

Analysis of the in vivo experiments

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2018-01-18

Contents

0.1	Loading packages	1
0.2	Data input and handling	2
0.2.1	Subsetting the data	2
0.2.2	Converting Animals to factor to fit the GLMM	3
0.3	Exploratory data analysis	3
0.3.1	Males Redness	3
0.3.2	Males Darkness	3
0.3.3	Female Darkness	4
0.3.4	Plotting the distribution of the data	5
1	Statisticsl analysis	5
1.1	Modelling Redness	5
1.1.1	Model diagnostics	5
1.1.2	Inference	6
1.2	Modelling Darkness	10
1.2.1	Diagnostics	10
1.2.2	Inference	10
1.3	Darkness	11
1.3.1	Checking model assumptions	11
1.3.2	Inference	12

```
R.Version()$version.string; R.Version()$platform
```

```
## [1] "R version 3.4.3 (2017-11-30)"
```

```
## [1] "x86_64-apple-darwin15.6.0"
```

```
set.seed(1001)#reproducibility
```

0.1 Loading packages

```
library(sjPlot)
```

```
packageVersion("sjPlot")
```

```
## [1] '2.3.2'
```

```
library(tidyverse)
```

```
packageVersion("tidyverse")
```

```
## [1] '1.1.1'
```

```
library(sciplot)
```

```
packageVersion("sciplot")
```

```
## [1] '1.1.1'
```

```
library(lsmeans)
```

```
packageVersion("lsmeans")
```

```
## [1] '2.26.3'
```

```
library(glmADMB)
packageVersion("glmADMB")
```

```
## [1] '0.8.5'
```

```
library(R2admb)
packageVersion("R2admb")
```

```
## [1] '0.7.16'
```

```
library(lme4)
packageVersion("lme4")
```

```
## [1] '1.1.13'
```

0.2 Data input and handling

```
data<-read.csv2("fish.csv", h=T)
head(data);str(data)
```

```
##   Animal Treatment Time Sex Darkness Redness
## 1      1         CT  0h  M 56.41569 131.17
## 2      1         CT  1h  M 49.52549 133.30
## 3      1         CT  2h  M 54.94118 132.69
## 4      1         CT  3h  M 42.18824 135.37
## 5      2         CT  0h  M 58.93333 133.35
## 6      2         CT  1h  M 52.45490 133.65

## 'data.frame':   80 obs. of  6 variables:
##  $ Animal   : int  1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","YOH": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Time      : Factor w/ 4 levels "0h","1h","2h",...: 1 2 3 4 1 2 3 4 1 2 ...
##  $ Sex       : Factor w/ 2 levels "F","M": 2 2 2 2 2 2 2 2 2 2 ...
##  $ Darkness  : num  56.4 49.5 54.9 42.2 58.9 ...
##  $ Redness   : num  131 133 133 135 133 ...
```

0.2.1 Subsetting the data

```
rednessmale<- filter(data,Sex=="M")
head(rednessmale)
```

```
##   Animal Treatment Time Sex Darkness Redness
## 1      1         CT  0h  M 56.41569 131.17
## 2      1         CT  1h  M 49.52549 133.30
## 3      1         CT  2h  M 54.94118 132.69
## 4      1         CT  3h  M 42.18824 135.37
## 5      2         CT  0h  M 58.93333 133.35
## 6      2         CT  1h  M 52.45490 133.65
```

```
darknessmale<- filter(data, Sex=="M")
darknessfemale<- filter(data, Sex=="F")
```

