Experimental Design

Module 10

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John Tukey

“The combination of some data and an aching desire for an answer does not ensure that a reasonable answer can be extracted from a given body of data”

Sunset salvo. The American Statistician 40 (1). Online at http://www.jstor.org/pss/2683137

https://en.wikiquote.org/wiki/John_Tukey

Scientific Inquiry

1. Observation
2. Research Question
3. Hypothesis
4. Prediction
5. Experimental test

Experimental design allows you to go from a question to a statistical result that answers that question

Gaining knowledge in Ecology

Observation

Hypothesis

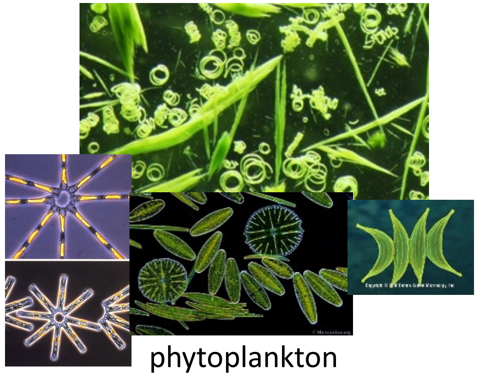
Experiment

Gaining knowledge in Ecology

Observation:

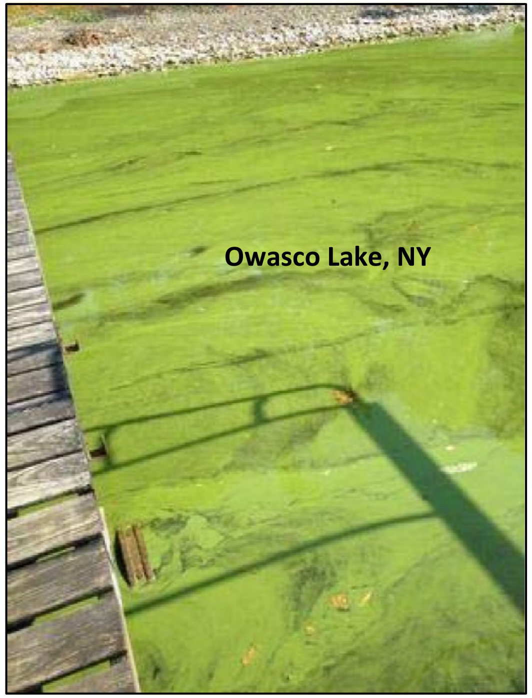
What are the patterns in nature?

Phytoplankton “blooms” in lakes



phytoplankton

Lake Erie

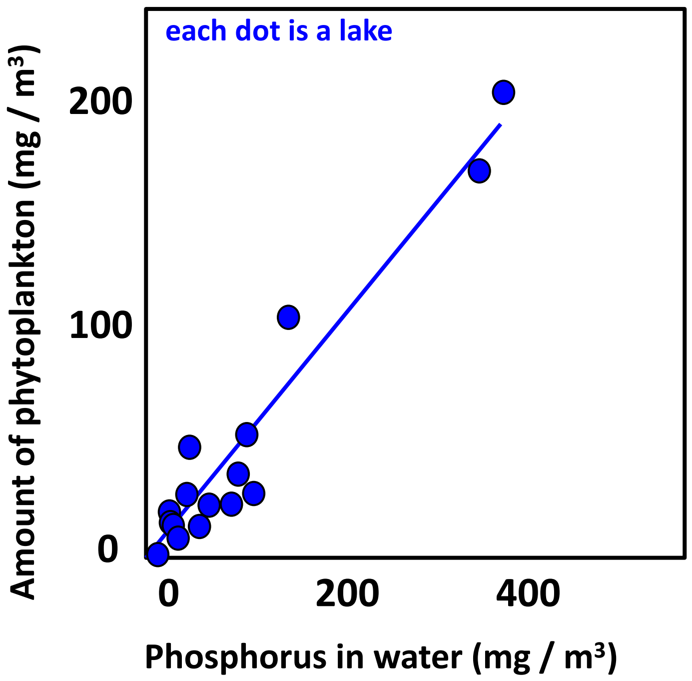


Owasco Lake, NY

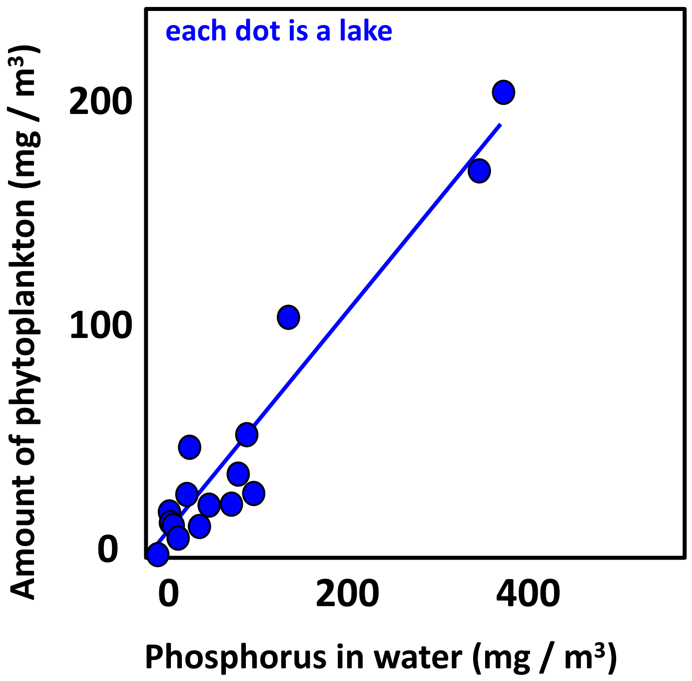
Honeoye Lake, NY

Nutrient pollution *Eutrophication*

From city wastewater treatment facilities
and from agricultural runoff



Observation:
a pattern in Nature
survey of 15 lakes



Observation:
a pattern in Nature
survey of 15 lakes

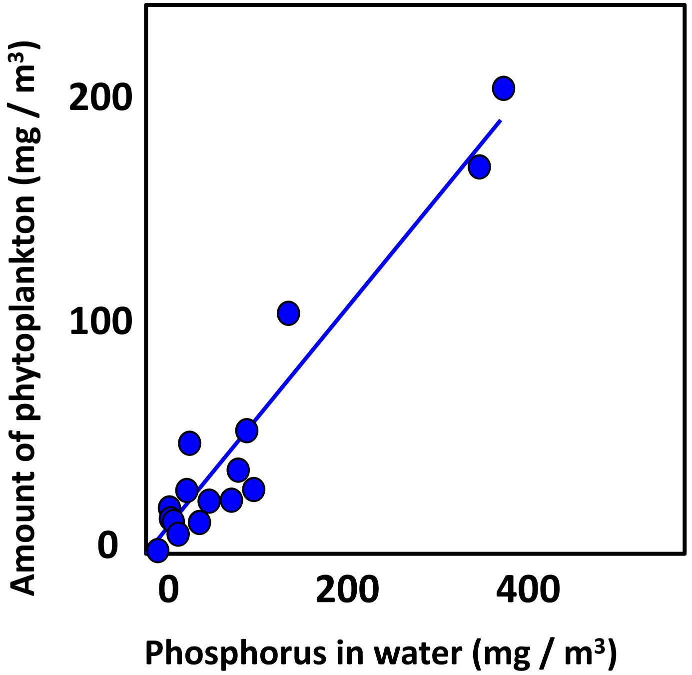
Hypothesis:
Phosphorus enrichment causes phytoplankton to bloom

