Pigs in a bucket combined analyses

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Garbage can analyses

This was an experiment designed to explore how carrion biomass and vertebrate scavenegers modulate decomposition rates. Individual pig carcasses were exposed to other decomposing carrion at increasing levels of biomass with and without vertebrate exclusion. After a time period, these individual carcasses werer returned to This is a factorial design crossing exposure biomass with vertebrate exclusion. There are six observations, one for each combination of carrion biomass and vertebrate exclusion (i.e., no replication).

The permutation based anova essentially rerranges the values of variables many times to generate a distribution of the test statistic. This test statistic is F for anova (or pseudo-f here), which is a ratio of between and among group variation. We need an approach that fits our data and goals: a nonparametric, permutation-based ancova-like analysis fit for saturated models.Section 2.6 of the supporting document for the lmPerm package provides such an anlysis (Wheeler 2016). The result of this analysis is significant for both treatments and their interaction.

outstanding question:

How do I choose between lmp vs aovp? The results do not agree.

Load packages and bring in the data

```
## -- Attaching packages ------------------ tidyverse 1.3.0 --

## v ggplot2 3.3.0     v purrr   0.3.3
## v tibble  3.0.3     v dplyr   0.8.3
## v tidyr   1.0.0     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.4.0

## Warning: package 'tibble' was built under R version 3.6.2

## -- Conflicts --------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()

## Warning: package 'RVAideMemoire' was built under R version 3.6.2

## *** Package RVAideMemoire v 0.9-79 ***

## Simple Bootstrap Routines (1.1-7)
```

```
## Package 'sm', version 2.2-5.6: type help(sm) for summary information

## Warning: package 'multcomp' was built under R version 3.6.2

## Loading required package: mvtnorm

## Loading required package: survival

## Warning: package 'survival' was built under R version 3.6.2

## Loading required package: TH.data

## Loading required package: MASS

##
## Attaching package: 'MASS'

## The following object is masked from 'package:sm':
##
##     muscle

## The following object is masked from 'package:dplyr':
##
##     select

##
## Attaching package: 'TH.data'

## The following object is masked from 'package:MASS':
##
##     geyser

## The following object is masked from 'package:sm':
##
##     geyser

## Loading required package: carData

##
## Attaching package: 'car'

## The following object is masked from 'package:dplyr':
##
##     recode

## The following object is masked from 'package:purrr':
##
##     some
```

Calculate the change in biomass

```r
d <- d.tmp %>%
    mutate(LOST_BIO = abs(PIG_FINAL-PIG_START))
```

Final model using ancova with permutation

```r
mod3 <- lmp(LOST_BIO~FENCE*EXPOSURE, data=d)
```

```
## [1] "Settings:  unique SS : numeric variables centered"
```

```r
summary(mod3)
```

```
##
## Call:
## lmp(formula = LOST_BIO ~ FENCE * EXPOSURE, data = d)
##
## Residuals:
##        1        2        3        4        5        6
##  0.10501 -0.13520  0.03019 -0.11094  0.14283 -0.03189
##
## Coefficients:
##                 Estimate Pr(Exact)
## FENCE1             1.190   0.00556 **
## EXPOSURE           0.003   0.00278 **
## FENCE1:EXPOSURE    0.001   0.01944 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1788 on 2 degrees of freedom
## Multiple R-Squared: 0.998,   Adjusted R-squared: 0.995
## F-statistic: 330.7 on 3 and 2 DF,  p-value: 0.003017
```

```r
summary(mod3)$r.squared
```

```
## [1] 0.997988
```

```r
Anova(mod3)
```

```
## Anova Table (Type II tests)
##
## Response: LOST_BIO
##                 Sum Sq Df F value   Pr(>F)
## FENCE           8.4966  1 265.767 0.003742 **
## EXPOSURE       21.8033  1 681.990 0.001463 **
## FENCE:EXPOSURE  1.4149  1  44.257 0.021857 *
## Residuals       0.0639  2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Fence increased biomass lost by 1.19 kg
# 1 kg increase in biomass exposure increased biomass lost by 0.003 kg
# That slope is 0.001 higher in Fence (0.004 kg)

sem <- function(x, na.rm=FALSE){
        out <- sd(x, na.rm = na.rm)/sqrt(length(x))
        return(out)
}

d %>% group_by(FENCE) %>%
    summarise(mean = mean(LOST_BIO),
                          sd = mean(LOST_BIO),
                          se = sem(LOST_BIO))
```

