Experimental design

Photo: Ben Weigel

Mark Lenz

An ISOS Lecture • Doing Science • November 5th 2013

GEOMAR

Hint #1: Identify your study design!

Hint #1: Identify your study design!

Data collection and statistical testing

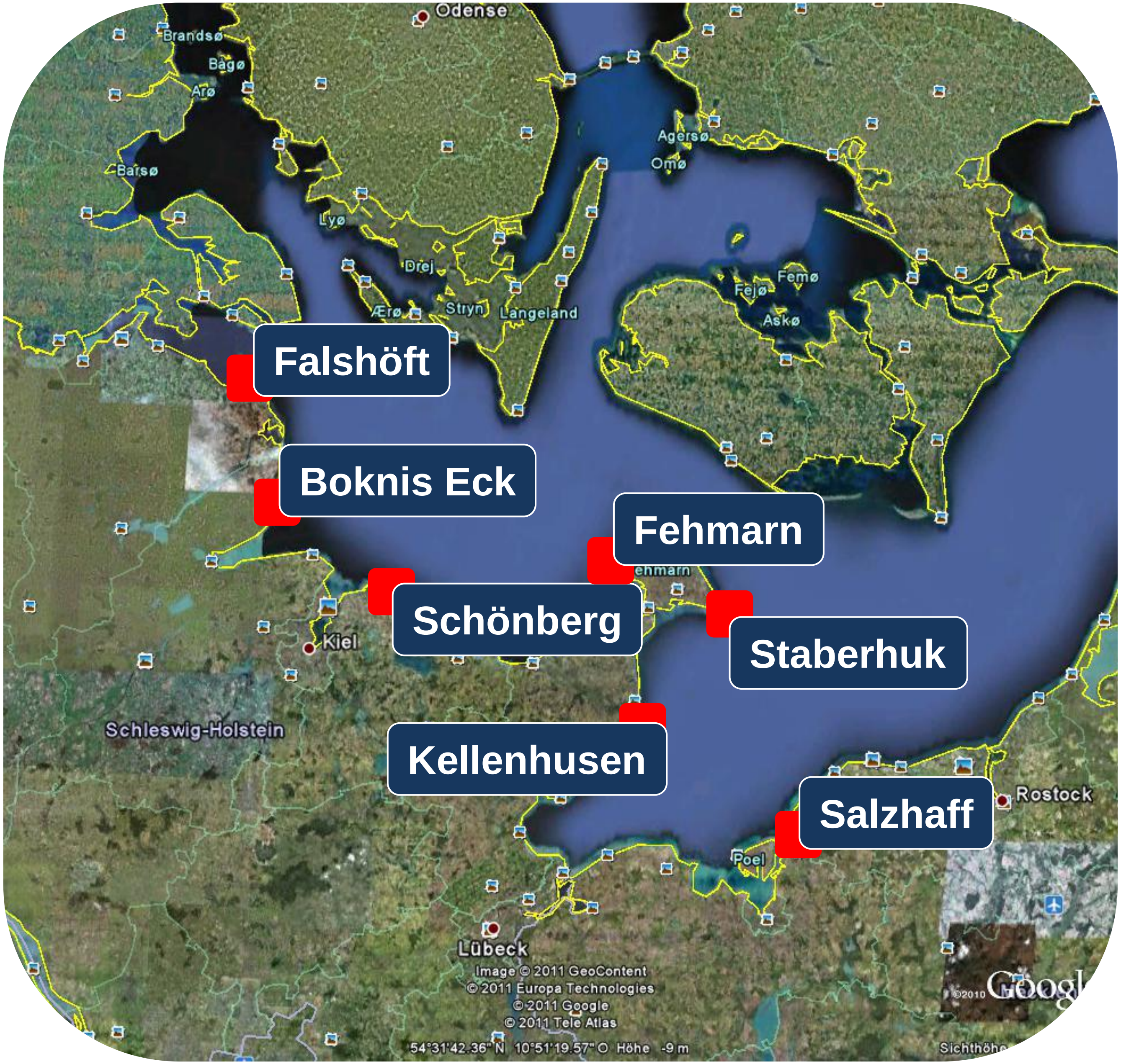
Observational scenario

- hypotheses are formulated *a posteriori* on base of data exploration
- number of data points collected is high
- half of the data should be used for exploration, the other half for testing
- predictors are mostly continuous

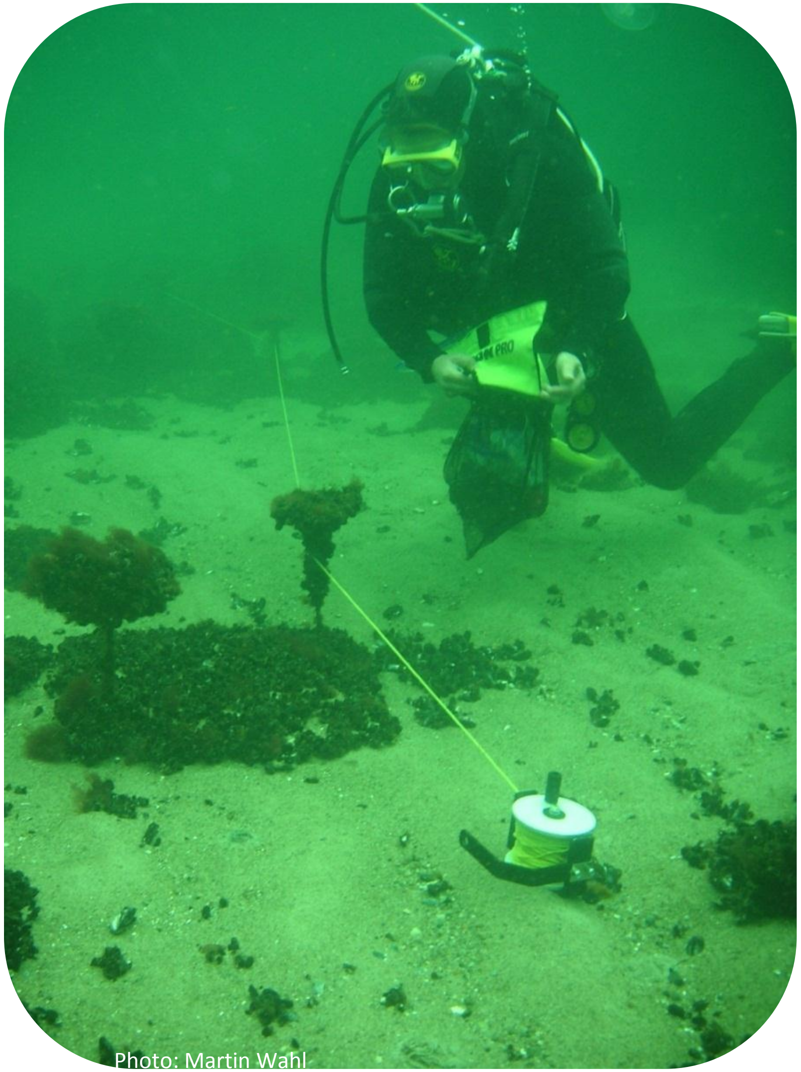
Experimental scenario

- *a priori* formulated hypotheses
- number of data points collected is low
- data can be fully used for testing
- predictors are mostly categorical

Hint #1: Identify your study design!



