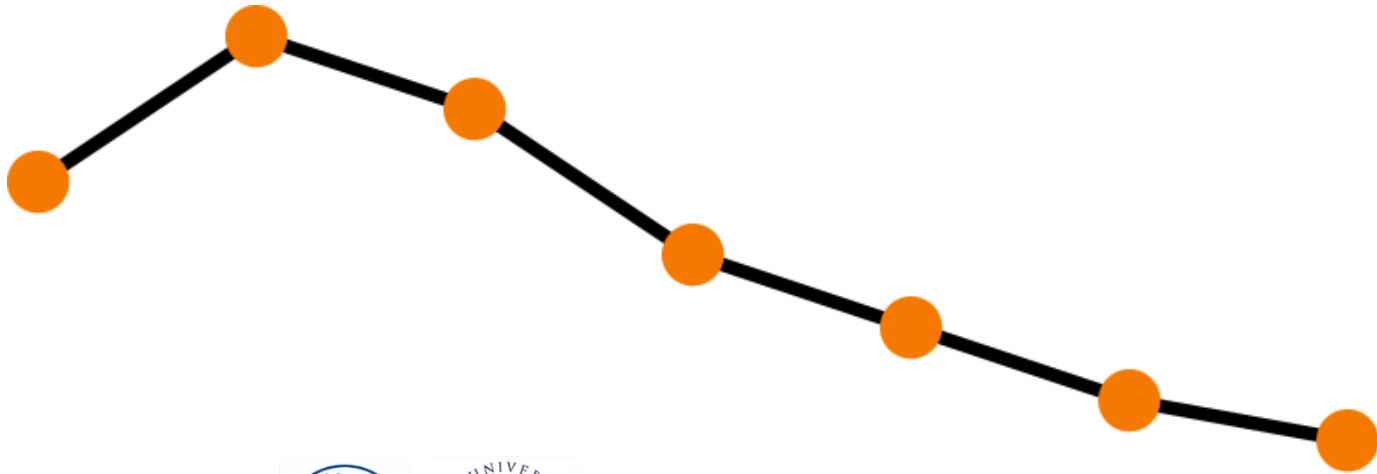


Lecture 9.3

Experimental design VI

Replication, pseudoreplication and repeated measures



浙江大学爱丁堡大学联合学院
ZJU-UoE Institute

Nicola Romanò
nicola.romano@ed.ac.uk
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Learning objectives

At the end of this lecture you will be able to:

- Determine what the experimental unit in an experiment is
- Describe the concept of pseudoreplication and explain the issues associated with it.
- Describe and give examples of repeated measures and nested designs.

What is an experimental unit?

An "experimental unit" is typically thought of as one member of a set of objects that are initially equivalent, with each object then subjected to one of several experimental treatments.

Wikipedia

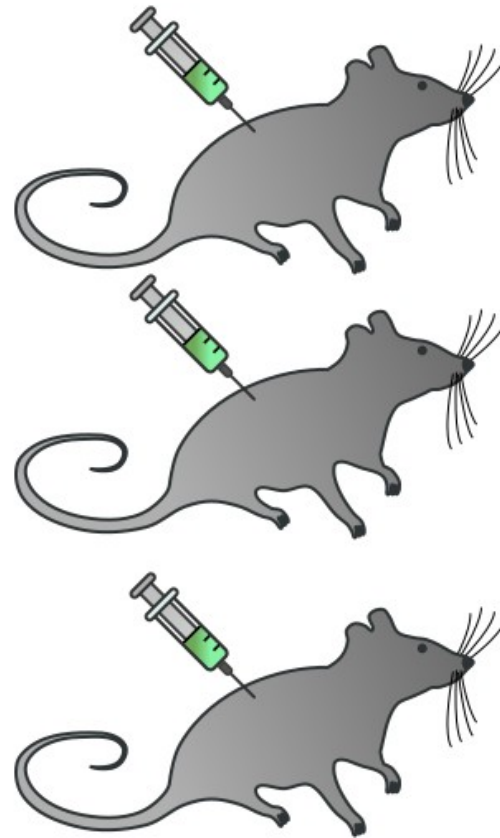
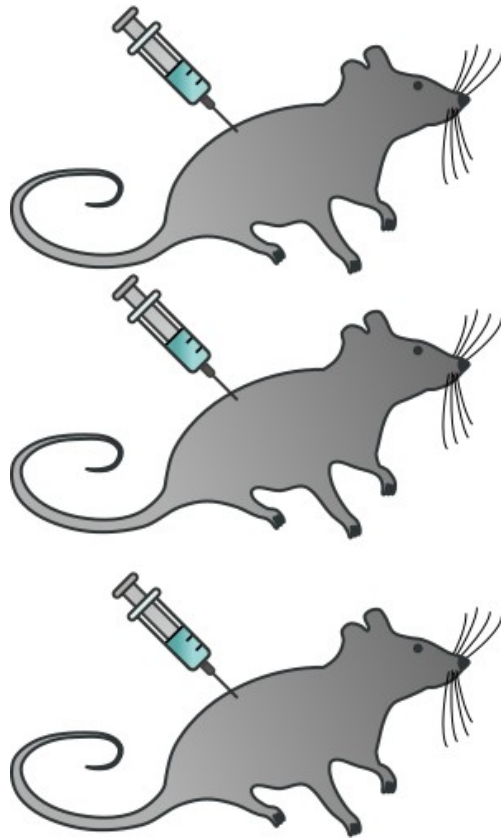
The experimental unit is the entity subjected to an intervention independently of all other units.