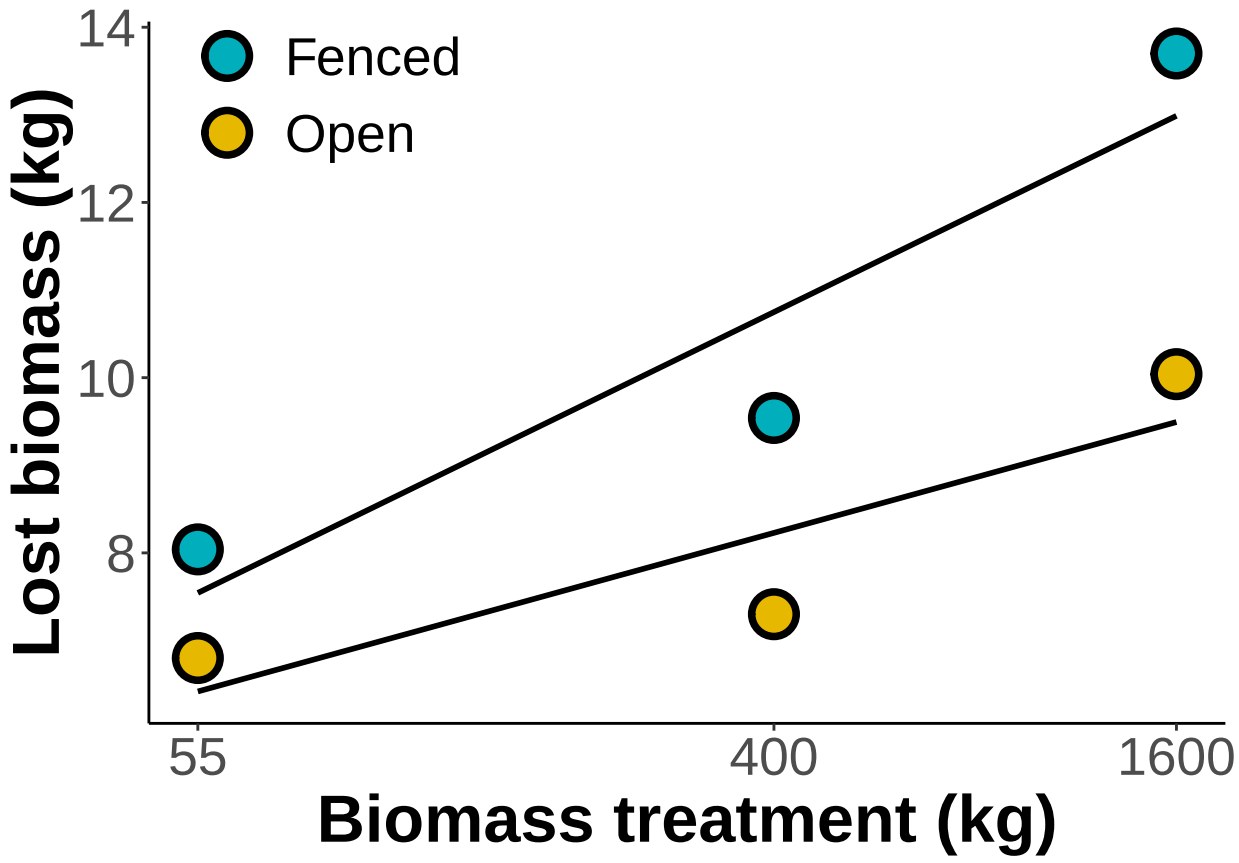
```
## # A tibble: 2 x 4
##   FENCE  mean    sd    se
##   <fct> <dbl> <dbl> <dbl>
## 1 fence 10.4  10.4   1.69
## 2 open   8.05  8.05  1.01
```

```
d %>% group_by(EXPOSURE) %>%
    summarise(mean = mean(LOST_BIO),
                          sd = mean(LOST_BIO),
                          se = sem(LOST_BIO))
```

```
## # A tibble: 3 x 4
##   EXPOSURE  mean    sd    se
##      <int> <dbl> <dbl> <dbl>
## 1       55  7.42  7.42 0.620
## 2      400  8.42  8.42 1.12
## 3     1600 11.9  11.9  1.83
```

Visualize the model

```
## `geom_smooth()` using formula 'y ~ x'
```




```
## `geom_smooth()` using formula 'y ~ x'
```

The y axis is the absolute value in change in biomass, and the x axis is log-transformed. The labels are back-transformed for interpretability.

Fly trapping analyses

In the original field experiment (i.e., not the additional carcassses referenced in the analysis above), the researchers trapped flies to demonstrate the difference in abundance days/weeks sost-deployment associated with biomass and the exclusion of vertebrate scavengers (Fenced).

I devloped a model with biomass (continuous) and exclusion (categorical) as fixed effects. In this experiment, we are not interested in the effect of time on fly abundance, beyond the need to account for variance in the model. Thus, I set date as a random effect.

Load packages and bring in the data

```
library(tidyverse)
library(lme4)
```

```
## Warning: package 'lme4' was built under R version 3.6.2
```

```
## Loading required package: Matrix
```

```
##
## Attaching package: 'Matrix'

## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack

## Registered S3 methods overwritten by 'lme4':
##   method                          from
##   cooks.distance.influence.merMod car
##   influence.merMod                car
##   dfbeta.influence.merMod         car
##   dfbetas.influence.merMod        car

##
## Attaching package: 'lme4'

## The following object is masked from 'package:RVAideMemoire':
##
##     dummy
```

```
library(lubridate)
```

```
##
## Attaching package: 'lubridate'

## The following object is masked from 'package:base':
##
##     date
```

```
library(car)
library(DHARMa)
```

```
## Warning: package 'DHARMa' was built under R version 3.6.2

## This is DHARMa 0.3.4. For overview type '?DHARMa'. For recent changes, type news(package = 'DHARMa')
```

```
library(emmeans)
library(MuMIn)

d <- read.csv("Data/flies.csv")
head(d)
```

```
##   Day Month Year Site Pig Fence Duration Biomass Obs1tot   Obs1rate Obs2tot
## 1  13  July 2016    1   Y     F     1440      55      29 0.02013889      44
## 2  13  July 2016    2   Y     F     1440     130      68 0.04722222     102
## 3  13  July 2016    3   Y     F     1440     400     128 0.08888889     112
## 4  13  July 2016    4   Y     F     1440     800     263 0.18263889     207
## 5  13  July 2016    5   Y     F     1440    1600     294 0.20416667     251
## 6  13  July 2016    1   Y     0     1440      55      36 0.02500000      27
```

```
##     Obs2rate Obs3tot Obs3rate Obs4tot Obs4rate    SUMrate NumObs    AVGrate
## 1 0.03055556      NA       NA      NA       NA 0.05069444      2 0.02534722
## 2 0.07083333      NA       NA      NA       NA 0.11805556      2 0.05902778
## 3 0.07777778      NA       NA      NA       NA 0.16666667      2 0.08333333
## 4 0.14375000      NA       NA      NA       NA 0.32638889      2 0.16319444
## 5 0.17430556      NA       NA      NA       NA 0.37847222      2 0.18923611
## 6 0.01875000      NA       NA      NA       NA 0.04375000      2 0.02187500
```

```
summary(d)
```