Hint #1: Identify your study design!

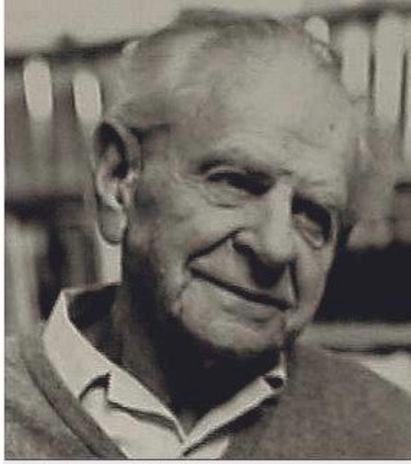


Observational studies cannot prove causalities!

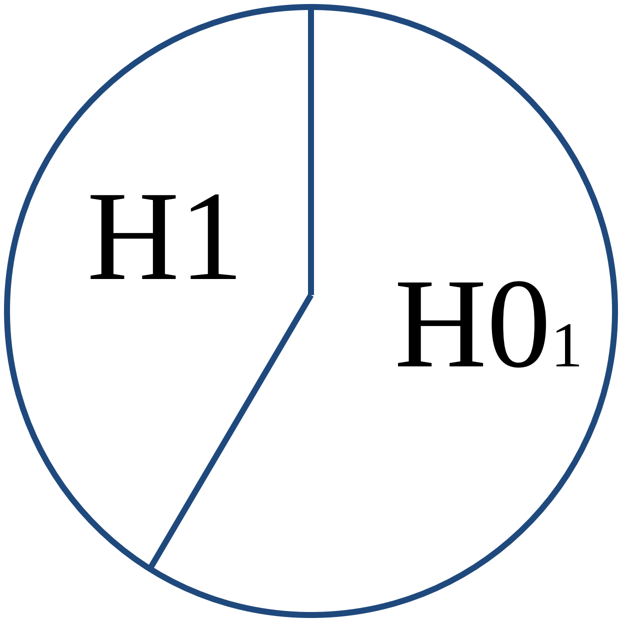
Carefully screen the literature and discuss your subject with other researchers!

Hint #2: Identify your hypotheses!

Hint #2: Identify your hypotheses!


Sir Karl Popper (1902-1994) Quelle: www.plato.stanford.ed

Reinforcement by falsification of the null hypothesis



$H0_1$

not falsified → ~~H1~~ falsified

falsified → H1 supported

Important: Perfect complementarity!

Hint #2: Identify your hypotheses!

Job description

Work: design, prepare and run one single experiment

Budget: whole project is 1 billion US Dollars

Time for preparation: 16 years

Distance to experimental site: 300 000 000 km

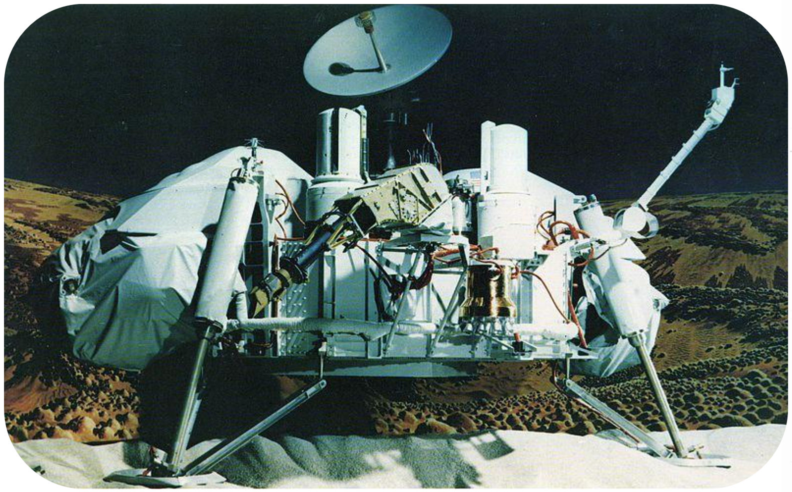
Mission: Find life on Mars

Problem: The job is done

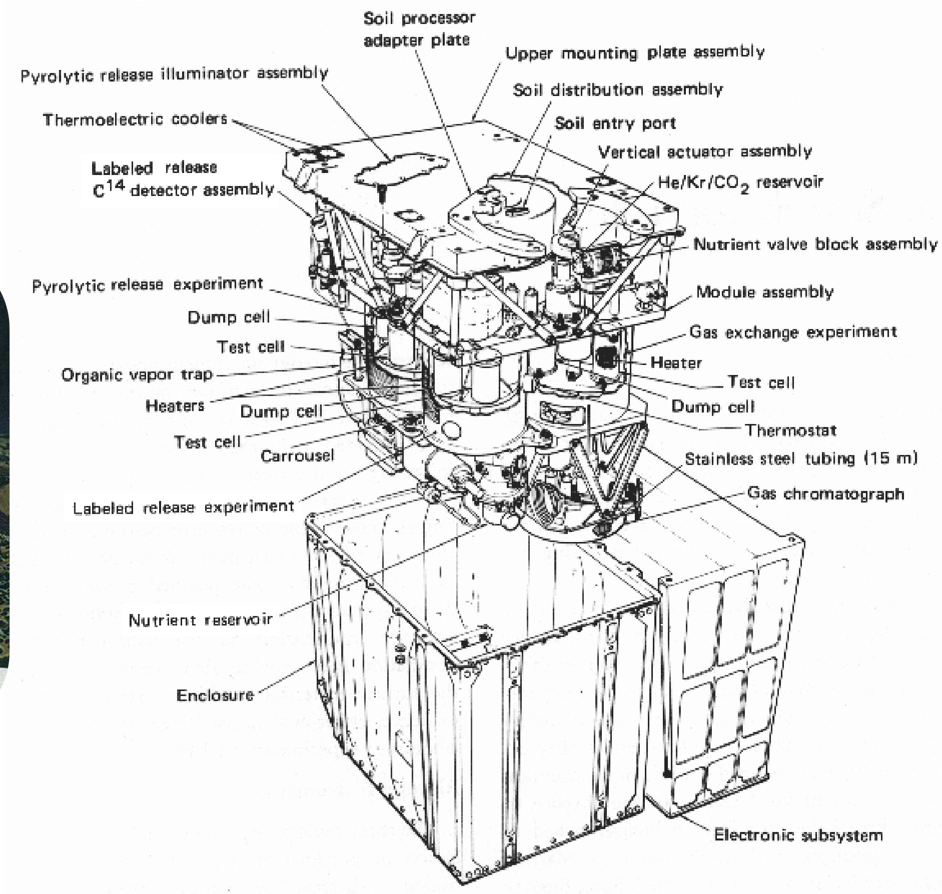

Source: Wikipedia

Hint #2: Identify your hypotheses!