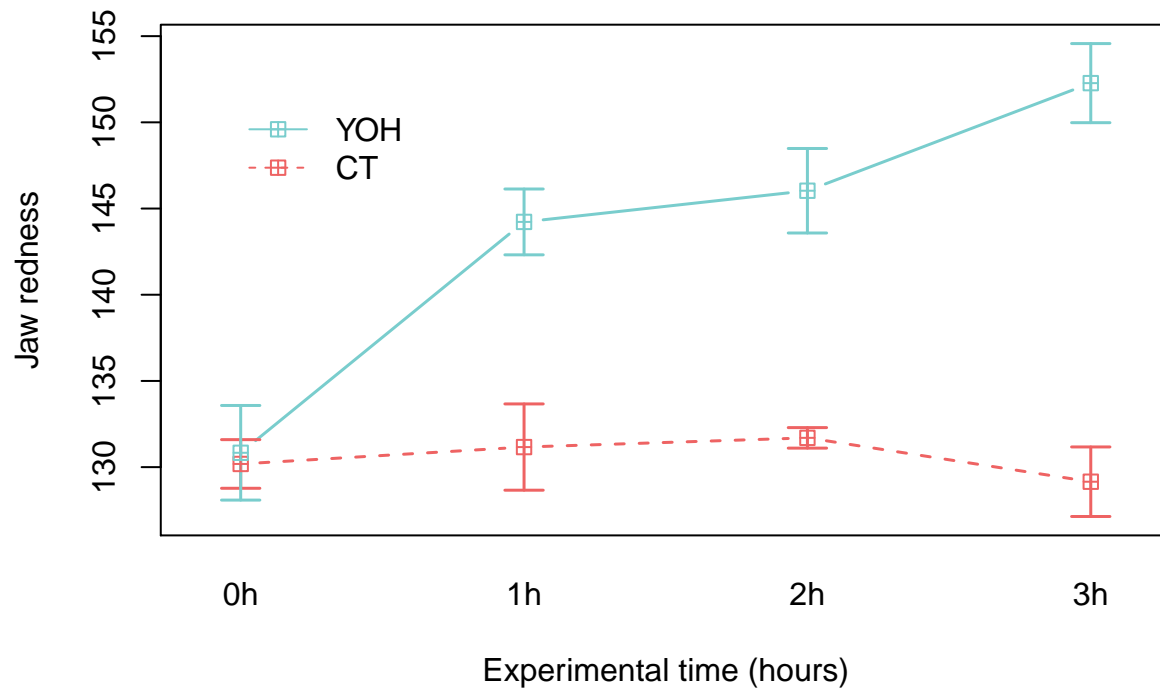
0.2.2 Converting Animals to factor to fit the GLMM

```
data$Animal<-factor(data$Animal)
rednessmale$Animal<-factor(rednessmale$Animal)
darknessmale$Animal<-factor(darknessmale$Animal)
darknessfemale$Animal<-factor(darknessfemale$Animal)
```

0.3 Exploratory data analysis

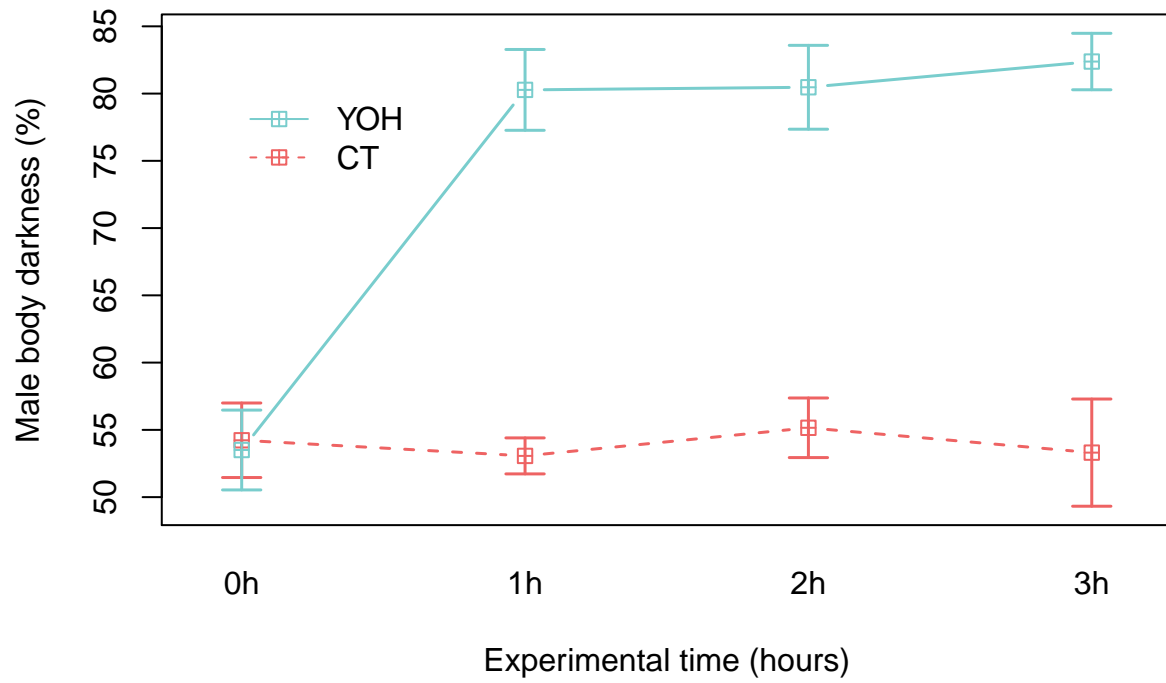
0.3.1 Males Redness

```
lineplot.CI(Time, Redness, Treatment, data=rednessmale, cex = 1,
             xlab = "Experimental time (hours)", ylab = "Jaw redness", cex.lab = 1, x.legend = 1,
             col = c("#EE6363", "#79CDCE"), pch = c(12,12), lwd = 1.5)
```



