Analysis of the in vitro experiments

Lilian Franco-Belussi, Classius De Oliveira, and Helen Nilsson Sköld

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Contents

0.1 R version

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

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

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

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

0.2 Loading packages

```r
library(sjPlot)
packageVersion("sjPlot")
```

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

```r
library(sciplot)
packageVersion("sciplot")
```

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

```r
library(lsmeans)
packageVersion("lsmeans")
```

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

```r
library(glmmADMB)
packageVersion("glmmADMB")
```

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

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

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

```r
library(ordinal)
packageVersion("ordinal")
```

```
## [1] '2015.6.28'
```

```r
library(car)
packageVersion("car")
```

```
## [1] '2.1.5'
```

```r
library(RVAideMemoire)
packageVersion("RVAideMemoire")
```

```
## [1] '0.9.66'
```

```r
library(tidyverse)
packageVersion("tidyverse")
```

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

1 Data input and handling

Here, we're going to import all the data, separately for each experiment with different hormones.

1.0.1 Noradrenalinee (NA)

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

```
##   Animal Treatment Time Sex Black Red
## 1      1        CT   0h   M     5   5
## 2      1        CT   1h   M     5   5
## 3      1        CT   2h   M     5   5
## 4      1        CT   3h   M     5   5
## 5      2        CT   0h   M     5   4
## 6      2        CT   1h   M     5   4

## '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","NA10uM": 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 2 ...
##  $ Black    : int  5 5 5 5 5 5 5 5 5 4 4 ...
##  $ Red      : int  5 5 5 5 4 4 4 4 4 4 4 ...
```

1.0.2 Melanocyte-Concentrating Hormone (MCH)

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

```
##   Animal Treatment Time Sex Black Red
## 1      1        CT   0h   M     5   4
## 2      1        CT   1h   M     5   4
## 3      1        CT   2h   M     5   4
## 4      1        CT   3h   M     5   4
## 5      2        CT   0h   M     4   4
## 6      2        CT   1h   M     4   4

## '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","MCH100mM": 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 2 ...
##  $ Black    : int  5 5 5 5 4 4 4 4 5 5 ...
##  $ Red      : int  4 4 4 4 4 4 4 4 4 4 4 ...
```

1.0.3 Melatonin (MEL)

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

```
##   Animal Treatment Time Sex Black Red
## 1      1          CT  0h   M     4   4
## 2      1          CT  1h   M     4   4
## 3      1          CT  2h   M     4   4
## 4      1          CT  3h   M     4   4
## 5      2          CT  0h   M     3   4
## 6      2          CT  1h   M     4   4

## '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","MEL10uM": 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 ...
##  $ Black    : int  4 4 4 4 3 4 4 4 4 4 ...
##  $ Red      : int  4 4 4 4 4 4 4 4 4 4 ...
```

1.0.4 Melanocyte Stimulating Hormone (MSH)

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

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1          CT  0h   M     5   4   0.10
## 2      1          CT  1h   M     4   4   0.11
## 3      1          CT  2h   M     4   4   0.13
## 4      1          CT  3h   M     4   4   0.11
## 5      2          CT  0h   M     4   4   0.09
## 6      2          CT  1h   M     4   4   0.10

## 'data.frame':    80 obs. of  7 variables:
##  $ Animal   : int  1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","MSH5uM": 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 ...
##  $ Black    : int  5 4 4 4 4 4 4 4 4 4 ...
##  $ Red      : int  4 4 4 4 4 4 4 4 3 3 ...
##  $ Yellow   : num  0.1 0.11 0.13 0.11 0.09 0.1 0.08 0.08 0.09 0.12 ...
```

1.0.5 Prolactin (PRL)

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

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1          CT  0h   M     5   4   0.11
## 2      1          CT  1h   M     5   4   0.10
## 3      1          CT  2h   M     4   4   0.13
## 4      1          CT  3h   M     5   4   0.14
## 5      2          CT  0h   M     5   3   0.09
## 6      2          CT  1h   M     5   3   0.09

## 'data.frame':    80 obs. of  7 variables:
##  $ Animal   : int  1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","PRL0.150IU": 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 ...
## $ Black    : int  5 5 4 5 5 5 4 5 4 4 ...
## $ Red      : int  4 4 4 4 3 3 3 3 3 3 ...
## $ Yellow   : num  0.11 0.1 0.13 0.14 0.09 0.09 0.1 0.11 0.09 0.11 ...
```

