(Re-)introduction to experimental design and analysis

BIOL2022 – **B**iology **E**xperimental **D**esign and **A**nalysis (**BEDA**)

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THE UNIVERSITY OF SYDNEY

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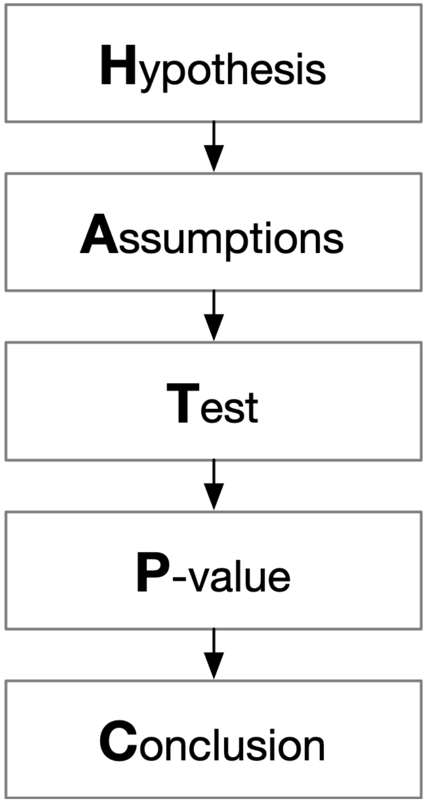
Learning outcomes

You should:

1. Understand the importance of *planning* in experimental design and analysis.
2. Appreciate the *iterative* nature of the process.
3. Be able to use visualisation to *guide* the development of a model.
4. Be able to *define* a simple empirical model involving two variables.