The National Centre for the Replacement, Refinement & Reduction of animals in research (NC3R)

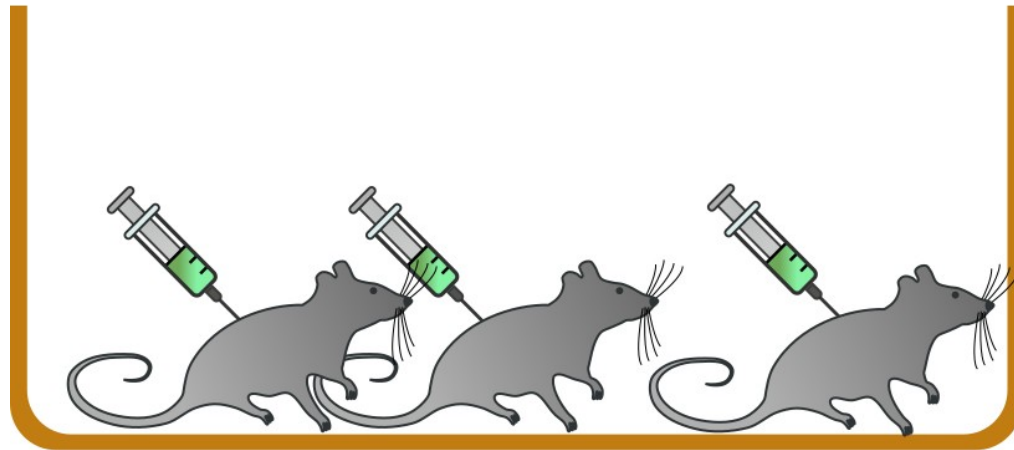
The experimental units are the smallest divisions of the collection of the experimental subjects which have been randomly assigned to the different conditions in the experiment and which have responded independently of each other for the duration of the experiment, or which, if allowed to interact during the experimental period, have had the influence of all extraneous variables controlled through randomization.

Peckam et al., 1969

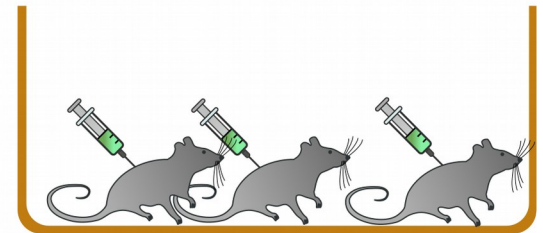
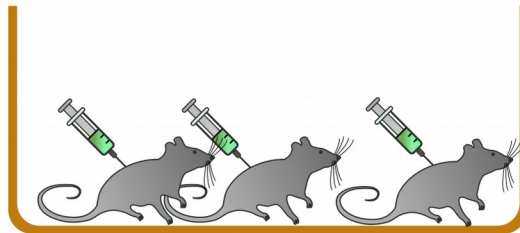
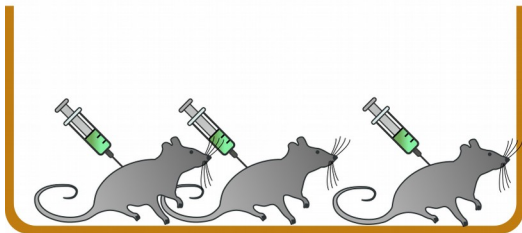
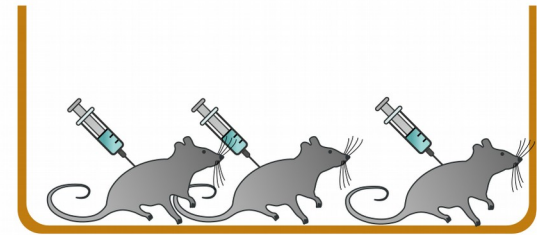
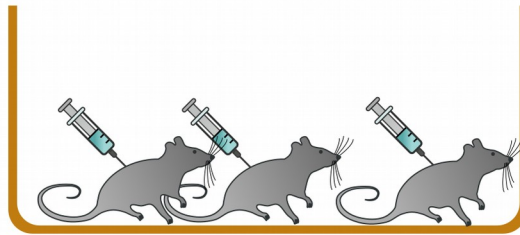
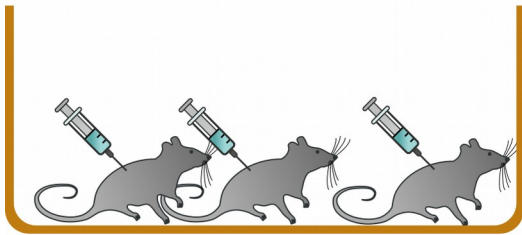
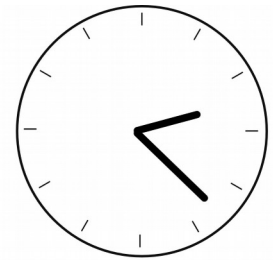
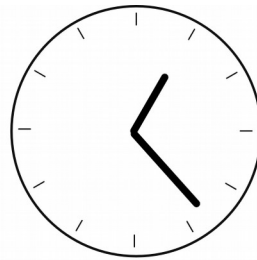
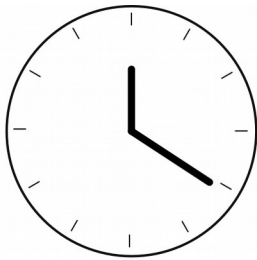
So... what is the experimental unit?