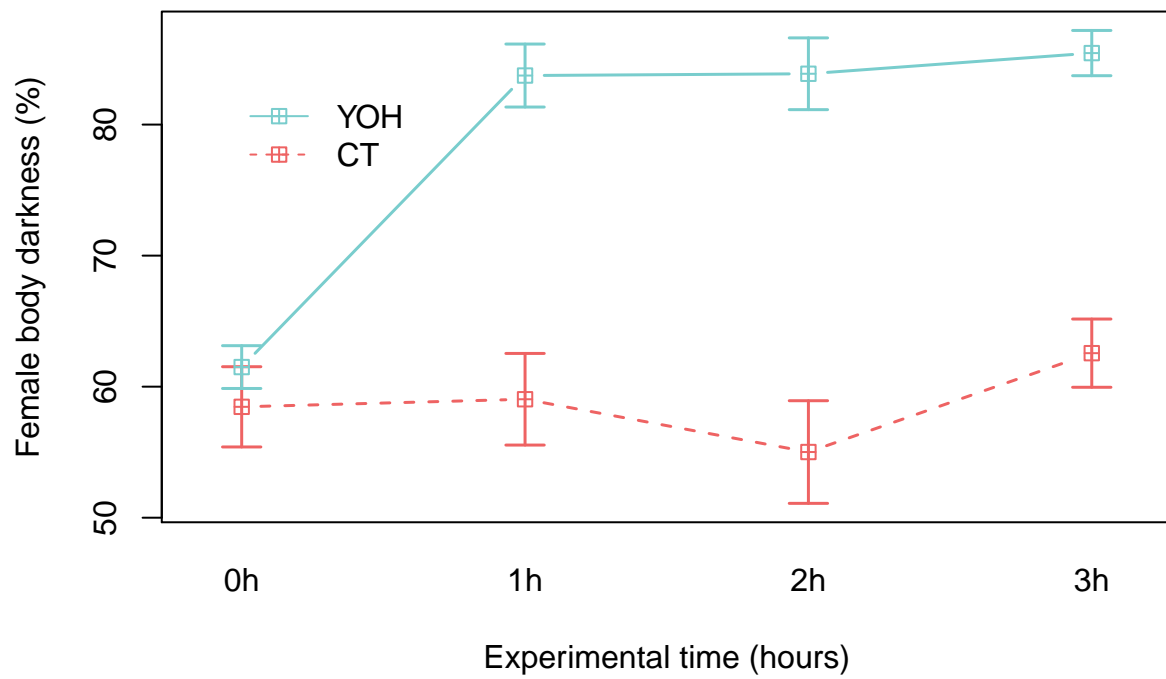
0.3.2 Males Darkness

```
lineplot.CI(Time, Darkness, Treatment, data=darknessmale, cex = 1,
             xlab = "Experimental time (hours)", ylab = "Male body darkness (%)", cex.lab = 1, x.legend = 1,
             col = c("#EE6363", "#79CDCE"), pch = c(12,12), lwd = 1.5)
```