Gaining knowledge in Ecology

Hypothesis:

Testable **explanation for an observed pattern in nature**

Hypothesis: *P causes phytoplankton to bloom*



***Experiment* on whole lake:**

Is P more important than N or C in regulating phytoplankton biomass?

David Schindler

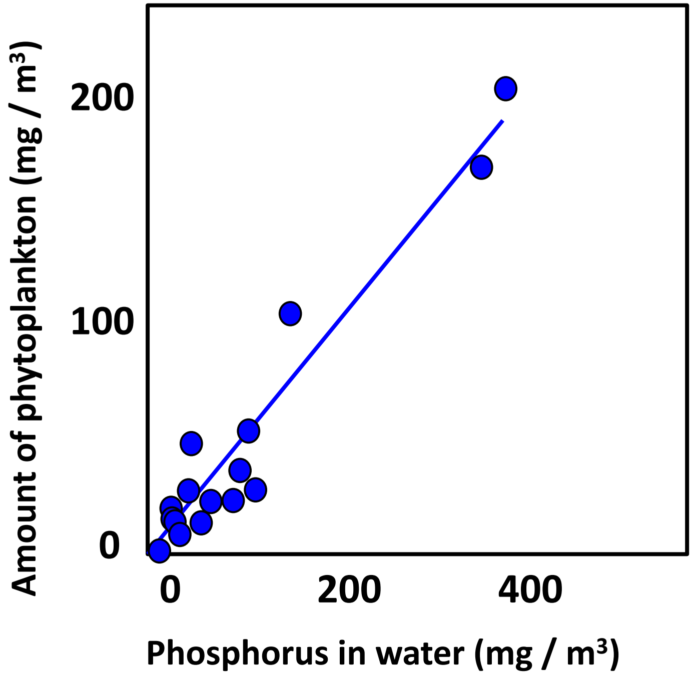
Gaining knowledge in Ecology

Experiment:

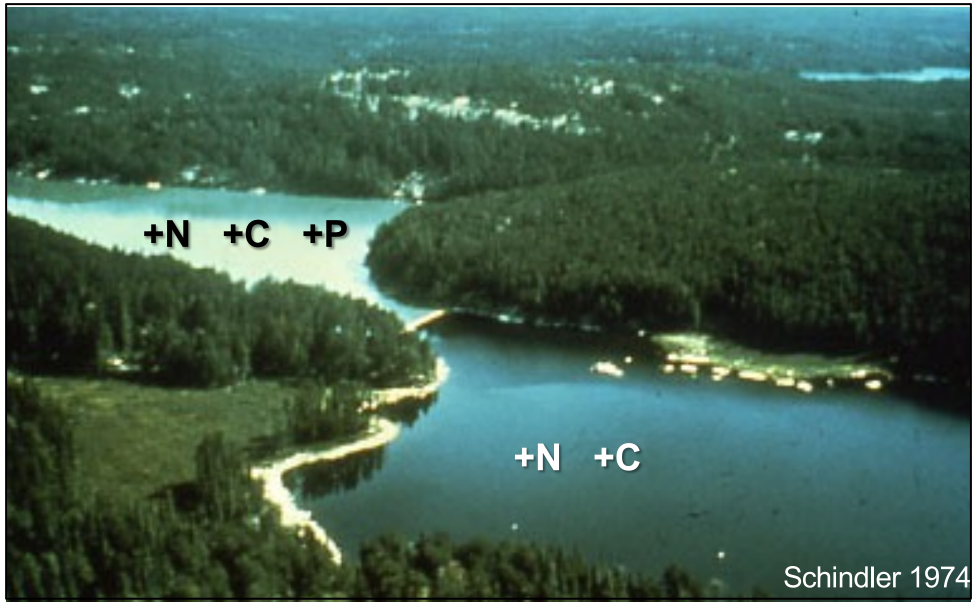
“A carefully designed human intervention into the ordinary course of nature.”

Alix Cooper (2011 American Scientist 99:343)

Hypothesis: P causes phytoplankton to bloom



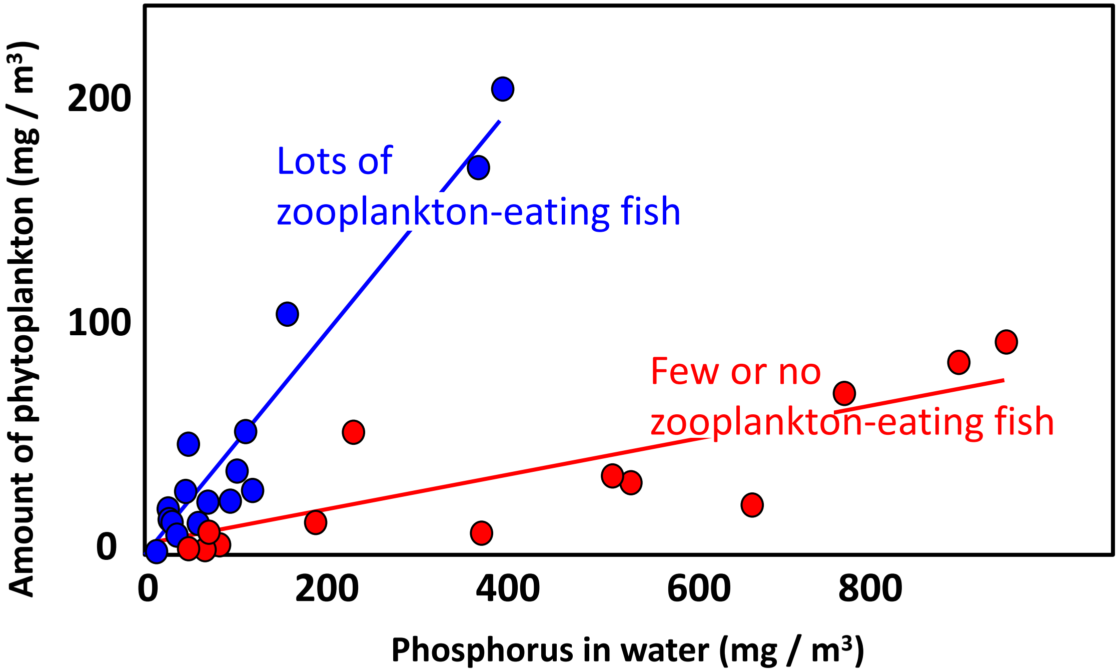
Whole-lake experiment



Adding P <u>did</u> cause phytoplankton to bloom
[adding N and C did not]