So... what is the experimental unit?



So... what is the experimental unit?



Replication

Replication is the repetition of an experimental condition so that the variability associated with the phenomenon can be estimated.

Two main types of replicates you should be concerned with:

Biological replicates: repetition of the same measurement on different experimental units.

Helps describing the variability in the measured parameter in the population.

Technical replicates: repetition of the same measurement on the same experimental unit and in the same condition.

Take into account the variability due to the experimenter/experimental method.

Replication – an example

We want to evaluate and compare the concentration of cortisol in patients suffering from Addison's disease (primary adrenal insufficiency) vs control subjects.

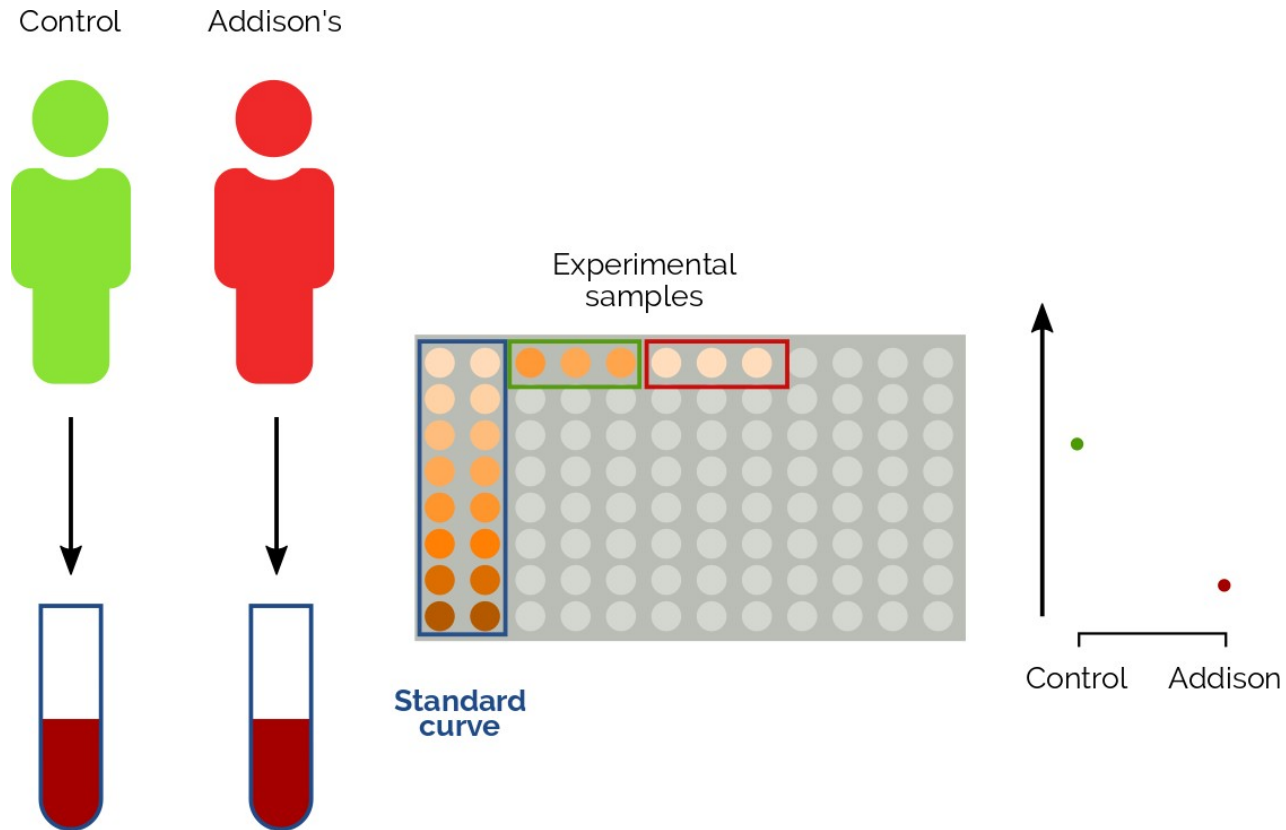
We take a morning (9AM) blood sample from each subject and measure the concentration of corticosterone by ELISA.