1.0.6 Noradrenaline and Melanocyte Stimulating Hormone (NAMSH)

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

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     2   2   0.11
## 2      1        CT   1h   M     2   2   0.12
## 3      1        CT   2h   M     2   1   0.13
## 4      1        CT   3h   M     2   1   0.12
## 5      2        CT   0h   M     2   2   0.12
## 6      2        CT   1h   M     2   1   0.11

## 'data.frame':    80 obs. of  7 variables:
## $ Animal   : int  1 1 1 1 2 2 2 2 3 3 ...
## $ Treatment: Factor w/ 2 levels "CT","NA3h+MSH": 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 ...
## $ Black    : int  2 2 2 2 2 2 2 2 2 2 ...
## $ Red      : int  2 2 1 1 2 1 1 1 2 2 ...
## $ Yellow   : num  0.11 0.12 0.13 0.12 0.12 0.11 0.1 0.15 0.13 0.14 ...
```

1.0.7 Noradrenalinee and Prolactin (NAPRL)

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

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     3   1   0.10
## 2      1        CT   1h   M     3   1   0.11
## 3      1        CT   2h   M     3   1   0.13
## 4      1        CT   3h   M     3   1   0.11
## 5      2        CT   0h   M     1   1   0.12
## 6      2        CT   1h   M     1   1   0.13

## 'data.frame':    80 obs. of  7 variables:
## $ Animal   : int  1 1 1 1 2 2 2 2 3 3 ...
## $ Treatment: Factor w/ 2 levels "CT","NA3h+PRL": 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 ...
## $ Black    : int  3 3 3 3 1 1 1 1 1 1 ...
## $ Red      : int  1 1 1 1 1 1 1 1 1 1 ...
## $ Yellow   : num  0.1 0.11 0.13 0.11 0.12 0.13 0.11 0.12 0.11 0.1 ...
```

1.1 Data manipulation

Since we're not interested in the differences between sexes in the response to hormones, we'll run separate models for males and females. To do this, first we have to subset our data to contain only the respective rows for each sex, in order to use them to make plots and the statistical models. We'll do it separately for each colouration as well, begining with the `Black` colour.

1.1.1 Black colour of Males

```r
maleNA<- filter(hormNA,Sex=="M")
head(maleNA)
```

```
##   Animal Treatment Time Sex Black Red
## 1      1        CT   0h   M     5   5
## 2      1        CT   1h   M     5   5
## 3      1        CT   2h   M     5   5
## 4      1        CT   3h   M     5   5
## 5      2        CT   0h   M     5   4
## 6      2        CT   1h   M     5   4
```

```r
maleMCH<- filter(hormMCH,Sex=="M")
head(maleMCH)
```

```
##   Animal Treatment Time Sex Black Red
## 1      1        CT   0h   M     5   4
## 2      1        CT   1h   M     5   4
## 3      1        CT   2h   M     5   4
## 4      1        CT   3h   M     5   4
## 5      2        CT   0h   M     4   4
## 6      2        CT   1h   M     4   4
```

```r
maleMEL<- filter(hormMEL,Sex=="M")
head(maleMEL)
```

```
##   Animal Treatment Time Sex Black Red
## 1      1        CT   0h   M     4   4
## 2      1        CT   1h   M     4   4
## 3      1        CT   2h   M     4   4
## 4      1        CT   3h   M     4   4
## 5      2        CT   0h   M     3   4
## 6      2        CT   1h   M     4   4
```

```r
maleMSH<- filter(hormMSH,Sex=="M")
head(maleMSH)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     5   4   0.10
## 2      1        CT   1h   M     4   4   0.11
## 3      1        CT   2h   M     4   4   0.13
## 4      1        CT   3h   M     4   4   0.11
## 5      2        CT   0h   M     4   4   0.09
## 6      2        CT   1h   M     4   4   0.10
```

```r
malePRL<- filter(hormPRL,Sex=="M")
head(malePRL)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     5   4   0.11
## 2      1        CT   1h   M     5   4   0.10
## 3      1        CT   2h   M     4   4   0.13
## 4      1        CT   3h   M     5   4   0.14
## 5      2        CT   0h   M     5   3   0.09
## 6      2        CT   1h   M     5   3   0.09
```
```
maleNAMSH<- filter(hormNAMSH,Sex=="M")
head(maleNAMSH)
```
```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     2   2   0.11
## 2      1        CT   1h   M     2   2   0.12
## 3      1        CT   2h   M     2   1   0.13
## 4      1        CT   3h   M     2   1   0.12
## 5      2        CT   0h   M     2   2   0.12
## 6      2        CT   1h   M     2   1   0.11
```
```
maleNAPRL<- filter(hormNAPRL,Sex=="M")
head(malePRL)
```
```
##   Animal Treatment Time Sex Black Red Yellow
## 1      1        CT   0h   M     5   4   0.11
## 2      1        CT   1h   M     5   4   0.10
## 3      1        CT   2h   M     4   4   0.13
## 4      1        CT   3h   M     5   4   0.14
## 5      2        CT   0h   M     5   3   0.09
## 6      2        CT   1h   M     5   3   0.09
```