<u>Further Observation</u>: Refining the Pattern in Nature:

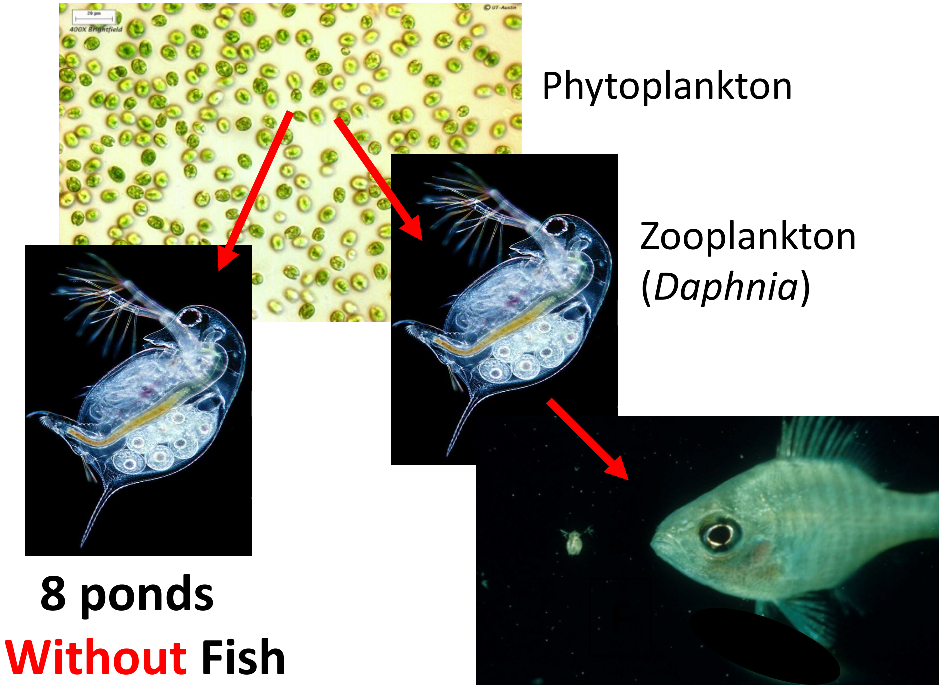
lakes with more zooplankton-eating fish
have more phytoplankton



<u>Hypothesis</u>:
Fish predation *indirectly* affects phytoplankton density

Experiment: Manipulate Food-Chain Length

Cornell Experimental Ponds Facility



Phytoplankton

Zooplankton (*Daphnia*)

