

Statistical Tests for Ecology

Why bother?

Sometimes when we look at data it is easy to spot if there is a definite relationship or not. Sometimes however it is not so clear cut. Statistical tests help us to decide if the relationship does exist by removing both the uncertainty and subjectivity from the decision.

How do I start?

All statistical tests start with the formulation of a **null hypothesis (H_0)**. This hypothesis states that there is no significant relationship or pattern within our data. During an investigation we assume that the null hypothesis is true and we only change our mind, and reject the null hypothesis, if there is strong evidence to show otherwise. The statistical test helps you decide if the evidence is strong enough.

Any pattern within data can always be due to chance rather than an actual relationship. The **significance level** is used to measure how strong the evidence needs to be before we reject the null hypothesis. In biology we work with a 5% significance level ($P = 0.05$). What this means, is that we only reject our null hypothesis if the probability of the pattern in our data being caused solely by chance is less than 5%. This allows us to say that there is at least a 95% probability that the data is statistically significant and that it shows a true relationship.

Student's t test

We use this test to compare the means of two samples and can be used even if they have different numbers of replicates. In simple terms, the *t*-test compares the actual difference between two means in relation to their variation (the spread of the data about the mean).

You can carry out a t test if:

- You have collected measurements from 2 or more sites/populations and are interested in the differences between them.
- The data are normally distributed (symmetrical about the mean).
- Data are unmatched (not collected in pairs)
- You are using small sets of data (less than 30 in each data set).

Worked example.

If you wanted to find out if there was a significant difference in the soil temperature of the North and South facing slopes, you could carry out a t test.

Our null hypothesis would be:

There is no difference between the soil temperatures on the two sides of the dale.

In our investigation, six soil temperature measurements were taken from each slope:

Soil temperature: North facing slope (degrees C)	$(x_1 - \bar{x}_1)^2$	Soil temperature: South facing slope (degrees C)	$(x_2 - \bar{x}_2)^2$
10	4.71	16	0.45
14	3.35	18	1.77
12	0.03	16	0.45
12	0.03	17	0.11
13	0.69	16	0.45
12	0.03	17	0.11
$\Sigma x_1 = 73$	$\Sigma(x_1 - \bar{x}_1)^2 = 8.84$	$\Sigma x_2 = 100$	$\Sigma(x_2 - \bar{x}_2)^2 = 3.34$
n = 6		n = 6	
$\bar{x}_1 = 12.17$		$\bar{x}_2 = 16.67$	

1. For each data set, add up all of your data (Σx), then calculate the mean (\bar{x}) by dividing this sum (Σx) by the number of samples (n).
2. For each number, subtract the mean and square the result $(x - \bar{x})^2$
3. Add up all of the squared differences to find $\Sigma (x - \bar{x})^2$
Find the average of the squared differences by dividing by n-1:

$$S^2 = \frac{\Sigma (x - \bar{x})^2}{n - 1}$$

North facing slope

$$S_1^2 = \frac{8.84}{6 - 1}$$

$$= 1.768$$

South facing slope

$$S_2^2 = \frac{3.34}{6 - 1}$$

$$= 0.668$$

4. Now find the t value for the data using the formula:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

$$\begin{aligned}
&= \frac{|12.17 - 16.67|}{\sqrt{\frac{1.768}{6} + \frac{0.668}{6}}} \\
&= \frac{4.5}{\sqrt{0.294 + 0.111}} \\
&= \frac{4.5}{0.636} \\
&= 7.075
\end{aligned}$$

Interpreting the result

First of all work out the degrees of freedom ($v = \text{the number of samples} - 2$) for our test this is 10.