1.1.2 Black colour of Females

```
femaleNA<- filter(hormNA, Sex=="F")
head(femaleNA)
```
```
##   Animal Treatment Time Sex Black Red
## 1      6        CT   0h   F     5  NA
## 2      6        CT   1h   F     4  NA
## 3      6        CT   2h   F     4  NA
## 4      6        CT   3h   F     4  NA
## 5      7        CT   0h   F     4  NA
## 6      7        CT   1h   F     4  NA
```
```
femaleMCH<- filter(hormMCH, Sex=="F")
head(femaleMCH)
```
```
##   Animal Treatment Time Sex Black Red
## 1      6        CT   0h   F     5  NA
## 2      6        CT   1h   F     5  NA
## 3      6        CT   2h   F     5  NA
## 4      6        CT   3h   F     5  NA
## 5      7        CT   0h   F     4  NA
## 6      7        CT   1h   F     4  NA
```
```
femaleMEL<- filter(hormMEL, Sex=="F")
head(femaleMEL)
```

```
##   Animal Treatment Time Sex Black Red
## 1     6        CT   0h   F     3  NA
## 2     6        CT   1h   F     3  NA
## 3     6        CT   2h   F     3  NA
## 4     6        CT   3h   F     3  NA
## 5     7        CT   0h   F     4  NA
## 6     7        CT   1h   F     4  NA
```

```
femaleMSH<- filter(hormMSH, Sex=="F")
head(femaleMSH)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1     6        CT   0h   F     4  NA   0.13
## 2     6        CT   1h   F     5  NA   0.15
## 3     6        CT   2h   F     5  NA   0.12
## 4     6        CT   3h   F     5  NA   0.12
## 5     7        CT   0h   F     4  NA   0.12
## 6     7        CT   1h   F     4  NA   0.17
```

```
femalePRL<- filter(hormPRL, Sex=="F")
head(femalePRL)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1     6        CT   0h   F     5  NA   0.11
## 2     6        CT   1h   F     5  NA   0.10
## 3     6        CT   2h   F     5  NA   0.12
## 4     6        CT   3h   F     5  NA   0.11
## 5     7        CT   0h   F     5  NA   0.12
## 6     7        CT   1h   F     5  NA   0.09
```

```
femaleNAMSH<- filter(hormNAMSH, Sex=="F")
head(femaleNAMSH)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1     1        CT   0h   F     2  NA   0.01
## 2     1        CT   1h   F     2  NA   0.01
## 3     1        CT   2h   F     2  NA   0.03
## 4     1        CT   3h   F     2  NA   0.04
## 5     2        CT   0h   F     2  NA   0.02
## 6     2        CT   1h   F     2  NA   0.02
```

```
femaleNAPRL<- filter(hormNAPRL, Sex=="F")
head(femaleNAPRL)
```

```
##   Animal Treatment Time Sex Black Red Yellow
## 1     6        CT   0h   F     3  NA   0.03
## 2     6        CT   1h   F     2  NA   0.02
## 3     6        CT   2h   F     2  NA   0.02
## 4     6        CT   3h   F     2  NA   0.02
## 5     7        CT   0h   F     2  NA   0.02
## 6     7        CT   1h   F     2  NA   0.03
```

1.2 Exploratory data analysis

Before jumping into statistical inferential modelling, it's good practice to look at the data, doing exploratory data analysis (see Zuur et al. 2016 MEE). Here, we'll take advantage of the sciplot package to easily plot our

data. We'll produce plots separately for each sex, hormone, and colouration type.

