# Analysis and Visualisation of Complex Agro-Environmental Data

## Lesson 05

- Introduction to statistical inference
- Point and interval estimation
- Hypothesis testing
- Working examples and exercise







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- 1. Introduction to statistical inference
- 2. Point and interval estimation
- 3. Hypothesis testing
- 4. Working examples and exercise

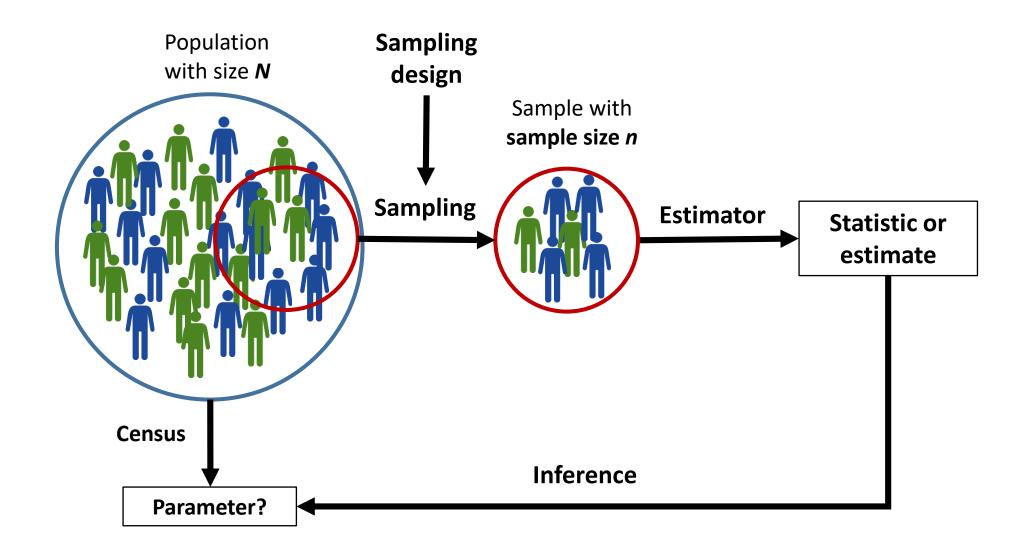
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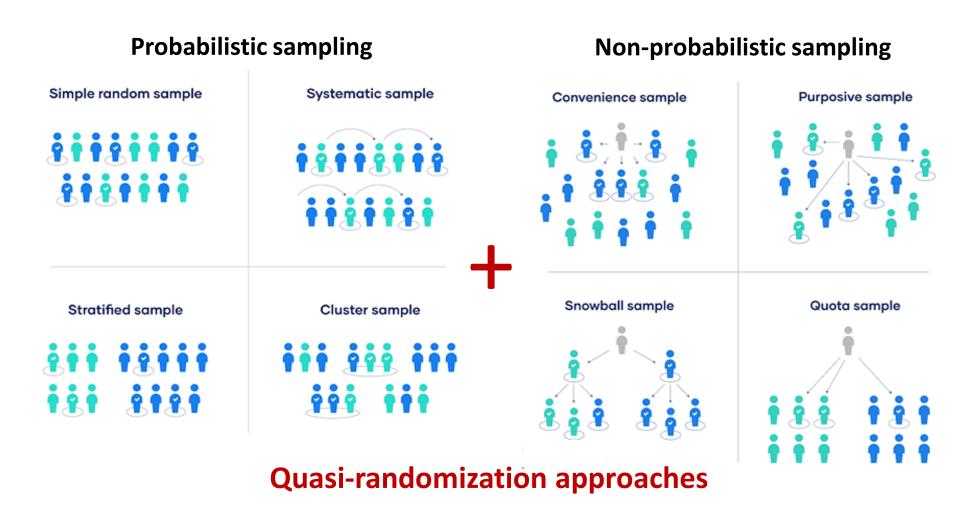
The process of drawing conclusion about unknown population properties, using a sample drawn from a population.

**Population** - a set of similar items or events which is of interest for some question or experiment.

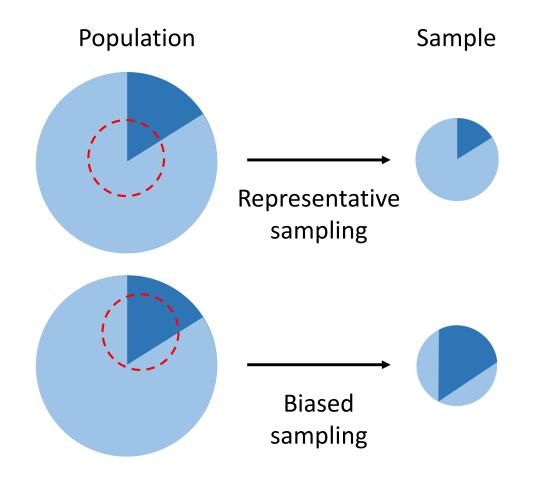
- Needs to be defined in beforehand according to the questions to be addressed. Usually involves defining the *targets*, *time frame* or *locations*.
- Examples: farms from the 'Alentejo Litoral' NUT3; fire events from 2000 to 2020; brown trout populations from the Tagus catchment).