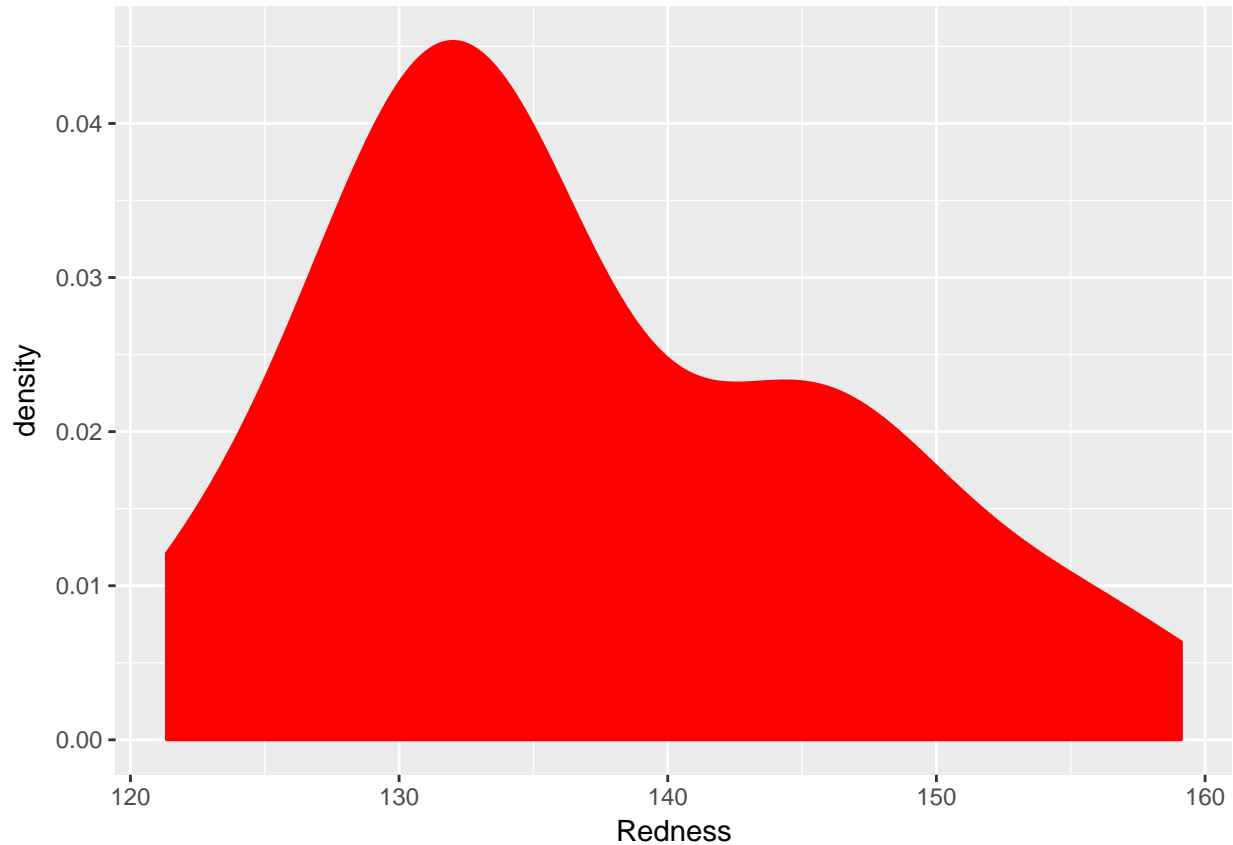
0.3.3 Female Darkness

```
lineplot.CI(Time, Darkness, Treatment, data=darknessfemale, cex = 1,
  xlab = "Experimental time (hours)", ylab = "Female body darkness (%)", cex.lab = 1, x
  col = c("#EE6363", "#79CDCE"), pch = c(12,12), lwd = 1.5)
```



0.3.4 Plotting the distribution of the data

```
ggplot(rednessmale, aes(Redness)) +  
  geom_density(colour="red", fill="red") +  
  theme(legend.position="none")
```



1 Statisticsl analysis

Statistical models for males:

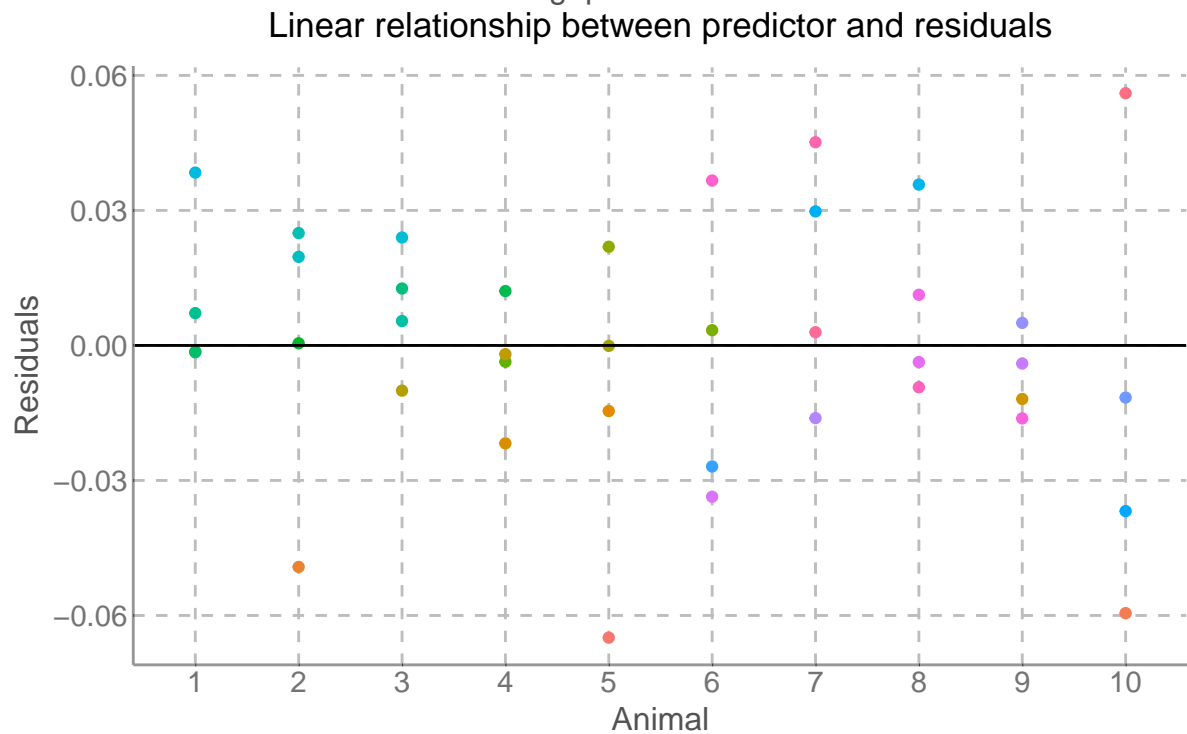
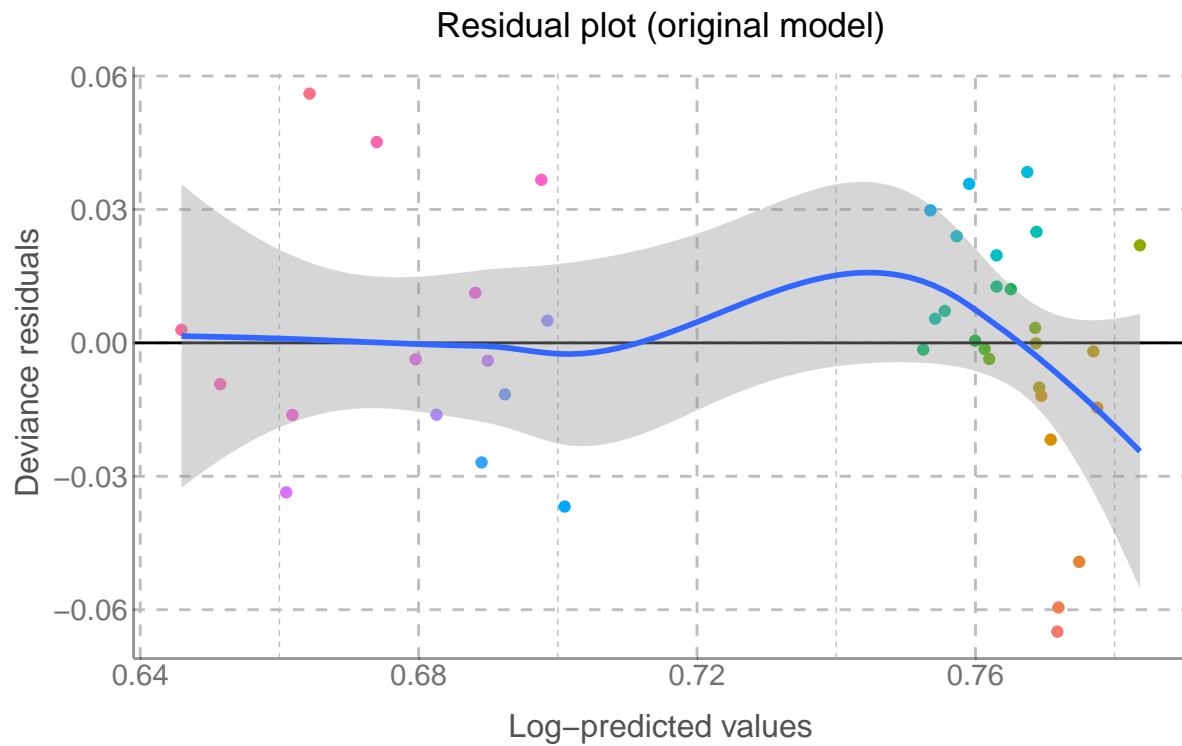
1.1 Modelling Redness

Since there's a big variation in the data 121.34, 159.12, the model failed o converge. Therefore, to improve model convergence we re-scaled the data by dividing it by 100. This doesn't change the results overrall nor the data, just change the scale in which they are showed.

```
mod1<-glmer(Redness/100~Treatment*Time+(1|Animal), family= Gamma, data=rednessmale)
```