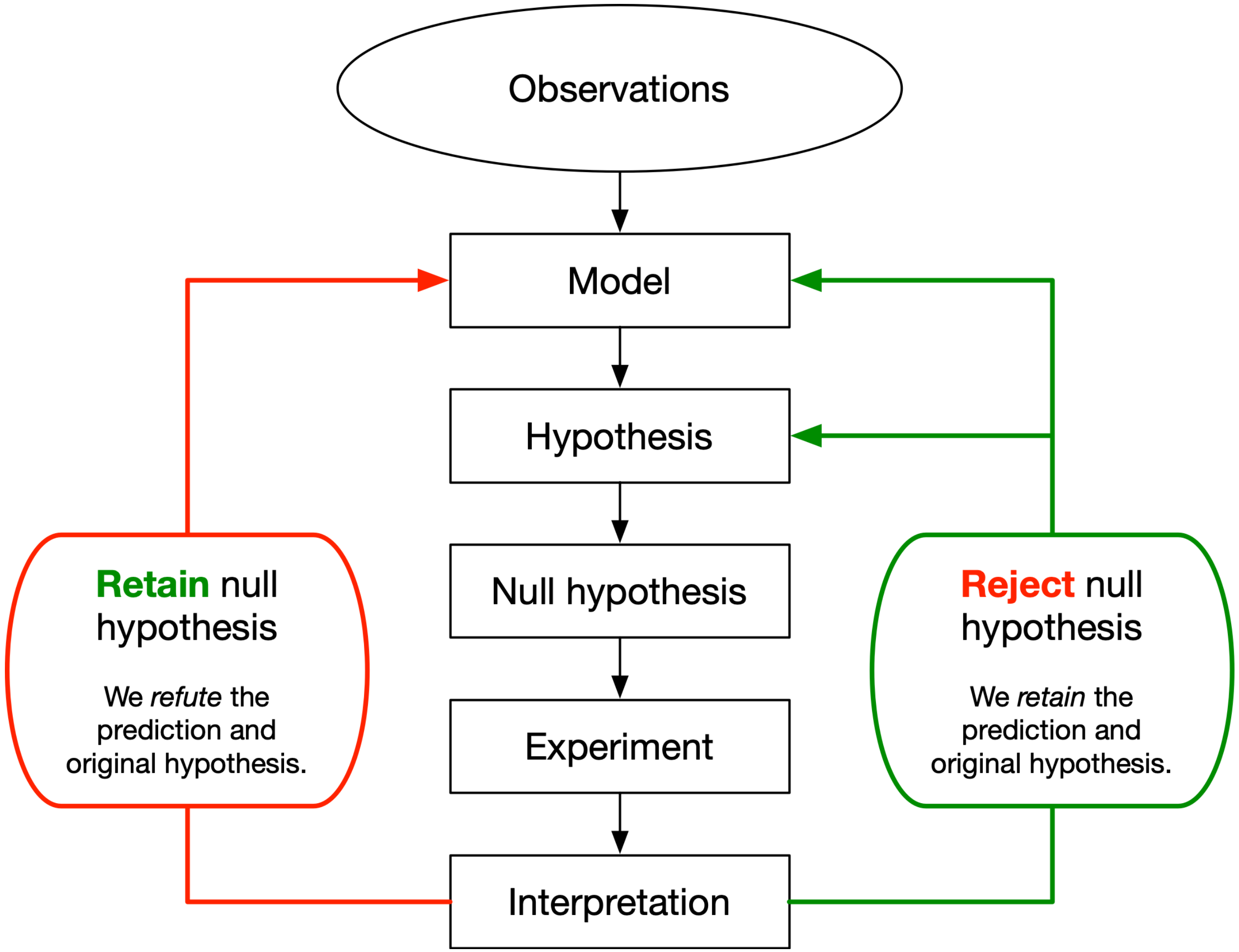
Workflow

HATPC[1]



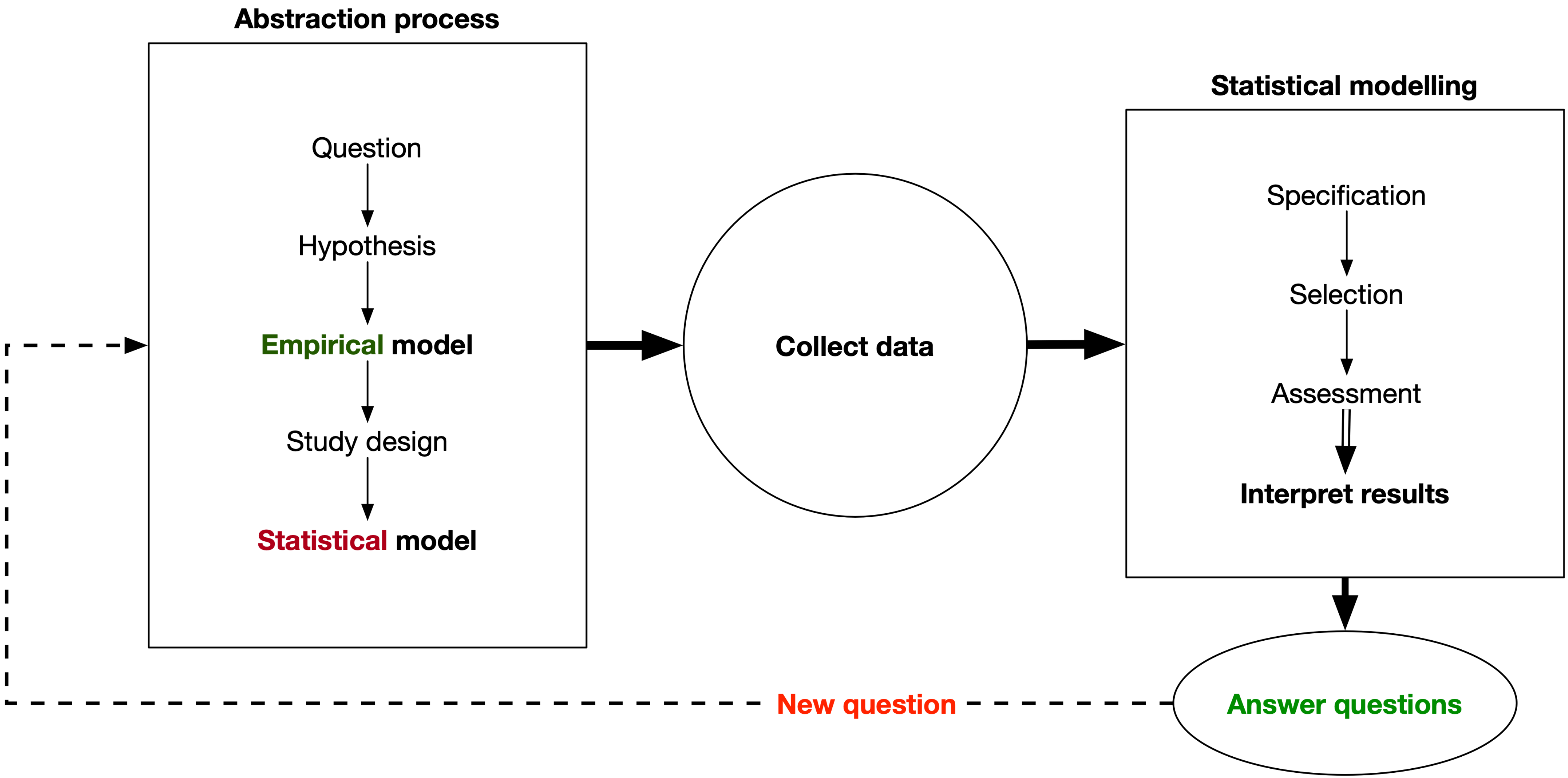
[1]Used in DATA1001, ENVX1002 and other units. The University of Sydney.

