

# One-Way Analysis of Variance (ANOVA)

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## Purpose

While the T-test is useful to compare the means of two samples, many biology experiments involve the parallel measurement of three or more different treatment conditions or levels. For example, rather than simply test the effect of the presence or absence of plants on the ability of goldfish to find and eat *Daphnia*, scientists might design an experiment in which *Daphnia* and goldfish are placed together in tanks with either no plants, underwater plants, or plants on the surface of the water. It is possible to perform three *t*-Tests to discover if any of the three treatments is significantly different from any other. However, the more *t*-Tests that are performed on experimental data, the greater the chance that one of the tests will produce a statistically significant result by accident. A more careful strategy is to analyze all of the treatments together in one go, and the one-way analysis of variance (ANOVA) makes a single analysis possible. ANOVA allows for statistical tests concerning the means of three or more treatment conditions or levels. Specifically, ANOVA provides a test of the null statistical hypothesis ( $H_0$ ) that the population means for three or more groups are equal ( $\mu_1 = \mu_2 = \mu_3 = \dots \mu_n$ ) and that any observed differences between the samples means ( $\bar{x}_1, \bar{x}_2, \bar{x}_3, \dots \bar{x}_n$ ) have occurred by chance. If at least one of the means differs significantly from all the others, then  $H_0$  is rejected and the alternative hypothesis ( $H_1$ ) is supported. In other words, one of the treatments (variations of the independent variable) is significantly different from the others. Ultimately, ANOVA compares the variance *between* the treatment means (called the “mean square between”:  $MS_B$ ) to the average variance *within* all the treatments (called the “mean square within”:  $MS_W$ ). If the variance between the treatment means is large and the variance within all the treatments is small, it is likely that the difference between the means reflects the differential treatment of the samples. However, if there is too much variance within each sample, it cannot be safely concluded that any observed differences between the treatment means are statistically significant.

## Equation and Sample Problem

The test statistic that is calculated for the ANOVA is called the F-ratio. The F-ratio ( $F$ ) is named for Ronald Fisher, the statistician who invented ANOVA in the 1920s.  $F$  is calculated by determining the ratio of the variance between the treatment means ( $MS_B$ ) to the average variance within all the treatments ( $MS_W$ ).

$$F = \frac{MS_B}{MS_W}$$

## Sample problem and steps for calculating mean squares and $F$ :

Students wanted to test the hypothesis that phosphorus is a limiting factor for algal growth. The students predicted that growing algae in three different phosphate solutions would produce a treatment effect of increasing chlorophyll concentration in the algae with increasing phosphorus concentration. The null hypothesis for this experiment is that there is no effect of phosphorus concentration on algal growth. The data the students collected are in Table 1 below.

**Table 1.** Chlorophyll concentration ( $\text{mg}/\text{m}^3$ ) after two weeks of growing algae in three different phosphorus treatments of 10, 15, and 20  $\text{mg}/\text{m}^3$ .

Sample #	Total Phosphorus Concentration ( $\text{mg}/\text{m}^3$ )		
	10	15	20
Chlorophyll Concentration ( $\text{mg}/\text{m}^3$ )			
1	2.9	5.7	11.3
2	3.7	6.1	9.8
3	2.9	4.9	10.6
4	4.0	5.5	10.9
5	4.1	5.4	10.4
6	3.6	5.9	12.0
7	3.4	5.1	10.9
8	3.7	4.7	10.1
9	3.0	6.1	9.7
10	2.7	6.9	11.1
11	4.1	7.2	11.4
12	3.9	6.1	10.2
Treatment Totals	42	69.6	128.4
Treatment Means	$\bar{x}_{10} = 3.5$	$\bar{x}_{15} = 5.8$	$\bar{x}_{20} = 10.7$
		<b>Grand Total</b>	240
		<b>Grand Mean</b>	$\bar{x}_G = 6.7$

- Determine the treatment totals (sums), treatment means, the grand total, and grand mean.
  - Treatment totals = 42, 69.6, and 128.4
  - Treatment means = 3.5, 5.8, and 10.7
  - Grand Total of all scores = 240
  - Grand Mean = 6.7
- Determine the degrees of freedom ( $df$ ).
  - Between groups  $df = (\text{number of groups} - 1) = (3 - 1) = 2$
  - Within groups  $df = (\text{the number of treatments}) \times (df \text{ for each treatment}) = 3(12 - 1) = 33$
- Determine the total variation among all the values in the experiment ( $SS_{total}$ ). This is done by calculating the sum of the squares of the difference of each score from the grand mean.
  - Square each value and sum all the squares to get the Sum of Squares ( $SS$ ).  

$$SS = 2.9^2 + 3.7^2 + 2.9^2 + 4.0^2 \dots + 11.4^2 + 10.2^2 = 1,938.92$$
  - Square the Grand Total and divide it by the total number of observations.  

$$\frac{\text{Grand Total}^2}{\text{Total \# of Observations}} = \frac{240^2}{36} = 1,600$$

c. Subtract the answer in “3b” from the answer in “3a” (SS) to get  $SS_{total}$ .