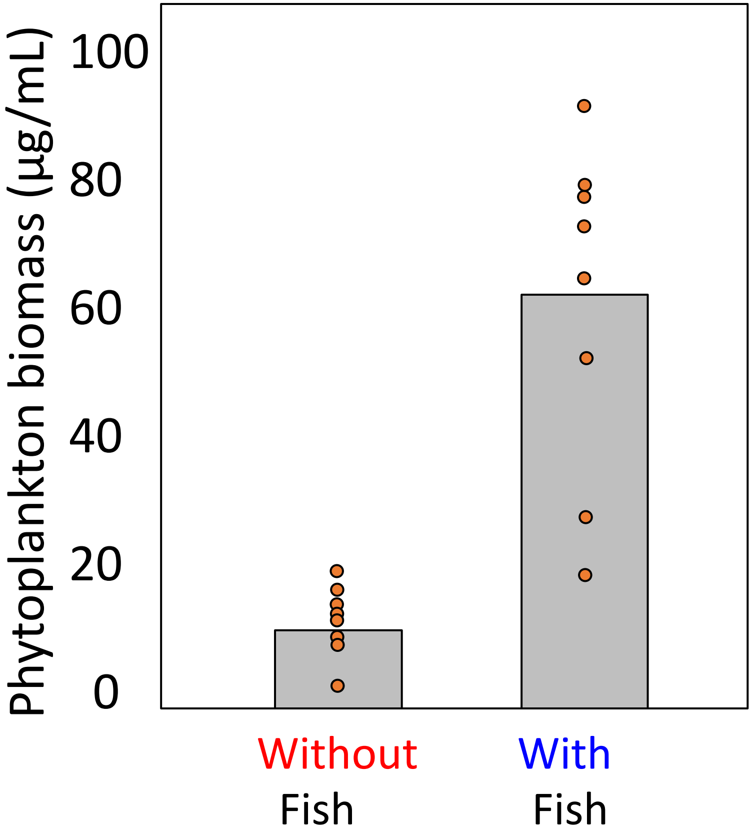
8 ponds
Without Fish

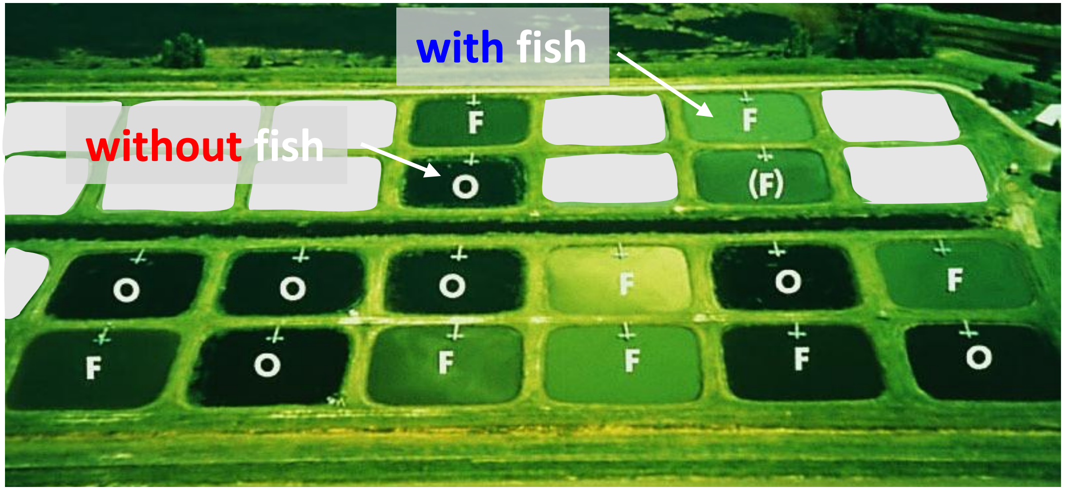
8 ponds
With Fish

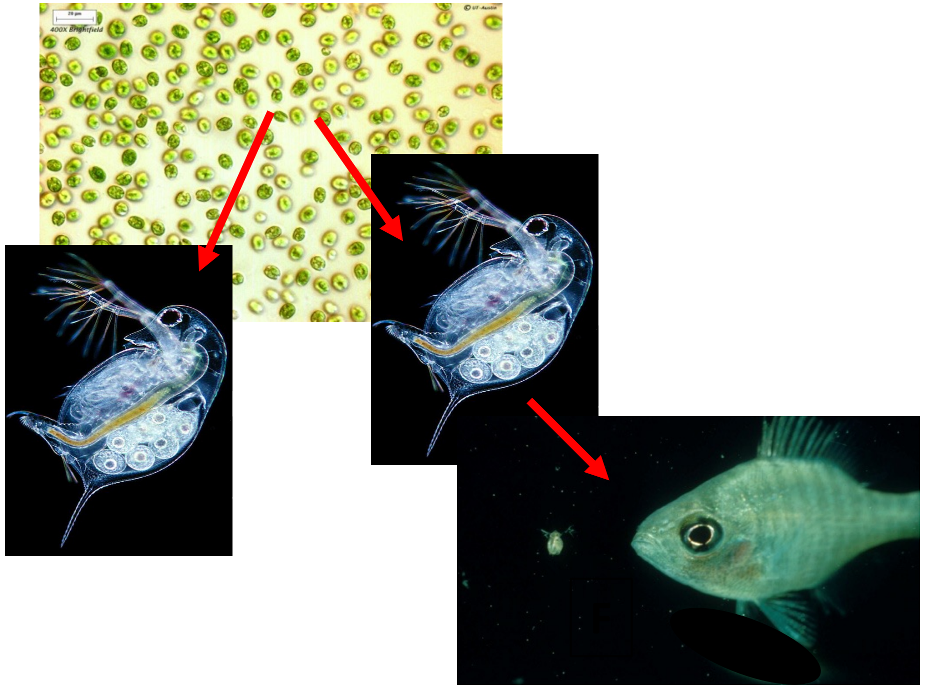
Hambright, Hairston, Howarth, Schaffner

Ponds with fish are much greener (more phytoplankton)

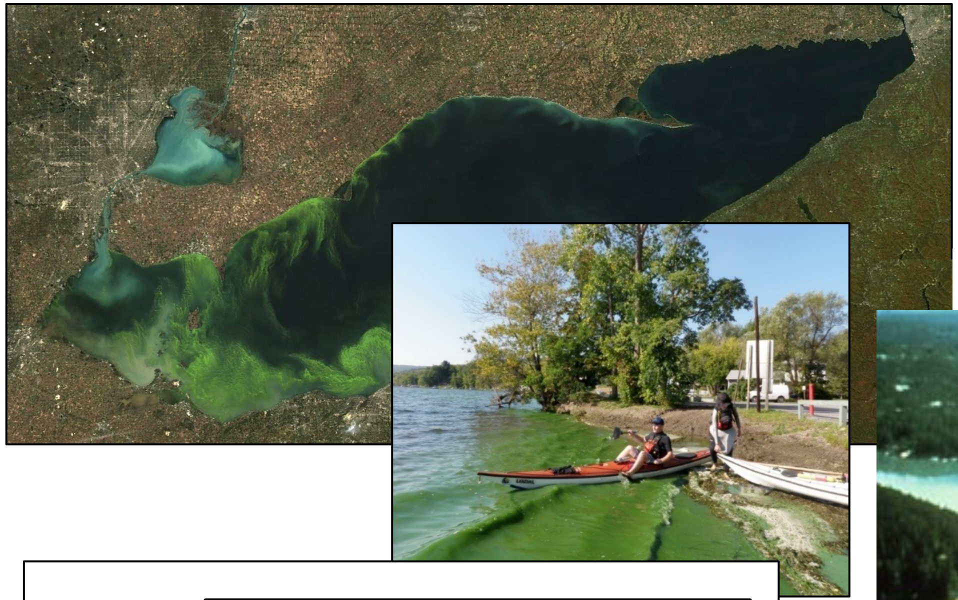
Presence of zooplankton-eating fish
does increase phytoplankton bloom