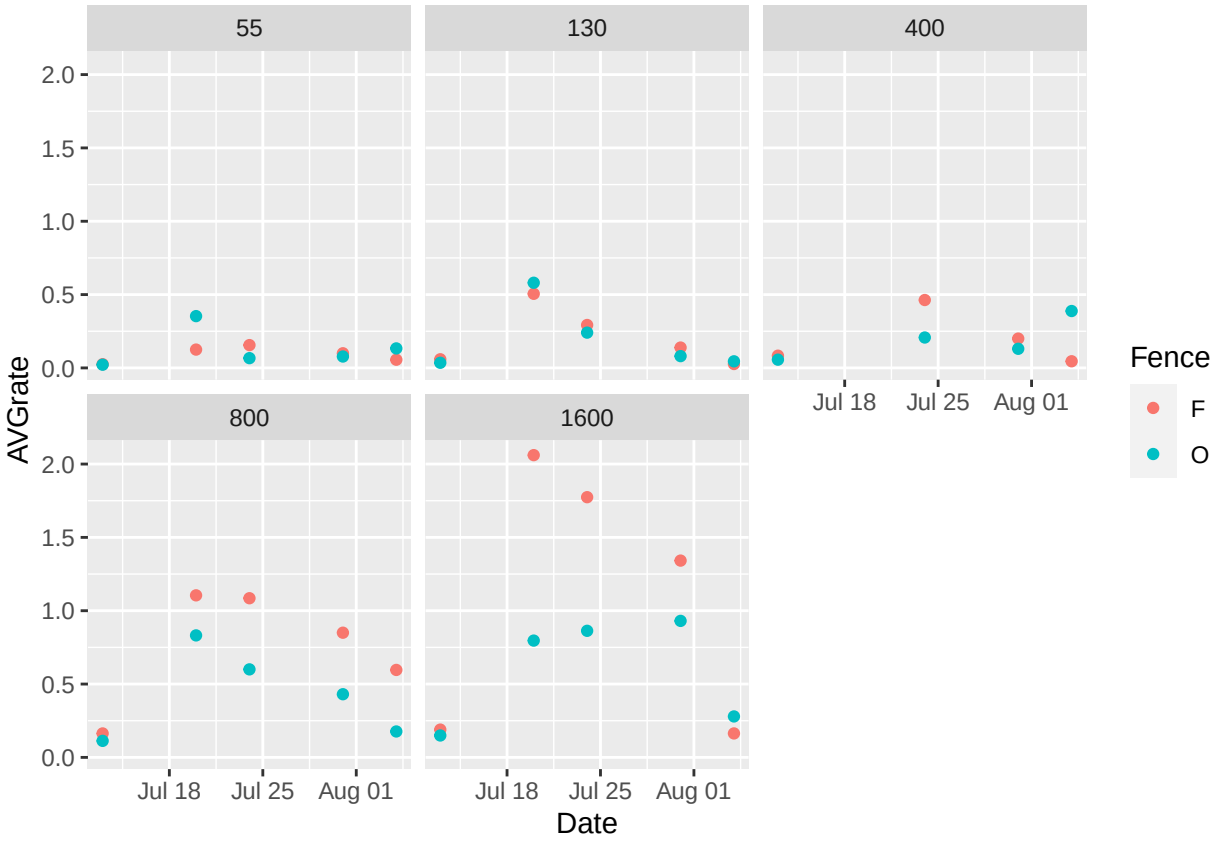
```
##       Day           Month         Year           Site   Pig    Fence
##  Min.   : 4.0   August:10   Min.   :2016   Min.   :1   Y:50   F:25
##  1st Qu.:13.0   July  :40   1st Qu.:2016   1st Qu.:2          O:25
##  Median :20.0               Median :2016   Median :3
##  Mean   :18.4               Mean   :2016   Mean   :3
##  3rd Qu.:24.0               3rd Qu.:2016   3rd Qu.:4
##  Max.   :31.0               Max.   :2016   Max.   :5
##
##     Duration         Biomass        Obs1tot          Obs1rate
##  Min.   :  29.0   Min.   :  55   Min.   :  0.00   Min.   :0.00000
##  1st Qu.:  90.0   1st Qu.: 130   1st Qu.: 14.00   1st Qu.:0.07284
##  Median : 103.5   Median : 400   Median : 29.50   Median :0.17515
##  Mean   : 382.5   Mean   : 597   Mean   : 60.29   Mean   :0.36570
##  3rd Qu.: 165.5   3rd Qu.: 800   3rd Qu.: 87.50   3rd Qu.:0.48429
##  Max.   :1440.0   Max.   :1600   Max.   :294.00   Max.   :1.74444
##  NA's   :2                       NA's   :2        NA's   :2
##     Obs2tot          Obs2rate          Obs3tot          Obs3rate
##  Min.   :  0.00   Min.   :0.00000   Min.   :  0.00   Min.   :0.0000
##  1st Qu.:  8.75   1st Qu.:0.05623   1st Qu.: 13.75   1st Qu.:0.1626
##  Median : 30.00   Median :0.15584   Median : 37.00   Median :0.3556
##  Mean   : 59.96   Mean   :0.36116   Mean   : 56.32   Mean   :0.5558
##  3rd Qu.: 88.50   3rd Qu.:0.46353   3rd Qu.: 79.25   3rd Qu.:0.7839
##  Max.   :251.00   Max.   :2.07778   Max.   :352.00   Max.   :3.0609
##  NA's   :2        NA's   :2         NA's   :12       NA's   :12
##     Obs4tot          Obs4rate          SUMrate          NumObs
##  Min.   :  2.00   Min.   :0.02000   Min.   :0.0000   Min.   :0.00
##  1st Qu.:  4.75   1st Qu.:0.03306   1st Qu.:0.2239   1st Qu.:3.00
##  Median : 14.50   Median :0.15556   Median :0.5889   Median :3.00
##  Mean   : 30.85   Mean   :0.34289   Mean   :1.2573   Mean   :3.08
##  3rd Qu.: 34.25   3rd Qu.:0.42244   3rd Qu.:1.7370   3rd Qu.:4.00
##  Max.   :126.00   Max.   :1.40000   Max.   :6.1826   Max.   :4.00
##  NA's   :30       NA's   :30
##     AVGrate
##  Min.   :0.02187
##  1st Qu.:0.08264
##  Median :0.18294
##  Mean   :0.39981
##  3rd Qu.:0.58452
##  Max.   :2.06087
##  NA's   :2
```

```
d$Date <- paste(d$Year, d$Month, d$Day, sep="-") %>% ymd() %>% as.Date()
```