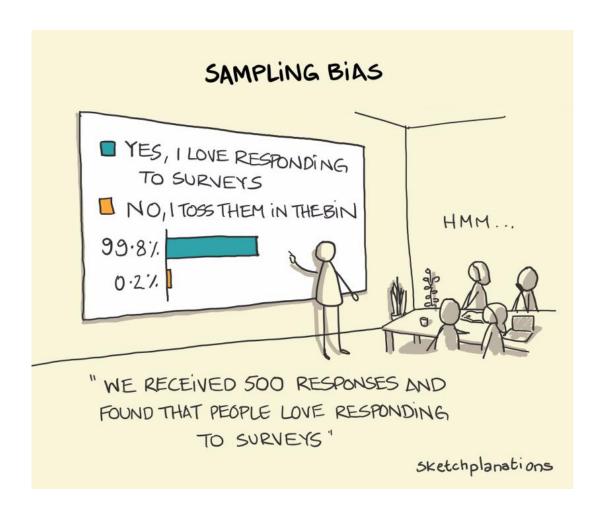
## Sampling design



**Sampling bias** – Measurements are systematically off-target or sample is not representative of population of interest



## **Sampling bias**



Parameters, estimators and estimates

#### **Parameter**

An *unknown* quantity of interest (e.g. mean farm size; proportion of burned area; population size of brown trout) – usually considered to be fixed (NOTE: Bayesian approaches are an exception: parameters are viewed as random variables).

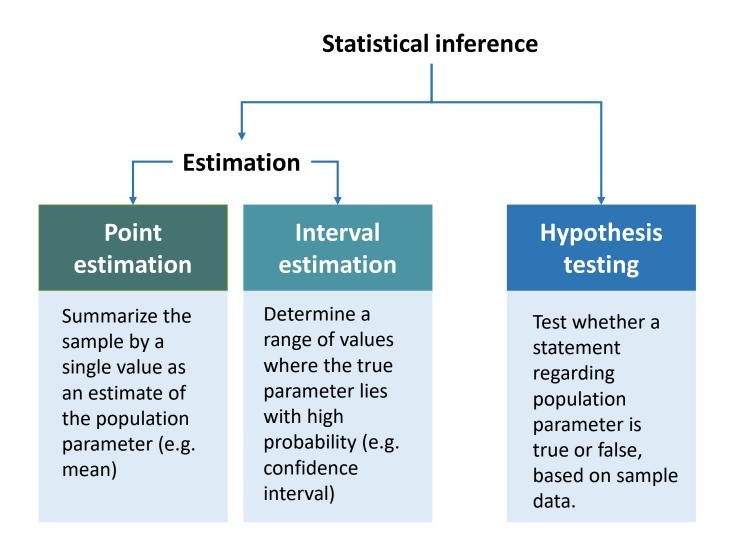
## **Estimate (statistic)**

The value returned from the estimator (e.g. sample mean value). Usually a **random variable** (i.e. follows a probability distribution – **sampling distribution**).

#### **Estimator**

A function based on sample values that estimates the parameter (e.g. sample mean, sample proportion, etc).

Parameter	Estimate	Estimator
Mean (μ)	$ar{X}$	$\frac{\sum_{i=1}^{n} x_i}{n}$
Median	Sample median	$x_{(n+1)/2}$ if $n$ odd $(x_{n/2} + x_{(n/2)+1})/2$ if n even
Variance ( $\sigma^2$ )	S <sup>2</sup>	$\sum_{i=1}^{n} \frac{(x_i - \bar{X})^2}{n - 1}$
Standard Deviation ( $\sigma$ )	S	$\sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{X})^2}{n-1}}$
Median absolute deviation (MAD)	Sample MAD	$median[ x_i - median ]$
Coefficient of Variation (CV)	Sample CV	$\frac{s}{\bar{X}} \times 100$
Standard Error of $ar{X}$ ( $\sigma_{ar{X}}$ )	$\mathcal{S}_{ar{X}}$	$\frac{s}{\sqrt{n}}$
95% confidence interval for $\mu$		$\bar{X} - 1.96\sigma_{\bar{X}} \le \mu \le \bar{X} + 1.96\sigma_{\bar{X}}$



## Lesson #5

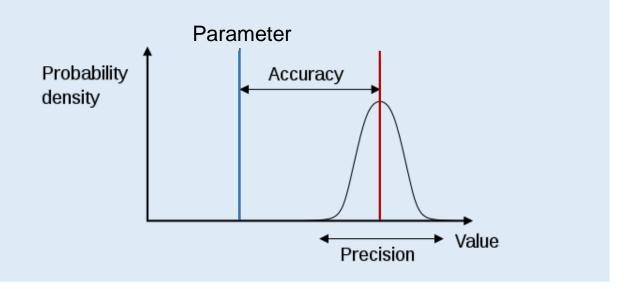
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#### **Point estimation**

Mean, median, mode, proportion, ...