Logical framework[2]



[2]Underwood AJ (1997) Experiments in Ecology: Their Logical Design and Interpretation using Analysis of Variance. Cambridge University Press, Cambridge.

Experimental design workflow[3]



[3]Fox, G. A., S. Negrete-Yankelevich, and V. J. Sosa. (2015). Ecological statistics: contemporary theory and application. Oxford University Press, USA.

One thing in common…

Planning is *fundamental*

There is no magical statistical method that will make up for a poorly designed study.

$$🗑️\ \textit{Garbage in} \rightarrow \textit{Garbage out}\ 💩$$

Why do we care?

> *“To call in a statistician after the experiment has been done may be no more than asking him to perform a post-mortem examination: he may be able to say what the experiment died of.”*
>
> *“That’s not an experiment you have there, that’s an experience.”*

– Ronald Fisher

There is no one-size-fits-all

- The process is *iterative* and *non-linear*.
- Different academic disciplines have different approaches but the core principles are the same.
- The key steps are:
    1. Formulate the idea, problem, or question to be addressed.
    2. Think **critically** about what data is needed to answer the question.
    3. Develop a suitable **model** which helps in planning the components of the study.

Model?

A digital elevation model that simplifies a 3D terrain in 2D. Licensed from Adobe.

- **Models simplify complex data** – by capturing the underlying relationships between variables.
- They *condense* information – which allows us to formulate hypotheses and make predictions.
- Just like physical models (e.g. world map), models in statistics are **abstractions** that overlook some details of reality.

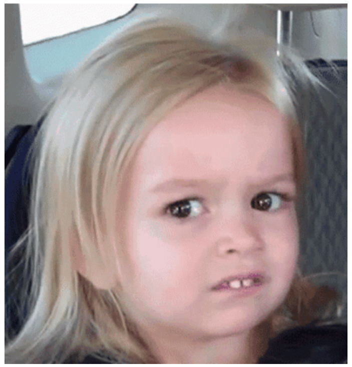
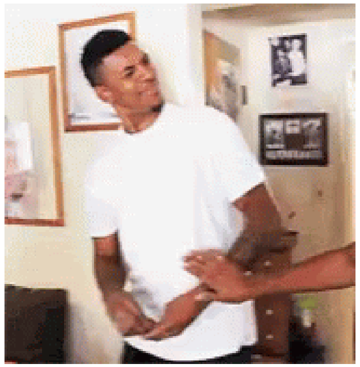
So how do we model data?