Visualize the data

```
ggplot(d, aes(y=AVGrate, x=Date, color=Fence))+
    geom_point()+
    facet_wrap(~Biomass)
```

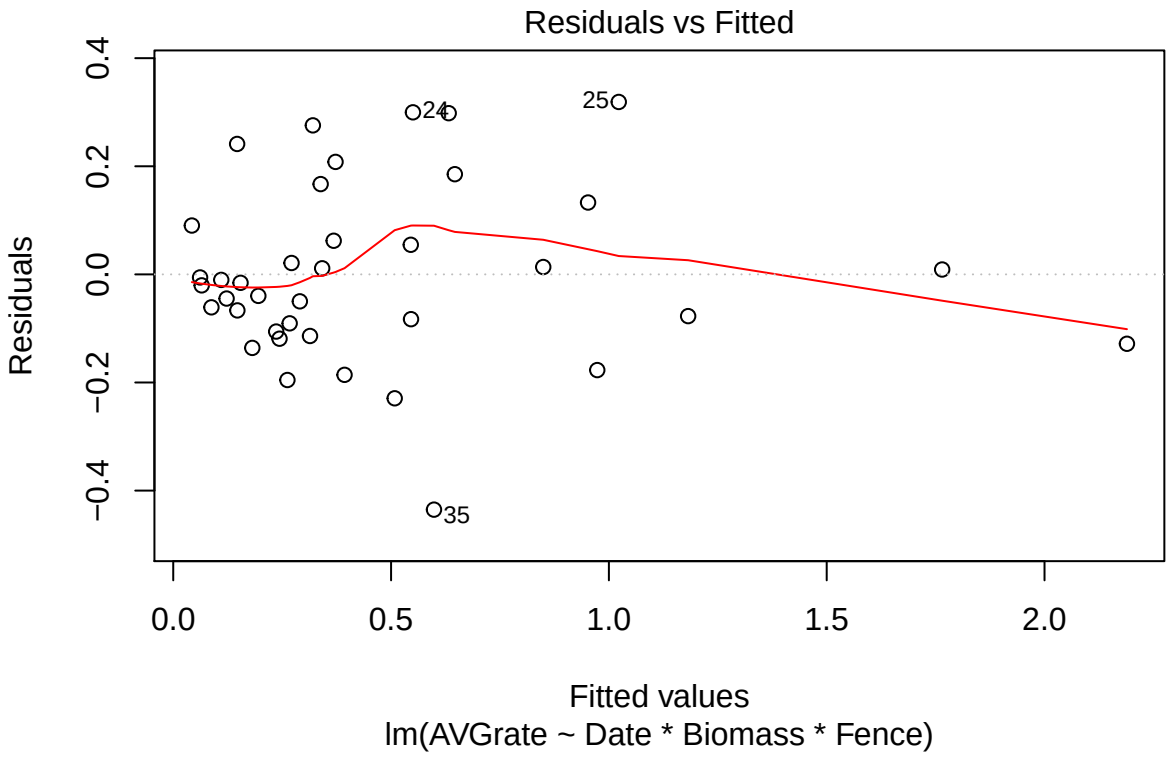
```
## Warning: Removed 2 rows containing missing values (geom_point).
```