1.1.1 Model diagnostics

```
sjp.glmer(mod1, type = "ma")
```



1.1.2 Inference

```
summary(mod1)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
```

```

## Family: Gamma ( inverse )
## Formula: Redness/100 ~ Treatment * Time + (1 | Animal)
## Data: rednessmale
##
##      AIC      BIC    logLik deviance df.resid
##   -123.6   -106.7     71.8   -143.6      30
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.27997 -0.44910 -0.02627  0.51967  2.04904
##
## Random effects:
## Groups Name Variance Std.Dev.
## Animal (Intercept) 9.717e-05 0.009858
## Residual 7.764e-04 0.027864
## Number of obs: 40, groups: Animal, 10
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)    0.768389   0.011491   66.87 < 2e-16 ***
## TreatmentYOH   -0.003729   0.016319   -0.23 0.819252
## Time1h         -0.005738   0.012779   -0.45 0.653395
## Time2h         -0.008831   0.012753   -0.69 0.488674
## Time3h          0.006124   0.012878    0.48 0.634413
## TreatmentYOH:Time1h -0.065216 0.017657   -3.69 0.000221 ***
## TreatmentYOH:Time2h -0.070705 0.017592   -4.02 5.84e-05 ***
## TreatmentYOH:Time3h -0.113721 0.017533   -6.49 8.81e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) TrtYOH Time1h Time2h Time3h TYOH:T1 TYOH:T2
## TreatmntYOH -0.704
## Time1h      -0.560  0.394
## Time2h      -0.561  0.395  0.505
## Time3h      -0.556  0.391  0.500  0.501
## TrtmnYOH:T1  0.405 -0.568 -0.724 -0.365 -0.362
## TrtmnYOH:T2  0.407 -0.570 -0.366 -0.725 -0.363  0.527
## TrtmnYOH:T3  0.408 -0.572 -0.367 -0.368 -0.734  0.529  0.531

```

`sjt.glmer(mod1, show.family = TRUE, show.dev = TRUE, show.hoslem = TRUE, show.se = TRUE, show.ci = FALSE)`

Redness/100

Estimate

std. Error

p

Fixed Parts

(Intercept)

2.16

0.02

<.001

TreatmentYOH

1.00

0.02

.819

Time1h

0.99

0.01

.653

Time2h

0.99

0.01

.489

Time3h

1.01

0.01

.634

TreatmentYOH:Time1h

0.94

0.02

<.001

TreatmentYOH:Time2h

0.93

0.02

<.001

TreatmentYOH:Time3h

0.89

0.02
<.001
Random Parts
00, Animal

0.000
NAnimal

10
ICCAAnimal

0.111
Observations

40
Deviance

0.028
Hosmer-Lemeshow-X2

-.050; p=1.000
Family

Gamma (inverse)

```
pairs(lsmmeans(mod1, ~ Treatment|Time, adjust="tukey"))#post-hoc tests
```

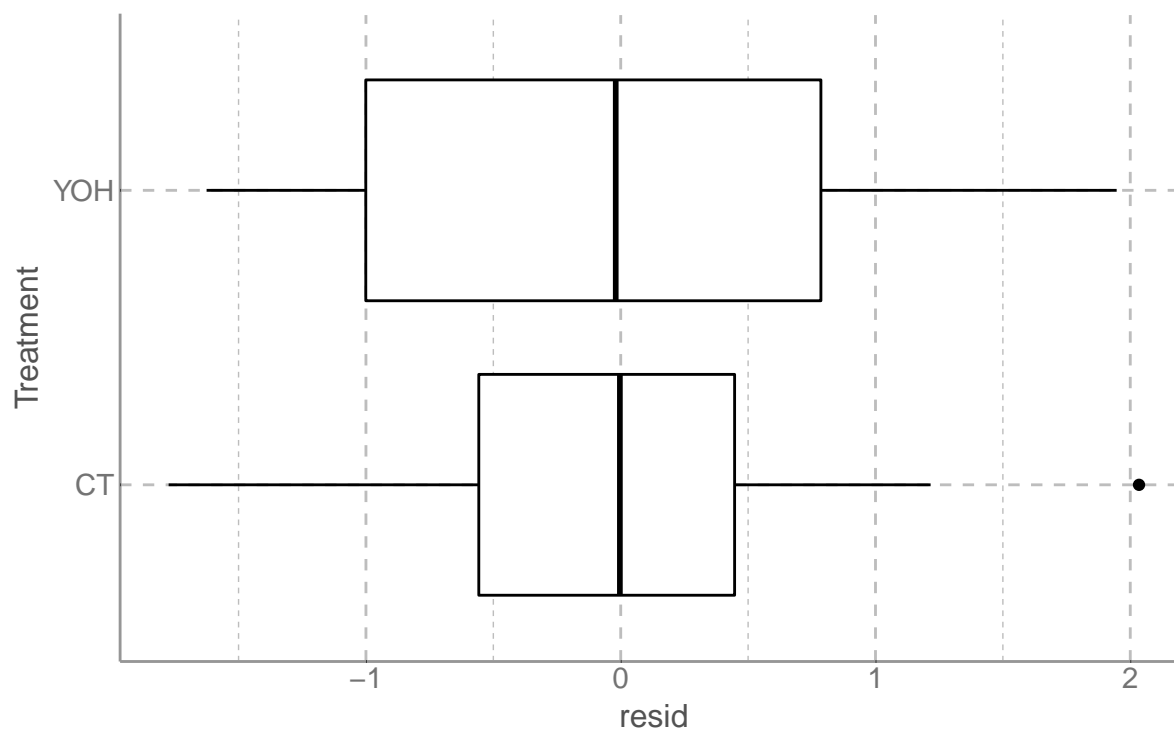
```
## Time = 0h:
## contrast estimate SE df z.ratio p.value
## CT - YOH 0.00372905 0.01631918 NA 0.229 0.8193
##
## Time = 1h:
## contrast estimate SE df z.ratio p.value
## CT - YOH 0.06894502 0.01583279 NA 4.355 <.0001
##
## Time = 2h:
## contrast estimate SE df z.ratio p.value
## CT - YOH 0.07443450 0.01575979 NA 4.723 <.0001
##
## Time = 3h:
## contrast estimate SE df z.ratio p.value
## CT - YOH 0.11745038 0.01569454 NA 7.484 <.0001
```