We need to consider (amongst other things...)

What are the sources of variability?

What are the sources of error?

Case 1



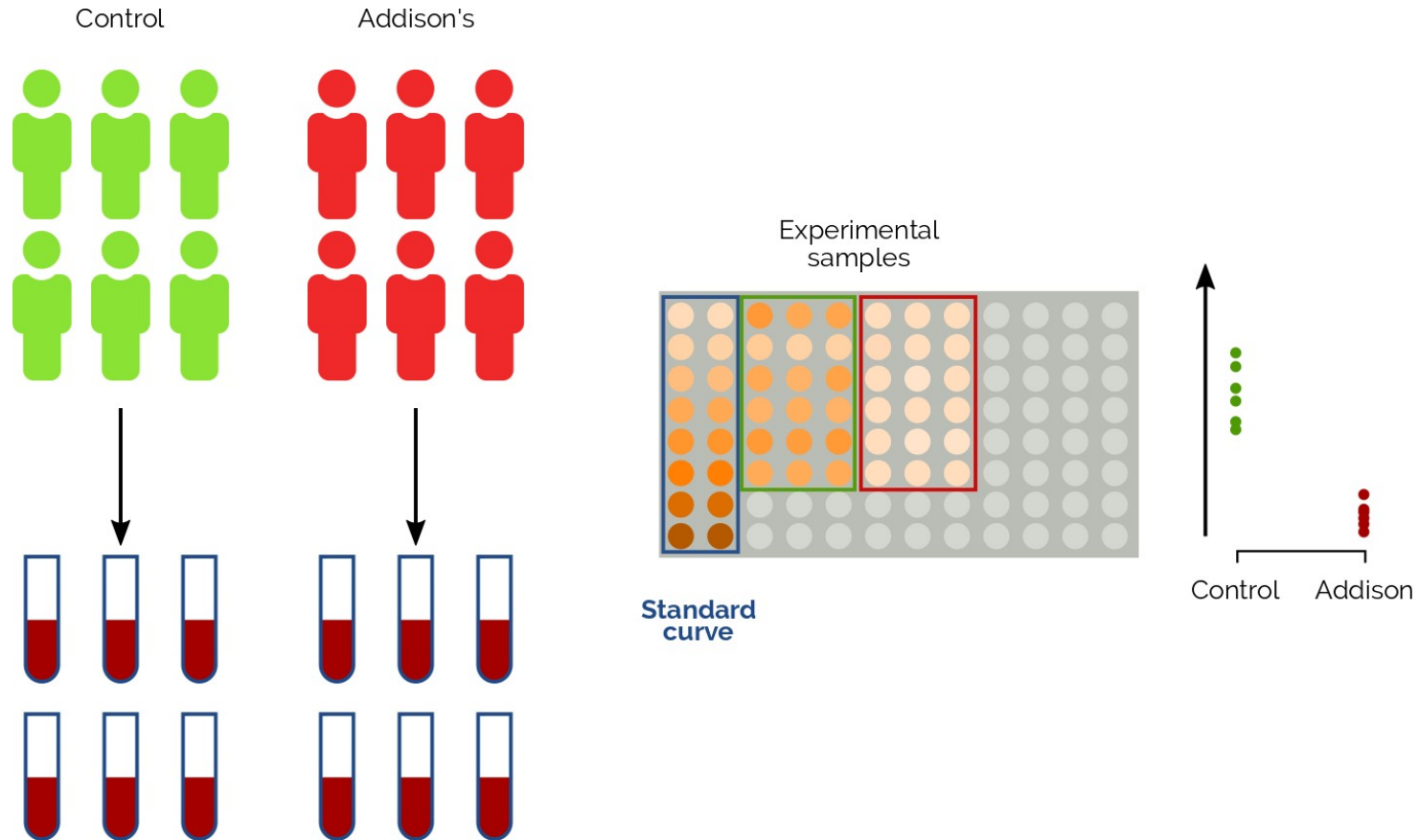
What type of replicates do we have?

What sources of variation are we considering here?

What is the n the we should report under this graph?

What statistical test should we use in this case?

Case 2



What type of replicates do we have?

What sources of variation are we considering here?

What is the n the we should report under this graph?

What statistical test should we use in this case?

What n should I report?

I examine the morphology of cells from metastatic areas from cancer patients.

Patient	Metastatic areas	Number of cells analysed
1	4	246+189+224+181
2	3	160+209+250
3	2	190+223
4	2	129+257
5	4	179+255+206+253
TOTAL	15	3151

What n should I report?

