effect of condition and temperature (a difference between Fig. 1 and 2 in the orange coloured temperature-increase-associated activity, as well as a difference between the figures in the green-coloured temperature-decrease-associated activity).

Also, the flies used for this experiment were reared at a constant 22 degrees C, which might also have some unexpected effects on their circadian rhythm. Please note, though, that the lab protocol only asks the students to look at the relationship between temperature and activity, and so does not ask the students to take into account the different conditions of increasing and decreasing temperatures.

Fig. 1 Effect of ambient temperature on distance moved in Condition A: Increase-Decrease

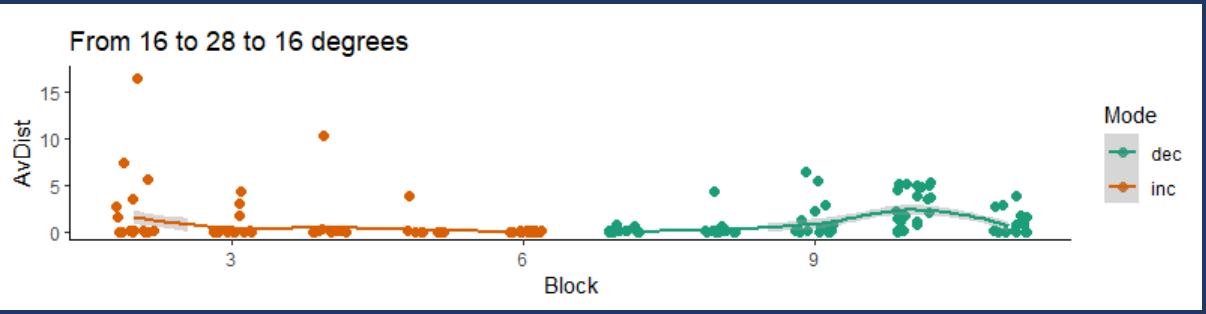
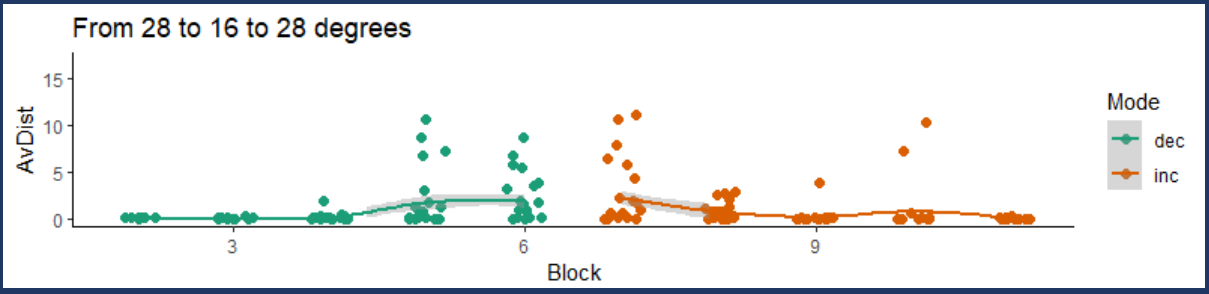


Fig. 2 Effect of ambient temperature on distance moved in Condition B: Decrease-Increase



The main finding (negative association between activity and temperature) is apparent without the need for doing any sophisticated statistics, and so it is up to you whether you would like your students to simply describe their findings based on plots and trend lines, or whether you would like them to conduct statistical analyses. Data analyses could simply be done using correlations, or you could get them to build more complicated linear models that take into account repeated measures of individual flies, the different conditions etc. One option would be to ask students to conduct only correlations, but for you to facilitate a healthy discussion about how such analyses could be improved upon by including random effects, interaction terms, etc.

Take-home messages post-discussion

Hopefully your students have touched upon some or all of the following points:

- Behavioural data is highly variable (among-subject variation)
- Increasing the sample size gives more reliable results
- Increasing the sampling effort (e.g. longer video recordings) might help to reduce among-sample variation
- Manual decoding of behaviour can be subjective
- Automated data recording may also include errors (e.g. record movement when a fly is only cleaning itself)
- Predicting animal behaviour is not trivial: hypothesis are often not supported
- Unexpected results are common in behavioural studies
- Many factors can interact to affect animal behaviour and so studies simply testing the effect of a single factor may not be appropriate (in this case behaviour was probably affected by the circadian rhythm, the time of day, the temperature the flies were reared at, the condition they were treated at, the use of repeated measures of a relatively small sample size etc.)
- Future studies would need to take many more variables into account

LAB REPORT

This practical is suitable for a full, summative lab report, written either individually or in groups. Alternatively, you can consider a smaller write-up appropriate for peer to peer formative assessment.

Summative lab report

A full lab report can be written as group work, or individually, and should contain all the typical sections of a lab report (Introduction, Methods, Results, Discussion). If you would like your students to properly analyse the data, you would need to provide further guidance and support for them to choose the right statistical tests. They would also need training in using a statistical software like R or SPSS. Simple statistics can also be done in Excel.

Formative peer review: report structure in bullet points

A smaller submission that students assess for each other could take the form of outlining the structure of a lab report, and for each of the sections (Introduction, Methods, Results, Discussion) they provide a list of bullet points of what they would include in that section, if they were to write the full report.

After they have handed in their report outlines, assign two or three assessors per assignment. Ask students to suggest clear actions and recommendations while also acknowledging the strengths of the submitted work. Remind them that assessing others' work can be a great way of learning and improving one's own work. It can also be educational for them to mark their own reports.

AUTHOR

Please outline the structure of your report to your group.

- Present sections in chronological order
- Provide a brief summary of the content of each section

ASSESSOR

For each report outline you are asked to 1) identify at least one strength and 2) propose at least one suggestion or action. Things you may consider:

- Does the report contain all required sections?
- Are the sections presented in a logical order?
- Does the report contain key information e.g., a clear hypothesis and aim?
- Is there anything that is missing?

REFERENCES

Bywalez, W., Menegazzi, P., Rieger, D., Schmid, B., Helfrich-Förster, C., & Yoshii, T. (2012). The Dual-Oscillator System of *Drosophila melanogaster* Under Natural-Like Temperature Cycles. *Chronobiology International, 29*(4), 395-407. https://doi.org/10.3109/07420528.2012.668505

ACKNOWLEDGEMENTS

This resource was supported by an ASAB Education Grant awarded to Dr Lena Grinsted. The ASAB Education Committee would like to thank Violet Gibson and Dr Lena Grinsted for their time and effort in developing this resource. Our thanks also go to Bill Budenberg and Shanelle Kohler of Zantiks for providing the video footage of Drosophila.