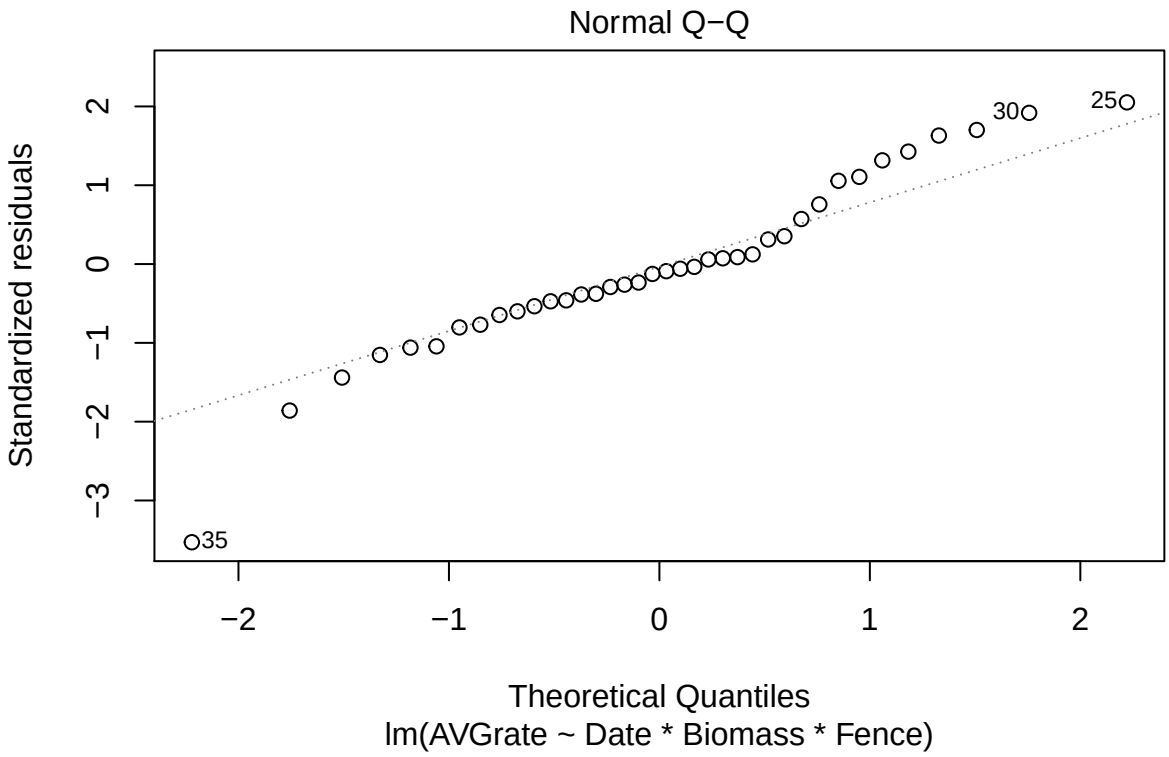


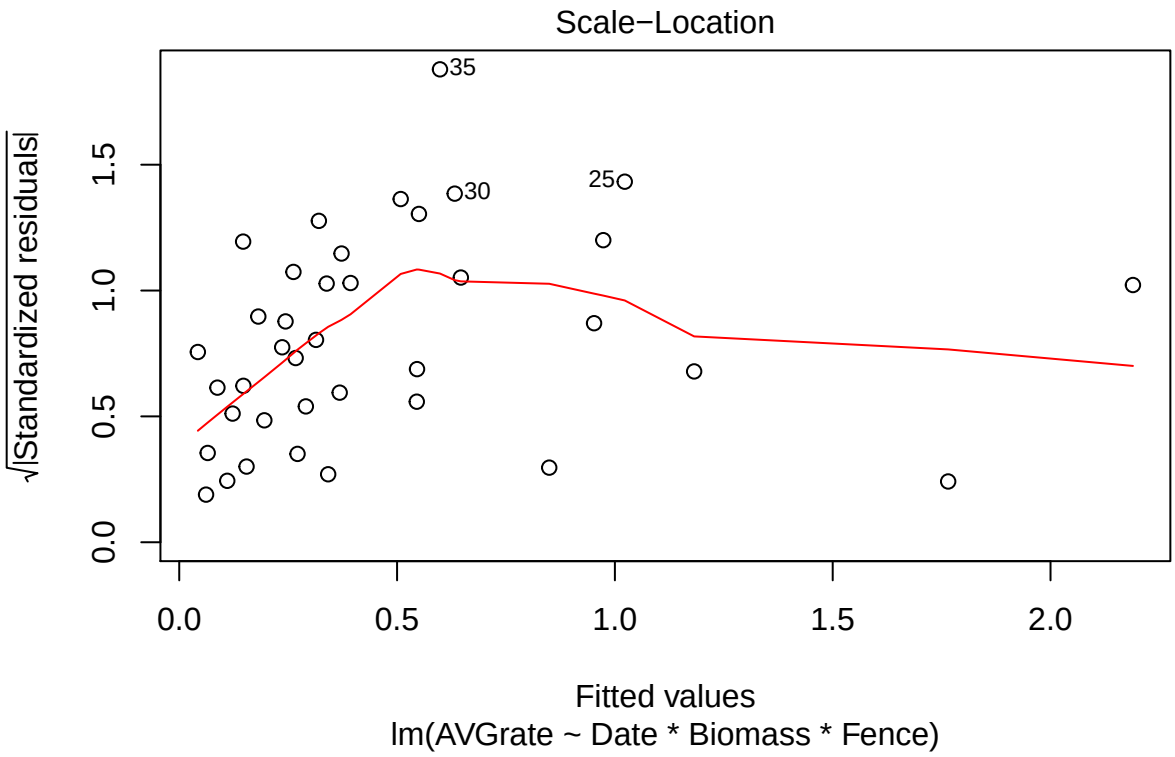
Model the interacton between fencing and the relationship between biomass and flies trapped

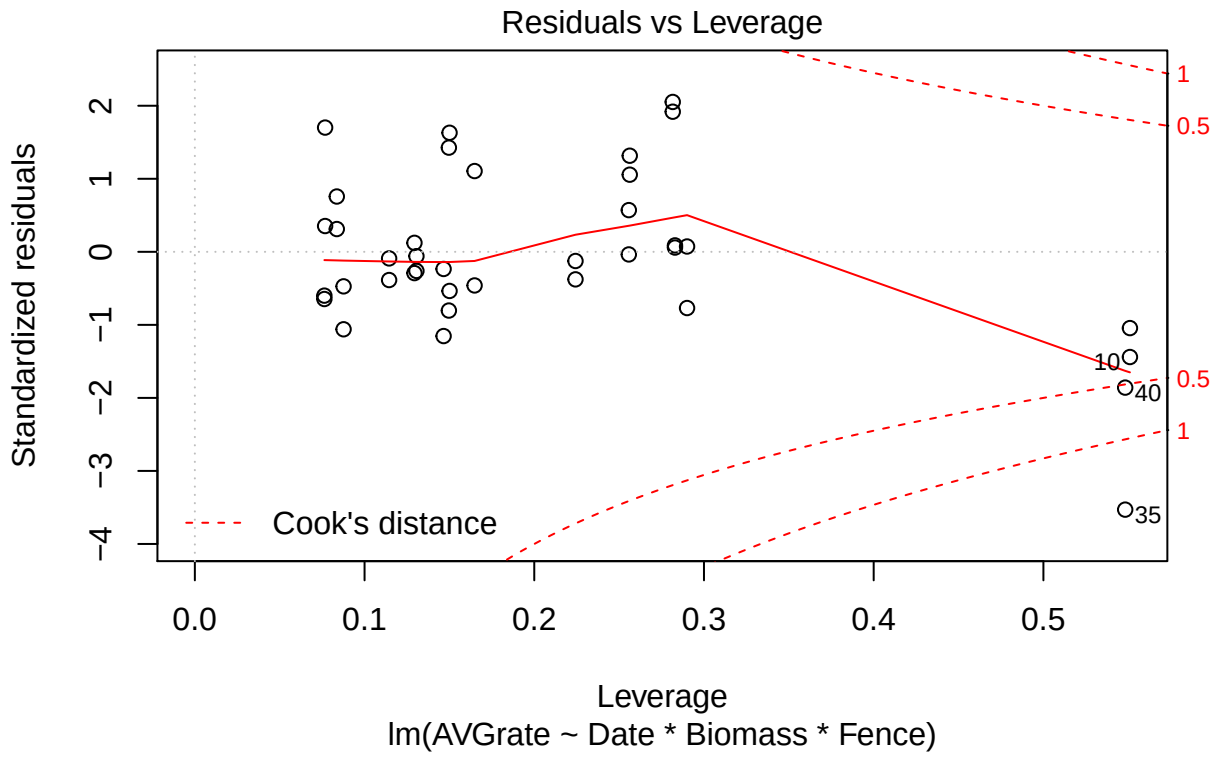
```
d.tmp <- filter(d, !Date=='2016-07-13')

# Simple model
mod3 <- lm(AVGrate ~ Date * Biomass * Fence, data = d.tmp)
plot(mod3)
```