- a. 5
- b. 15
- c. 3151

Pseudoreplication

Pseudoreplication: the process of (maliciously or not) artificially inflating the number of samples or replicates in a study by treating correlated observations as if they were independent.

Adapted from Wikipedia

Pseudoreplication in the literature...

Pseudoreplication is extremely common... more than you would imagine!
Big issue in current biomedical research.

Ecological Monographs, 54(2), 1984, pp. 187–211
© 1984 by the Ecological Society of America

PSEUDOREPLICATION AND THE DESIGN OF ECOLOGICAL FIELD EXPERIMENTS¹

STUART H. HURLBERT
*Department of Biology, San Diego State University,
San Diego, California 92182 USA*

~ 50% of studies considered
had some form of pseudoreplication

The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis?

Stanley E Lazic ✉

BMC Neuroscience 2010 11:5

12% of studies considered (in a single
issue of *Nature Neuroscience*) had
some form of pseudoreplication.
36% were suspected (not enough info
reported)

Widespread issue. Not limited to neuroscience!

Nested design

- Also called **hierarchical** or **multilevel** design
- Contain (noise) factors that are structured in a hierarchical manner
- Extremely common in biology
- Very commonly incorrectly analysed!
- Example:
 - Cage → Mouse → β -cells → Secretion vesicles
 - Forest → Tree → Seed

Repeated measure design

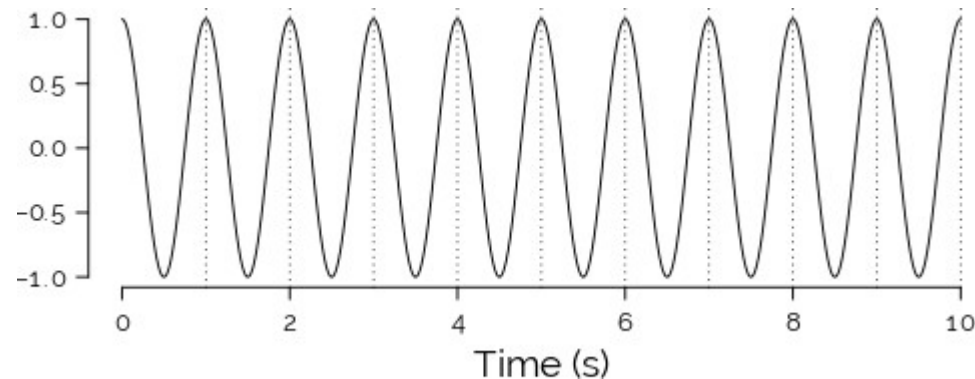
A design where we take measurements from the same experimental unit in several occasions.

Two most common situations

- Repeated measures over time ← probably most common in biomed. sciences
- Repeated measure over space

Repeated measures... but how often?

Real signal (1 Hz frequency)



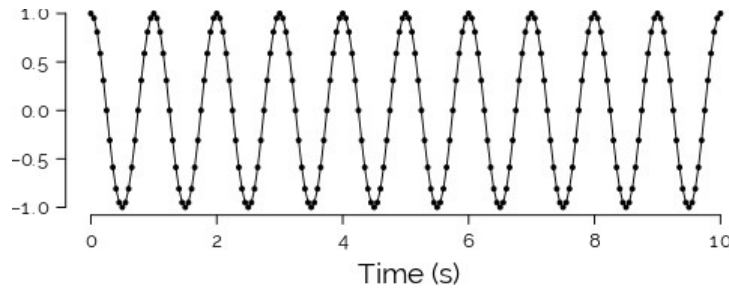
Let's assume we have a signal with a frequency of 1Hz (1 cycle/second).

How often should we sample to get enough information on the periodic signal?

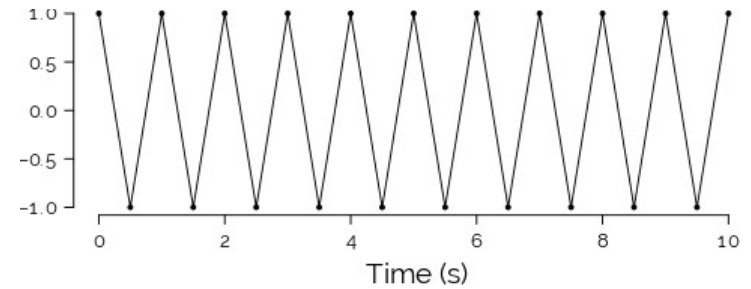
Repeated measures... but how often?