Estimation accuracy versus precision:

- accuracy is related with systematic errors or bias;
- precision is related with the statistical error variability.

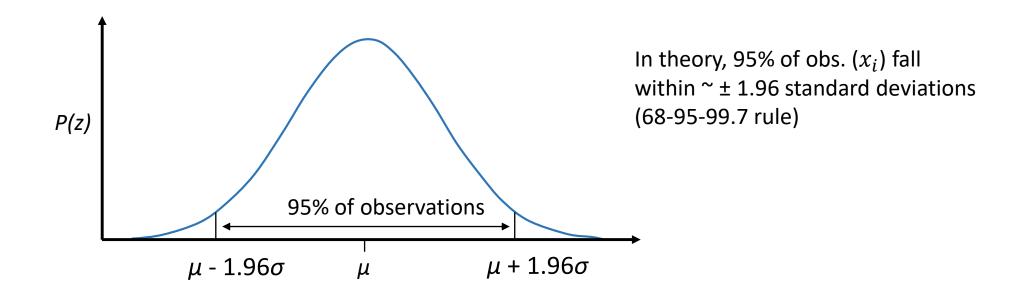


Multiple population bias simulation: <a href="https://markkurzejaumich.shinyapps.io/multiple\_population\_bias/">https://markkurzejaumich.shinyapps.io/multiple\_population\_bias/</a>

#### **Interval estimation**

confidence intervals for the mean

Population with **mean** =  $\mu$  and **standard deviation** =  $\sigma$ :



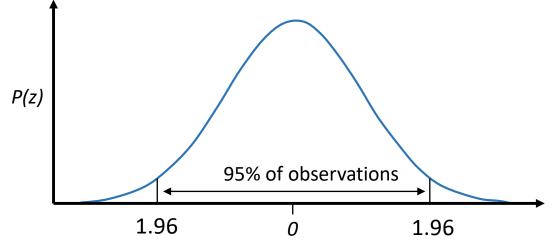
#### **Interval estimation**

#### **Standard z-score**

$$z = \frac{x_i - \mu}{\sigma}$$

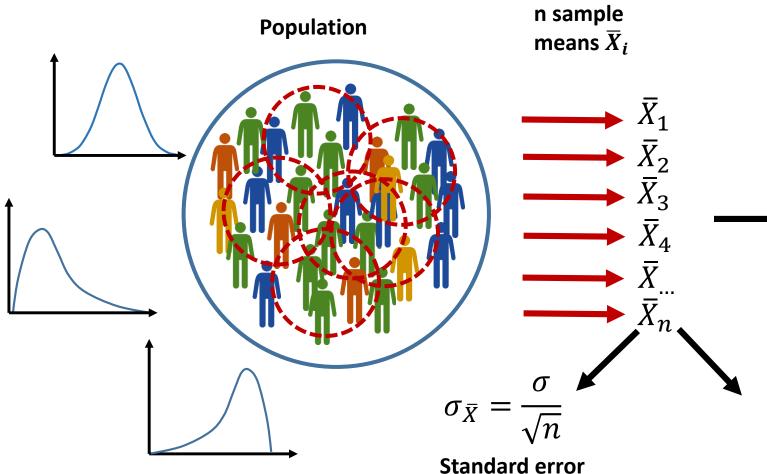
- measures how unusual is an observation
- converts any normal distribution to a Standard
   Normal Distribution or z-distribution N(0,1)

#### **Standard Normal Distribution** ( $\mu = 0$ and $\sigma = 1$ ):



Theoretical values (provided in tables or sofware) are derived from this distribution.

Confidence intervals for the mean – in **theory**:



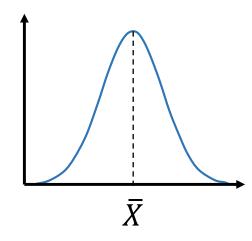
(St. dev. of the sample means;

 $\sigma$  = st. dev. of the population )

#### **Central Limit Theorem:**

- $\bar{X}$  approaches a normal distribution for increasing n
- $\overline{X}$  is a good estimator of population mean