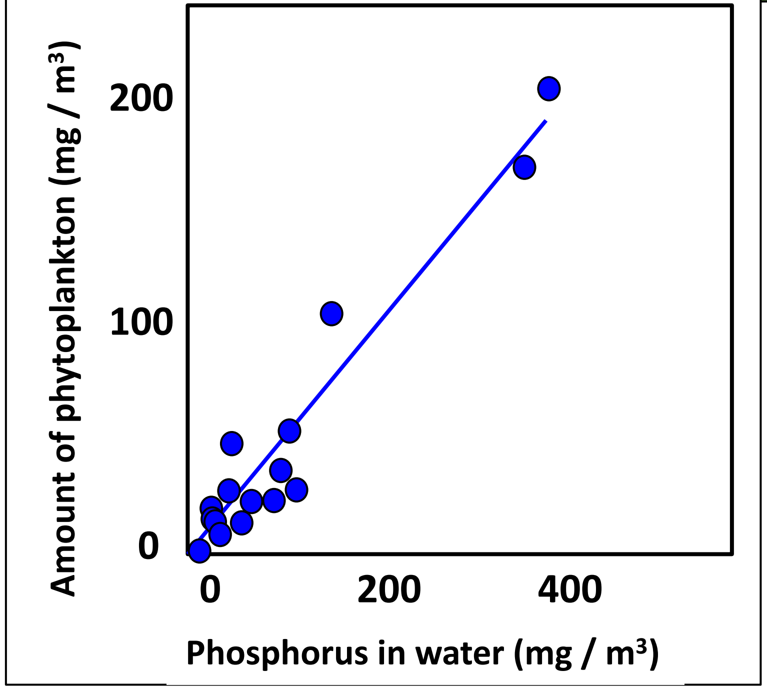


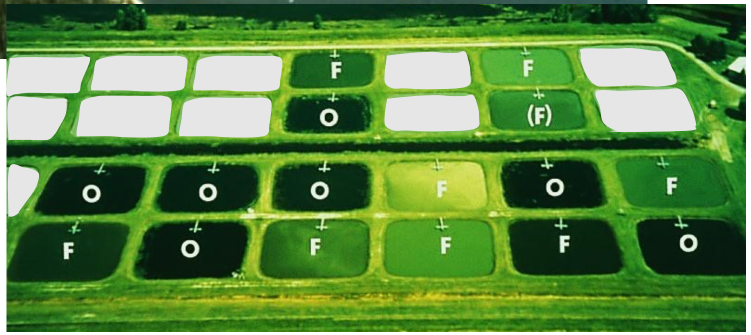




Observation
Hypothesis
Experiments
Understanding

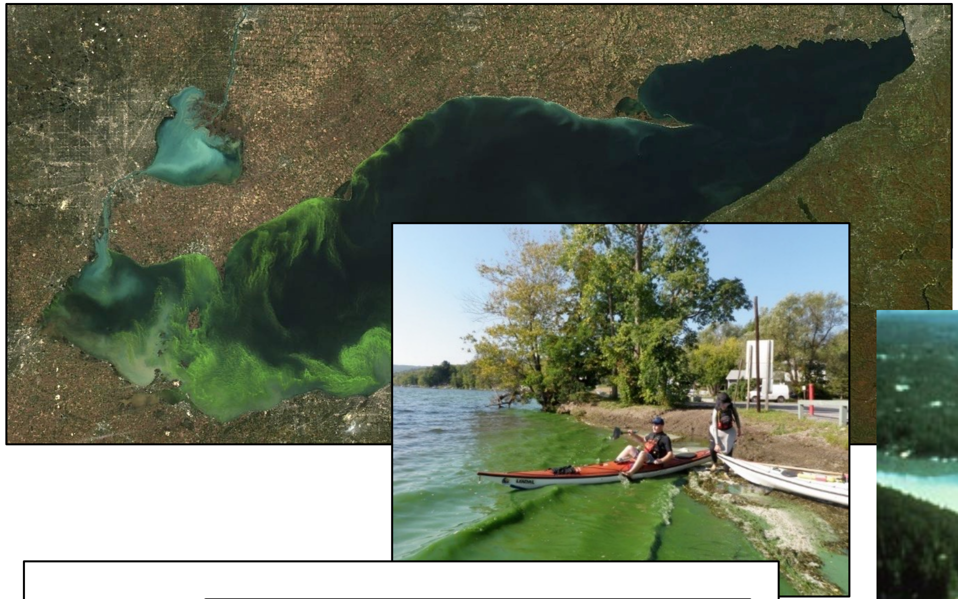


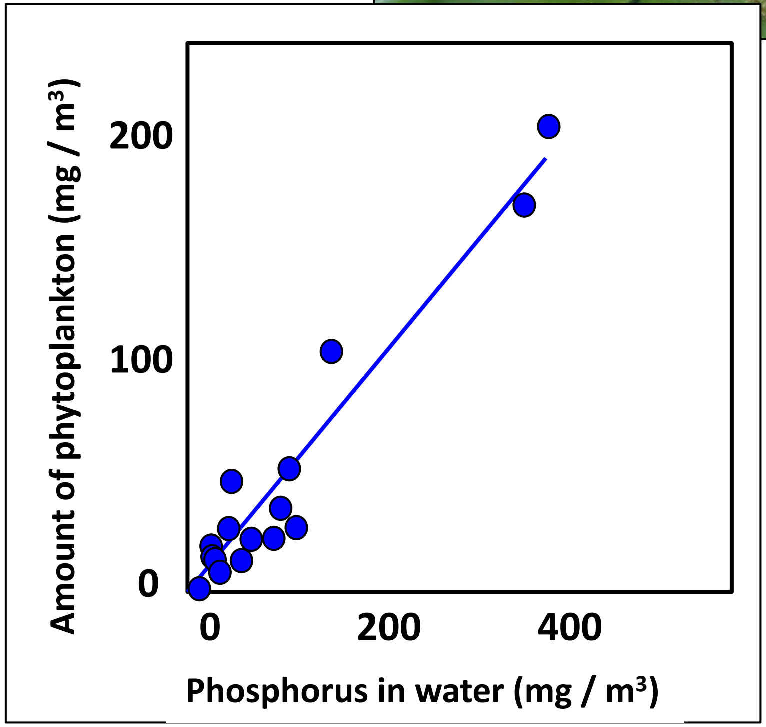


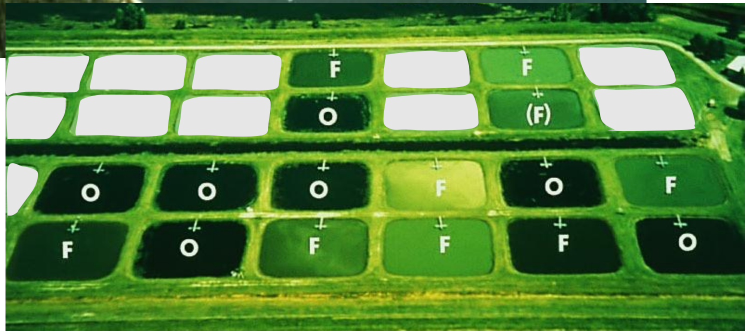


Observation
Hypothesis
Experiments
Understanding

Ecology!







Two Types of Investigation

- **Observational Studies**
  - Measurements made on populations or environments that *already exist*.
  - Differences between groups are caused by *unknown* forces or processes.
  - Sample naturally occurring groups or populations.

- **Manipulative Experiments**
  - Populations or environments are *created* by experimenter in a specified way
    - 2 or more *treatments*.
  - Individuals are *assigned to* treatments randomly