Working down the $p = 0.05$ column and across the 10 degrees of freedom row, we get a critical value of 2.228

Significance value p =						
v	0.20	0.10	0.05	0.02	0.01	0.001
1.	3.078	6.314	12.70	31.82	63.65	318.3
2.	1.886	2.920	4.303	6.965	9.925	22.32
3.	1.638	2.353	3.182	4.541	5.841	10.21
4.	1.533	2.132	2.776	3.747	4.604	7.173
5.	1.476	2.015	2.571	3.365	4.032	5.893
6.	1.440	1.943	2.447	3.143	3.707	5.208
7.	1.415	1.895	2.365	2.998	3.499	4.782
8.	1.397	1.860	2.306	2.896	3.355	4.499
9.	1.383	1.833	2.262	2.821	3.250	4.296
10.	1.372	1.812	2.228	2.764	3.169	4.143
11.	1.363	1.796	2.201	2.718	3.106	4.024
12.	1.356	1.782	2.179	2.681	3.055	3.929

If our calculated value is bigger than the critical value we reject our null hypothesis. In rejecting our hypothesis of no difference, we are saying that there is indeed a significant difference between the means of the two sets of data.

If our calculated value is smaller than our critical value we have to accept our null hypothesis, that there is indeed no difference between the means of the two data sets.

In choosing the 5% significance level we are saying that we would expect to be correct in accepting or rejecting our null hypothesis 95% of the time. We might get a different result due to chance 5% of the time.

In this example, our calculated value of 7.075 is greater than the critical value of 2.228. We can therefore reject our null hypothesis and be 95% certain that there is indeed a significant difference between the soil depths on the two slopes.

Spearman's rank correlation coefficient

We use this to test the strength of the correlation between two variables (i.e. how does one variable change as the other one changes?). You can carry out this test if:

- You have collected quantitative data
- The data are not normally distributed (equally spread about the mean).
- Have at least 8 pairs of data.

Worked Example:

If you want to find out if soil depth on the slopes is correlated to moss cover you can carry out a spearman's rank correlation.

The null hypothesis would be:

There is no relationship between the soil depth and moss cover.

Soil depth cm	23	25	32.5	13.5	3.5	13	13	11.5	7.5	6.5	6	5
Rank 1	10	11	12	9	1	7.5	7.5	6	5	4	3	2
Moss %	9	5	12	10	1	6	0	1	0	5	2	3
Rank 2	10	7.5	12	11	3.5	9	1.5	3.5	1.5	7.5	5	6
Difference between ranks R1-R2 	0	3.5	0	-2	-2.5	-1.5	6	2.5	3.5	-3.5	-2	-4
d²	0	12.25	0	4	6.25	2.25	36	6.25	12.25	12.25	4	16

1. Make a table from your data to list your two variables (rows 1 and 3).

2. Rank both sets of data from lowest to highest. The lowest number on row 1 gets a rank of 1, the second lowest gets 2 and so on. Do this for both sets of data individually.
3. If two values in a data set are the same (tied ranks) they must be awarded the mean (average) of their ranks. In the example above two samples have 13 cm of soil depth. These then share ranks 7 and 8. The mean rank is therefore 7.5 and the next highest value on the table will get rank 9.
4. Calculate the differences in the pairs of ranks i.e. rank 1-rank 2 (row 5).
5. Square the differences to remove negative values (row 6).
6. Add up the last row of numbers to find the sum of the differences:

$$\Sigma d^2 = 111.5$$

7. Calculate Spearman's Rank (r) using the equation:

$$R = 1 - \frac{6\Sigma d^2}{n(n^2 - 1)}$$

n is the number of samples you have taken

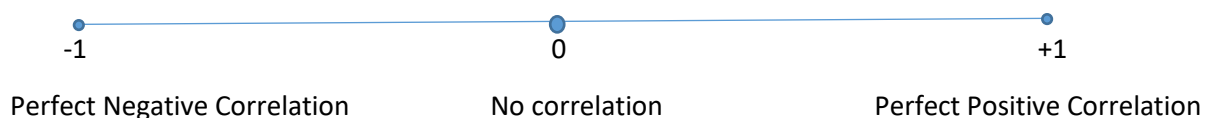
$$R = 1 - \frac{6 \times 111.5}{12(144 - 1)}$$

$$R = 1 - \frac{669}{1716}$$

$$R = 0.6101$$

What does this value mean?