1.2.1 Black color of males

1.2.1.1 Noradrelanine

```
lineplot.CI(Time, Black, Treatment, data=maleNA, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
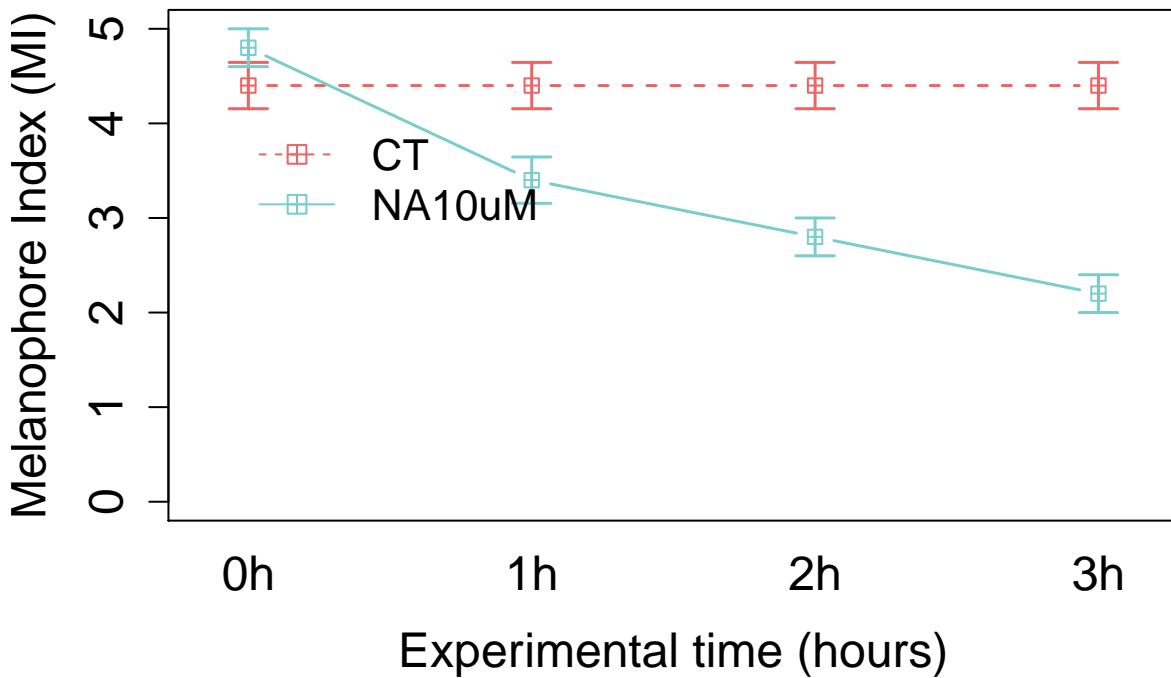

1.2.1.2 Melanocyte-Concentrating Hormone