First, some history

Traditional statistics

If you have studied statistics before, you might have been taught that:

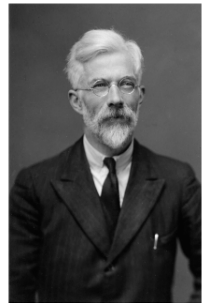
- Each statistical technique is distinct and separte from the others, and so, e.g. you need to choose the right one.
- To make sense of it all, you probably need to refer to a flowchart, e.g. like this.

Needless complexity – why?

Karl Pearson, 1857-1936, from Wikipedia

VS



Ronald Fisher, 1890-1962, from Wikipedia

- The disagreements (to put it mildly) between two superstar statisticians **Karl Pearson** and **Ronald Fisher** shaped the development of statistical techniques such as t-tests, ANOVA, and regression.
- While these techniques have similar mathematical roots, the two had *different* views on the role of mathematical models in statistical inference.
- These differences *may* have contributed to the teaching of statistical techniques that are often presented as **distinct** and **separate** from each other.
- J. Lenhard, Models and Statistical Inference: The Controversy between Fisher and Neyman–Pearson. The British Journal for the Philosophy of Science 57, 69–91 (2006).

Dropping the complexity

- It turns out that *most* statistical techniques are based on the **same underlying principles**.
- We can easily observe this with modern statistical software – without doing the math – like R (**more on this next week**).
- It makes learning statistics simpler as well – a **model-centric** approach.

Empirical modelling

The process of developing a model that relies on **data** to make predictions, rather than mathematical theory.

The model-centric approach

… is not a new idea.

> Effective data analysis requires us to consider **vague concepts**, concepts that can be made definite in many ways. To help understand many definite concepts, we need to **go back to more primitive and less definite concepts and then work our way forward**.

– Mosteller and Tukey (1977)[1]

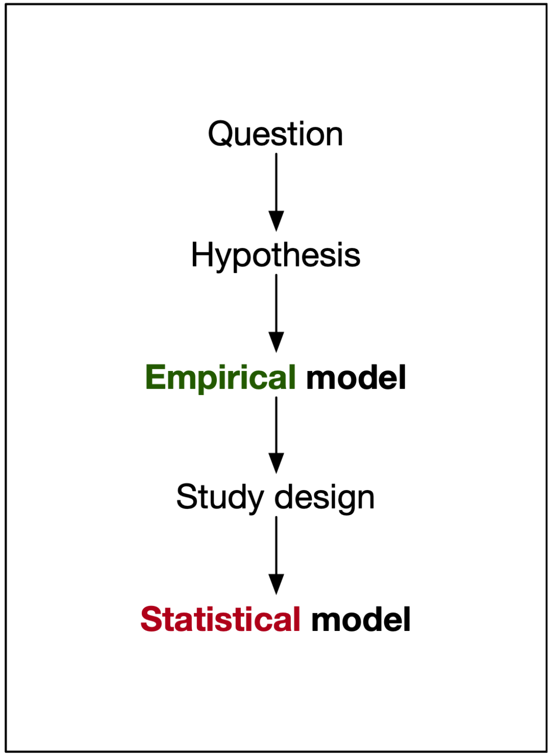
It doesn't have to be *the perfect model* from the start:

- Model the data, even if we don't have a clear idea.
- **Use** the model to *guide* the planning of the study design.
- Iterate the model as we learn *more* about the **limitations** of our study and finalise the **statistical** model.

1. Mosteller, F., & Tukey, J. W. (1977). Data analysis and regression: A second course in statistics. Addison-Wesley.

Model building basics - a suggested workflow

Abstraction process



1. **Formulate the question**: What are you trying to predict?
2. **Generate a hypothesis**: What relationships do you expect to see?
3. **Prototype a model**: What relationships exist between the variables?
4. **Design the study**: replicate, randomise, and control.
5. **Finalise the model**: refine the model based on the study design.

Example - sea urchins

Abstraction process

Question

↓

Hypothesis

↓

Empirical model

↓

Study design

↓

Statistical model

Abstraction process

Question

↓

Hypothesis

↓

Empirical model

↓

Study design

↓

Statistical model

Context

1. Urchins inhabit many coastal ecosystems.
2. Some are keystone species.
3. Understanding the health of urchin populations provides insights into the health of the ecosystem.

