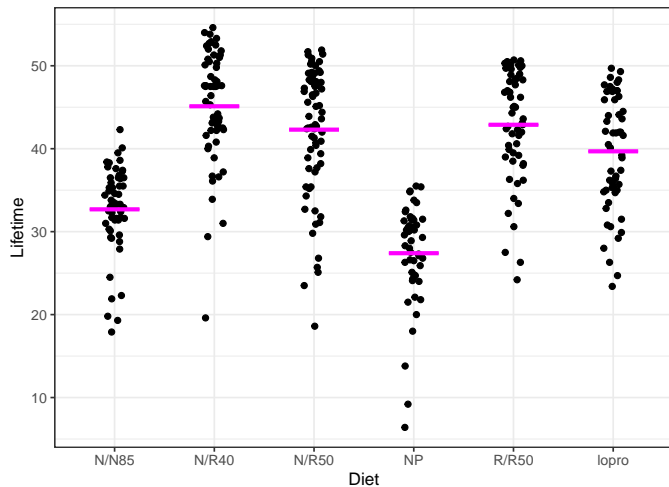


R07 - Contrasts

STAT 5870 (Engineering)
Iowa State University

August 28, 2024

Diet Effect on Mice Lifetimes



ANOVA and Regression Models

ANOVA model:

$$Y_{ij} \stackrel{ind}{\sim} N(\mu_j, \sigma^2)$$

with Y_{ij} being the lifetime for the i th mouse on the j th diet for $j = 0, 1, 2, 3, 4, 5$.

Regression model:

$$Y_i \stackrel{ind}{\sim} N(\beta_0 + \beta_1 X_{i,1} + \dots + \beta_p X_{i,p}, \sigma^2)$$

where Y_i is the lifetime for the i th mouse and $X_{i,j}$ is an indicator for the i th mouse being on the j th diet.

Reparameterized model since

$$\mu_0 = \beta_0 \quad \text{and} \quad \mu_j = \beta_0 + \beta_j$$

for $j > 0$.

Scientific questions

Here are a few example scientific questions:

1. What is the effect of pre-wean calorie restriction on mean lifetimes?
2. What is the difference in mean lifetimes between mice on a 40 kcal diet compared to those on a 50 kcal diet?
3. What is the effect of high calorie vs low calorie diets on mean lifetimes?

We can compute **contrasts**:

$$\gamma_1 = \mu_{R/R50} - \mu_{N/R50}$$

$$\gamma_2 = \mu_{N/R40} - \frac{1}{2}(\mu_{N/R50} + \mu_{R/R50})$$

$$\gamma_3 = \frac{1}{4}(\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro}) \\ - \frac{1}{2}(\mu_{NP} + \mu_{N/N85})$$

Contrasts

A **linear combination** of group means has the form

$$\gamma = C_1\mu_1 + C_2\mu_2 + \dots + C_J\mu_J$$

where C_j are known coefficients and μ_j are the unknown population means.

A linear combination with $C_1 + C_2 + \dots + C_J = 0$ is a **contrast**.

Contrast interpretation is usually best if $|C_1| + |C_2| + \dots + |C_J| = 2$, i.e. the positive coefficients sum to 1 and the negative coefficients sum to -1.

Inference on Contrasts

Contrast

$$\gamma = C_1\mu_1 + C_2\mu_2 + \cdots + C_J\mu_J \quad \text{with} \quad \hat{\gamma} = C_1\bar{Y}_1 + C_2\bar{Y}_2 + \cdots + C_J\bar{Y}_J$$

with standard error

$$SE(\hat{\gamma}) = \hat{\sigma} \sqrt{\frac{C_1^2}{n_1} + \frac{C_2^2}{n_2} + \cdots + \frac{C_J^2}{n_J}}.$$

p -values for $H_0 : \gamma = g_0$ vs $H_A : \gamma \neq g_0$ and posterior probabilities (i.e. $2P(\gamma > 0|y)$ or $2P(\gamma < 0|y)$):

$$t = \frac{g - g_0}{SE(g)}, \quad p = 2P(T_{n-J} < -|t|).$$

Two-sided equal-tail $100(1 - \alpha)\%$ confidence/credible intervals:

$$g \pm t_{n-J, 1-\alpha/2} SE(g).$$

Contrasts for mice lifetime dataset

For these contrasts:

1. Mean lifetimes for N/R50 and R/R50 diet are different.
2. Mean lifetimes for N/R40 is different than for N/R50 and R/R50 combined.
3. Mean lifetimes for high calorie (NP and N/N85) diets is different than for low calorie diets combined.