```
lineplot.CI(Time, Black, Treatment, data=maleMCH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
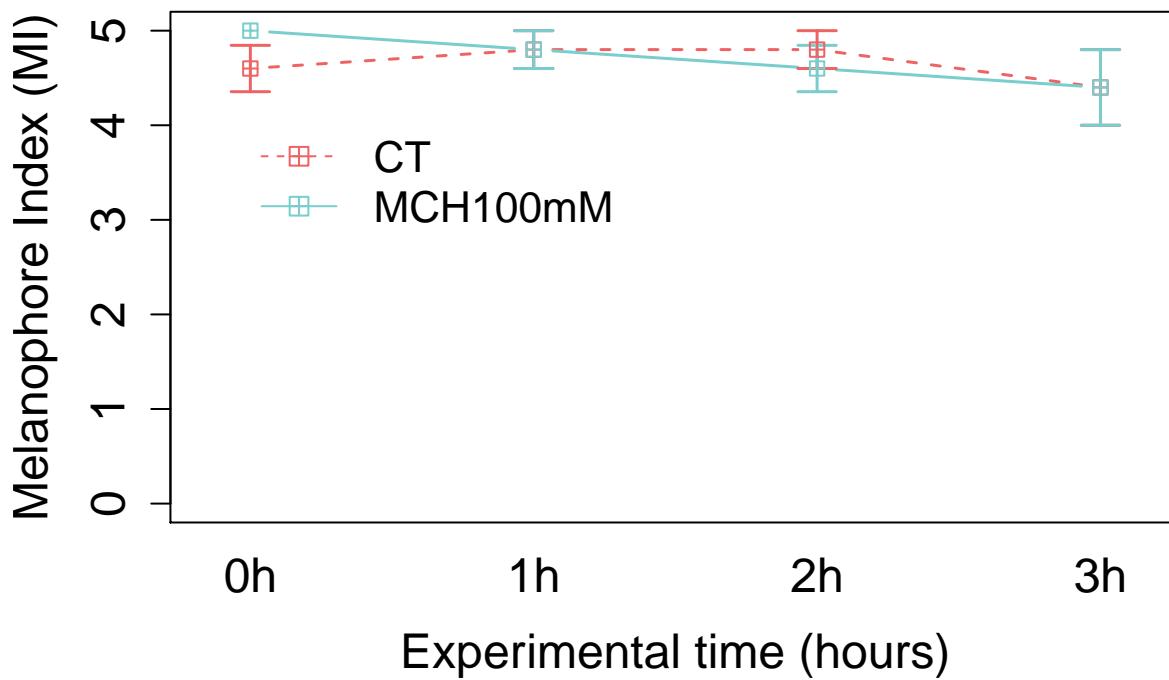
1.2.1.3 Melatonin

```
lineplot.CI(Time, Black, Treatment, data=maleMEL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
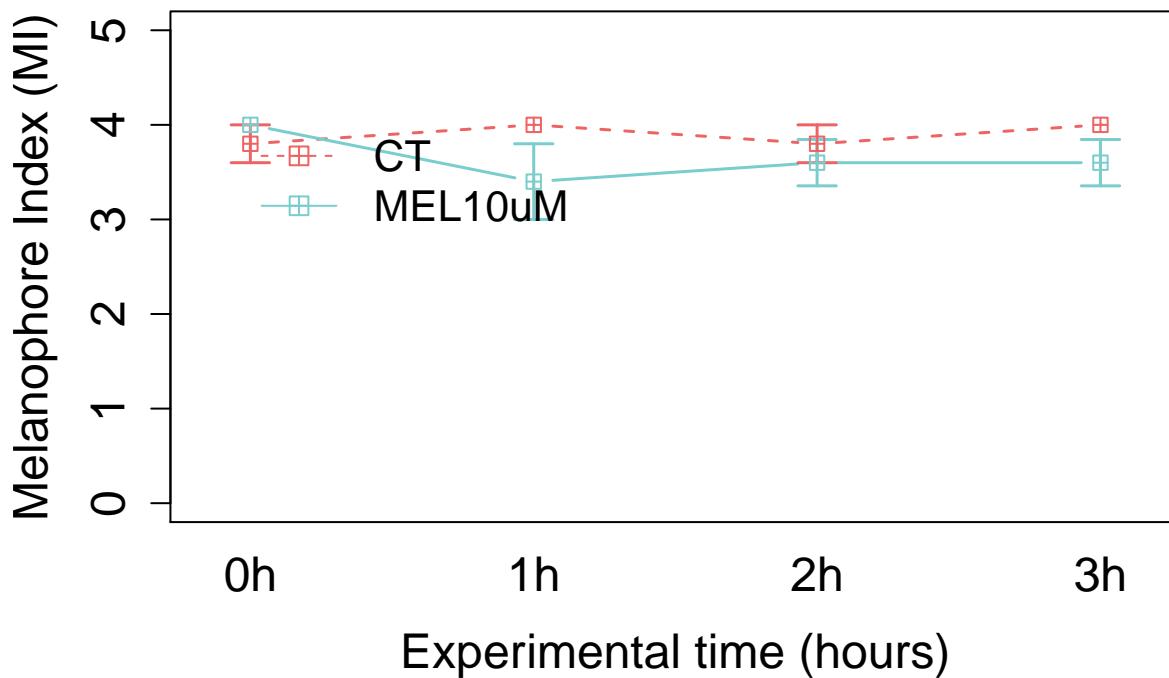