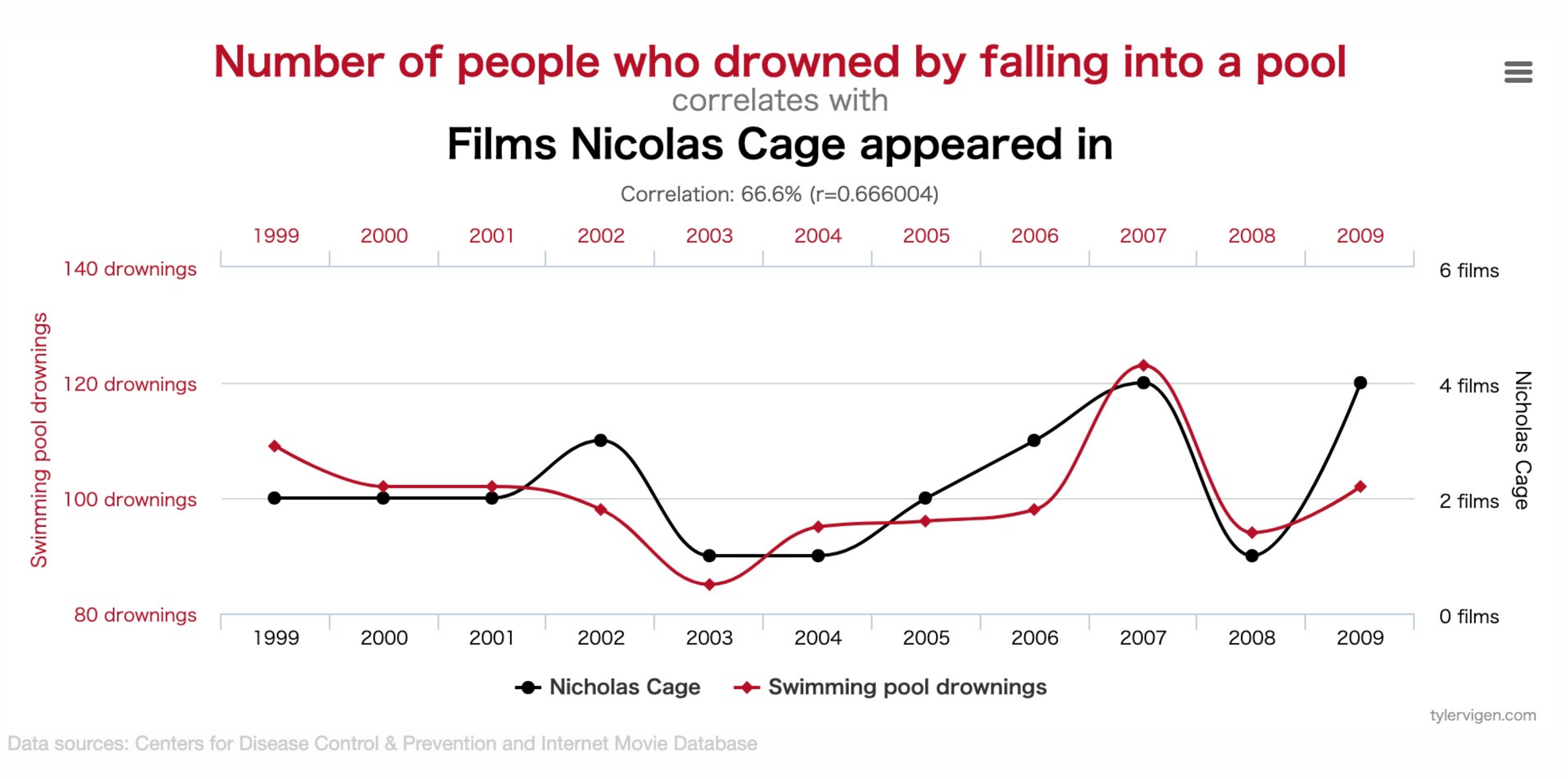
Both types of study are useful – used in different ways. Observational studies can often refine the scientific question, and followed up by a manipulative experiment

Correlation does not indicate Causation

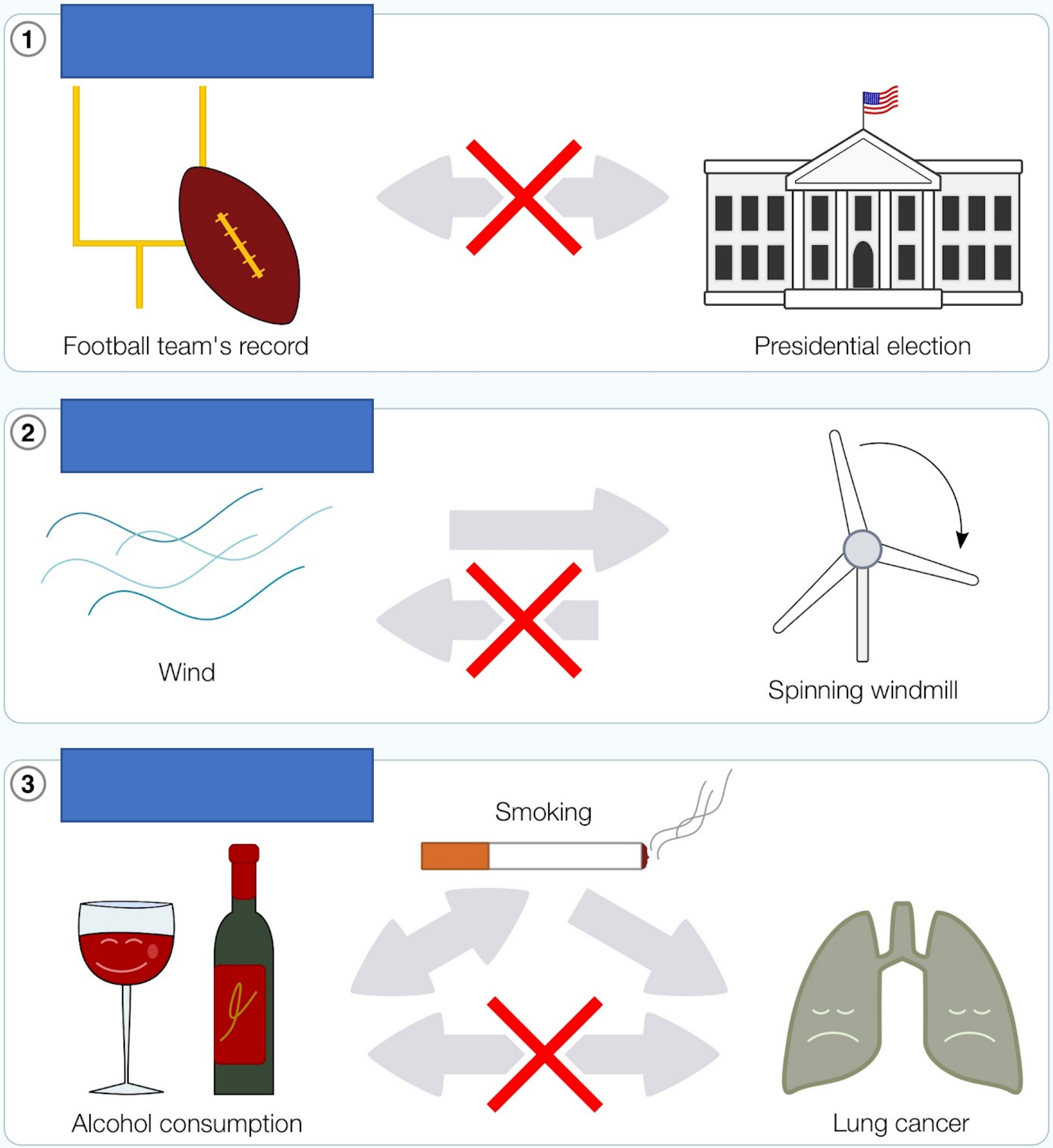
1. Spurious association – no causal relationships
2. Reverse causality
3. A third, unmeasured, variable
4. Masking variable

