Lab 10 – Repeated Measures

October 22 & 23, 2018
FANR 6750

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Overview

Design

- We randomly assign each "subject" to a treatment
- We record the response to the treatment over time

Sources of variation

- Treatment
- Time
- Treatment-time interaction
- Random variation among subjects
- Random variation within subjects

Approaches

Univariate

- This is just a split-plot analysis with adjusted p-values
- Adjustments: Greenhouse-Geisser or Huynh-Feldt methods
- In **R**, you must do a MANOVA to obtain these adjusted P-values

MANOVA

- Testing based on Wilks' lambda or Pillai's trace
- This is usually followed by a profile analysis

Mixed effects model with (ARMA) correlation structure

- This can be done using `lme`
- We might cover this later

The plant data

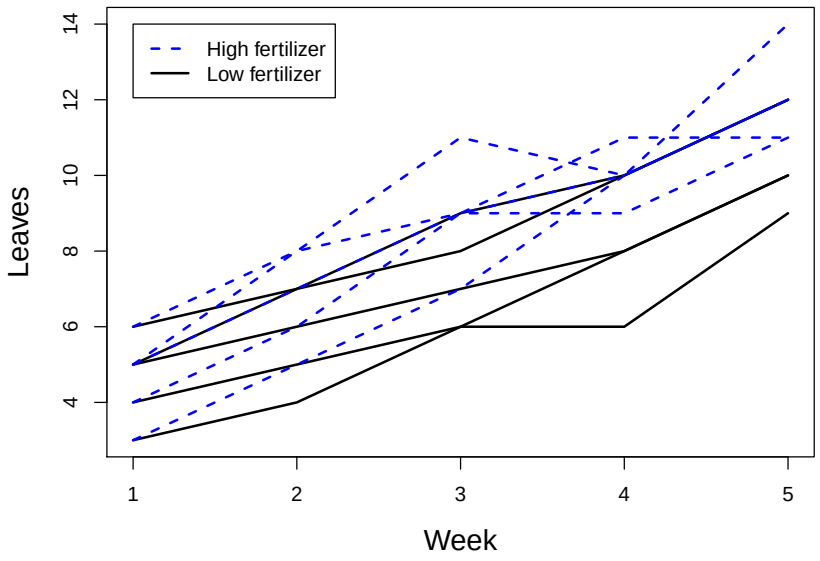
```
plantData <- read.csv("plantData.csv")
plantData$plant <- factor(plantData$plant)
plantData$week <- factor(plantData$week)
str(plantData)

## 'data.frame': 50 obs. of  4 variables:
##  $ plant     : Factor w/ 10 levels "1","2","3","4",..: 1 1 1 1 1 2 2 2 2 2 ...
##  $ fertilizer: Factor w/ 2 levels "H","L": 2 2 2 2 2 2 2 2 2 2 ...
##  $ week      : Factor w/ 5 levels "1","2","3","4",..: 1 2 3 4 5 1 2 3 4 5 ...
##  $ leaves    : int  4 5 6 8 10 3 4 6 6 9 ...
```

```
head(plantData, n=8)

##   plant fertilizer week leaves
## 1     1          L    1      4
## 2     1          L    2      5
## 3     1          L    3      6
## 4     1          L    4      8
## 5     1          L    5     10
## 6     2          L    1      3
## 7     2          L    2      4
## 8     2          L    3      6
```

Leaf Growth



Univariate split-plot approach

```
aov1 <- aov(leaves ~ fertilizer*week + Error(plant),
            data=plantData)
```

```
summary(aov1)

##
## Error: plant
##            Df Sum Sq Mean Sq F value Pr(>F)
## fertilizer  1  16.82   16.82   2.604  0.145
## Residuals   8  51.68    6.46
##
## Error: Within
##                 Df Sum Sq Mean Sq F value Pr(>F)
## week             4 267.40   66.85 158.225 <2e-16 ***
## fertilizer:week  4   5.08    1.27   3.006 0.0326 *
## Residuals       32  13.52    0.42
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

We need to adjust the p-values for the time and interaction effects
In **R**, this requires reformatting the data and running a MANOVA

Format data for MANOVA

```
plantData2 <- reshape(plantData, idvar="plant",
                      timevar="week", v.names="leaves",
                      direction="wide")
```