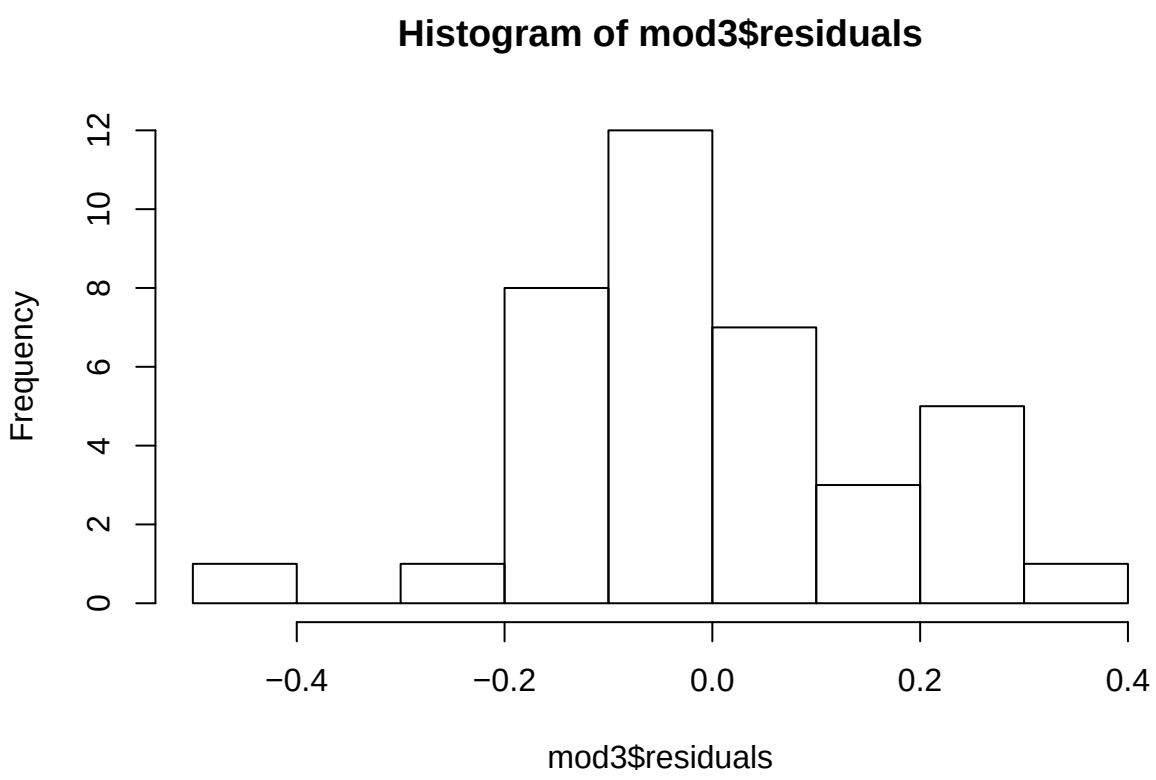

Residuals vs Fitted
Residuals
0.4
0.2
0.0
−0.2
−0.4
24
25
35
0.0
0.5
1.0
1.5
2.0
Fitted values
lm(AVGrate ~ Date * Biomass * Fence)


Normal Q−Q
Standardized residuals
Theoretical Quantiles
lm(AVGrate ~ Date * Biomass * Fence)
35
30
25
−3
−2
−1
0
1
2


Scale−Location
35
30
25
√|Standardized residuals|
0.0
0.5
1.0
1.5
0.0
0.5
1.0
1.5
2.0
Fitted values
lm(AVGrate ~ Date * Biomass * Fence)




```
hist(mod3$residuals, breaks=10)
```

