Fundamentals of a statistical test
Experimental Design

Statistical Tests

Procedure of a statistical test

• Conceive a hypothesis (H_A)
• Define the null hypothesis (H_0)
• Choose the level of rejection of the null hypothesis (\alpha)
• Choose an adequate statistical test
• Obtain a statistic (actually, a number!)
• Compute the “probability” (P) of the statistic

\[ P \geq \alpha : H_0 \text{ retained (} H_A \text{ rejected)} \]
\[ P < \alpha : H_A \text{ retained (} H_0 \text{ rejected)} \]
Hypothesis testing in action

*Actinia equina* and *Actinia fragacea*
After some observations you come up with an hypothesis

\((H_A)\) The number of tentacles of \textit{Actinia fragacea} differs from that of \textit{Actinia equina}
Make a trip to Berlengas (Islands)

Catch some anemones and count their tentacles...
Two samples obtained at Berlengas

<table>
<thead>
<tr>
<th>equina</th>
<th>fragacea</th>
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<tbody>
<tr>
<td>126</td>
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</table>

\[ \bar{X} \]

132 148

\[ n \]

6 6

WAIT!

By using these two samples, are we actually testing the hypothesis that the number of tentacles in *A. fragacea* is different from that of *A. equina*?
Experimental Design

Statistical Tests

Two samples obtained at Berlengas

Distribution of Actinia equina + fragacea
Rephrase the hypothesis

(Hₐ) The number of tentacles of *Actinia fragacea* differs from that of *Actinia equina* in Portugal

or

(Hₐ) The number of tentacles of *Actinia fragacea* differs from that of *Actinia equina* at Berlengas
Formalize the hypothesis

\( (H_A) \) The average number of tentacles of \( A. \ fragacea \ (\mu_1) \) differs from that of \( A. \ equina \ (\mu_2) \) at Berlengas

\[ H_A : \mu_1 \neq \mu_2 \quad \quad H_0 : \mu_1 = \mu_2 \]

Set the \textit{rejection level} at \( \alpha = 0.05 \) (more on that later)
Procedure of a statistical test

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\[
P \geq \alpha: H_0 \text{ retained (} H_A \text{ rejected)}
\]
\[
P < \alpha: H_A \text{ retained (} H_0 \text{ rejected)}
\]
Let’s invent a statistical test...

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$\bar{X}$

\[
\begin{array}{c|c|c}
\text{equina} & \text{fragacea} \\
126 & 150 \\
138 & 156 \\
132 & 150 \\
144 & 126 \\
120 & 156 \\
132 & 150 \\
\hline
\bar{X} & 132 & 148 \\
n & 6 & 6
\end{array}
\]

$H_A: \mu_1 \neq \mu_2 \quad H_0: \mu_1 = \mu_2$

$Blah$ statistic

$\bar{x}_1 - \bar{x}_2$

$Blah = 132 - 148$

$Blah = -16$

What do we know about $Blah$?

NOTHING!
Student’s $t$-test

$$t = \frac{X_1 - X_2}{\sqrt{\frac{1}{2} (S_1^2 + S_2^2) \cdot \frac{2}{n}}}$$

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$
Procedure of a statistical test

- Conceive a hypothesis ($H_A$)
- Define the null hypothesis ($H_0$)
- Choose the level of rejection of the null hypothesis ($\alpha$)
- Choose an adequate statistical test
- Obtain a statistic (actually, a number!)
- Compute the “probability” ($P$) of the statistic

$P \geq \alpha$: $H_0$ retained ($H_A$ rejected)
$P < \alpha$: $H_A$ retained ($H_0$ rejected)
We need to compute sample variances for the \( t \)-test

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}
\]

Variance of a sample

\[
s^2 = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n-1}
\]

\[
s^2 = \frac{\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2}{n-1}
\]
Experimental Design

Statistical Tests

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\[ \bar{X} = \frac{\sum X}{n} \]

\[ s^2 = \frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n-1} \]

\[ s^2_1 = \frac{104904 - \frac{792^2}{6}}{6-1} = \frac{360}{5} = 72 \]

\[ s^2_2 = \frac{132048 - \frac{888^2}{6}}{6-1} = \frac{642}{5} = 124.8 \]
Experimental Design

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<td>$\bar{X}$</td>
<td>132</td>
<td>148</td>
</tr>
<tr>
<td>$s^2$</td>
<td>72</td>
<td>124.8</td>
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<tr>
<td>$n$</td>
<td>6</td>
<td>6</td>
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$$s^2 = \frac{\sum_{i=1}^{n} X_i^2 - \frac{(\sum_{i=1}^{n} X_i)^2}{n}}{n-1}$$

$$s^2_1 = \frac{104904 - \frac{792^2}{6}}{6-1} = \frac{360}{5} = 72$$

$$s^2_2 = \frac{132048 - \frac{888^2}{6}}{6-1} = \frac{642}{5} = 124.8$$
### Experimental Design