$$H_0 : \gamma = 0 \quad H_A : \gamma \neq 0 :$$

$$\gamma_1 = \mu_{R/R50} - \mu_{N/R50}$$

$$\gamma_2 = \mu_{N/R40} - \frac{1}{2}(\mu_{N/R50} + \mu_{R/R50})$$

$$\gamma_3 = \frac{1}{4}(\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro}) \\ - \frac{1}{2}(\mu_{NP} + \mu_{N/N85})$$

	N/N85	N/R40	N/R50	NP	R/R50	lopro
early rest - none @ 50kcal	0.00	0.00	-1.00	0.00	1.00	0.00
40kcal/week - 50kcal/week	0.00	1.00	-0.50	0.00	-0.50	0.00
lo cal - hi cal	-0.50	0.25	0.25	-0.50	0.25	0.25

Fit the Multiple Regression Model

```
m = lm(Lifetime ~ Diet, data = Sleuth3::case0501)
summary(m)
```

Call:

```
lm(formula = Lifetime ~ Diet, data = Sleuth3::case0501)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-25.5167	-3.3857	0.8143	5.1833	10.0143

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	32.6912	0.8846	36.958	< 2e-16 ***
DietN/R40	12.4254	1.2352	10.059	< 2e-16 ***
DietN/R50	9.6060	1.1877	8.088	1.06e-14 ***
DietNP	-5.2892	1.3010	-4.065	5.95e-05 ***
DietR/R50	10.1945	1.2565	8.113	8.88e-15 ***
Dietlopro	6.9945	1.2565	5.567	5.25e-08 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 6.678 on 343 degrees of freedom

Multiple R-squared: 0.4543, Adjusted R-squared: 0.4463

F-statistic: 57.1 on 5 and 343 DF, p-value: < 2.2e-16

Estimate Group Means

```
library("emmeans")  
em = emmeans(m, ~ Diet)  
em
```

Diet	emmean	SE	df	lower.CL	upper.CL
N/N85	32.7	0.885	343	31.0	34.4
N/R40	45.1	0.862	343	43.4	46.8
N/R50	42.3	0.793	343	40.7	43.9
NP	27.4	0.954	343	25.5	29.3
R/R50	42.9	0.892	343	41.1	44.6
lopro	39.7	0.892	343	37.9	41.4

Confidence level used: 0.95

```
K_list
```

```
$`early rest - none @ 50kcal`
```

```
[1] 0 0 -1 0 1 0
```

```
$`40kcal/week - 50kcal/week`
```

```
[1] 0.0 1.0 -0.5 0.0 -0.5 0.0
```

```
$`lo cal - hi cal`
```

```
[1] -0.50 0.25 0.25 -0.50 0.25 0.25
```

```
co = contrast(em, K_list)
```

```
# p-values (and posterior tail probabilities)
```

```
co
```

contrast	estimate	SE	df	t.ratio	p.value
early rest - none @ 50kcal	0.589	1.19	343	0.493	0.6223
40kcal/week - 50kcal/week	2.525	1.05	343	2.408	0.0166
lo cal - hi cal	12.450	0.78	343	15.961	<.0001

```
# confidence/credible intervals
```

```
confint(co)
```

contrast	estimate	SE	df	lower.CL	upper.CL
early rest - none @ 50kcal	0.589	1.19	343	-1.759	2.94
40kcal/week - 50kcal/week	2.525	1.05	343	0.463	4.59
lo cal - hi cal	12.450	0.78	343	10.915	13.98

Summary

- Contrasts are linear combinations of means where the coefficients sum to zero
- t-test tools are used to calculate p-values and confidence intervals

Sulfur effect on scab disease in potatoes

The experiment was conducted to investigate the effect of sulfur on controlling scab disease in potatoes. There were seven treatments: control, plus spring and fall application of 300, 600, 1200 lbs/acre of sulfur. The response variable was percentage of the potato surface area covered with scab averaged over 100 random selected potatoes. A completely randomized design was used with 8 replications of the control and 4 replications of the other treatments.

Cochran and Cox. (1957) Experimental Design (2nd ed). pg96 and Agron. J. 80:712-718 (1988)

Scientific questions:

- Does sulfur have any impact at all?
- What is the difference between spring and fall application of sulfur?
- What is the effect of increased sulfur application?

Data