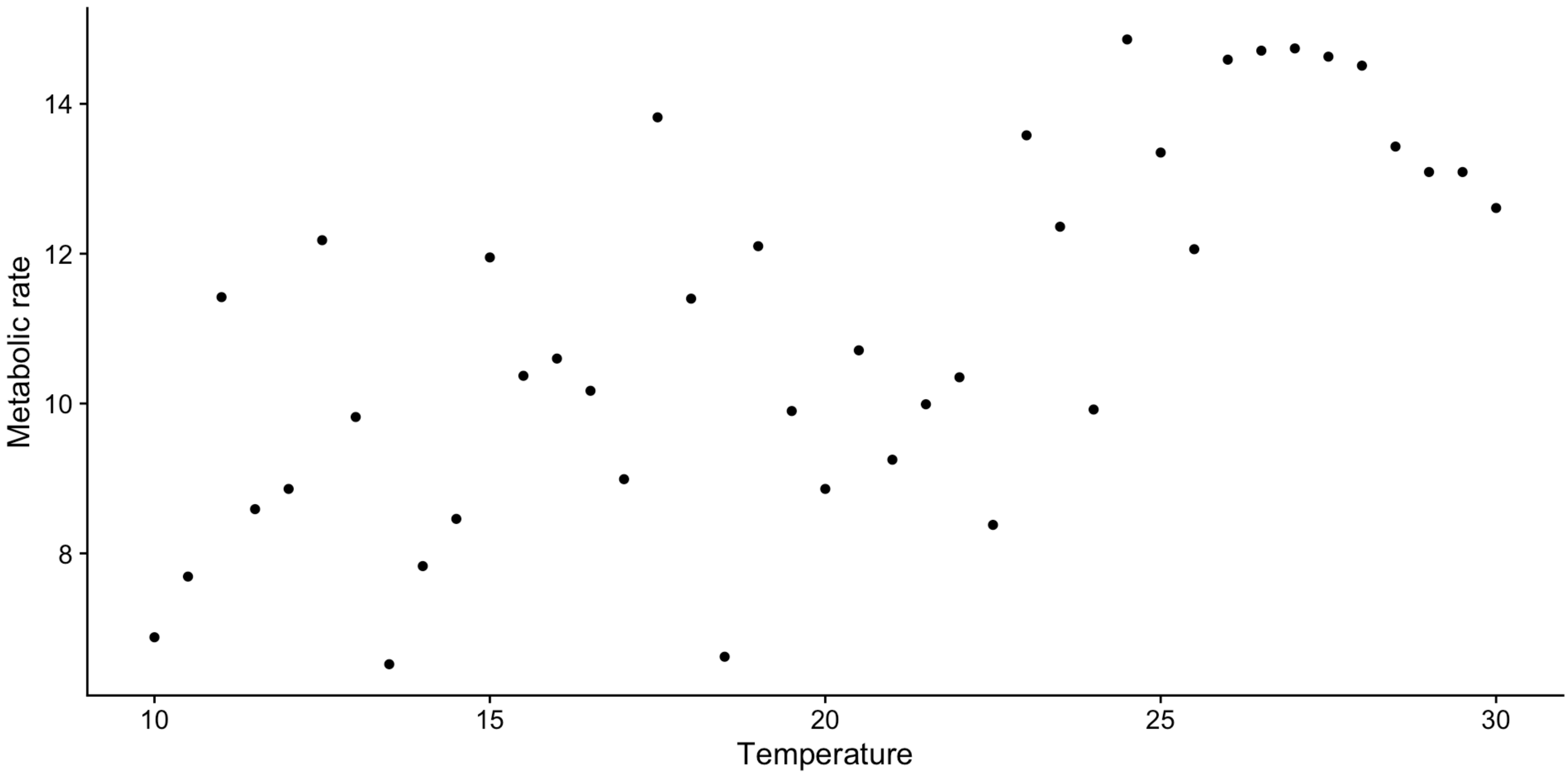
> **Tip**
>
> It is best to test for *simple* relationships first, before moving on to more complex ones, for complex questions.