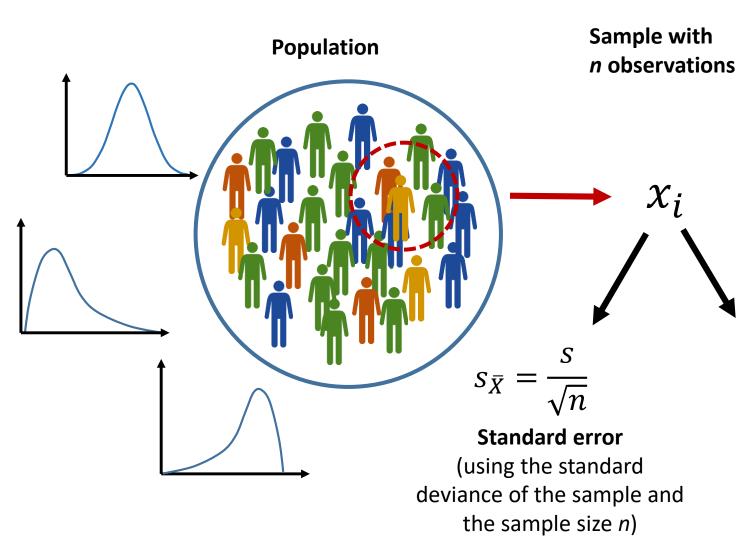
#### **Sampling distribution:**



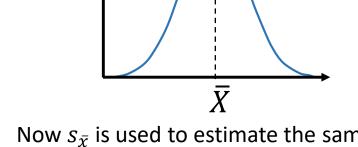
- Convert  $\bar{X}_i$  to N(0,1):  $z = \frac{X_i \mu}{\sigma_{\bar{x}}}$
- 95% of the sample means falls between:

$$\bar{X} - 1.96\sigma_{\bar{X}} \le \mu \le \bar{X} + 1.96\sigma_{\bar{X}}$$

Confidence intervals for the mean – in **practice**:



It is possible to estimate features of the **sampling distribution** based in one sample only:



- Now  $s_{\bar{x}}$  is used to estimate the sampling distribution:
  - $t = \frac{\bar{X} \mu}{s_{\bar{X}}}$  , that follows a **t-distribution**
- 95% of the sample means falls between:

$$\bar{X} - t_{0.05(n-1)} s_{\bar{X}} \le \mu \le \bar{X} + t_{0.05(n-1)} s_{\bar{X}}$$

Interval estimation - confidence intervals for the mean

**95% confidence interval** of a mean from a normally distributed sample:

$$\bar{X} - t_{0.05(n-1)} s_{\bar{X}} \le \mu \le \bar{X} + t_{0.05(n-1)} s_{\bar{X}}$$

- n-1 is also termed degree of freedom (df) for each df there is a different t-distribution
- $t_{0.05(n-1)}$  the value from the *t*-distribution with *n-1 df* (for a confidence of 0.95 that a sample interval computed that way, will contain the population mean).

#### **Degrees of freedom**

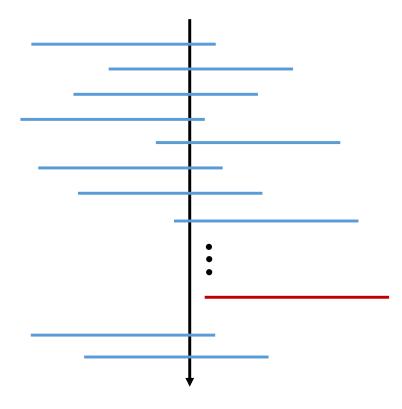
The number of observations in our sample that are "free to vary" when we are estimating the variance <=> knowing the mean and n-1 observations, the last observation is fixed (i.e., it is possible to determine). Rule:  $df = number \ of \ observations - number \ of \ parameters$  included in the formula for the variance.

For **large sample sizes:**  $t \rightarrow z$  and the confidence interval estimated with z is a good approximation:  $\bar{X} - 1.96\sigma_{\bar{X}} \le \mu \le \bar{X} + 1.96\sigma_{\bar{X}}$ 

Interval estimation - confidence intervals for the mean

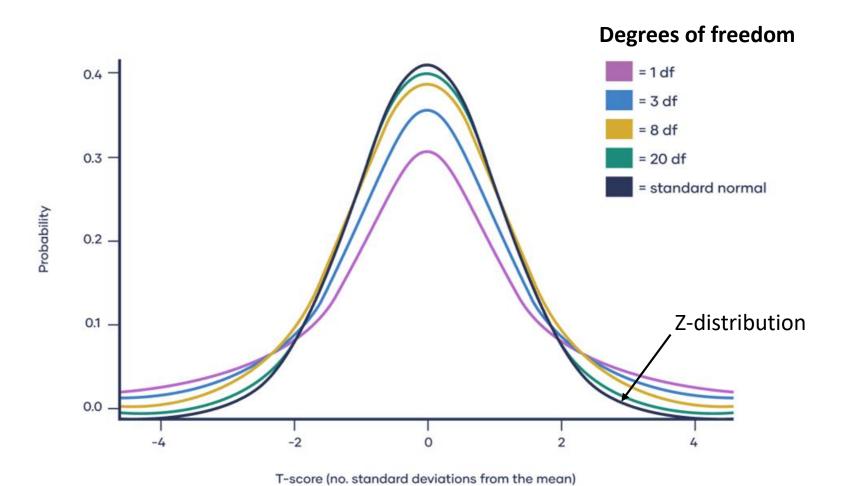
# More correct interpretation of confidence interval:

95% of the intervals of repeated samples will cover the true mean value (**not** the probability of the true mean value to be within the interval).