1.2.1.4 Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Black, Treatment, data=maleMSH, cex = 1,
        xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
        col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
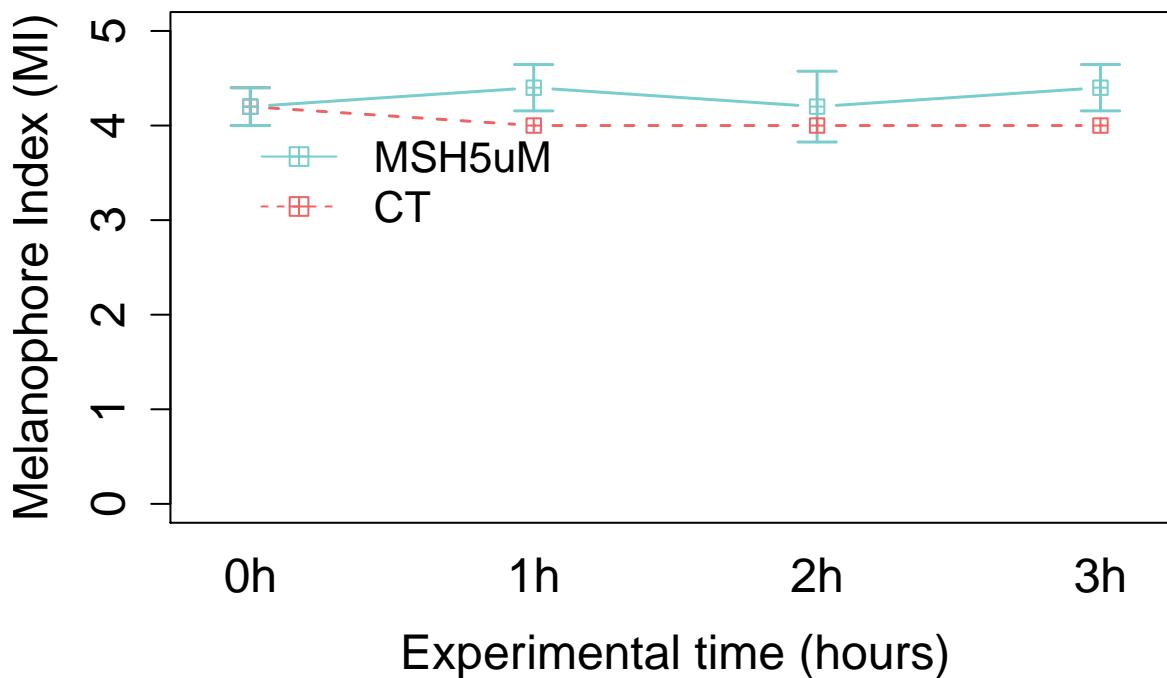

1.2.1.5 Prolactin