Is there life on Mars?



Source: Wikipedia



Source: Wikipedia

The labeled release experiment was positive

The gas chromatography/mass spectroscopy was negative

Think carefully about all possible outcomes of your experiment!

Hint #3: Know the terminology!

Photo: Ben Weigel

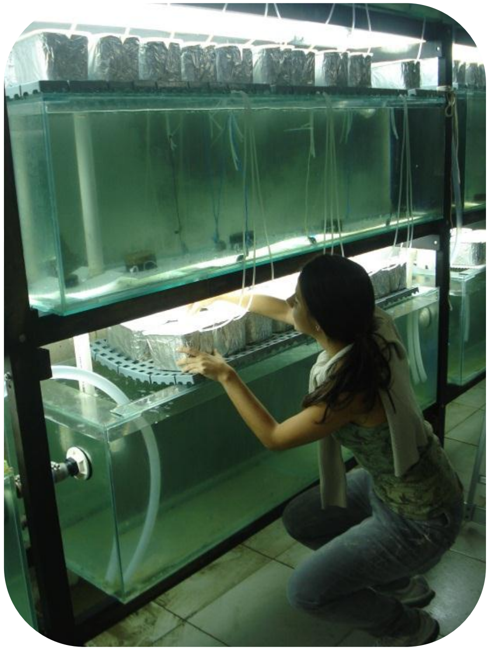
Hint #3: Know the terminology!

Population =
totality of all units characterized by a variable

Sample =
actually analyzed part of the population

(Experimental) Unit =
replicate = parallel = sampling unit

Hint #3: Know the terminology!



14

Hint #3: Know the terminology!

Treatment = Factor

pCO_2

Treatment levels =
Samples

pre-industrial	2013	2100
Tank 1	Tank 1	Tank 1
Tank 2	Tank 2	Tank 2
Tank 3	Tank 3	Tank 3
Tank 4	Tank 4	Tank 4

This is one sample!

Sampling unit
= Experimental unit

Sample size = number of sampling units (n)
In this case n = 4 for each sample

The number of samples is 3

Total number of replicates is N = 12

Since n is the same in all samples, this design is balanced

Put a name to all physical and conceptual components of your experiment!

Hint #4: Know your variables!

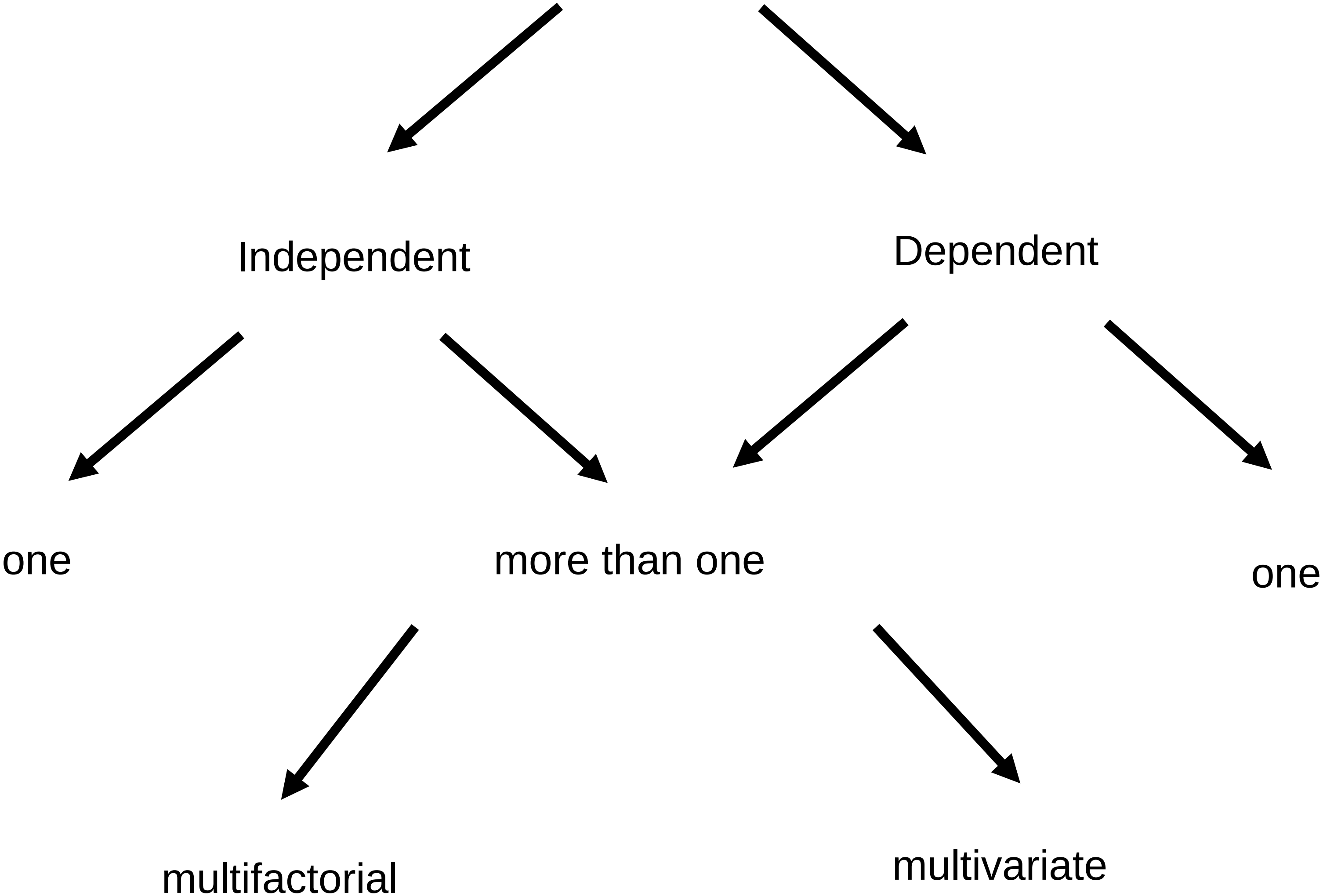
Hint #4: Know your variables!

Variables in a data set

- Independent
  Predictor
  Grouping
  Explanatory
  Factor
  Covariate
  - Categorical
  - Continuous
- Dependent
  Response
  - Categorical
  - Continuous

Hint #4: Know your variables!

Variables in a data set



Hint #4: Know your variables!

Data quality levels
Limpets in intertidal horizons

Horizon	Nominal	Ordinal	Derived	Interval
1	Y = 1	1	61%	30
2	Y = 1	3	1%	1
3	Y = 1	2	38%	19
4	N = 0	4	0%	0

Possible Transformation

Identify the character of your independent and dependent variables! Count them!