True parameter value

## *t*-distribution for different df



#### Interval estimation - confidence intervals for the variance

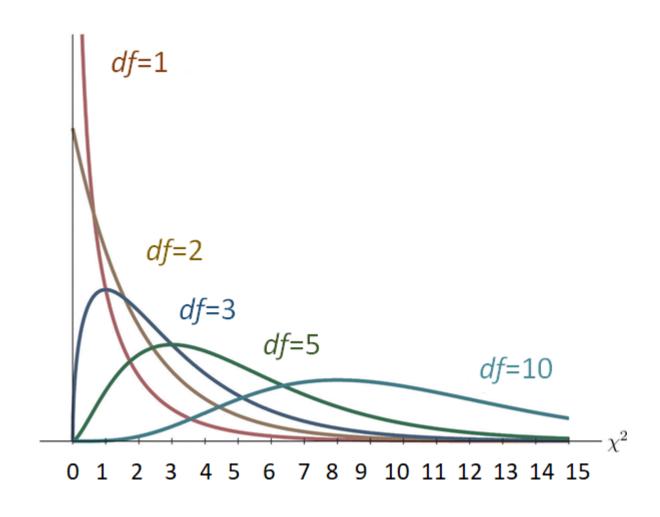
- The Central Limit Theorem does not apply to sample variance
- The probability distribution of the sample variance follows a  $\chi^2$  distribution
- Confident intervals for variances are based on the  $\chi^2$  distribution:

$$\chi^2 = \frac{(x-\mu)^2}{\sigma^2}$$
, corresponding to the square of the standard z score

- $\chi^2$  is always positive, ranging from 0 to  $\infty$ .
- Right skewed, approaching normality as df increases
- Variance confidence interval is given by

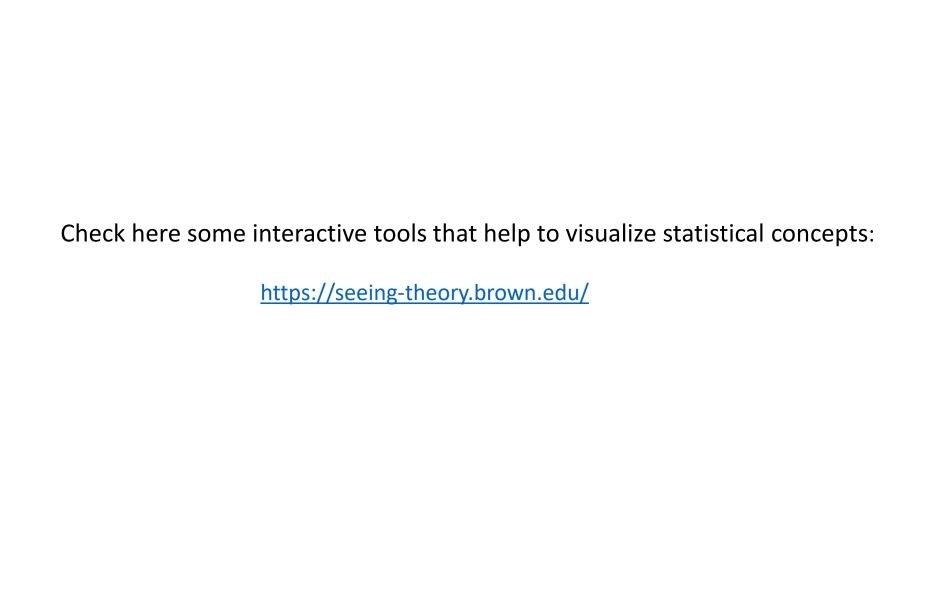
$$\frac{s^2(n-1)}{\chi^2_{0,025(n-1)}} \le \sigma^2 \le \frac{s^2(n-1)}{\chi^2_{0,975(n-1)}}$$
  $\chi^2_{0.025}$  value below which 2.5% of all  $\chi^2$  fall  $\chi^2_{0.975}$  value above which 2.5% of all  $\chi^2$  fall

 $\chi^2$  distribution for different df



Main methods of parameter estimation:

- Maximum Likelihood (MLE) the estimator that maximizes a likelihood function
- Ordinary Least Squares (OLS) the estimator that minimizes the sum of the squared differences between each value and the parameter,
- Resampling methods estimating standard errors and confidence intervals by subsampling the original sampling:
  - **Bootstrap** p samples of size n with replacement (good to estimate bias)
  - Jacknife sampling by sequentially removing each observation
- **Bayesian inference** estimation an alternative to the above classical or frequentist statistical inference that incorporates prior knowledge about the population, as degrees-of-belief.