```
Warning: There was 1 warning in 'mutate()'.
i In argument: 'application = fct_explicit_na(application)'.
Caused by warning:
! 'fct_explicit_na()' was deprecated in forcats 1.0.0.
i Please use 'fct_na_value_to_level()' instead.
```

	inf	trt	row	col	sulfur	application	treatment
1	9	F3	4	1	300	fall	F3
2	12	0	4	2	0	(Missing)	0
3	18	S6	4	3	600	spring	S6
4	10	F12	4	4	1200	fall	F12
5	24	S6	4	5	600	spring	S6
6	17	S12	4	6	1200	spring	S12
7	30	S3	4	7	300	spring	S3
8	16	F6	4	8	600	fall	F6
9	10	0	3	1	0	(Missing)	0
10	7	S3	3	2	300	spring	S3
11	4	F12	3	3	1200	fall	F12
12	10	F6	3	4	600	fall	F6
13	21	S3	3	5	300	spring	S3
14	24	0	3	6	0	(Missing)	0
15	29	0	3	7	0	(Missing)	0
16	12	S6	3	8	600	spring	S6
17	9	F3	2	1	300	fall	F3
18	7	S12	2	2	1200	spring	S12
19	18	F6	2	3	600	fall	F6
20	30	0	2	4	0	(Missing)	0
21	18	F6	2	5	600	fall	F6
22	16	S12	2	6	1200	spring	S12

Design

**Completely randomized design
potato scab experiment**

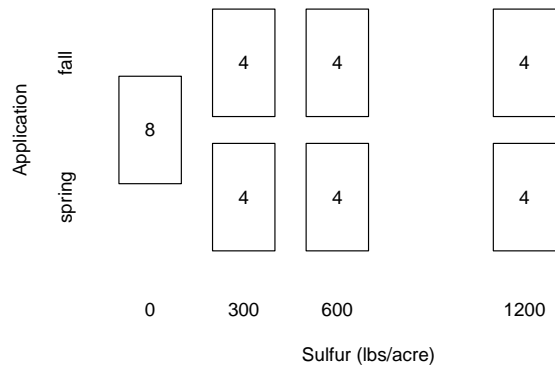
4	F3	O	S6	F12	S6	S12	S3	F6
3	O	S3	F12	F6	S3	O	O	S6
2	F3	S12	F6	O	F6	S12	F3	F12
1	S3	O	S12	S6	O	F12	O	F3
	1	2	3	4	5	6	7	8

row

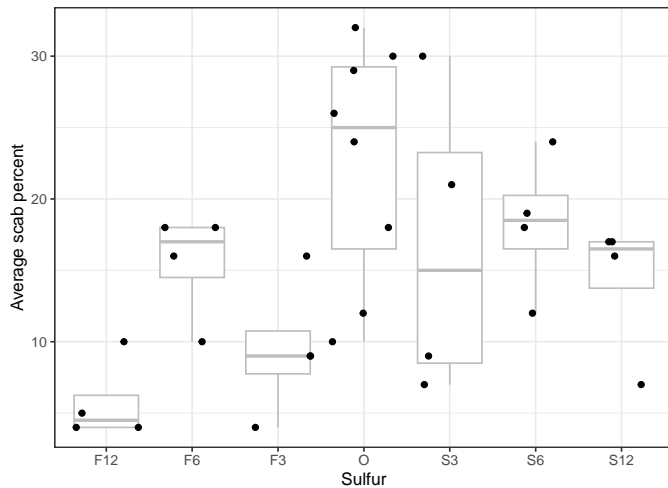
col

Design

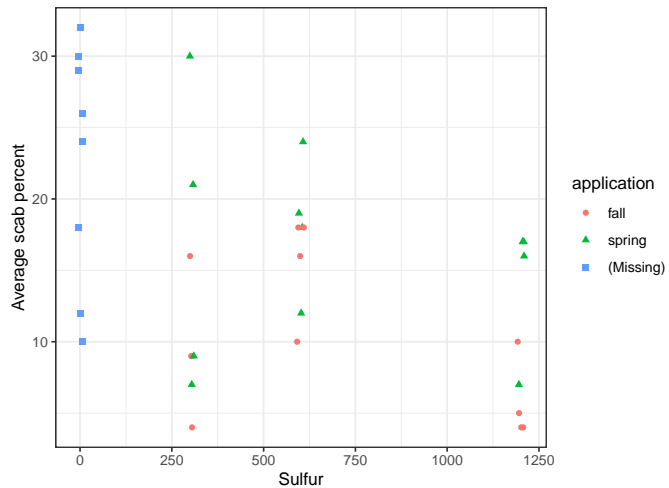
Treatment visualization



Data

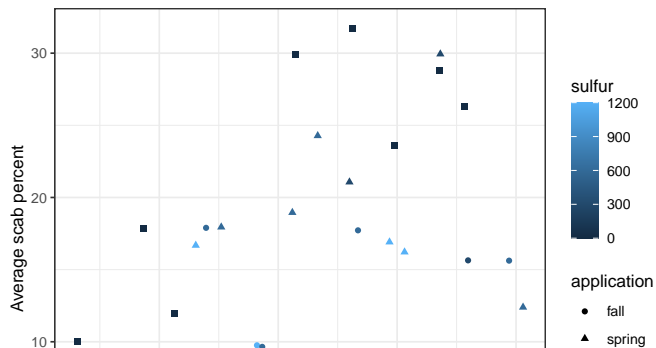


Data

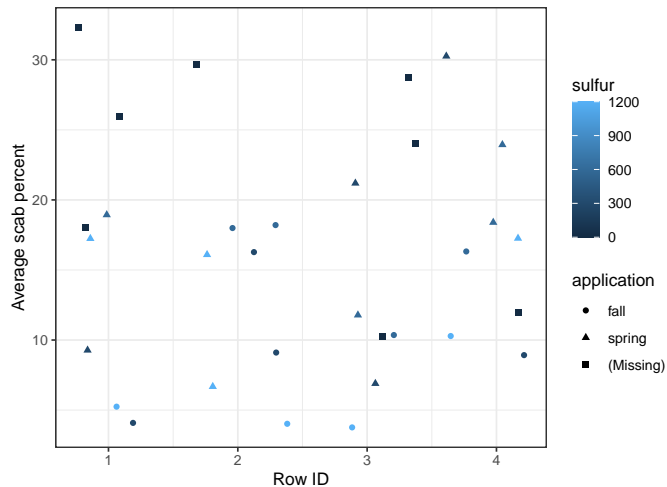


Data

Warning: 'qplot()' was deprecated in ggplot2 3.4.0.
This warning is displayed once every 8 hours.
Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was generated.