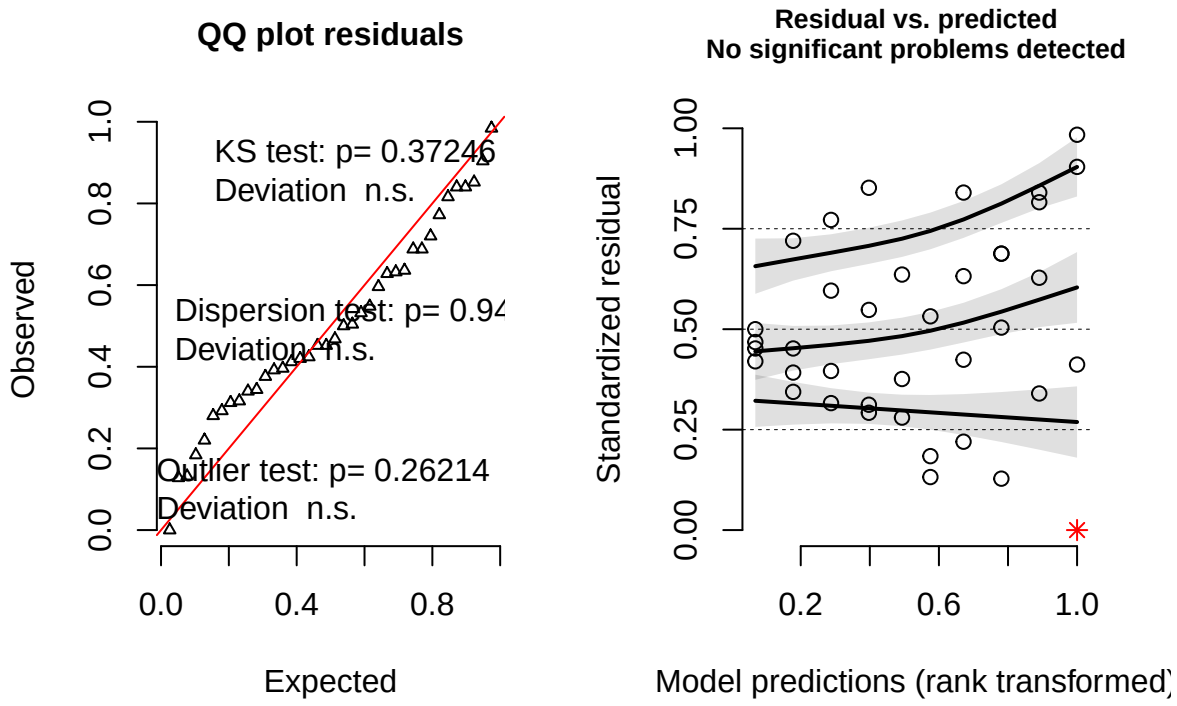



```
anova(mod3)
```

```
## Analysis of Variance Table
##
## Response: AVGrate
##                    Df Sum Sq Mean Sq  F value    Pr(>F)
## Date                1 1.6704  1.6704  49.6539 7.821e-08 ***
## Biomass             1 4.2316  4.2316 125.7862 2.964e-12 ***
## Fence               1 0.3948  0.3948  11.7349 0.0017980 **
## Date:Biomass        1 0.4991  0.4991  14.8368 0.0005728 ***
## Date:Fence          1 0.1711  0.1711   5.0870 0.0315573 *
## Biomass:Fence       1 0.6296  0.6296  18.7151 0.0001548 ***
## Date:Biomass:Fence  1 0.3110  0.3110   9.2442 0.0048675 **
## Residuals          30 1.0092  0.0336
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Mixed effects model
mod4 <- lmer(AVGrate ~ Biomass * Fence + (1|Date), data = d.tmp)

mod4.sim <- simulateResiduals(fittedModel = mod4, n = 250)
plot(mod4.sim)
```




```
Anova(mod4)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: AVGrate
##                 Chisq Df Pr(>Chisq)
## Biomass       68.8248  1  < 2.2e-16 ***
## Fence          6.4084  1   0.011358 *
## Biomass:Fence 10.4785  1   0.001208 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Model results

```
## emmeans, correcting for bias (only needed for GLMMs) by calculating the total SD of the random effec
modSD <- VarCorr(mod4) %>% as.data.frame() %>% summarize(totSD=sum(vcov)) %>% mutate(totSD=sqrt(totSD))

emmeans(mod4, pairwise~Fence, type="response", bias.adj=T, sigma=modSD$totSD)
```

```
## NOTE: Results may be misleading due to involvement in interactions

## $emmeans
##  Fence emmean    SE  df lower.CL upper.CL
```

```
##  F      0.596 0.127 3.7    0.231    0.961
##  O      0.392 0.127 3.7    0.027    0.758
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate     SE df t.ratio p.value
##  F - O       0.204 0.0805 31 2.531   0.0166
##
## Degrees-of-freedom method: kenward-roger
```

```
# flies caught per minute is 0.204 (SE = 0.0805) higher in fence than control
# fence mean is 0.596 (LCL = 0.231, UCL = 0.961)
# open mean is 0.392 (LCL = 0.027, UCL = 0.758)
emtrends(mod4, pairwise~Fence, var = 'Biomass')
```

```
## $emtrends
##  Fence Biomass.trend       SE df lower.CL upper.CL
##  F          0.000804 9.85e-05 31 0.000603 0.001004
##  O          0.000353 9.85e-05 31 0.000152 0.000554
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate       SE df t.ratio p.value
##  F - O    0.000451 0.000139 31 3.237   0.0029
##
## Degrees-of-freedom method: kenward-roger
```

```
# each 1 kg of biomass increased mean flys caught per minute by 0.000451
# (SE = 0.000139) for fence in comparison to control.
# fence per unit (kg) increase in flies is 0.000804 (LCL = 0.000603, UCL = 0.001004)
# open per unit (kg) increase in flies is 0.000353 (LCL = 0.000152, UCL = 0.000554)

r.squaredGLMM(mod4) # fixed efects = 0.557324, entire model = 0.759619
```

```
## Warning: 'r.squaredGLMM' now calculates a revised statistic. See the help page.
```

```
##           R2m      R2c
## [1,] 0.557324 0.759619
```

```
VarCorr(mod4) # random effects = 0.22769, unexplained variance = 0.24820
```

```
##  Groups   Name        Std.Dev.
##  Date     (Intercept) 0.22769
##  Residual             0.24820
```

Visualize the model