Effect of changing sampling rate

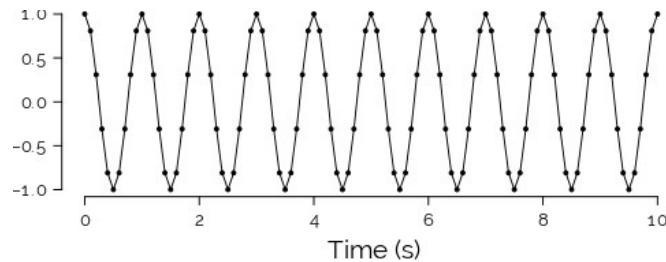
20 Hz



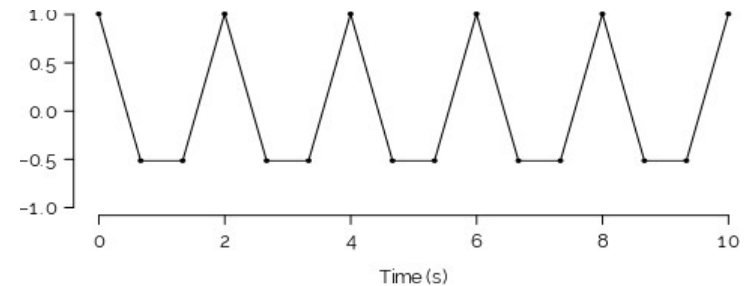
2 Hz



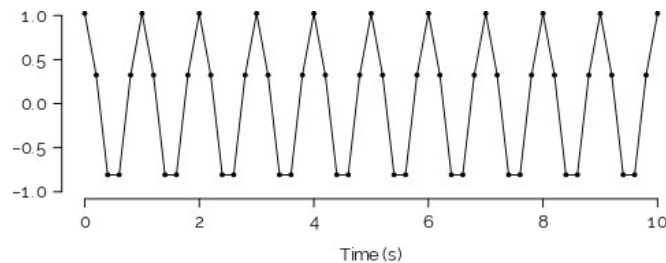
10 Hz



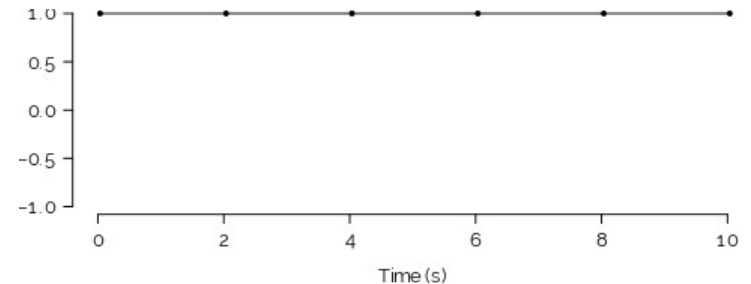
1.5 Hz



5 Hz



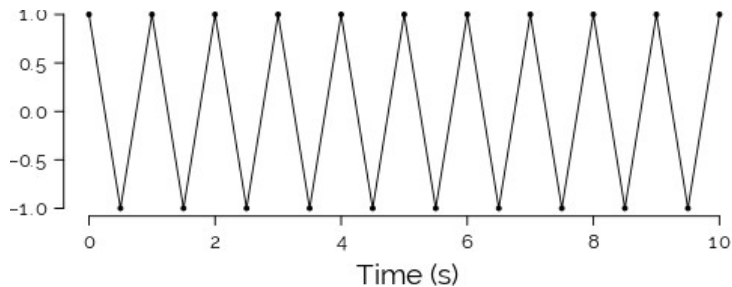
1 Hz



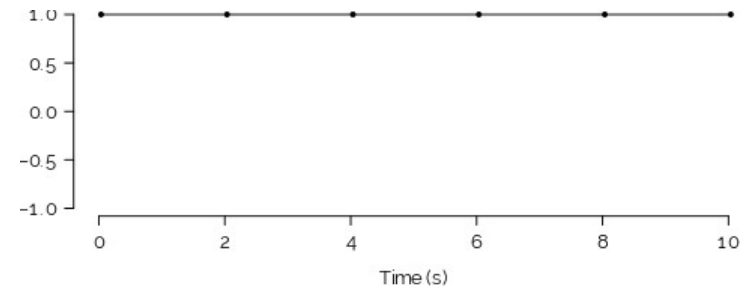
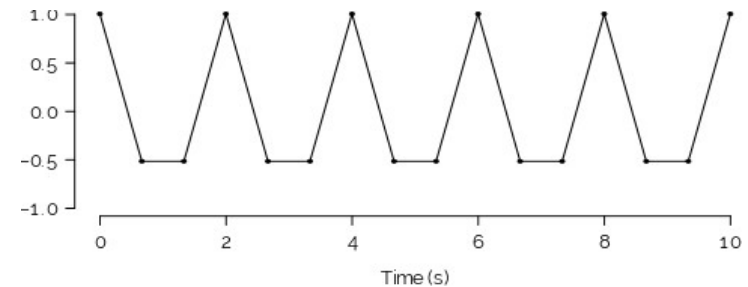
The Nyquist rate

For a periodic signal of frequency f , the minimum sampling rate that allows to correctly determine periodicity is $2f$ (**Nyquist rate**).