Data



Model

Y_{ij} : avg % of surface area covered with scab for plot i in treatment j for $j = 1, \dots, 7$.

Assume $Y_{ij} \stackrel{ind}{\sim} N(\mu_j, \sigma^2)$.

Hypotheses:

- Difference amongst any means:
One-way ANOVA F-test
- *Any effect*:
Contrast: control vs sulfur
- *Fall vs spring*:
Contrast: fall vs spring applications
- *Sulfur level*:
Contrast: linear trend

Contrasts

- *Sulfur effect*: Any sulfur vs none

$$\begin{aligned}\gamma &= \frac{1}{6}(\mu_{F12} + \mu_{F6} + \mu_{F3} + \mu_{S3} + \mu_{S6} + \mu_{S12}) - \mu_O \\ &= \frac{1}{6}(\mu_{F12} + \mu_{F6} + \mu_{F3} + \mu_{S3} + \mu_{S6} + \mu_{S12} - 6\mu_O)\end{aligned}$$

- *Fall vs spring*: Contrast comparing fall vs spring applications

$$\begin{aligned}\gamma &= \frac{1}{3}(\mu_{F12} + \mu_{F6} + \mu_{F3}) + 0\mu_O - \frac{1}{3}(\mu_{S3} + \mu_{S6} + \mu_{S12}) \\ &= \frac{1}{3}[1\mu_{F12} + 1\mu_{F6} + 1\mu_{F3} + 0\mu_O - 1\mu_{S3} - 1\mu_{S6} - 1\mu_{S12}]\end{aligned}$$

Contrasts (cont.)

- Sulfur linear trend

- The group sulfur levels (X_j) are 12, 6, 3, 0, 3, 6, and 12 (100 lbs/acre)
- and a linear trend contrast is $X_j - \bar{X}$

X_i	12	6	3	0	3	6	12
$X_i - \bar{X}$	6	0	-3	-6	-3	0	6

$$\gamma = 6\mu_{F12} + 0\mu_{F6} - 3\mu_{F3} - 6\mu_O - 3\mu_{S3} + 0\mu_{S6} + 6\mu_{S12}$$

Trt	F12	F6	F3	O	S3	S6	S12	Div
Sulfur v control	1	1	1	-6	1	1	1	6
Fall v Spring	1	1	1	0	-1	-1	-1	3
Linear Trend	6	0	-3	-6	-3	0	6	1