#### Statistical Tests

$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{1}{2}(S_1^2 + S_2^2)} \cdot \sqrt{\frac{2}{n}}}$

$t = \frac{132 - 148}{\sqrt{\frac{1}{2}(72 + 124.8)} \cdot \sqrt{\frac{2}{6}}}$

$t = \frac{-16}{9.91967 \cdot 0.57735} = -2.79372$

$v = 2n - 2 = 10$ degrees of freedom

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$\bar{X}$ | 132 | 148 |
$s^2$ | 72 | 124.8 |

$n$ | 6 | 6 |
Experimental Design

Statistical Tests

\[ t = -2.79372 \]

(ignore the signal)

\[ \nu = 10 \]
Procedure of a statistical test

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$$P \geq \alpha: H_0 \text{ retained (} H_A \text{ rejected)}$$

$$P < \alpha: H_A \text{ retained (} H_0 \text{ rejected)}$$
Experimental Design

Statistical Tests

\[ \alpha = 0.05 \]
\[ n = 6 \]
\[ v = 10 \]

\[ t_{\text{crit}} = 2.228 \]
\[ t = 2.79372 \]
\[ p < 0.05 \]

Reject H\(_0\): samples came from different populations!
Procedure of a statistical test

- Conceive a hypothesis (Hₐ)
- Define the null hypothesis (H₀)
- Choose the level of rejection of the null hypothesis (α)
- Choose an adequate statistical test
- Obtain a statistic (actually, a number!)
- Compute the “probability” (P) of the statistic

\[ P \geq \alpha: H₀ \text{ retained (Hₐ rejected)} \]
\[ P < \alpha: Hₐ \text{ retained (H₀ rejected)} \]
The logic behind Student’s t-test

There is a model...
Assumptions

- Assume that the two samples come from the same population (that is, $H_0$ is true)
- Assume that observations are independent (that is, they do not influence each other)
- Assume that variances of samples are homogeneous
- Assume that the variable of interest (number of tentacles) has a normal distribution
Assumptions

- Assume that the two samples come from the same population (that is, $H_0$ is true)
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The normal distribution

**Probability density function (PDF)** of the normal distribution

\[
f(X) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(X-\mu)^2}{2\sigma^2}}
\]

\(\mu = 20\)

\(\sigma^2 = 20\)
The normal distribution (in excel)

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<thead>
<tr>
<th></th>
<th>A</th>
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</table>

Drag cell A4 down to cell A204
Drag cell B4 down to cell B204

Make a bar-chart graph from range B4:B204
The normal distribution (in excel, but the easy way: use function NORM.DIST)

<table>
<thead>
<tr>
<th></th>
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Make a bar-chart graph from range B4:B204
Assumptions

- Assume that the two samples come from the same population (that is, $H_0$ is true)
- Assume that observations are independent (that is they do not influence each other)
- Assume that variances of samples are homogeneous
- Assume that the variable of interest (number of tentacles) has a normal distribution
Sample $N$ times the normal distribution taking at each step two samples with $n$ replicates and performing a $t$-test

...
A big dilemma: how do you sample from the normal distribution?

Think this way: how do you generate a sample of size $n=6$ from a normally distributed population with an average of $\mu=20$ and a variance of $\sigma^2=6$?
Experimental Design

Recall that the normal distribution depends on two parameters: an average ($\mu$) and a variance ($\sigma^2$)

$$f(X) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(X-\mu)^2}{2\sigma^2}}$$

But the variance depends on the average!

$$\sigma^2 = \frac{\sum_{i=1}^{n} (X_i - \mu)^2}{n}$$
Sampling the normal distribution

**Cumulative distribution function** [CDF] of the normal distribution

\[ f(X) = \frac{1}{2} \left[ 1 + \text{erf} \left( \frac{X - \mu}{\sqrt{2} \sigma^2} \right) \right] \]

From the graph

\[ f(2.0) \text{ with } \mu=0 \text{ and } \sigma^2=5.0 \approx 0.8 \]

What does this probability mean?
Experimental Design

Statistical Tests

Probability of obtaining values less than or equal to 2

80% of the area under curve

\[
\int_{-\infty}^{2} PDF_{\text{normal}} \quad \int_{-\infty}^{+\infty} PDF_{\text{normal}}
\]
We need the inverse of the CDF of the normal distribution!