```
plantData2

##    plant fertilizer leaves.1 leaves.2 leaves.3 leaves.4 leaves.5
## 1      1          L        4        5        6        8       10
## 6      2          L        3        4        6        6        9
## 11     3          L        6        7        9       10       12
## 16     4          L        5        7        8       10       12
## 21     5          L        5        6        7        8       10
## 26     6          H        4        6        9        9       11
## 31     7          H        3        5        7       10       12
## 36     8          H        6        8       11       10       14
## 41     9          H        5        7        9       10       12
## 46    10          H        5        8        9       11       11
```


MANOVA and adjusted P-values

```
manova1 <- manova(cbind(leaves.1, leaves.2, leaves.3,
                        leaves.4, leaves.5) ~ fertilizer,
                  data=plantData2)
```

```
anova(manova1, X=~1, test="Spherical")

## Analysis of Variance Table
##
##
## Contrasts orthogonal to
## ~1
##
## Greenhouse-Geisser epsilon: 0.5882
## Huynh-Feldt epsilon:        0.8490
##
##             Df        F num Df den Df   Pr(>F)   G-G Pr  H-F Pr
## (Intercept)  1 158.2249      4     32 0.000000 0.000000 0.00000
## fertilizer   1   3.0059      4     32 0.032613 0.066622 0.04224
## Residuals    8
```

The last 3 columns are p-values corresponding to the effects of time (Intercept) and interaction (fertilizer). No adjustment is necessary for the main effect so you can use the p-value from `aov`.

An aside – sphericity

Technically, adjusted p-values and MANOVA aren't necessary if the assumption of sphericity holds. However, we recommend doing the adjustments (or the MANOVA) anyway because the test of sphericity has low power.

Sphericity is the multivariate analogue of the homogeneity of variance assumption of ANOVA.

Here is how you test the assumption:

```
mauchly.test(manova1, X=~1)

##
##  Mauchly's test of sphericity
##  Contrasts orthogonal to
##  ~1
##
##
## data:  SSD matrix from manova(cbind(leaves.1, leaves.2, leaves.3, leaves.4, lea
## W = 0.099297, p-value = 0.1062
```

We fail to reject the null hypothesis, so sphericity can be assumed.

Compare aov and manova results

```
summary(aov1)

##
## Error: plant
##           Df Sum Sq Mean Sq F value Pr(>F)
## fertilizer  1  16.82   16.82   2.604  0.145
## Residuals   8  51.68    6.46
##
## Error: Within
##                Df Sum Sq Mean Sq F value Pr(>F)
## week            4 267.40   66.85 158.225 <2e-16 ***
## fertilizer:week  4   5.08    1.27   3.006 0.0326 *
## Residuals      32  13.52    0.42
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
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## Residuals    8
```


Multivariate tests – Wilks' lambda

An alternative to the adjusted p-value approach is to do a multivariate analysis relaxing the assumptions about the structure of the variance-covariance matrix. We're already most of the way there.

```
anova(manova1, X=~1, test="Wilks")

## Analysis of Variance Table
##
##
## Contrasts orthogonal to
## ~1
##
##             Df    Wilks approx F num Df den Df    Pr(>F)
## (Intercept)  1 0.008487  146.042      4      5 2.308e-05 ***
## fertilizer   1 0.144772    7.384      4      5   0.02503 *
## Residuals    8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

As before, we conclude that the effect of fertilizer changes over time

Multivariate tests – Pillai's trace

Pillai's trace is an alternative to Wilks' lambda. In this case, it returns the same p-values as Wilks' test.

```
anova(manova1, X=~1, test="Pillai")

## Analysis of Variance Table
##
##
## Contrasts orthogonal to
## ~1
##
##             Df  Pillai approx F num Df den Df    Pr(>F)
## (Intercept)  1 0.99151  146.042      4      5 2.308e-05 ***
## fertilizer   1 0.85523    7.384      4      5   0.02503 *
## Residuals    8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Profile analysis

Profile analysis requires calculating the differences (ie, the number of leaves grown each week).

```
manova2 <- manova(
    cbind(leaves.2-leaves.1, leaves.3-leaves.2,
          leaves.4-leaves.3, leaves.5-leaves.4) ~
    fertilizer, data=plantData2)
```


Profile Analysis

During which intervals do the growth rates differ?