The closer R is to 1, the stronger the positive correlation, the closer this R is to -1, the stronger the negative correlation.



How to test the significance

The value of R must now be looked up on a significance table:



n (number of pairs)	Probability that your result occurred by chance				
	0.1	0.05	0.025	0.01	0.005
4	1.0000	1.0000	1.0000	1.0000	1.0000
5	0.7000	0.9000	0.9000	1.0000	1.0000
6	0.6571	0.7714	0.8286	0.9429	0.9429
7	0.5714	0.6786	0.7857	0.8571	0.8929
8	0.5476	0.6429	0.7381	0.8095	0.8571
9	0.4833	0.6000	0.6833	0.7667	0.8167
10	0.4424	0.5636	0.6485	0.7333	0.7818
11	0.4182	0.5273	0.6091	0.7000	0.7545
12	0.3986	0.5035	0.5874	0.6713	0.7273
13	0.3791	0.4780	0.5604	0.6484	0.6978
14	0.3670	0.4593	0.5385	0.6220	0.6747
15	0.3500	0.4429	0.5179	0.6000	0.6536

For our 12 pairs of data, and at a confidence limit of $p = 0.05$, the critical value is 0.5035

Conclusion

Our value for R (0.6101) is above this critical value, so we can be confident that there is less than 5% probability that our results occurred due to chance. We can therefore reject our null hypothesis and conclude that the data do show a positive correlation: Soil depth and % cover of moss are indeed positively correlated.

If our value was lower than the critical value then we would have to accept the null hypothesis because there would be greater than 5% probability that our results were due to chance alone.

Simpsons Index of Diversity

We use this test to find out how diverse a community is. It is better than simply finding the species richness (number of different species) as it takes into account the abundance (size) of every species within that community. This, therefore, reduces the impact of species which are not well represented within the community on the diversity index and hence bases the most value on the most viable (strong) populations within the community. As species richness and evenness increase, so does the diversity index.

To work out the diversity index you need to have:

- Collected data regarding the numbers of individuals present rather than cover.

Worked example:

If you wanted to compare the diversity of plants growing on the north and south facing slopes we would calculate the diversity index for each slope.

Note, this test does not require the formulation of a null hypothesis, unless you want afterwards to test the significance of the difference in values found.

We will work out the index for one slope here as an example.

Species	Number of plants in quadrat (n)	n(n-1)
Grass, broad-leaved	2	2
Grass, narrow-leaved	70	4830
Heath Bedstraw	3	6
Ladies Bedstraw	2	2
Lesser Celandine	0	0
Milkwort	2	2
Moss	9	72
Mouse-ear Hawkweed	0	0
Other	0	0
Ribwort Plantain	2	2
Salad Burnet	1	0
Thistle	2	2
Thyme	0	0
Tormentil	5	20
Violet	2	2
Total =	100	4940
	N = 100	$\Sigma n(n-1) = 4940$

1. Make a table with your data to show the number of each species found
2. Add up the total number of species found in the first column = N
3. For each species multiply its number (n) by (n-1).
4. Find the sum of the column for n(n-1)
5. Use the formula to calculate the index:

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

$$D = 1 - \frac{4940}{100(99)}$$

$$D = 1 - 0.499$$

$$D = 0.501$$

What does this value mean?

The value of D ranges from 0 (no diversity) to 1 (infinite diversity). Clearly, on its own the value of 0.501 does not mean that much to us. We use this test to compare the diversity of habitats. So, for example, you were comparing the diversity of the north and south facing slopes in a limestone dale:

North facing slope = 0.501

South facing slope = 0.555

Conclusion

Then the south facing slope is slightly more diverse than the north facing slope.