Manipulative experiments can be helpful in distinguishing correlation from causation

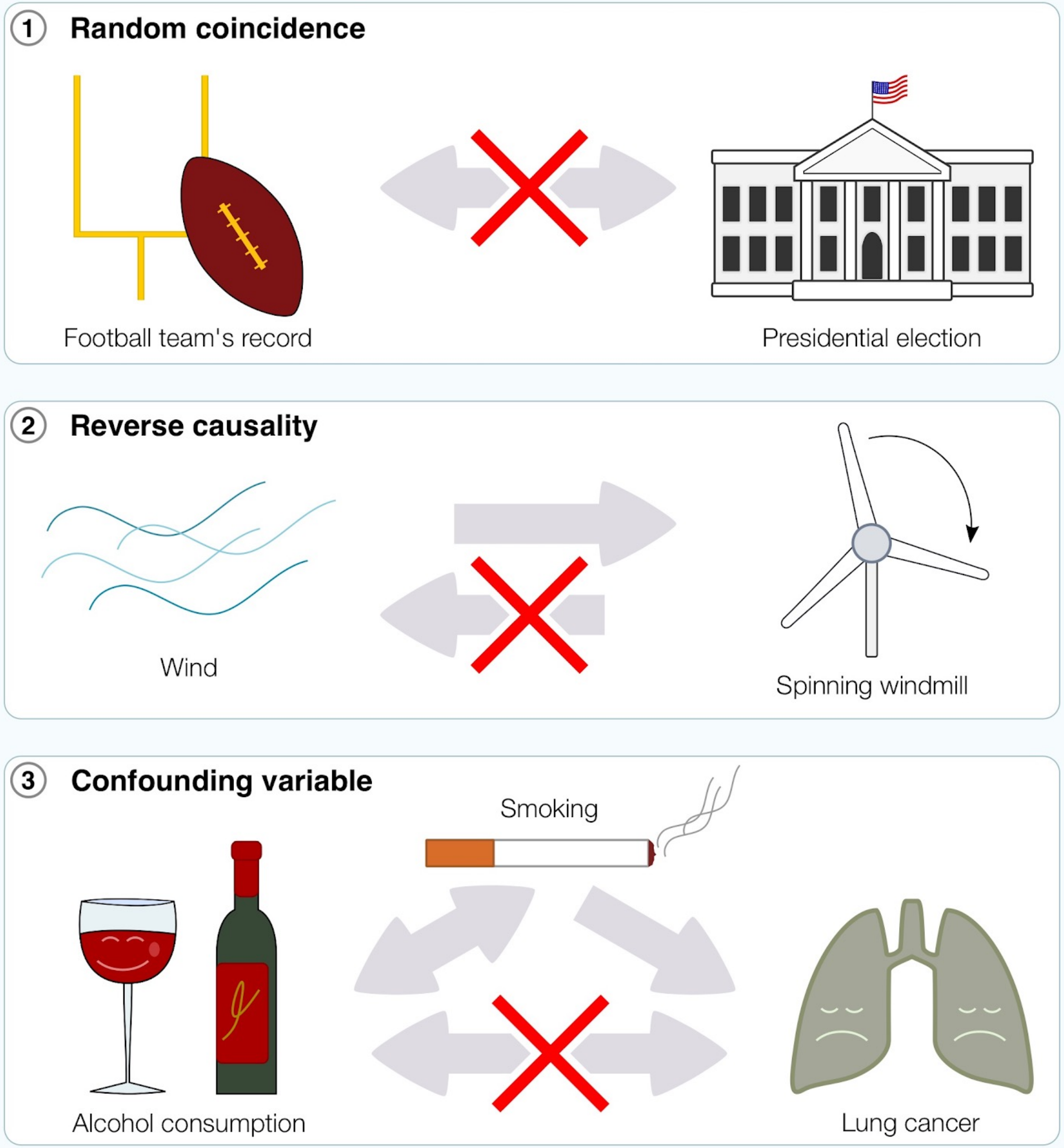
Correlation does not indicate Causation



https://www.tylervigen.com/spurious-correlations



https://sitn.hms.harvard.edu/flash/2021/when-correlation-does-not-imply-causation-why-your-gut-microbes-may-not-yet-be-a-silver-bullet-to-all-your-problems/



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Fisher's Three Elements to Experimental Design

1) **Replication**

2) Randomisation

3) Blocking

Replication has **3 functions:**

1. Provides an **estimate of <u>experimental error</u>**.
2. Improves **<u>precision</u>**
3. Increased breadth of experimental **<u>inference</u>** = wider applicability of our results.

Recognising true replication from pseudo-replication

- Mistaking data that have the potential to share error (variation that’s not scientifically interesting to us) for true replicates mislead
  - false positives or Type I statistical errors where we mistakenly reject the null hypothesis

Fisher’s Three Elements to Experimental Design

1) Replication

2) **Randomisation**

3) Blocking

Randomisation:

- Ensures there is **NO** association between groups or treatments of interest and any other part of the experiment
- Improves accuracy and reduces bias

In experimental design, randomisation doesn't just mean random sampling of the individual things of interest!

What's important here is that each experimental unit has an equal chance of ending up in either treatment / of being included in an observational study

How to get a random sample? And what if e.g., in study with human subjects, you invite a random sample, but a non-random subset responds?

Fisher’s Three Elements to Experimental Design

1) Replication

2) Randomisation

3) **Blocking**

Blocking:

- reduces within-group variability (“noise”).
- Allows you to align groups of measured experimental units with some known source of variation, allowing us to account for that variation, rather than letting it inflate the error (residual)
- Most useful when you know about of factor that contributes variation but is not directly of interest.

“To consult the statistician after an experiment is finished is often merely to ask him to conduct a post mortem examination. He can perhaps say what the experiment died of.”

Ronald A. Fisher. Presidential Address to the First Indian Statistical Congress, 1938. Sankhya 4, 14-17.
https://en.wikiquote.org/wiki/Ronald_Fisher

Learning Question

Two students want to test the effect of **temperature** (25 ℃ versus 30 ℃) on **running speed** in flies. Each student has 10 temperature cabinets and randomly allocates different numbers of flies to each cabinet. Five cabinets are set to 25 ℃ and five are set to 30 ℃.