Hint #5: Understand your stats!

Photo: Sarah Piehl

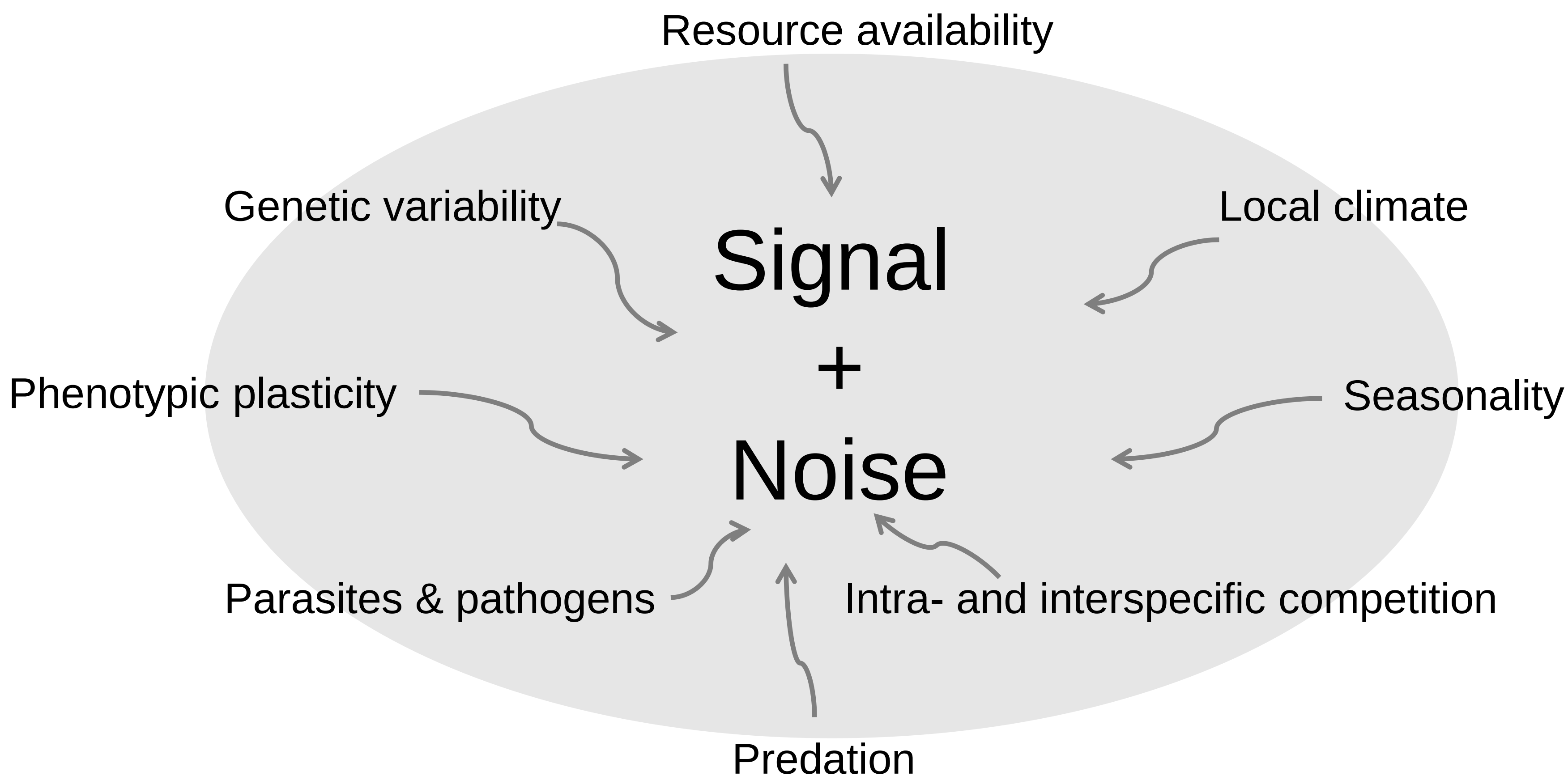
Hint #5: Understand your stats

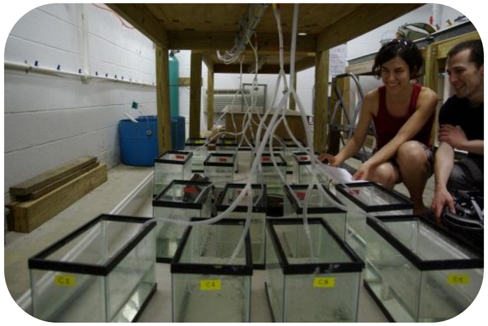
Why do we need stats?

Sources of variation:

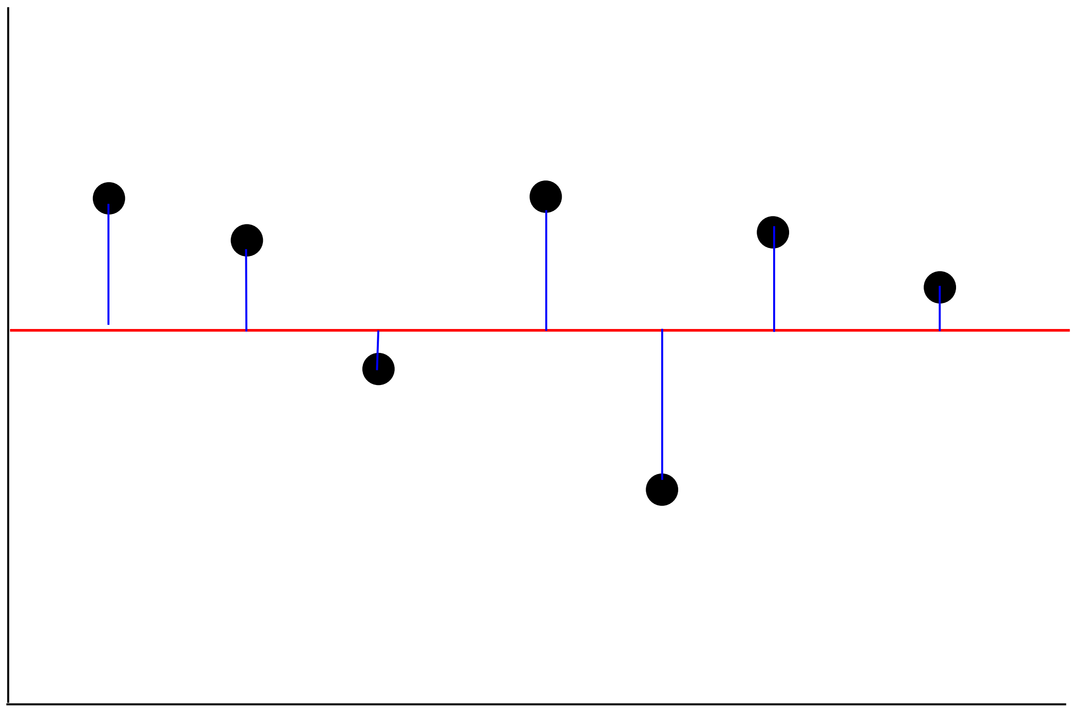
- spatial heterogeneity
- temporal variability (life cycles, rhythms..)
- genotypic variability
- phenotypic plasticity
- methodical problems