Abstraction (a quick example)

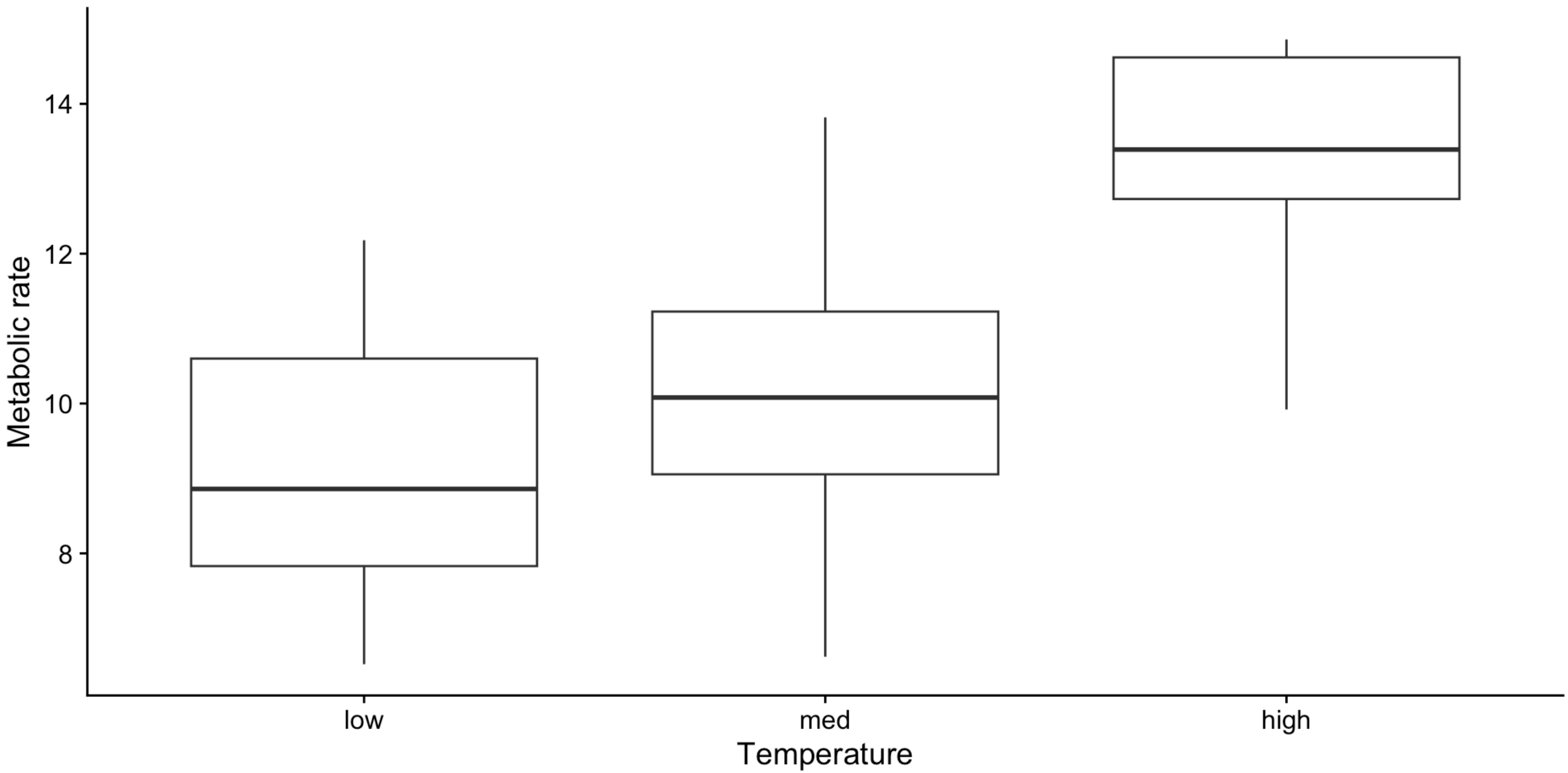
Suppose we want to first benchmark the metabolic rate of sea urchins at different temperatures since health is related to metabolic rate.