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## Main steps:

Steps	Actions	Decisions	
1	Define a <b>null hypothesis</b> H <sub>0</sub>	Usually an hypothesis of <b>no effect</b> (no differences).	
2	Select a <b>test statistic</b> that measures deviations from $H_0$ with a known sampling distribution: $statistic = \frac{estimate-null\ value}{standard\ error}$	<ul> <li>t test (z-test when n is big)</li> <li>F test (more than 2 means)</li> <li>χ² test for 2 categorical variables</li> <li>Other non-parametric tests</li> </ul>	
3	Specify an a priori maximum <b>error</b> significance level $P(\text{reject } H_0 \mid H_0 \text{ is True})$	<ul><li>0.01 level</li><li>0.05 level</li></ul>	
Collect the sample(s) and compute statistic and <i>p</i> -value		Critical value from $z$ , $t$ , $F$ , $\chi^2$ tables (or software)	
5	Arrive at <b>decision</b>	<ul> <li>if p &lt; 0.05, then reject H<sub>0</sub>;</li> <li>if p &gt; 0.05, then conclude there is no evidence that H<sub>0</sub> is false and retain it.</li> </ul>	

**P-value** = P (data |  $H_0$ ) - the probability of observing our sample data, or data more extreme, under repeated identical experiments if the  $H_0$  is true

Types I and II errors; true/false positives and negatives; power

Observed

#### Type I error

 $\alpha = P \text{ (reject } H_0 \mid H_0 \text{ is True)}$ 

#### Type II error

 $\beta$  = P (fail to reject  $H_0 \mid H_0$  is not True)

#### Power

 $1 - \beta = P$  (reject  $H_0 \mid H_0$  is not True)

#### **Power analysis**

Process to assess whether a given study design is likely to yield meaningful findings

#### Real

		<i>H<sub>o</sub></i> is TRUE	<b>H</b> <sub>o</sub> is FALSE	
	Reject H <sub>0</sub>	Type I error (false positive rate)	Correct outcome - <b>Power</b> (true positive rate)	
	Fail to reject $H_0$	Correct outcome (true negative rate)	Type II error (false negative rate)	

Which kind of error, type I or type II, is more importante in applied sciences?

Relevance of Type I and Type II errors

- Type I error is more conservative since it detects the effect (or pattern)
  of something that is not occurring.
- Type II errors imply the failure to detect an effect (or pattern) that in fact occurs => more relevant for applied sciences such as environmental and human health sciences.

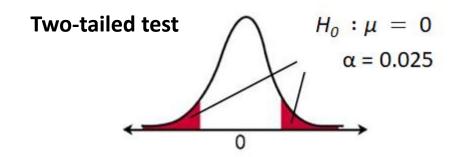
## Parametric hypothesis tests for single population

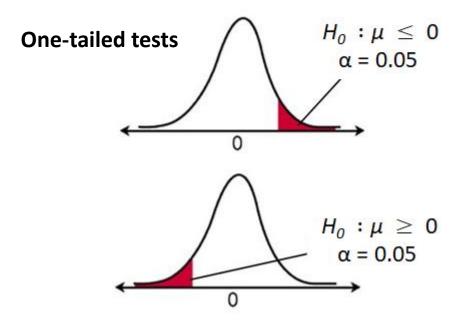
Example: does the population mean equal zero?

#### One-sample *t* test:

- 1.  $H_0: \mu = 0$  (two-tailed test),  $H_0: \mu \leq 0$  or  $H_0: \mu \geq 0$  (one tailed test)
- 2. Take a probability sample from population
- 3. Compute t statistic:  $t = \frac{\bar{x} \mu}{s_{\bar{x}}}$
- 4. Compare t value with the sampling distribution of t at e.g.  $\alpha = 0.05$  with n-1 df

The equivalent of checking whether the 95% confidence interval for  $\mu$  overlaps zero! (Compare this with the confidence interval estimation explained above).