```r
d.NA <- d.tmp[!is.na(d.tmp$AVGrate),]

sem <- function(x, na.rm = FALSE) {
  out <-sd(x, na.rm = na.rm)/sqrt(length(x))
  return(out)
}

d.means <- d.NA %>%
                              group_by(Biomass,Fence) %>%
                              summarise(mean = mean(AVGrate*60),
                                  sd = sd(AVGrate*60),
                                  se = sem(AVGrate*60))

# Get values for back-transformed axis labels
log(55) # 4.007333
```

```
## [1] 4.007333
```

```r
log(130) # 4.867534
```

```
## [1] 4.867534
```

```r
log(400) # 5.991465
```

```
## [1] 5.991465
```

```r
log(800) # 6.684612
```

```
## [1] 6.684612
```

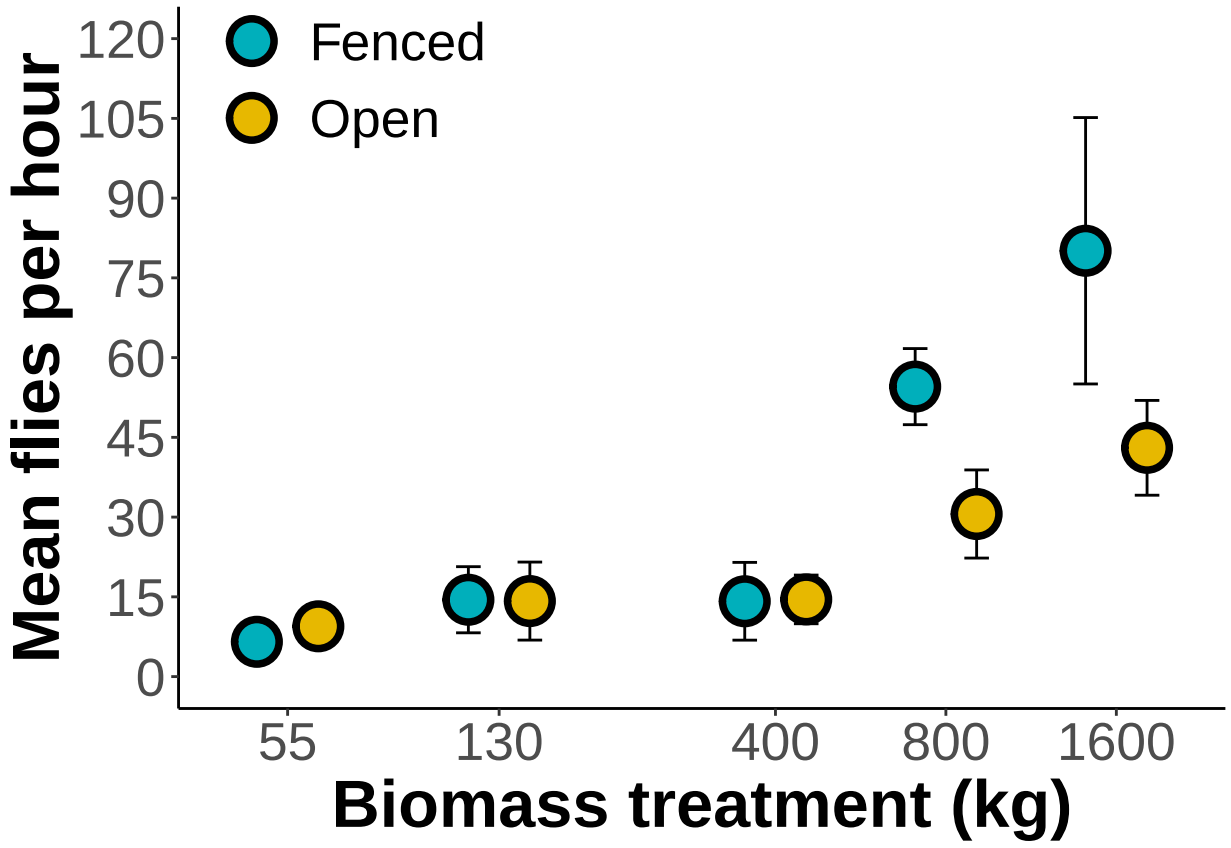
```r
log(1600) # 7.377759
```

```
## [1] 7.377759
```

```r
levels(d.means$Fence) <- c("Fenced","Open")

ggplot(d.means, aes(x=log(Biomass),y=mean,color=Fence,fill=Fence))+
    geom_errorbar(aes(ymin=mean-se,ymax=mean+se),
                                  position = position_dodge(width = 0.5),color='black', width=0.2)+
    geom_point(size=6.5,shape=21,stroke=2,position=position_dodge(width = 0.5),color='black')+
    scale_color_manual(values=c("#00AFBB", "#E7B800"))+
    scale_fill_manual(values=c("#00AFBB", "#E7B800"))+
    scale_x_continuous(name="Biomass treatment (kg)",
                                          breaks=c(4.007333,4.867534,5.991465, 6.684612, 7.377759),
                                          labels=c("55", "130", "400", "800", "1600"))+
    scale_y_continuous(name="Mean flies per hour",
                                          breaks=c(0.0*60,0.25*60,0.50*60,0.75*60,1.00*60,1.25*60,
                                                   1.5*60,1.75*60,2*60),
                                          limits = c(0,2*60))+
```

```
    theme_classic()+
    theme(legend.title = element_blank())+
    theme(legend.position = c(0.17, 0.92))+
    theme(text = element_text(size = 25))+
    theme(axis.title.x = element_text(face="bold"))+
    theme(axis.title.y = element_text(face="bold", vjust=0.7))+
     guides(fill=guide_legend(
                keyheight=0.4,
                default.unit="inch"))
```




```
ggsave('flies_drop_first.jpg', width = 6, height = 6, dpi = 300)
```