2 Hz – Nyquist rate



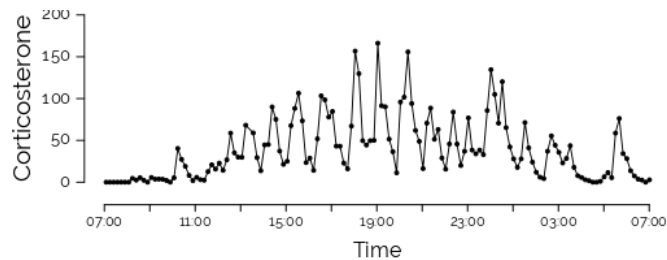
Lower frequencies lead to **aliasing**



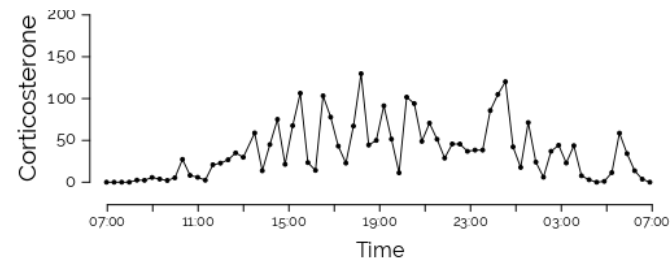
A real-life example

Corticosterone secretion from the rat adrenal gland

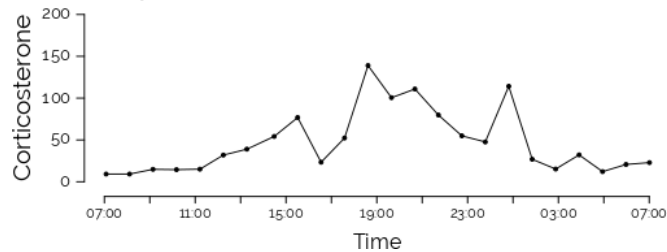
1 sample / 10 min



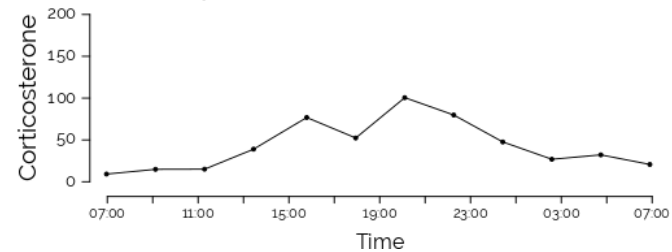
1 sample / 20 min



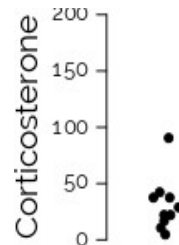
1 sample / hour



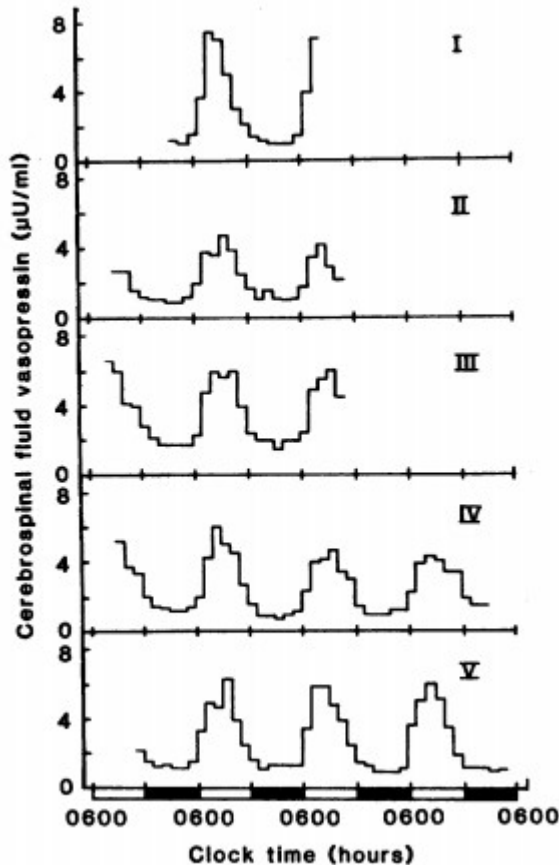
1 sample / 2 hours



10 independent measurements



Example



Reppert and colleagues collected samples from 5 cats to measure AVP concentration over the day.

They took 1 sample/hour over the course of several days

What is the experimental unit?

What type of design is this?