Hint #5: Understand your stats





A simple statistical model: the sample mean



= amount of unexplained variance (residuals)

= amount of explained variance (model)

Hint #5: Understand your stats

$$\text{test statistic} = \frac{\text{variance explained by the fertilizer}}{\text{variance not explained by the fertilizer}}$$

the t-statistic:

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

$\overline{X}$ = mean

S = standard deviation

n = sample size

t = 8.69

Hint #5: Understand your stats

Effect size

Inside t-test:

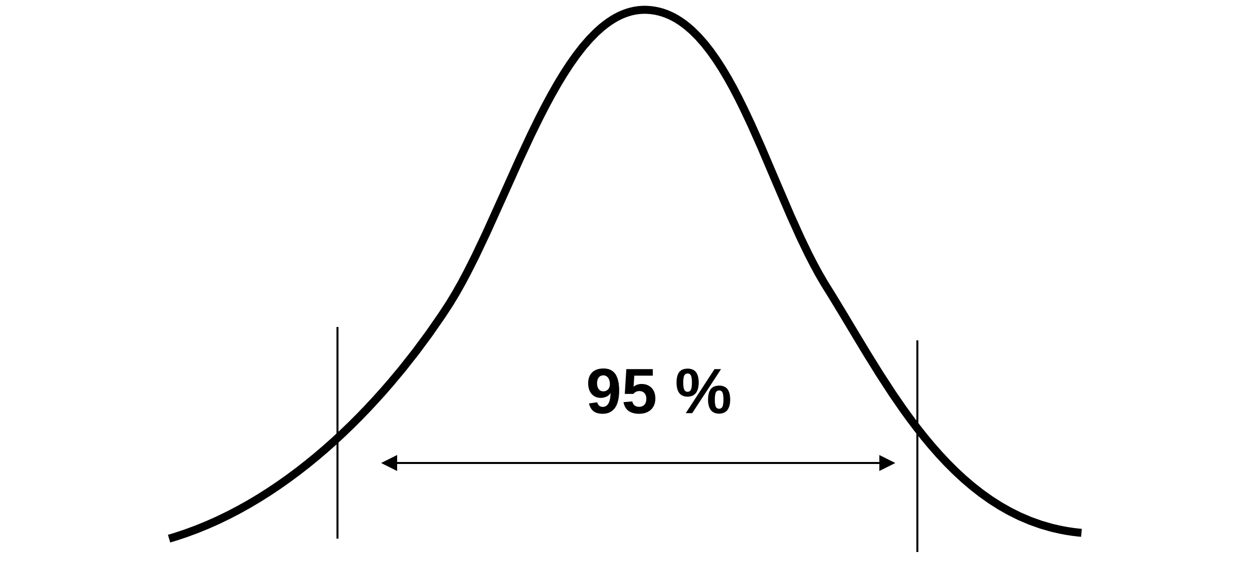
Amount of unexplained variation

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Sample size

Hint #5: Understand your stats

Where do p-levels grow?



A distribution of t, calculated under the assumption that H_0 is true.

Where in this distribution is our observed value of t located?

If it is outside the 95% range, we assume that it is not random.

28

Error? What error?

Type I error: Rejecting the nullhypothesis though it is right. We believe there is an effect, but there is not. This is the α-level, it is by convention $p \leq 0.05$ (5 %, Fisher‘s criterion).

Type II error: Accepting the nullhypothesis though it is wrong. We believe there is no effect, but there is one. This is the β-level, it should not be larger than 0.2.

29

Hint #5: Understand your stats

In simple words….

Type I error: We see something, where there is nothing

Type II error: We overlook the effect

Test power $1 - \beta$: The probability to detect an existing effect.
It should be at 0.8.

Hint #5: Understand your stats

Assumptions of statistical tests

Independency between samples
(unless it is a repeated measures study)

Independency within samples

Random sampling

Non-collinearity between independent variables

Homogenous variances

Normality of data

Stats are easy, but data can be difficult!

There is no easy way out: Read textbooks, join stats courses!

Hint #6: Avoid pseudoreplication

Hint #6: Avoid pseudoreplication

Factor: CO_2 partial pressure with 4 levels

10 tanks per level

20 mussel individuals per tank

2 valves per mussel

Pseudoreplicated:

