

## Multi-factorial ANOVA Practice Exercises

### Questions

- 1) A laboratory experiment was conducted to determine if there were significant differences in the cell doubling time (in hrs) of two randomly selected strains of bloom-forming phytoplankton cells when exposed to four different N:P nutrient concentration ratios (labeled A, B, C and D). Equal aliquots of the two phytoplankton strains were randomly and independently assigned to 32 flasks containing sterile seawater; 16 flasks per strain. Then, each nutrient treatment was randomly added to 8 flasks containing the phytoplankton cells in seawater (i.e., 8 flasks x 4 nutrient additions = 32 flasks total). Are there significant differences in the cell doubling times among strains or among phytoplankton exposed to different nutrient concentration ratios?
  
- 2) A field experiment was conducted to test for possible inter-specific competitive effects between an exotic tree species (Australian Pine) and a native tropical tree species (Gumbo-Limbo) as a function of elevation. Therefore, 10 Australian pine trees (exotic transplantation; labeled "E") and 10 Gumbo-Limbo trees were transplanted next to one another at each of 5 randomly chosen locations at each of three elevations (0m, 300m, 500m); thus, a total of 15 locations were used in the experiment. Similarly, 10 Gumbo-Limbo trees were transplanted next to another 10 Gumbo-Limbo trees at the same 15 locations to serve as a control for intra-specific competitive effects (control transplantation; labeled "C"). Later, the mean number of fruits per Gumbo-Limbo tree (i.e., mean of 10 trees per location) were determined to estimate the possible effect of competition on reproductive success. Are there significant differences in fruit production by Gumbo-Limbo trees in response to inter- or intra-specific competition at the three elevations tested? [YOU MAY ASSUME THAT ALL ASSUMPTIONS ARE MET FOR THIS QUESTION]
  
- 3) An experiment was conducted to determine if the growth of mouse embryos varied among embryos of three different initial sizes (S = small, M = medium, L = large) when exposed for different lengths of time (5 days, 10 days, 15 days, 20 days, 25 days, and 30 days) to a chemical suspected of being an endocrine disruptor. Three hundred and sixty (360) embryos were allocated to 36 growth chambers; ten embryos per chamber. Small embryos were randomly assigned to 12 chambers, medium embryos to another 12 chambers, and large embryos to the last 12 chambers. Two chambers for each embryo size were then randomly assigned to one of the 6 exposure treatments. After 30 days, the mean weight change (expressed as % growth) of the 10 embryos per chamber was determined. Does embryo size or duration of exposure to the hypothesized endocrine disruptor effect the growth of mouse embryos?  
[YOU MAY ASSUME THAT ALL ASSUMPTIONS ARE MET FOR THIS QUESTION]

**Question 1 Data**

Strain	Nutrient	Data	Strain	Nutrient	Data
1	A	3	2	A	5
1	B	4	2	B	6
1	C	7	2	C	9
1	D	7	2	D	8
1	A	6	2	A	5
1	B	5	2	B	6
1	C	8	2	C	8
1	D	8	2	D	9
1	A	3	2	A	3
1	B	4	2	B	4
1	C	7	2	C	5
1	D	47	2	D	3
1	A	3	2	A	6
1	B	3	2	B	6
1	C	6	2	C	7
1	D	8	2	D	8

**Question 2 Data**

Trt	Site	Elev	Data	Trt	Site	Elev	Data
E	1	0	13	C	1	0	26
E	2	0	19	C	2	0	14
E	3	0	21	C	3	0	21
E	4	0	16	C	4	0	29
E	5	0	11	C	5	0	33
E	6	300	21	C	6	300	32
E	7	300	14	C	7	300	19
E	8	300	16	C	8	300	26
E	9	300	21	C	9	300	30
E	10	300	13	C	10	300	28
E	11	500	24	C	11	500	32
E	12	500	15	C	12	500	27
E	13	500	22	C	13	500	25
E	14	500	19	C	14	500	27
E	15	500	17	C	15	500	31

**Question 3 Data**

Embryo Size	Days Exposed	% Growth
S	5	34
S	10	40
S	15	42
S	20	37
S	25	28
S	30	3
S	5	41
S	10	46
S	15	49
S	20	45
S	25	41
S	30	50
M	5	36
M	10	47
M	15	53
M	20	40
M	25	37
M	30	44
M	5	40
M	10	42
M	15	43
M	20	39
M	25	40
M	30	43
L	5	25
L	10	20
L	15	15
L	20	10
L	25	5
L	30	5
L	5	22
L	10	19
L	15	13
L	20	11
L	25	5
L	30	2

## Answers to Multifactorial ANOVA Questions

### Question 1:

Test required: 2x4 mixed model ANOVA  
Independent variable: fixed: N:P conc (4 levels); random: strain (2 levels)  
Dependent variable: cell doubling times  
Number & description of replicates: n = 4 replicate flasks per nutrient x strain treatment  
Assumption check: an outlier; assumptions not met; inverse transformation best

Results:

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>	<u>% Variance Explained</u>
Strain	1	2.246e <sup>-3</sup>	0.537	0.471	0%
Nutrient Conc	3	2.789 e <sup>-3</sup>	3.158	0.185	NA
Strain x Nutrient	3	8.833 e <sup>-3</sup>	2.111	0.125	21.7%
<u>Error</u>	<u>24</u>	<u>4.185 e<sup>-3</sup></u>			<u>78.3%</u>
Total	31				

Conclusion: No significant effect of nutrient concentration, strain, or their interaction on cell doubling times. Most of the variance in the response is explained by differences among replicate flasks, although 21.7% is due to the interaction between strain and nutrient concentration.

### Question 2:

Test required: 2-factor split-plot ANOVA  
Independent variable: whole-plot: elevation (3 levels); sub-plot: competition (2 levels); block: location (15 locations)  
Dependent variable: mean number of fruits per tree  
Number & description of replicates: 5 trees per elevation and competition trt  
Assumption check: assume all met

Results:

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Elevation	2	32.43	1.685	0.227
Site (elevation)	12	19.25	0.768	0.672
Competition	1	634.8	25.341	<0.0005
Elevation x Comp	2	1.3	0.052	0.950
<u>Error</u>	<u>12</u>	<u>25.05</u>		
Total	29			

Conclusion: Competition between two trees significantly effected the mean production of fruit per tree, surprisingly it increased fruit production. Elevation and the interaction between elevation and competition treatment did not effect fruit production.

### Question 3:

Test required: 3 x 6 model I ANOVA with multiple comparison test  
Independent variable: factor a: embryo size (3 levels); factor b: chemical exposure time (6 levels)  
Dependent variable: % growth of embryos  
Number & description of replicates: 2 replicate embryos per size and exposure treatment combination

Assumption check: assume all met

Results:

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>P</u>
Embryo size	2	3036	39.72	<0.005
Exposure period	5	140	1.833	0.157
Size x exposure	10	55	0.725	0.695
<u>Error</u>	<u>18</u>			
Total	36			

Tukey Test of Embryo size differences:      L      S      M

Conclusion: Embryo size significantly effected embryo growth, with larger embryos growing significantly faster than either the small or medium embryos whose growth did not differ from one another. The duration of chemical exposure did not affect growth, nor did the interaction between embryo size and chemical exposure.