```
K =
#
      F12 F6 F3  O S3 S6 S12
list("sulfur - control" = c( 1, 1, 1,-6, 1, 1,  1)/6,
      "fall - spring"   = c( 1, 1, 1, 0,-1,-1, -1)/3,
      "linear trend"    = c( 6, 0,-3,-6,-3, 0,  6)/1)
```

```
m = lm(inf ~ treatment, data = d)
anova(m)
```

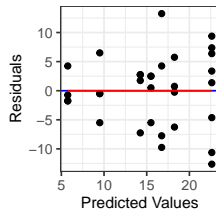
Analysis of Variance Table

Response: inf

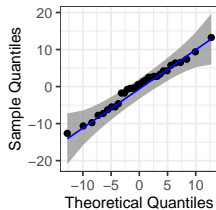
```
      Df Sum Sq Mean Sq F value Pr(>F)
treatment  6  972.34  162.057   3.6081 0.01026 *
Residuals 25 1122.88   44.915
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

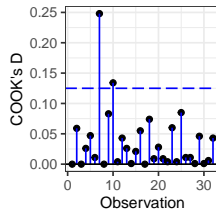
Residual Plot



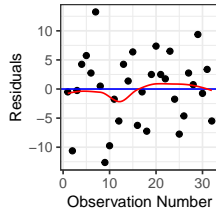
Q-Q Plot



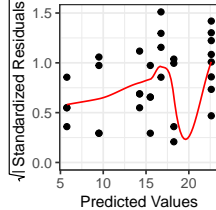
COOK's D Plot



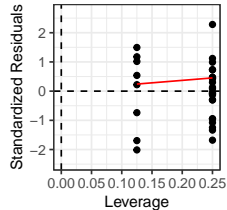
Index Plot



Location-Scale Plot



Residual-Leverage




```
em <- emmeans(m, ~treatment); em
```

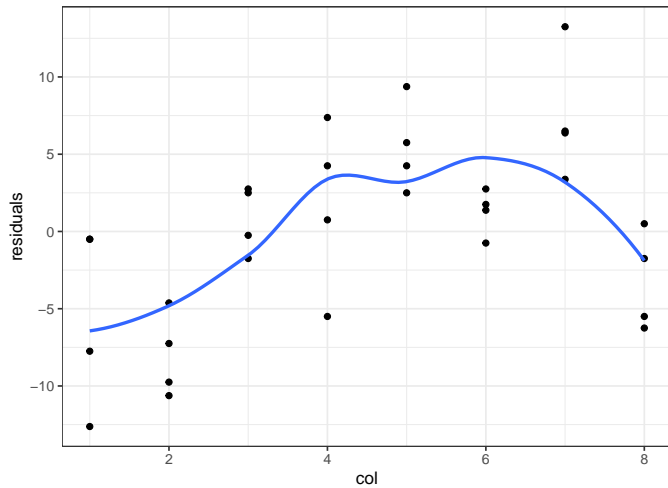
treatment	emmean	SE	df	lower.CL	upper.CL
F12	5.75	3.35	25	-1.15	12.7
F6	15.50	3.35	25	8.60	22.4
F3	9.50	3.35	25	2.60	16.4
0	22.62	2.37	25	17.74	27.5
S3	16.75	3.35	25	9.85	23.7
S6	18.25	3.35	25	11.35	25.2
S12	14.25	3.35	25	7.35	21.2

Confidence level used: 0.95

```
co <- contrast(em, K)
confint(co)
```

contrast	estimate	SE	df	lower.CL	upper.CL
sulfur - control	-9.29	2.74	25	-14.9	-3.657
fall - spring	-6.17	2.74	25	-11.8	-0.532
linear trend	-94.50	34.82	25	-166.2	-22.779

Confidence level used: 0.95



Summary

For this particular data analysis

- Significant differences in means between the groups (ANOVA $F_{6,25} = 3.61$ $p=0.01$)
- Having sulfur was associated with a reduction in scab % of 9 (4,15) compared to no sulfur
- Fall application reduced scab % by 6 (0.5,12) compared to spring application
- Linear trend in sulfur was significant ($p=0.01$)

- Concerned about spatial correlation among columns
- Consider a logarithm of the response
 - CI for F12 (-1.2, 12.7)
 - Non-constant variance (residuals vs predicted, sulfur, application)