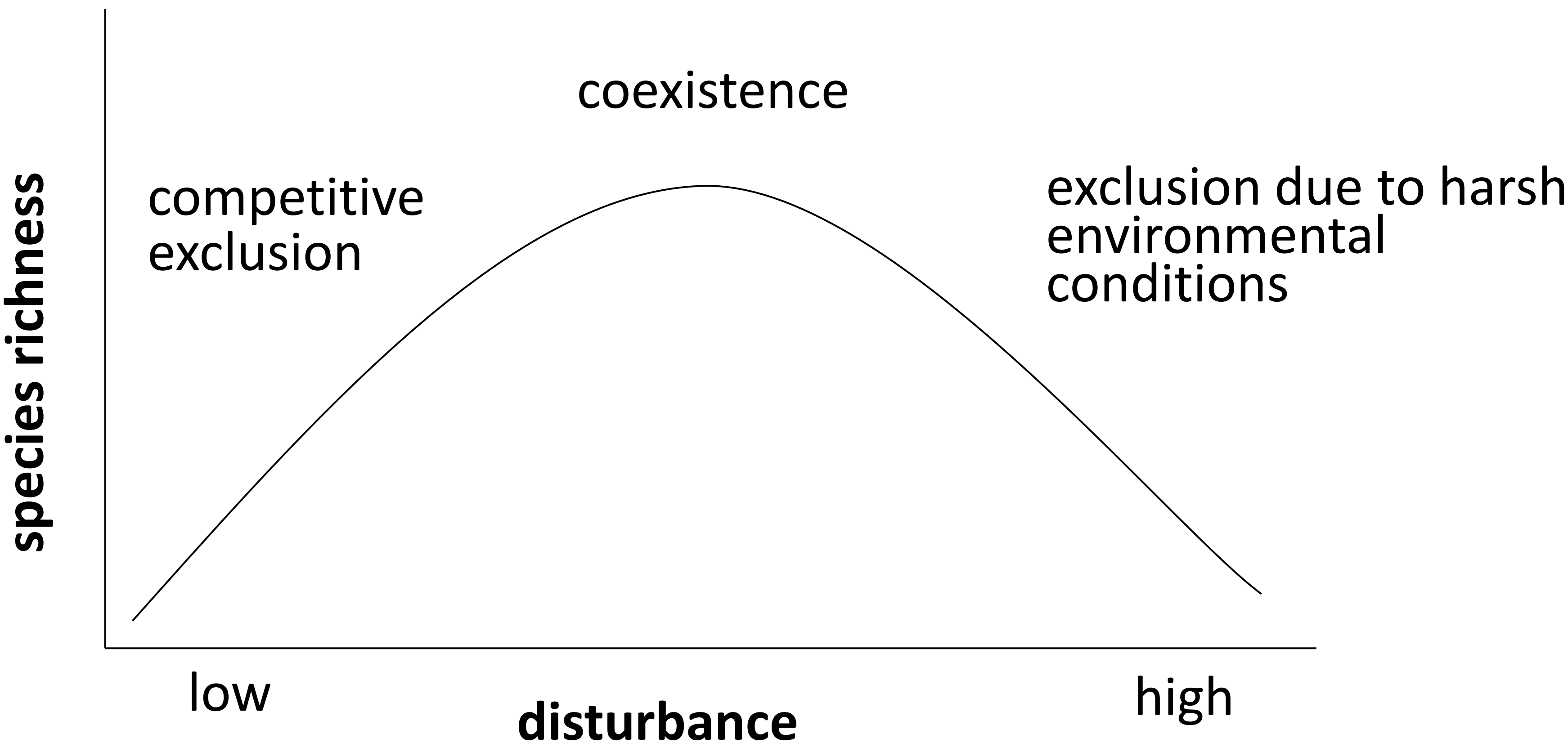
40 x 20 x 2 = 1600 = 1596 df

Replicated:

4 x (10-1) = 36 df

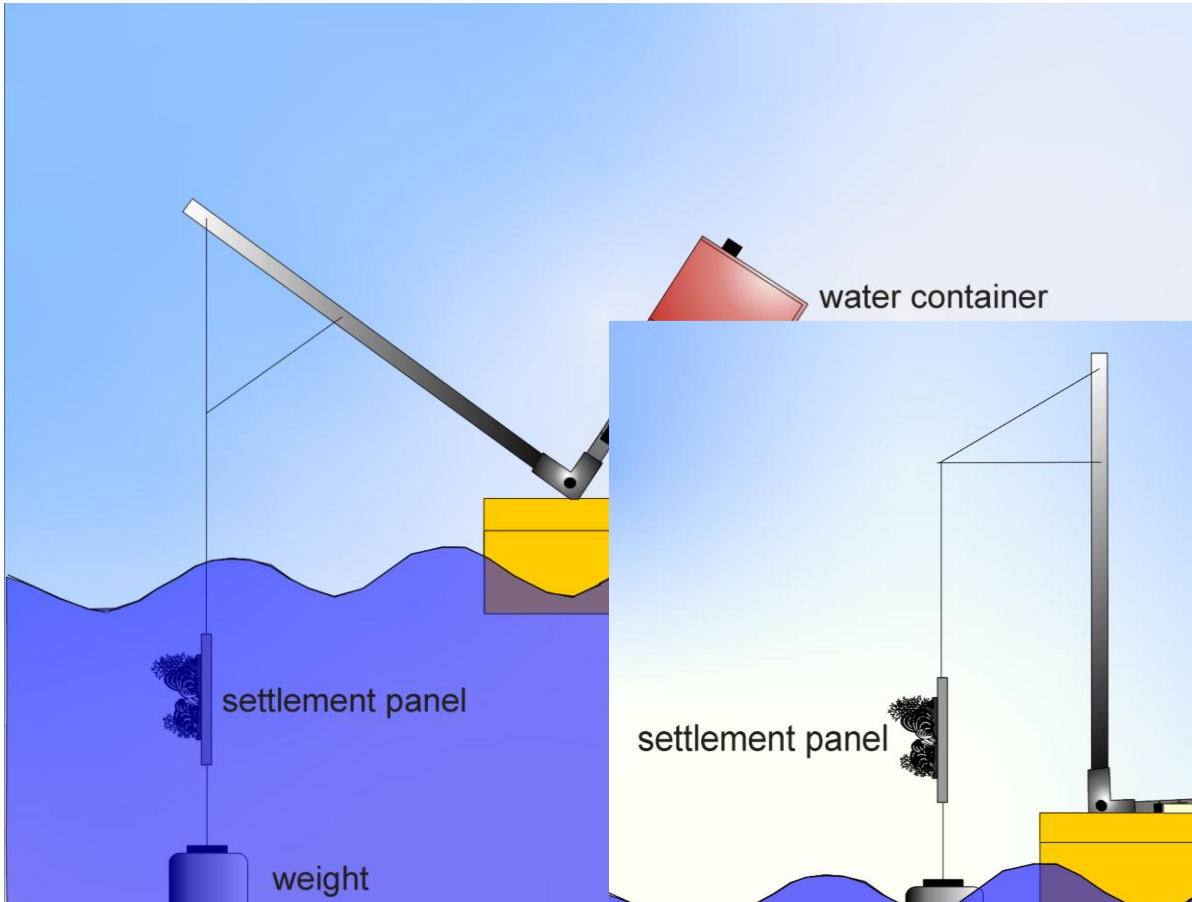
Hint #6: Avoid pseudoreplication

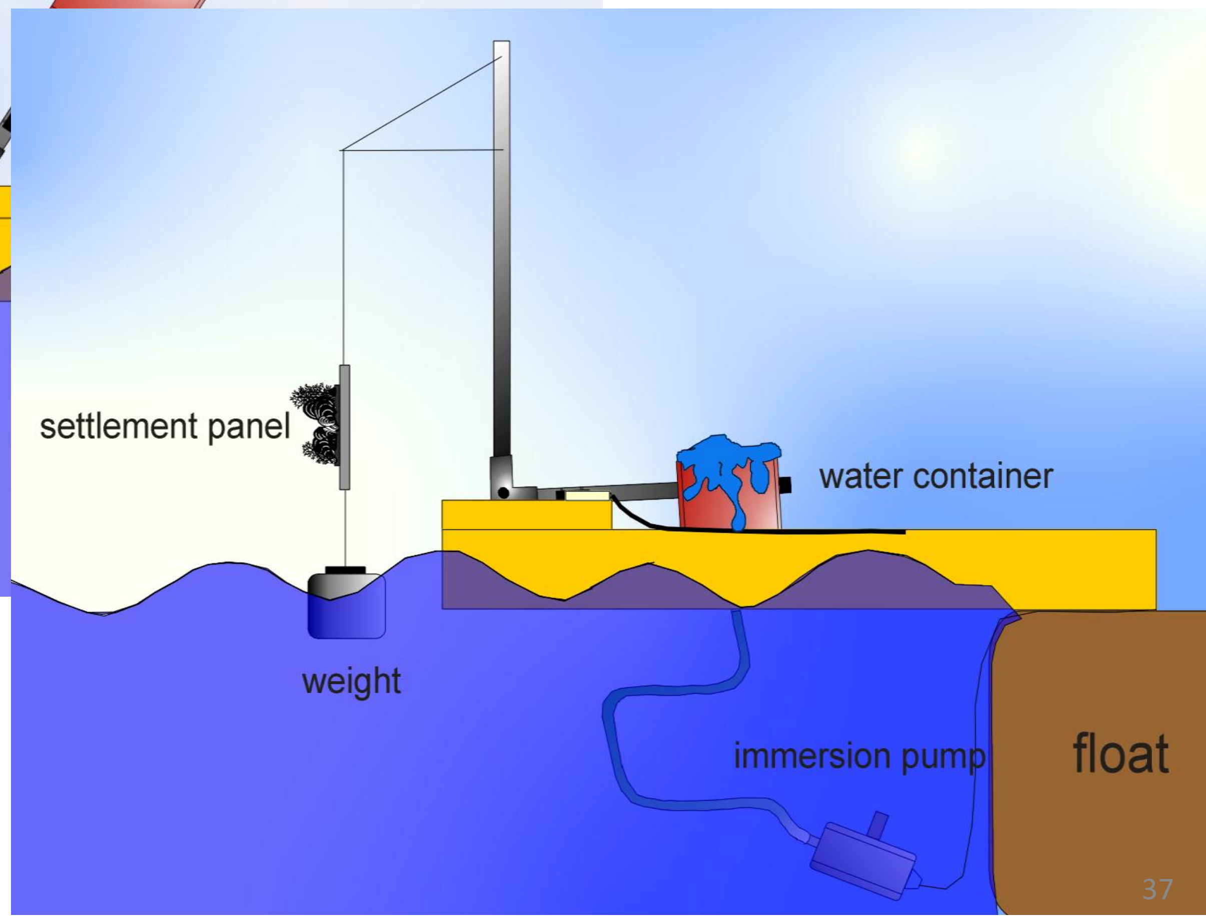
Intermediate Disturbance Hypothesis (IDH) in sensu Grime (1973) und Connell (1978)



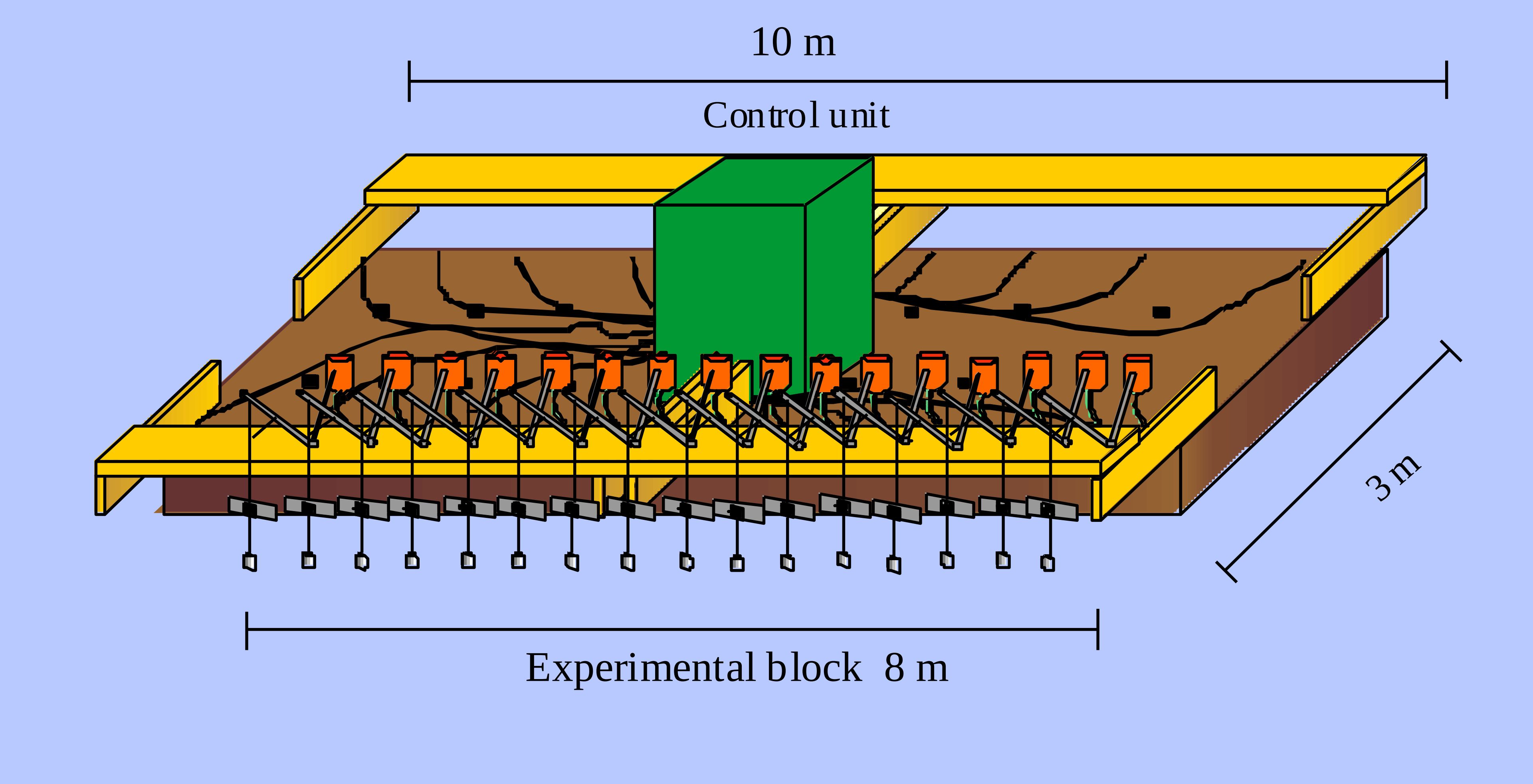

2. Woche
4. Woche
6. Woche
10. Woche
14. Woche
18. Woche

Hint #6: Avoid pseudoreplication





Hint #6: Avoid pseudoreplication



Hint #6: Avoid pseudoreplication

Hint #6: Avoid pseudoreplication

Sources of pseudoreplication:

1. Shared enclosure
2. Common environment
3. Relatedness
4. Pseudoreplicated stimuli
5. Repeated measures

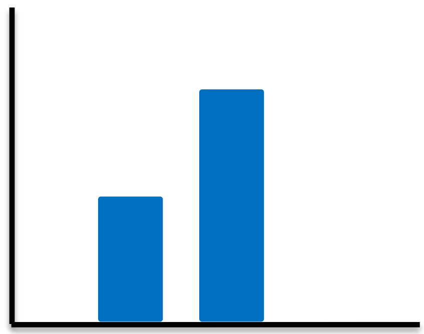
Discuss your experimental design and set-up with other researchers!