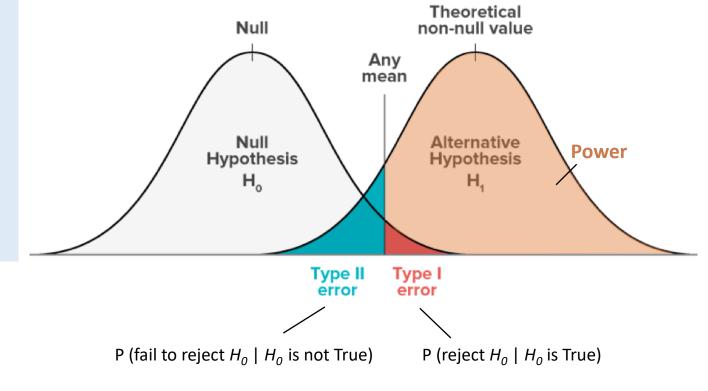


## Parametric hypothesis tests for 2 populations

Example: Is the population mean the same between two populations?

#### Two-sample *t* test:

- 1.  $H_0: \mu_1 = \mu_2$  (two-tailed test),  $H_0: \mu_1 \leq \mu_2$  or  $H_0: \mu_1 \geq \mu_2$  (one tailed test)
- 2. Take a probability sample from population
- 3. Compute t statistic:  $t = \frac{(\bar{x}_1 \bar{x}_2) (\bar{\mu}_1 \bar{\mu}_2)}{s_{\bar{x}_1} \bar{x}_2}$
- 4. Compare t value with the sampling distribution of t at e.g.  $\alpha = 0.05$  with n-1 df



Parametric hypothesis testing for more than 2 groups

**Analysis of Variance (ANOVA)** - a family of analyses related with regression which may also be used to test hypothesis about group (treatment) means.

Two main aims of classical ANOVA:

- To examine the **relative contribution of different sources of variation** (factors or predictive variables) to the total amount of variability in the response variable;
- To test the **null hypothesis** that population group or treatment means are equal.

## Parametric hypothesis testing for more than 2 groups

- Variations according to the number of factors envolved: one-way ANOVA, two-way ANOVA and N-way multivariate ANOVA.
- Other variants (e.g.):
  - Nested ANOVA for nested factors (e.g. sampling sites are nested within river catchments)
  - Repeated measures ANOVA when measurements are not independent (e.g. measuring the same individual throughout time).
- Post-hoc or multiple comparison tests most common: Tukey tests similar to t-test but corrects for multiple non-independent comparisons (includes a modified version for unequal sample sizes). Commonly used after ANOVA but can be used in their own.

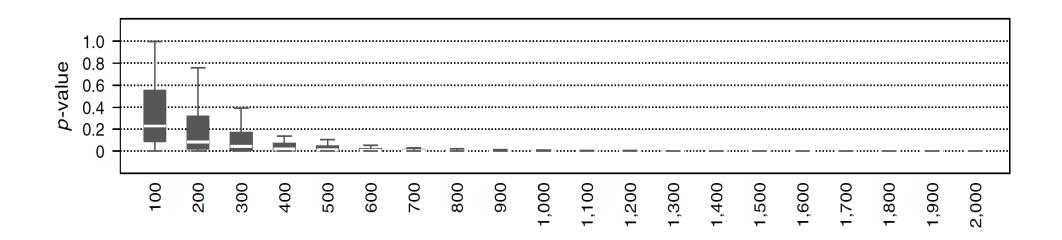
## Assumptions of parametric hypothesis testing

- **1. Normally distributed** populations but *t* test (and ANOVA) is robust to moderate violations results from normality tests are not recommended to discard these tests (better to assess through graphical methods). Data transformation might help.
- 2. Samples from populations with **equal variances** *t* test (and ANOVA) is also robust to moderate unequal variances if sample sizes are equal. The same data transformation also will help.
- 3. Observations are **sampled randomly** from clearly defined populations this will assure that observations are **independent and identically distributed** (*iid*).
- 4. There are **no outliers** strong effect on type I and II errors.

These assumptions are not met? => Non-parametric hypothesis testing

Hypothesis testing and big data

- Larger sample sizes are more likely to produce a statistically significant result.
- => even small and uninteresting effects can be statistically significant!



Check also here: https://www.bintel.io/blog/the-curse-of-big-data

Hypothesis testing and big data

Alternative approaches to classical hypothesis testing are needed:

- Shift the focus towards the **size of the estimated effect**, e.g. to assess if the estimated effect size has practical implications.
- Perform **sensitivity analysis** how does the estimated effect change when control variables are added and dropped.
- Use **Bayesian statistics**, which do not rely on arbitrary *p-values*

