Introduction to Analysis of Variance (ANOVA)
***Complete this exercise and hand in at the end of the lab**
Section______________________, Name______________________________

Analysis of variance is a useful statistical tool when you want to compare more than two groups or when there are two or more independent variables. Using a two-sample test (such as a t-test) to analyze multiple groups by testing different combinations multiple times increases is not recommended. Every time you add a test, you increase the possibility of making Type I error (i.e., wrongly concluding two groups are statistically different). As you all know, we use $\alpha=0.05$ to judge statistical significance. This "α" is actually the Type I error rate, meaning that we conclude that there is statistical significance when the possibility of mistakenly finding difference between two groups is less than 5% (everybody agrees 5% is pretty low risk). In other words, if you run two-sample tests 100 times, 5 out of those 100 t-tests likely conclude wrongly. Thus, by repeatedly using two-sample tests, you may eventually find a statistically significant difference between two groups even if they are not actually different from each other. This is why we want to use ANOVA to analyze multiple groups simultaneously.

Interestingly, ANOVA uses variances (rather than means) to test whether a significant portion of variance in the dataset is explained by group differences. In ANOVA, total variance consists of between-group variance (called Sum of Squares between Groups or SS_B) and within-group variance (called Sum of Squares within Groups or SS_W). Understanding the difference between SS_B and SS_W is a key to ANOVA. There are three important assumptions underlying ANOVA.

1. Samples must be random and independent.
2. Dataset must be normally distributed.
3. Variance is homogeneous across groups.

If you are preparing a manuscript for publication, you have to test whether assumptions 2 and 3 are met by using different statistical tests before you conduct the ANOVA. However, for 208 lab we assume that assumption 2 and 3 are met. You still have to ask whether assumption 1 is met based on your experimental design.

Example 1

There are 3 batches of radish seeds each weighing 1.5 g and grown under the following three experimental conditions.

Experiment treatments:

1. Seeds placed on DRY paper towels in LIGHT
2. Seeds placed on WET paper towels in LIGHT
3. Seeds placed on WET paper towels in DARK

There are 5 replicates in each treatment.

The photo below showed radish growth after 1 week. Then, all plant materials were completely dried in an oven overnight and plant biomass was measured in grams.

1. 2. 3.

The Null hypothesis is that light and water conditions do not affect biomass of radish seeds and seedling. This hypothesis predicts:
H_0: 1 = 2 = 3 (all 1.5 g)

Do you think the data will support the null hypothesis?

If not, what is your hypothesis?

Circle your prediction below and explain why you chose it.

A. 1>2>3
B. 1>3>2
C. 2>1>3
D. 2>3>1
E. 3>2>1
F. 2=3>1
G. 2>1=3

Ok, here are the data collected. You are ultimately calculating the F value, which will be compared with the F critical value to determine whether the proportion of variance explained by group differences is significant or not.

Replicates	Light, No water	Light, Water	No Light, water
1	1.503	1.791	1.334
2	1.372	1.432	1.210
3	1.450	1.713	1.230
4	1.504	1.604	1.260
5	1.447	1.587	1.350

Now, **let's calculate the Sum of Squares between Groups (SS_B)** by completing the table below. Make sure that you understand why this is called the "Sum of Squares between Groups"

Replicates	Light No water	Light Water	No Light water	
1	1.503	1.791	1.334	
2	1.372	1.432	1.210	
3	1.450	1.713	1.230	
4	1.504	1.604	1.260	
5	1.447	1.587	1.350	
# of replicate in each treatment (N_R)	5	5	5	N = 15
Sum of values in each treatment (Σxi)				
Treatment Mean				Grand Mean =
$(\text{Treatment Mean - Grand Mean})^2$				
$N_R \times (\text{Treatment Mean - Grand Mean})^2$				Total (SS_B) =

Explain what conditions make SS_B larger or smaller

Next, **let's calculate the Sum of Squares within Groups (SS_W)** by completing the table below. Make sure that you understand why this is called the "Sum of Squares within Groups"

Replicates	Light No water	Light Water	No Light water	
1	1.503	1.791	1.334	
2	1.372	1.432	1.210	
3	1.450	1.713	1.230	
4	1.504	1.604	1.260	
5	1.447	1.587	1.350	
# of replicate in each treatment (N_R)	5	5	5	N = 15
Treatment Mean				
$(1^* - \text{Treatment Mean})^2$				
$(2^* - \text{Treatment Mean})^2$				
$(3^* - \text{Treatment Mean})^2$				
$(4^* - \text{Treatment Mean})^2$				
$(5^* - \text{Treatment Mean})^2$				
Total				Total (SS_W) =

*These numbers indicate values in each replicate

Explain what conditions make SS_w larger or smaller

Calculate the **degrees of freedom** by completing the table below.