$$SS_{total} = 1,938.92 - 1,600 = 338.92$$

4. Determine the Sum of the Squares within the groups ( $SS_{within}$ ). To do this, sum the squares of the differences of each score from its group (treatment) mean. Square the quantity of each score minus its own column mean, then sum the values for all the subjects in the experiment:

$$SS_{within} = (2.9 - 3.5)^2 + (3.7 - 3.5)^2 + (2.9 - 3.5)^2 + \dots + (11.4 - 10.7)^2 + (10.2 - 10.7)^2 = 14.36$$

5. Determine the sum of the squares between the groups ( $SS_{between}$ ).

$$SS_{total} - SS_{within} = SS_{between}$$

$$338.92 - 14.36 = 324.56$$

6. Determine the mean squares between ( $MS_B$ ) and mean squares within ( $MS_W$ ) by dividing each by the  $df$  between and within, respectively.

$$MS_B = \frac{SS_{between}}{df_{between}} = \frac{324.56}{2} = 162.28$$

$$MS_W = \frac{SS_{within}}{df_{within}} = \frac{14.36}{33} = 0.435$$

7. Determine the  $F$  – ratio.

$$F = \frac{MS_B}{MS_W} = \frac{162.28}{0.435} = 373$$

8. Compare the observed F-ratio ( $F_{obs}$ ) with the critical value ( $F_{crit}$ ) of  $F$  at  $\alpha$  (alpha, the  $H_0$  rejection level) = 0.05 and the between (numerator) and within (denominator) degrees of freedom = 2 and 33, respectively:  $F_{2;33;0.05}$ .

9. Compare the  $F_{obs}$  to the critical  $F$  values in **Table 3** on the following page.

10. Create an ANOVA table to summarize the results. (**Table 2** is an example.)

**Table 2.** Results of one-way analysis of variance for the effect of phosphorus concentration on chlorophyll concentration.

<b>Source of Variation</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P-value</b>	<b>F<sub>crit</sub></b>
Between Treatments	324.56	2	162.28	373	P < 0.001	3.28
Within Treatments	14.36	33	0.435			
Total	338.92	35				

**Table 3: Critical values for  $F$  at a rejection level ( $\alpha$ ) of 0.05.**

Degrees of Freedom Within the Treatments	Degrees of Freedom (df) Between the Treatments									
	1	2	3	4	5	6	7	8	9	10
1	161.45	199.50	215.71	224.58	230.16	233.99	236.77	238.88	240.54	241.88
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91
$\infty$	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83

### Summary

**Table 3**, critical values for  $F$ , shows us the  $F$ -ratio the students could obtain by accident 5% of the time in an ANOVA test for various combinations of degrees of freedom between and within groups and treatments. In Table 3,  $F$  is 3.28 for data that include 2 and 33 degrees of freedom between and within groups, respectively. The calculated  $F$  in the ANOVA for the phosphorus-chlorophyll data is 373. This number is much higher than 3.28, so the probability ( $P$ ) of getting a value as large as 373 if  $H_0$  is true is less than 0.05. Therefore, the  $H_0$  (statistical null hypothesis) is rejected (that the population means are equal, and any difference between the sample means occurred by chance) and it is reasonable to conclude that the phosphorus treatment had a statistically significant effect on algae growth. Therefore, the experimental hypothesis has been supported by the results of this experiment and the ANOVA statistical test.

### Solve this Problem

A student observed that just after bean plants (*Phaseolus vulgaris*) germinate, their cotyledons slowly shrivel. She hypothesized that the young plants are using energy from their cotyledons to fuel extra growth and predicted that bean plants with either one or both cotyledons removed just after germination would be shorter after three weeks relative to bean plants with their cotyledons intact.

1. What is the null statistical hypothesis ( $H_0$ ) for this experiment?
2. Perform the ANOVA on the student's data to test the  $H_0$ .
3. Write a short conclusion about what the results of the ANOVA suggest about the student's experimental hypothesis and statistical hypothesis.

**Table 4.** Heights (cm) of bean plants with 0, 1, or 2 cotyledons removed, three weeks after germination.

Plant #	Number of Cotyledons Removed from Plant		
	0	1	2
1	10.3	9.7	6.5
2	9.6	10.2	4.6
3	10.0	6.8	7.9
4	9.8	8.3	8.1
5	10.1	7.8	5.8
6	8.4	9.9	5.9
7	12.4	10	6.0
8	7.9	8.0	7.1
9	8.8	7.8	5.2
Treatment Totals			
Treatment Means	$\bar{x}_0 =$	$\bar{x}_1 =$	$\bar{x}_2 =$
		Grand Total	
		Grand Mean	$\bar{x}_G =$

**Table 5.** ANOVA results of the effect of removing cotyledons on growth in bean plants.

Source of Variation	SS	df	MS	F	P-value	$F_{crit}$
Between Treatments						
Within Treatments						
Total						