- **Question**: How does temperature affect the metabolic rate of sea urchins?
- **Hypothesis**: If temperature increases, the metabolic rate of sea urchins will increase.
- **Model**: ? ? ?

The simplest model



The simplest model



Why does visualisation help?

- Visual models **define** the structure of the data and the relationships between variables.
    - ➟ Scatter plot: both variables are continuous, we can *perhaps* predict with a linear relationship.
    - ➟ Box plot: one variable is categorical and the other is continuous, we can *perhaps* see differences between categories.
- **Implications for study design**:
    - ➟ Scatter plot: measure metabolic rates at different temperatures. Aim: to predict.
    - ➟ Box plot: measure metabolic rates at fixed temperatures. Aim: to compare.
- Prepares for defining the model empirically – but it all doesn't *really* matter from that point of view… because both designs are still based on the *same* modelling framework!

What is a variable?

A **variable** is just anything you can measure or count.

In a study, we usually have two main types:

- **Response Variable**: The main outcome you are interested in.
    - ➠ *Example*: The **metabolic rate** of our sea urchins.
- **Predictor Variable**: Something you think might affect the response.
    - ➠ *Example*: The water **temperature** for the urchins.

Types of variables matter

How you measure a variable changes what you can do with it. A single concept, like temperature, can be treated in different ways.

Categorical (Groups)

Puts things into boxes.

- **Nominal**: Boxes with no order.
  - ➟ *Example*: `Colour` (red, blue).
- **Ordinal**: Boxes with a clear order.
  - ➟ *Example*: We can group precise temperatures into `Temperature Categories` (e.g., 'low', 'medium', 'high').

Continuous (Numbers)

A value on a scale.

- **Interval**: A scale with no true 'zero'.
  - ➟ *Example*: `Temperature` in Celsius is a number (e.g., 14.5°C). 0°C isn't 'no temperature'.
- **Ratio**: A scale with a true 'zero'.
  - ➟ *Example*: `Height` in cm. 0 cm is 'no height'.

You can often choose the type

It's easy to make a continuous variable categorical (e.g., turn exact `Height` measurements into 'short', 'tall' groups). It's much harder to go the other way.

This choice dictates your next steps:

- **Graphs**: Use a scatter plot for two continuous variables, but a box plot for a categorical and a continuous one.
- **Models**: The statistical test you choose depends entirely on your variable types.

Defining the model

- $y = f(x)$
- y is influenced by x.
- A **response** is influenced by a **predictor**.
- **Metabolic rate** is influenced by **temperature**.
- $\text{Metabolic rate} = f(\text{Temperature})$
- $\text{Metabolic rate} \sim \text{Temperature}$

The chosen model

$$\text{Metabolic rate} \sim \text{Temperature}$$

- Simple!
- Easy to interpret, modify (e.g. add more predictors) and refer to.
- **Not** a statistical model… yet.

Time to design the study

Lots of things to consider, where some of the following *might* be relevant:

- **Replication**: to account for variability.
- **Randomisation**: to address bias.
- **Control and blocking**: perhaps to account for confounding or other factors.
- **Sample size**: determines precision, power, and generalisability.
- **Interactions and covariates**: to account for complex relationships.
- …

Fortunately, the model-centric approach means that we can **iterate** the model as we learn more about the limitations of our study and finalise the statistical model.

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$$\text{Metabolic rate} \sim \text{Temperature}$$

$$\text{Metabolic rate} \sim \text{Temperature} + \text{body size}$$

$$\text{Metabolic rate} \sim \text{Temperature} + \text{body size} + \text{pH}$$

$$\text{Metabolic rate} \sim \text{Temperature} + \text{body size} + (1|\text{pH})$$

$$\text{Metabolic rate} \sim \text{Temperature} + \text{body size} + (1|\text{pH}/\text{Site})$$

All of these models can be incorporated within a unified statistical framework, specifically the **general linear model**.

Just the beginning...

We will cover more on models and study design in the next few weeks, but I hope you are less intimidated by the process!

Don't forget…

🗑️ $Garbage\ in \rightarrow Garbage\ out$ 💩

Thanks!