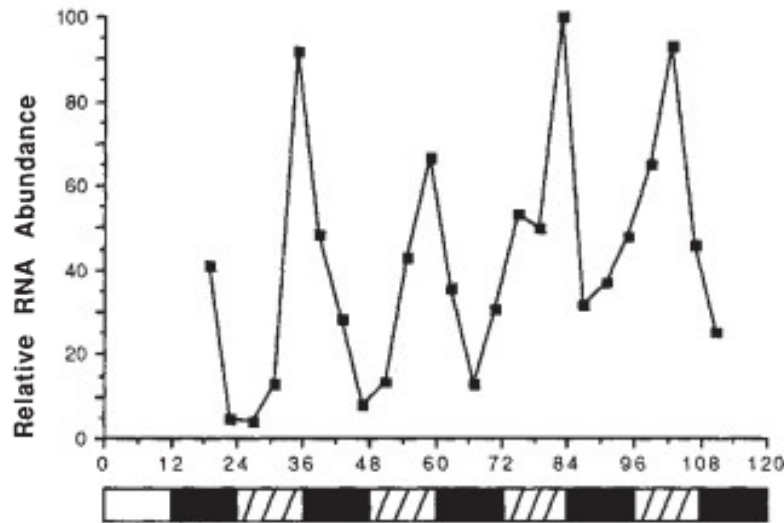
What n should be reported in this figure?

Any other comment?

AVP secretion *in vivo*

Reppert et al. 1981

Example



Hardin et al., 1990

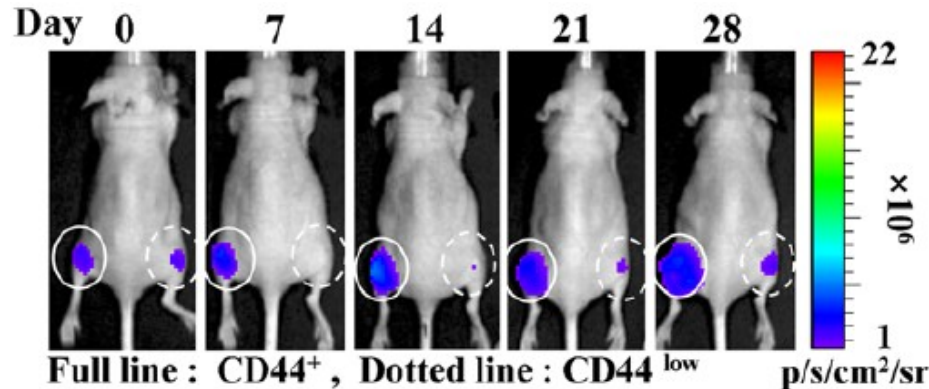
Hardin and colleagues collected samples from *Drosophila*, to measure *per* abundance over several days. They collected samples every 4 hours over the course of several day. Each time point they collected RNA from 100 flies, and measured RNA by Southern blot.

What is the experimental unit?

What type of design is this?

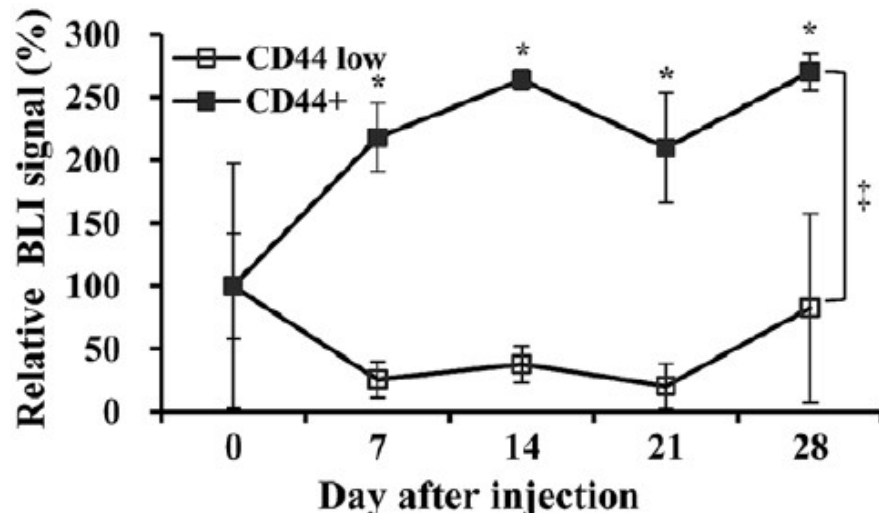
What n should be reported in this figure?

Example



Kim and colleagues implanted two different types of cancer cell (genetically modified so that they emit luminescence) in the left and right mammary gland of mice.

They then imaged the mice over four weeks to determine the growth of the tumoral mass.



What is the experimental unit?

What type of design is this?

What n should be reported in this figure?

Looking for a solution

Repeated measures and nested designs are at risk of pseudoreplication

Is this inevitable? Should I avoid nested designs? **Absolutely not!**

Nothing wrong with correctly analysed repeated measures or nested designs!

1. Always think carefully about the design of your experiment. What is your experimental unit?
2. Always be specific when reporting the number of samples. In a nested design, report n at different levels.
3. Use correct statistical methods, e.g. mixed effects models ← We will talk about this next week!