1.2 Modelling Darkness

```
mod2<-glmmadmb(Darkness/100~Treatment*Time+(1|Animal), family= "beta", data=darknessmale)
```

1.2.1 Diagnostics

```
augDat <- data.frame(darknessmale,resid=residuals(mod2,type="pearson"),
                     fitted=fitted(mod2))
ggplot(augDat,aes(x=Treatment,y=resid))+
  geom_boxplot()+
  coord_flip()
```



1.2.2 Inference

```
summary(mod2)
```

```
##
## Call:
## glmmadmb(formula = Darkness/100 ~ Treatment * Time + (1 | Animal),
##   data = darknessmale, family = "beta")
##
## AIC: -95.6
##
## Coefficients:
##               Estimate Std. Error z value Pr(>|z|)
## (Intercept)      0.1678    0.1133   1.48    0.14
## TreatmentYOH     -0.0283    0.1601  -0.18    0.86
## Time1h           -0.0466    0.1601  -0.29    0.77
```

```
## Time2h          0.0377      0.1603      0.24      0.81
## Time3h          -0.0331     0.1601     -0.21      0.84
## TreatmentYOH:Time1h 1.3352     0.2418     5.52 3.4e-08 ***
## TreatmentYOH:Time2h 1.2571     0.2422     5.19 2.1e-07 ***
## TreatmentYOH:Time3h 1.4307     0.2447     5.85 5.0e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=10
## Random effect variance(s):
## Group=Animal
##          Variance      StdDev
## (Intercept) 1.125e-07 0.0003355
##
## Beta dispersion parameter: 61.763 (std. err.: 13.72)
##
## Log-likelihood: 57.7771
```

```
pairs(lsmeans(mod2, ~ Treatment|Time))
```

```
## Time = 0h:
## contrast      estimate      SE df z.ratio p.value
## CT - YOH  0.02832475 0.1601100 NA   0.177  0.8596
##
## Time = 1h:
## contrast      estimate      SE df z.ratio p.value
## CT - YOH -1.30683870 0.2935229 NA  -4.452 <.0001
##
## Time = 2h:
## contrast      estimate      SE df z.ratio p.value
## CT - YOH -1.22876288 0.2956331 NA  -4.156 <.0001
##
## Time = 3h:
## contrast      estimate      SE df z.ratio p.value
## CT - YOH -1.40240242 0.3054967 NA  -4.591 <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

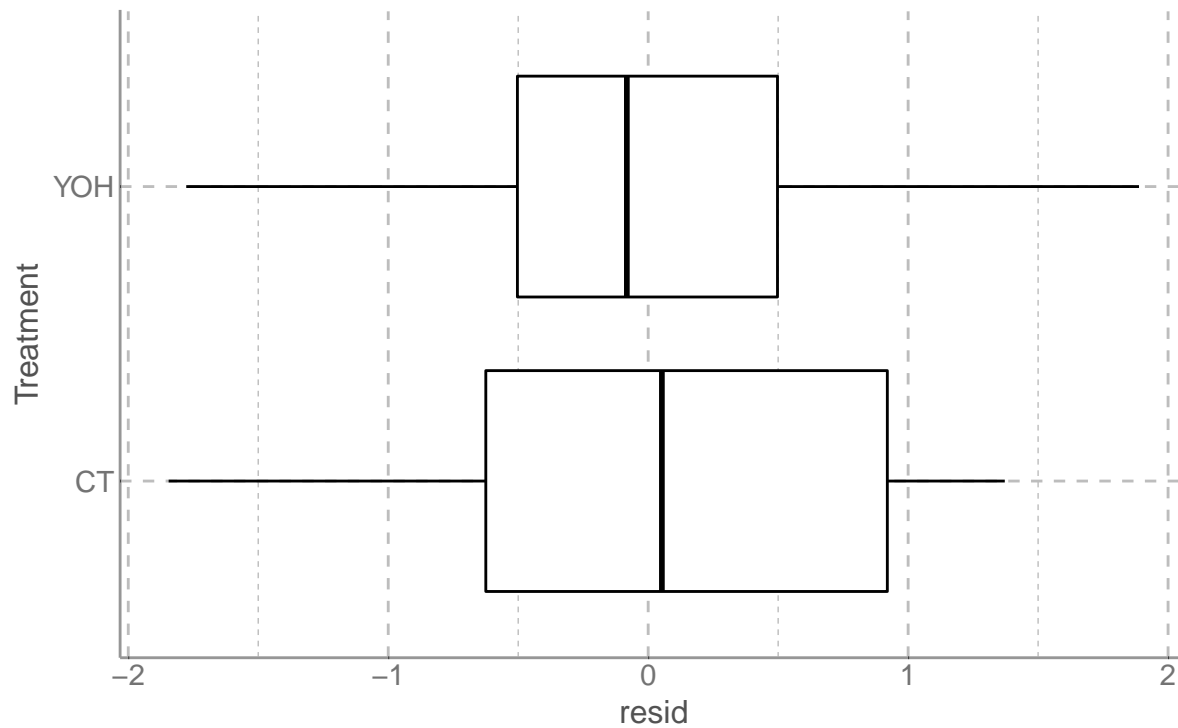
Models for females:

1.3 Darkness