That is, we need a function that takes a probability and returns a value $X$ from a normal distribution with mean=$\mu$ and variance=$\sigma^2$
This function exists and is called

Inverse CDF of the normal distribution
## Sampling the normal distribution in excel

<table>
<thead>
<tr>
<th></th>
<th>A</th>
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</tbody>
</table>

Drag cells A4:B4 down to Row 9

Compute AVERAGE(B4:B9)
Compute STDEV(B4:B9)
Get back to the assumptions

- Assume that the two samples come from the same population (that is, $H_0$ is true)
- Assume that observations are independent (that is they do not influence each other)
- Assume that variances of samples are homogeneous
- Assume that the variable of interest (number of tentacles) has a normal distribution
If we draw two samples \((n=6\) each\) many times, preferably indefinitely, from a normal distribution with parameters \(\mu=10\) and \(\sigma^2=5\), and preform a \(t\)-test each time, we will end up with a good approximation to the distribution of \(t\).

Do that in the simulator!
Generate 1000 $t$-tests using samples of $n=6$ and a population mean of $\mu=10$ and variance of $\sigma^2=5$

What is the expected most common value of $t$?

Sort the $t$-values (ascending) and find out the ones corresponding to row 25 and row 975 (these are the 2.5% upper and lower tails of the distribution = 0.05)

Build an histogram of frequencies (using a scale ranging from -3.5 to 3.5 with intervals of 0.1)

Repeat for 10000 samples!
Simulated distribution of $t$
OK, but we used $\mu=10$ and $\sigma^2=5$! What if we have used a different mean or variance or both?

Should we know, a priori, the mean and variance of an hypothetical population?

Repeat with $\mu=5$ and $\sigma^2=10$
Wait! This distribution was already derived!

know as the **probability density function** (PDF) of *t*
Derived by WS Gosset (1908)

\[ f(x) = \frac{\Gamma\left(\frac{(\nu+1)/2}{2}\right)}{\sqrt{\pi \nu} \Gamma\left(\frac{\nu}{2}\right)} \left(1 + \frac{x^2}{\nu}\right)^{-\frac{(\nu+1)/2}{2}} \]

\[ \Gamma(z) \equiv \int_0^\infty t^{z-1} e^{-t} \, dt \quad -\infty < x < \infty \]

Popularized by RA Fisher as Student’s \( t \)-distribution
Experimental Design

Statistical Tests

\[ f(x) = \frac{\Gamma\left(\frac{\nu+1}{2}\right)}{\sqrt{\pi} \nu \Gamma\left(\frac{\nu}{2}\right)} \left(1 + \frac{x^2}{\nu}\right)^{-\frac{\nu+1}{2}} \]

\[ \Gamma(z) = \int_0^\infty t^{z-1} e^{-t} \, dt \quad -\infty < x < \infty \]

It has one parameter

\[ \nu = \text{degrees of freedom} \]

It does not depend neither on the mean nor on the variance of a population!
Recall that for the anemone tentacles’ data

\[ t = 2.79372 \]

\[ \nu = 10 \]
OK, so we know the PDF of $t$ for 10 degrees of freedom and we have got a $t$ statistic of 2.79372
Decision theory
• We’ve got a test (Student’s $t$-test)
• We’ve got the data
• We’ve got a statistic ($t=2.79372$, with $\nu=10$)
• We know the distribution of $t$ for $\nu=10$, when the null hypothesis is true
Get back to the assumptions

- Assume that the two samples come from the same population (that is, \( H_0 \) is true)
- Assume that observations are independent (that is they do not influence each other)
- Assume that variances of samples are homogeneous
- Assume that the variable of interest (number of tentacles) has a normal distribution
When should we reject the null hypothesis?

\[ H_0 : \mu_1 = \mu_2 \]

**Type I Error**

\[ \alpha = 0.05 \]

Probability of rejecting \( H_0 \) when it is true

Probability of detecting false differences between two samples that came from the same population
Experimental Design

When should we reject the null hypothesis?

- If $t_{\text{observed}} > t_{\text{critical}}$ → Reject $H_0$
- If $t_{\text{observed}} < t_{\text{critical}}$ → Accept $H_0$
- If $t_{\text{observed}}$ falls inside rejection area → Reject $H_0$
- If $t_{\text{observed}}$ falls outside rejection area → Accept $H_0$
Experimental Design

Statistical Tests

$t_{\text{observed}}$ falls in rejection area

$t_{\text{observed}} > t_{\text{critical}}$

$(2.79372 > 2.228)$

$t_{\text{obs}} = 2.79372$

Reject $H_0$

There are differences between the number of tentacles of *A. equina* and *A. fragacea* at Berlengas Islands
Unilateral test (for a more specific hypothesis)

\[ H_A : \mu_1 > \mu_2 \quad H_0 : \mu_1 \leq \mu_2 \]

Rejecting \( H_0 \) if

\[ t_{\text{obs}} > t_{\text{crit}} \]

\( t_{\text{obs}} \) falls in rejection area

Critical value of \( t \) for \( \alpha = 0.05 \)

Probability distribution function of \( t \) for \( \nu = 10 \)
A few points to notice

- Rejection/Acceptance of the null hypothesis is an all-or-nothing process and is determined by the rejection level of the null hypothesis ($\alpha$) which must be set a priori.