# **Hypothesis testing in Python**

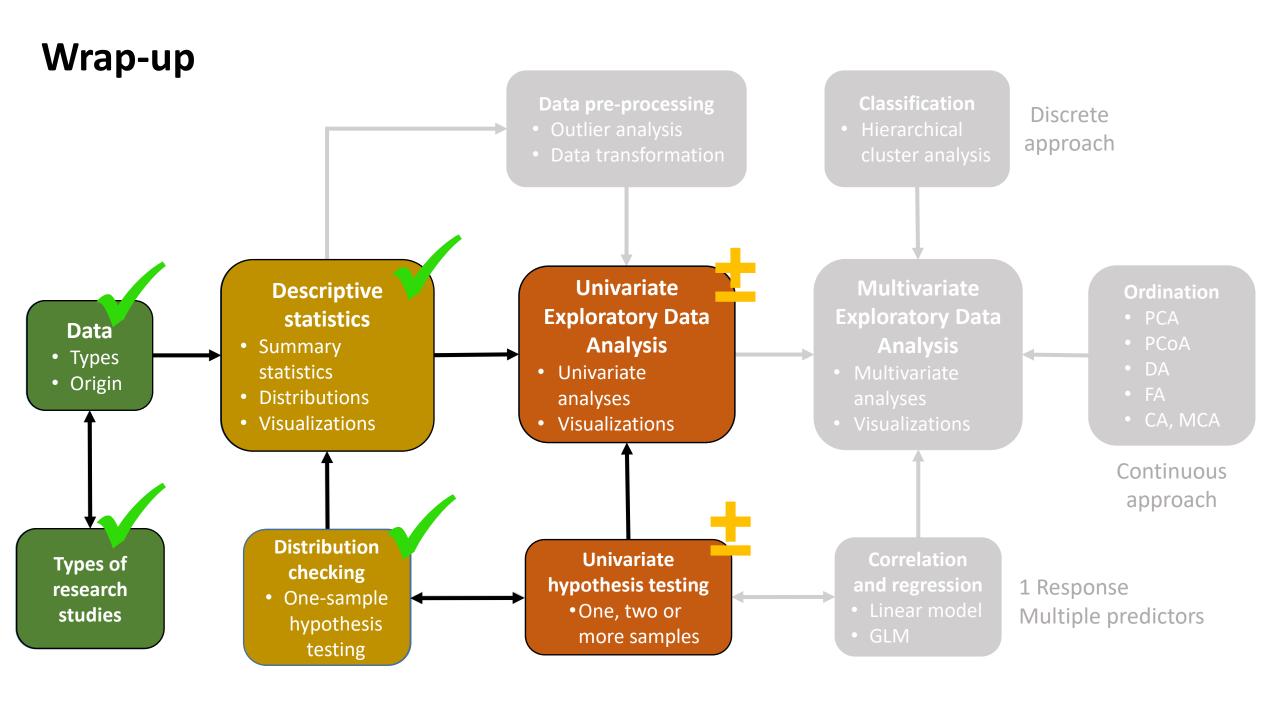
Null Hypothesis	Distributions	SciPy Functions for Test
The population mean has a given value.	Normal distribution (stats.norm), or Student's t distribution (stats.t)	stats.ttest_1samp
The means of two random variables are equal (independent or paired samples).	Student's t distribution (stats.t)	stats.ttest_ind, stats.ttest_rel
Two or more variables have equal variance in samples	F distribution (stats.f)	stats.barlett, stats.levene
Two or more groups have the same population mean (ANOVA).	F distribution	stats.f_oneway, stats.kruskal
The distribution of two random variables are equal.	Kolmogorov-Smirnov distribution	stats.kstest
	χ2 distribution (stats.chi2)	stats.chisquare
	χ2 distribution (stats.chi2)	stats.chi2_contingency
Two variables are not correlated.	Beta distribution (stats.beta, stasts.mstats.betai)	stats.pearsonr, stats.spearmanr

**Next class** 

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# References

Quinn, G., & Keough, M. (2002). Experimental Design and Data Analysis for Biologists. Cambridge: Cambridge University Press. doi:10.1017/CBO9780511806384

Johansson, R. (2019). Numerical Python. Scientific Computing and Data Science Applications with Numpy, SciPy and Matplotlib. 2<sup>nd</sup> ed. Apress. doi.org/10.1007/978-1-4842-4246-9

Navlani, A., Fandango, F., & Idris, I. (2021) Python Data Analysis: Perform data collection, data processing, wrangling, visualization, and model building using Python, 3rd ed. Packt Publishing.

## **Exercise 5**

In this exercise you will use again the dataset in EFIplus\_medit.zip to perform some hypothesis testing

- 1. Standardize, using z-score, the "Mean Annual Temperature" (Temp\_ann), calculate the new mean, SD and 95% confidence interval, and plot the histogram.
- 2. Test whether the means (or medians) of "Mean Annual Temperature" between presence and absence sites of *Salmo trutta fario* (Brown Trout) are equal using an appropriate test. Use both standardized and non-standardized values and compare results. Please state which is/are the null hypothesis of your test(s).
- 3. Test whether there are diferences in the mean elevation in the upstream catchment (Elevation\_mean\_catch) among the eight most sampled catchments. For which pairs of catchments are these diferences significant? Please state which is/are the null hypothesis of your test(s).
- 4. Which potential problems did you identified in the data that could limit the conclusions derived from the performed tests?