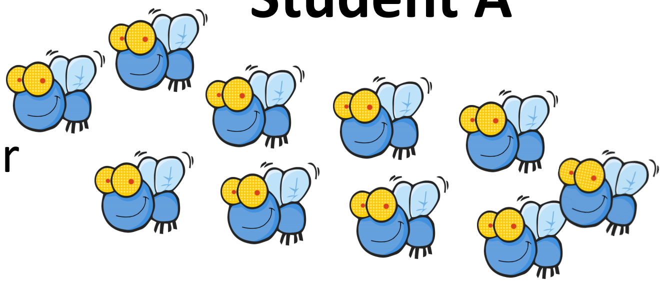
Cabinets at 25°C

Cabinets at 30°C

Student A measures 10 flies per cabinet while student B measures three flies per cabinet.

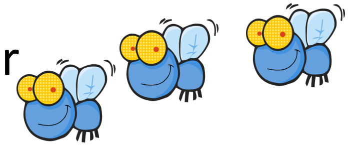
Student A

10 flies per cabinet



Student B

3 flies per cabinet



Learning Question

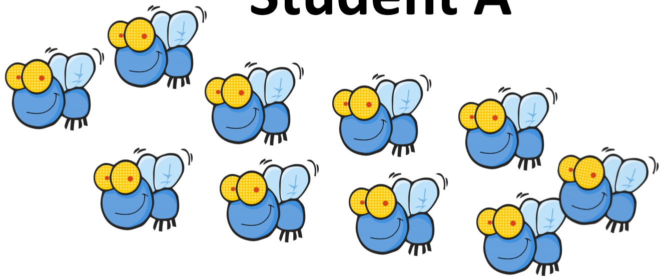
They then proceed to analyse their data using ANOVA.

Which student (A or B) has correctly identified the independent replicates in this experiment? (which student has implemented the correct ANOVA model?)

Five cabinets set to 25 ℃ and five cabinet set to 30 ℃.

Student A

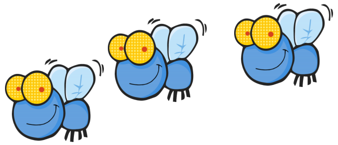
10 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1				
Error	98				
Total	99				

Student B

3 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1				
Error	8				
Total	9				

Learning Question

The **<u>CABINETS</u>** are the independent replicates

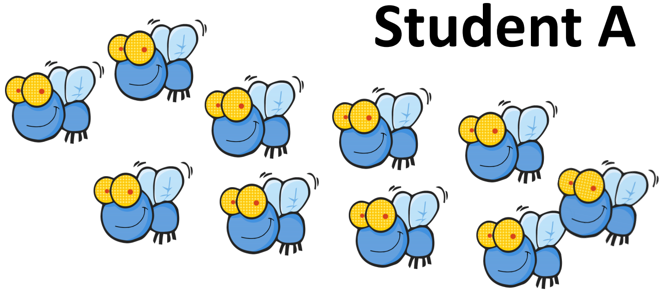
- Flies *within* the same cabinet share variation due factors other than temperature

Student A is counting individual flies as replicates and has **pseudoreplicated**

Student B looks like they have calculated the cabinet means

Student A

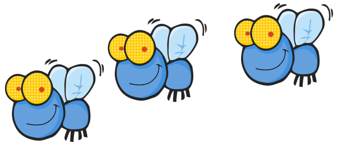
10 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1				
Error	98				
Total	99				

Student B

3 flies per cabinet



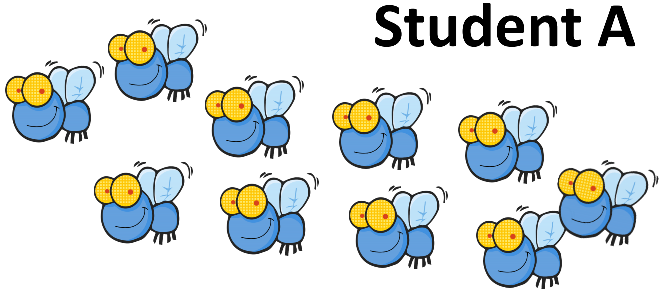
Effect	DF	SS	MS	F	P
Temperature	1				
Error	8				
Total	9				

Learning Question

Did fly speed vary with temperature?

Student A

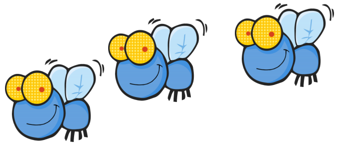
10 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1			4.1	0.0013
Error	98				
Total	99				

Student B

3 flies per cabinet



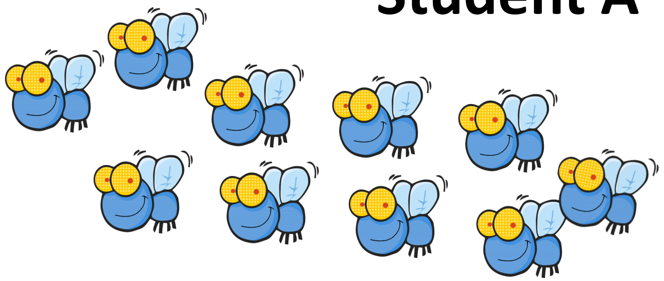
Effect	DF	SS	MS	F	P
Temperature	1			3.1	0.034
Error	8				
Total	9				

Learning Question

If both students worked on cabinet means who would have the more powerful experiment?

Student A

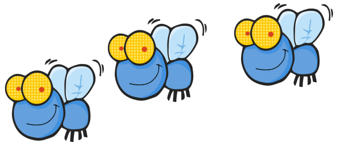
10 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1				
Error	8				
Total	9				

Student B

3 flies per cabinet



Effect	DF	SS	MS	F	P
Temperature	1				
Error	8				
Total	9				

Question

A researcher is interested in whether a species of flowering plant's seed production is affected by self versus cross pollination.

To test this, she was given a total of 200 plants and allocated them to either self- or cross-pollination treatments.

She allocated 50 plants to the self-pollination treatment and 150 to the cross pollination treatment.

After a period of weeks, she took five flowers from each plant and counted the number of seeds on each flower, recording these counts in a spreadsheet.

How many independent data points does the researcher have her data file?

A) 250

B) 1000

C) 200

A researcher conducted a study to assess the relationship between coffee bean production and the local abundance of bees near the coffee tree.

Bean production was significantly positively correlated with bee abundance.

The researcher concludes that increasing bee abundance has a positive effect on coffee bean production. **Is this a correct interpretation of the results?**

https://www.needpix.com/photo/729165/coffee-beans-ripe-agriculture-plant-raw-crop-grow-fruit-plantation

https://commons.wikimedia.org/wiki/File:Blue_Banded_Bee_(Amegilla_cingulata).jpg