- We will never know if the two samples come actually from different populations. What we know is that there is a 5% chance (for $\alpha=0.05$) of being wrong when we reject $H_0$.

- Computer packages will usually give you an exact probability. If it is smaller than $\alpha$ reject $H_0$. 

A few points to notice

• In the present case, the exact probability of the statistic is 0.018 (that is, the probability of obtaining a $t > 2.79372$ or a $t < -2.79372$)

• What would happen if the rejection level of the null hypothesis had been set to $\alpha=0.01$ instead of $\alpha=0.05$?

• We would have retained $H_0$! (no differences)
Spot the error?

Two out of the 4 sites showed significant differences with regard to genotype frequency composition and habitat (SFI, Wald $\chi^2 = 5.8$, $p = 0.05$; SBI, Wald $\chi^2 = 8.8$, $p = 0.01$; Table 2). The mussel populations at 3 sites (SFI, AI, and SBI) frequently had fewer *Mytilus trossulus* genotypes in the sun-exposed microhabitat than in the shaded microhabitat, and an increase in *M. galloprovincialis* and/or hybrid genotypes. Additionally, 3 sites (SFI, CSI, SBI) showed highly significant differences between years ($p < 0.0001$; see Table 2).
We control Type I Error (the probability of rejecting $H_0$ when it is true)

What about the probability of accepting $H_0$ when it is false?

**Type II Error**

$\beta$

$= \text{the probability of rejecting } H_A \text{ when it is true}$
### Statistical Tests

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<th>$H_0$ Rejected</th>
<th>$H_0$ Accepted</th>
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<tbody>
<tr>
<td>$H_0$ True</td>
<td>Type I Error ($\alpha$)</td>
<td>✔</td>
</tr>
<tr>
<td>$H_0$ False</td>
<td>✔</td>
<td>Type II Error ($\beta$)</td>
</tr>
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Power of a statistical test

Power = 1 - β

• If β is the probability of accepting $H_0$ when it is false, it is also the probability of rejecting $H_A$ when it is true.

• Hence, 1-β is the probability of accepting $H_A$ when it is true. This is called the **Power** of a statistical test.
Can we estimate $\beta$?

It is not easy...

- $\beta$ is inversely related with $\alpha$ (the higher the value of $\alpha$, the lower the value of $\beta$)
- $\beta$ depends on the magnitude of the effect between samples when $H_0$ is false
- $\beta$ depends on the number of replicates $n$ (the higher the $n$, the lower the $\beta$)
**Power and the magnitude of the effect**

(the latter is also known as **effect size**)

• Assume that you are comparing two putatively different populations (which are actually different, but you don’t know yet)

• Therefore, $H_0$ is false

• In the case of sea anemones, assume that on average *A. fragacea* has more 30 tentacles than *A. equina* (unstandardized magnitude of the effect $= 30$)
Power and the magnitude of the effect

Effect size = +30
Power and the magnitude of the effect

Effect size = +30

Distribution of $t$ when the null hypothesis is true

Distribution of $t$ when the Magnitude of the effect is +30

Power = $1 - \beta = 1 - 0.001 = 0.999$
Power and the magnitude of the effect

Effect size = +20

Distribution of $t$ when the Magnitude of the effect is +20

$\beta = 0.15$

Power = $1 - \beta = 1 - 0.15 = 0.85$
Power and the magnitude of the effect

Effect size = +10

Distribution of $t$ when the null hypothesis is true

$\beta = 0.60$

Magnitude of the effect is +10

$\mu = 0$

$\mu = +10$

$\alpha = 0.05$

Power = $1 - \beta = 1 - 0.60 = 0.40$
How to increase the power?

Power = 1 - β = 1 - 0.60 = 0.40
How to increase the power?

1) Increase $\alpha$

Power $= 1 - \beta = 1 - 0.40 = 0.60$
Experimental Design

How to increase the power?

2) Use unilateral tests

\[ H_A : \mu_1 > \mu_2 \]

\[ \beta = 0.40 \]

\[ \alpha = 0.05 \]

Distribution of t when the null hypothesis is true

Power = 1 - \beta = 1 - 0.40 = 0.60
How to increase the power?

3) Increase \( n \)

Distribution of \( t \) when the null hypothesis is true

\[
\text{Power} = 1 - \beta = 1 - 0.20 = 0.80
\]
Power Analysis