Hint #7: Identify your stats!

Photo: Florian von Bechtolsheim

Hint #7: Identify your stats

Comparing proportions (nominal data)



2 independent samples

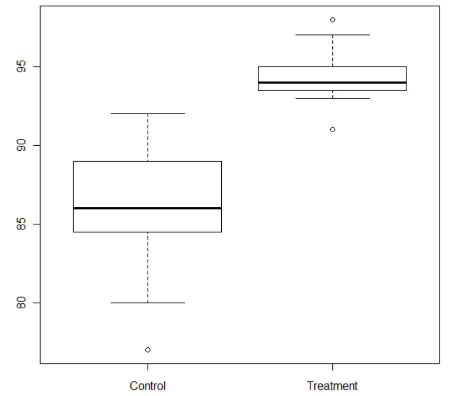
chi-square test

2 dependent samples

sign test

Hint #7: Identify your stats

Comparing medians (ordinal or interval data)



2 independent samples

Mann-Whitney-U test

> 2 dependent samples

Friedmann‘s ANOVA

2 dependent samples

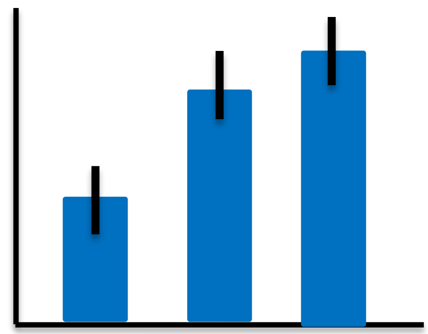
Wilcoxon signed-rank test

> 2 independent samples

Kruskal-Wallis ANOVA

Hint #7: Identify your stats

Comparing means (interval data)



2 independent samples

t-test

> 2 dependent samples

Repeated Measures ANOVA

2 dependent samples

paired t-test

> 2 independent samples

ANOVA

Find the right stats:

- What are my hypotheses?
- How many samples do I have?
- Are my samples independent or dependent?
- What is my data quality level?
- How are my data distributed (if interval)?
- How many independents do I have?
- Are they categorial or continuous?
- How many dependents do I have?
- Are they categorial or continuous?

Do it before you collect your data! Generate a fake data set and simulate the analysis!

Hint #8: Estimate your test power!

Hint #8: Estimate your test power!

Effect size

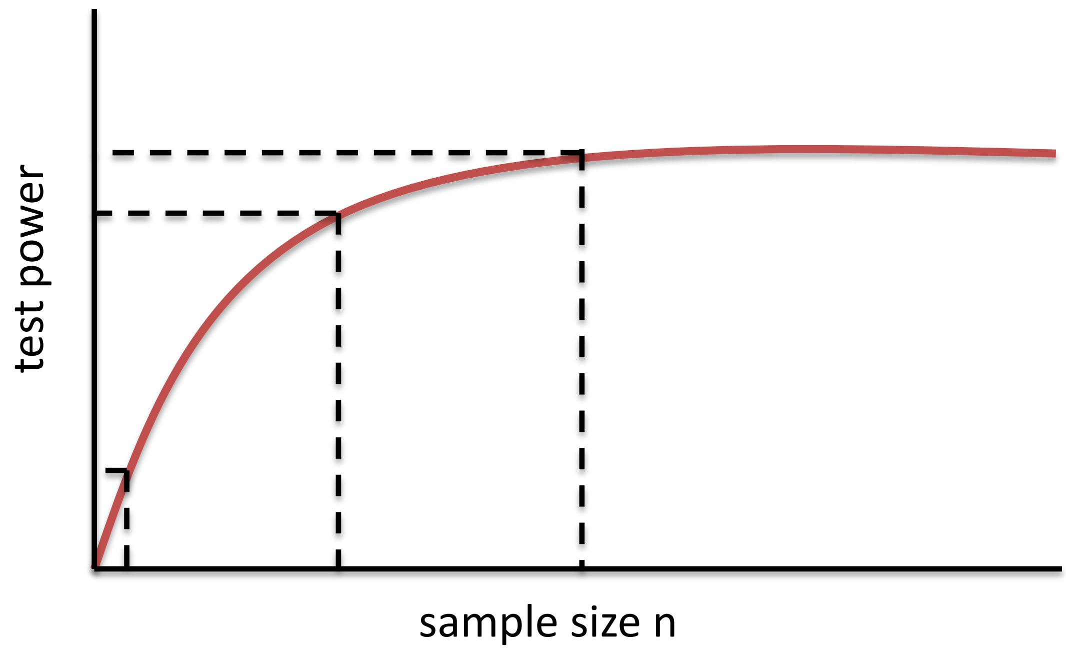
Amount of unexplained variation

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Sample size

Power analysis can estimate the test power that can be achieved with a given number of replicates !

Hint #8: Estimate your test power!



Power analysis can estimate the number of replicates that is needed to achieve a certain test power!

Hint #8: Estimate your test power!

A power analysis includes:

1. Observations per group (=n)

2. Delta (=difference between means)

3. Standard deviation

4. Significance level

5. Power level aimed for (should be at leat 0.8)

6. The kind of test you want to use

Hint #8: Estimate your test power!

Prospective Power Analysis: how can you know about effects sizes and variability?

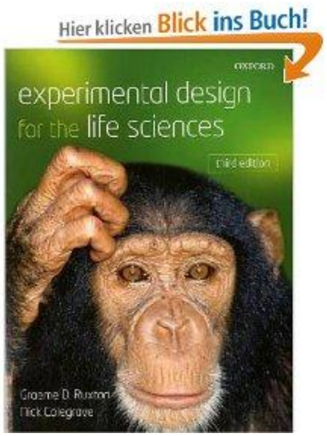
Literature

Pilot studies

Academic guess

Do it!

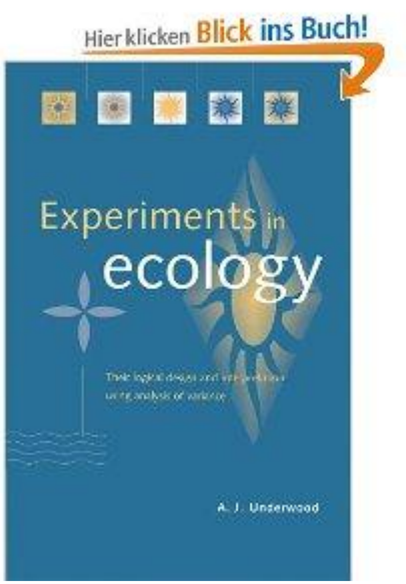
Finally…



Ruxton GD, Colegrave N (2010).

Experimental Design for the Life Sciences.

3rd Edition, Oxford University Press, New York



Underwood AJ (1996).

Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance.

Cambridge University Press