```
summary.aov(manova2)

##  Response 1 :
##             Df Sum Sq Mean Sq F value  Pr(>F)
## fertilizer   1    2.5     2.5    12.5 0.00767 **
## Residuals    8    1.6     0.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##  Response 2 :
##             Df Sum Sq Mean Sq F value Pr(>F)
## fertilizer   1    1.6     1.6     3.2 0.1114
## Residuals    8    4.0     0.5
##
##  Response 3 :
##             Df Sum Sq Mean Sq F value Pr(>F)
## fertilizer   1    0.1     0.1  0.0625 0.8089
## Residuals    8   12.8     1.6
##
##  Response 4 :
##             Df Sum Sq Mean Sq F value Pr(>F)
## fertilizer   1    0.1     0.1  0.0909 0.7707
## Residuals    8    8.8     1.1
```


Plot the growth rates

Calculate mean growth rate for each time interval

```
leavesMat <- plantData2[,3:7]
growthMat <- leavesMat[,2:5] - leavesMat[,1:4]
colnames(growthMat) <- paste("interval", 1:4, sep=".")
(lowFertilizer <- colMeans(growthMat[1:5,]))

## interval.1 interval.2 interval.3 interval.4
##        1.2        1.4        1.2        2.2

(highFertilizer <- colMeans(growthMat[6:10,]))

## interval.1 interval.2 interval.3 interval.4
##        2.2        2.2        1.0        2.0
```

Calculate the standard errors for these growth rates

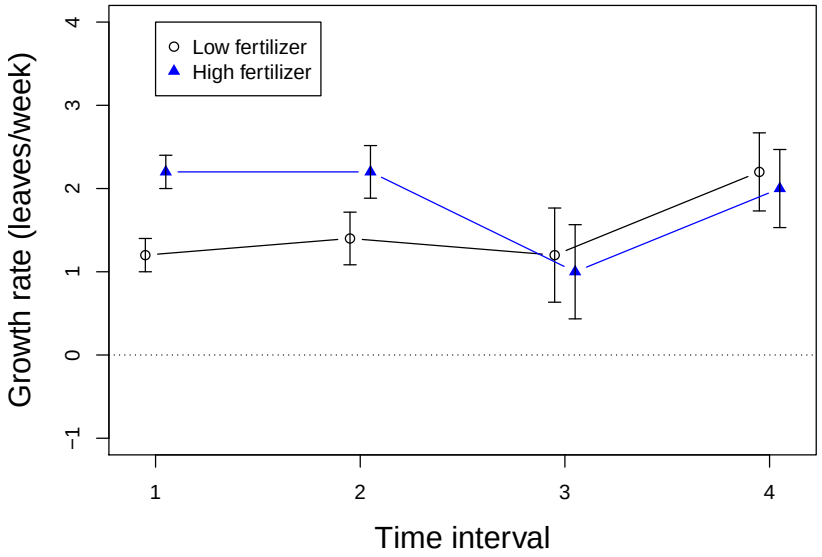
```
SE <- sqrt(diag(stats:::vcov.mlm(manova2)))
SE <- SE[names(SE)==":(Intercept)"] # Only use "intercept" SEs
unname(SE) ## Ignore the names

## [1] 0.2000000 0.3162278 0.5656854 0.4690416
```


Plot the growth rates

```
plot(1:4-0.05, lowFertilizer, type="b", xlim=c(0.9, 4.1),
     ylim=c(-1, 4), xaxp=c(1,4,3), cex.lab=1.5,
     xlab="Time interval", ylab="Growth rate (leaves/week)")
abline(h=0, lty=3)
arrows(1:4-.05, lowFertilizer-SE, 1:4-.05, lowFertilizer+SE,
       angle=90, code=3, length=0.05)
lines(1:4+0.05, highFertilizer, type="b", pch=17, col=4)
arrows(1:4+0.05, highFertilizer-SE, 1:4+0.05,
       highFertilizer+SE, angle=90, code=3, length=0.05)
legend(1, 4, c("Low fertilizer", "High fertilizer"),
       col=c("black", "blue"), pch=c(1,17))
```

Plot the growth rates



Assignment

A researcher wants to assess the effects of crowding on the growth of the dark toadfish (*Neophrynichthys latus*). 15 fish tanks are stocked with three densities of conspecifics. Five tanks have low density (1 fish), 5 tanks have medium density (5 fish), and 5 tanks have high density (10 fish). In each tank, the weight of one "focal fish" is recorded on 6 consecutive weeks. The data are in the file `fishData.csv`.

(1) Conduct the univariate repeated measures ANOVA using `aov`. Calculate the adjusted p-values using the Huynh-Feldt method. Does the effect of density change over time?

(2) Conduct a multivariate repeated measures ANOVA and use Wilks' lambda to test if the effect of density changes over time. What is your conclusion?

(3) Conduct a profile analysis. In which time intervals is the effect of density on growth rate significant?

Upload your self-contained R-script to ELC at least one day before your next lab