	Degrees of Freedom (df)
Between Groups = K - 1	
Within Groups = k (N_R-1)	
Total	

K = # of treatments
N_R = # of replicates

Here's the final stage of the ANOVA. **Complete the ANOVA table** below.

	SS	df	MS	F	F cv
Between Groups					
Within groups				---------------	---------------
Total			---------------	---------------	---------------

Mean Square (MS) = SS/df, $F = MS_B/MS_W$
F critical value (F cv) can be found from the next table

F Distribution Table

Degrees of Freedom for the F-Ratio numerator (columns); *Degrees of Freedom for the F-Ratio denominator* (rows)

	1	2	3	4	5	6	7	8	9	10
1	161.4	199.5	215.8	224.8	230.0	233.8	236.5	238.6	240.1	242.1
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
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19
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
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.03
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99

When your calculated F value is larger than F critical value (F cv), you would reject the null hypothesis and conclude that there is significant effect of the treatment on biomass of radish seedling.

What is your conclusion?

Now you know that there are differences in seedling mass among the treatments. However, you still don't know which groups are different from each other. When ANOVA suggests statistical significance among multiple groups, we have to conduct post hoc multiple-comparison tests. For post hoc tests, we use the Tukey test (aka honestly significant difference test or HSD test).

The Tukey test is based on the Q statistic which is defined as follows:

$$Q = \frac{Mean\ x - Mean\ y}{\sqrt{MS_W / N_R}}$$

MS_W is given in your ANOVA table. Mean x is the larger and mean y is the smaller treatment mean. N_R is # of replicates.

Calculate the **difference in mean between treatments** by completing the table below.

	Light, No water	Light, Water	No Light, water
Light, No water	--------	--------	--------
Light, Water		--------	--------
No Light, water			--------

Calculate the **Q for each pairwise comparison** by completing the table below.

	Light, No water	Light, Water	No Light, water
Light, No water	--------	--------	--------
Light, Water		--------	--------
No Light, water			--------

Find the Q critical value from the table on the next page.

Q cv =

When your calculated Q is larger than the Q cv, the pair is significantly different from one another.

Place * in the cells of the above table when pairs are significantly different from one another.

Did the results support your prediction? If not, discus why.

Q Distribution Table

	Number of means								
df_w	2	3	4	5	6	7	8	9	10
1	17.97	26.98	32.82	37.08	40.41	43.12	45.40	47.36	49.07
2	6.08	8.33	9.80	10.88	11.74	12.44	13.03	13.54	13.99
3	4.50	5.91	6.82	7.50	8.04	8.48	8.85	9.18	9.46
4	3.93	5.04	5.76	6.29	6.71	7.05	7.35	7.60	7.33
5	3.64	4.60	5.22	5.67	6.03	6.33	6.58	6.80	7.00
6	3.46	4.34	4.90	5.31	5.63	5.90	6.12	6.32	6.49
7	3.34	4.16	4.68	5.06	5.36	5.61	5.82	6.00	6.16
8	3.26	4.04	4.53	4.89	5.17	5.40	5.60	5.77	5.92
9	3.20	3.95	4.42	4.76	5.02	5.24	5.43	5.60	5.74
10	3.15	3.88	4.33	4.65	4.91	5.12	5.30	5.46	5.60
11	3.11	3.82	4.26	4.57	4.82	5.03	5.20	5.35	5.49
12	3.08	3.77	4.20	4.51	4.75	4.95	5.12	5.26	5.40
13	3.06	3.74	4.15	4.45	4.69	4.88	5.05	5.19	5.32
14	3.03	3.70	4.11	4.41	4.64	4.83	4.99	5.13	5.25
15	3.01	3.67	4.08	4.37	4.60	4.78	4.94	5.08	5.20
16	3.00	3.65	4.05	4.33	4.56	4.74	4.90	5.03	5.15
17	2.98	3.63	4.02	4.30	4.52	4.70	4.86	4.99	5.11
18	2.97	3.61	4.00	4.28	4.50	4.67	4.82	4.96	5.07
19	2.96	3.59	3.98	4.25	4.47	4.64	4.79	4.92	5.04
20	2.95	3.58	3.96	4.23	4.44	4.62	4.77	4.90	5.01
24	2.92	3.53	3.90	4.17	4.37	4.54	4.68	4.81	4.92
30	2.89	3.49	3.84	4.10	4.30	4.46	4.60	4.72	4.82
40	2.86	3.44	3.79	4.04	4.23	4.39	4.52	4.64	4.74
60	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65
120	2.80	3.36	3.69	3.92	4.10	4.24	4.36	4.47	4.56
∞	2.77	3.31	3.63	3.86	4.03	4.17	4.29	4.39	4.47