```
mod3<-glmmadmb(Darkness/100~Treatment*Time+(1|Animal), family= "beta", data=darknessfemale)
```

1.3.1 Checking model assumptions

```
augDat <- data.frame(darknessfemale,resid=residuals(mod3,type="pearson"),
                     fitted=fitted(mod3))
ggplot(augDat,aes(x=Treatment,y=resid))+
  geom_boxplot()+
  coord_flip()
```



1.3.2 Inference

```
summary(mod3)
```

```
##
## Call:
## glmmadmb(formula = Darkness/100 ~ Treatment * Time + (1 | Animal),
##   data = darknessfemale, family = "beta")
##
## AIC: -99.1
##
## Coefficients:
##               Estimate Std. Error z value Pr(>|z|)
## (Intercept)      0.341     0.116    2.93  0.0034 **
## TreatmentYOH      0.123     0.166    0.75  0.4562
## Time1h           0.024     0.154    0.16  0.8762
## Time2h          -0.139     0.153   -0.91  0.3644
## Time3h           0.172     0.155    1.11  0.2691
## TreatmentYOH:Time1h  1.152     0.237    4.85 1.2e-06 ***
## TreatmentYOH:Time2h  1.353     0.238    5.69 1.3e-08 ***
## TreatmentYOH:Time3h  1.135     0.242    4.68 2.8e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=10
## Random effect variance(s):
## Group=Animal
##               Variance StdDev
## (Intercept) 0.008762 0.09361
##
```

```
## Beta dispersion parameter: 68.861 (std. err.: 17.428)
##
## Log-likelihood: 59.5687
pairs(lsmeans(mod3, ~ Treatment|Time))

## Time = 0h:
## contrast estimate SE df z.ratio p.value
## CT - YOH -0.1233735 0.1655700 NA -0.745 0.4562
##
## Time = 1h:
## contrast estimate SE df z.ratio p.value
## CT - YOH -1.2751179 0.2923997 NA -4.361 <.0001
##
## Time = 2h:
## contrast estimate SE df z.ratio p.value
## CT - YOH -1.4759002 0.2946529 NA -5.009 <.0001
##
## Time = 3h:
## contrast estimate SE df z.ratio p.value
## CT - YOH -1.2588635 0.3079546 NA -4.088 <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```