```
lineplot.CI(Time, Black, Treatment, data=malePRL, cex = 1,
        xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
        col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
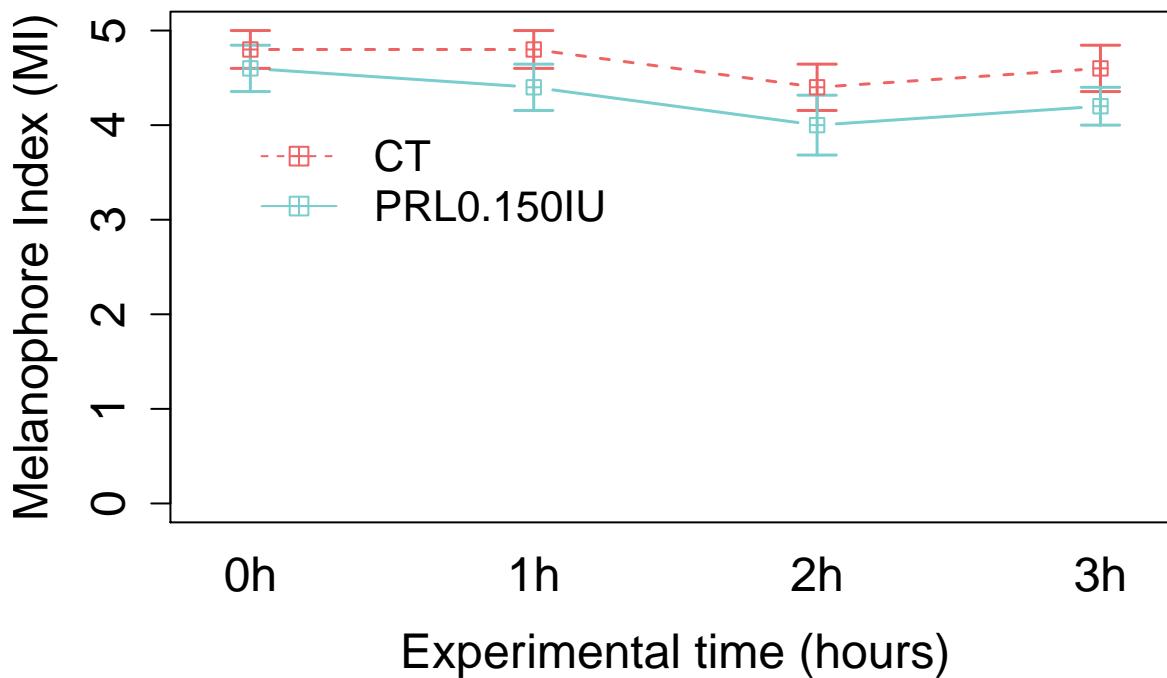
1.2.1.6 Noradrenaline and Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Black, Treatment, data=maleNAMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
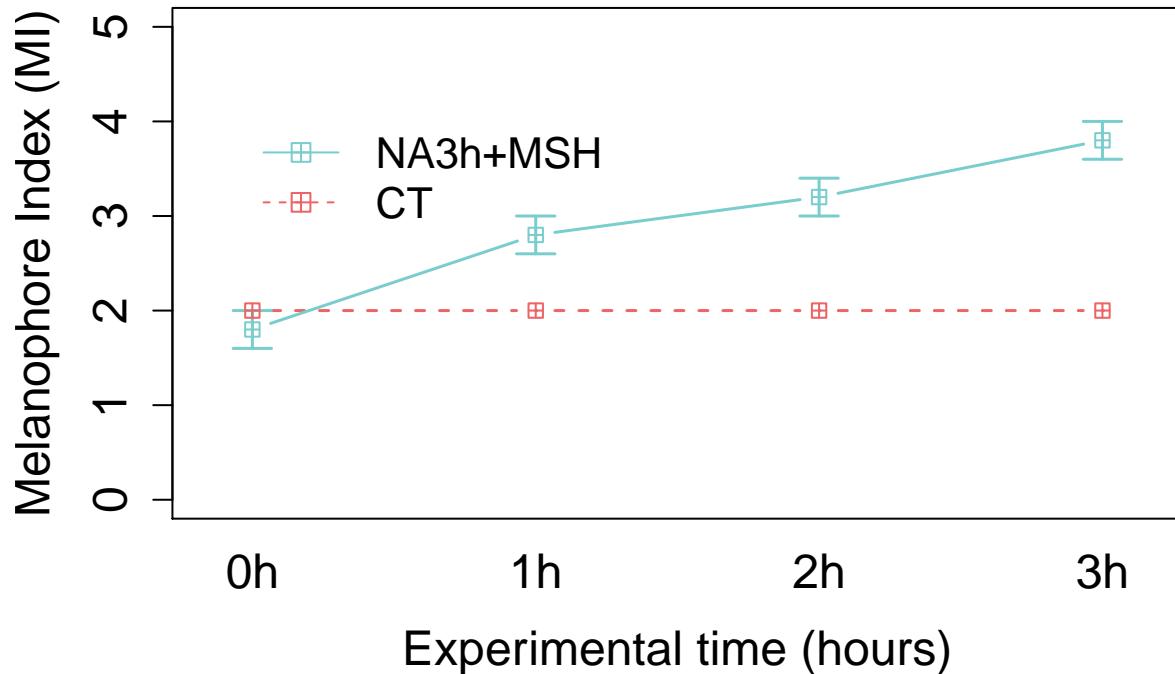

1.2.1.7 Noradrenaline and Prolactin

```
lineplot.CI(Time, Black, Treatment, data=maleNAPRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5,  ylim= c(0,5))
```
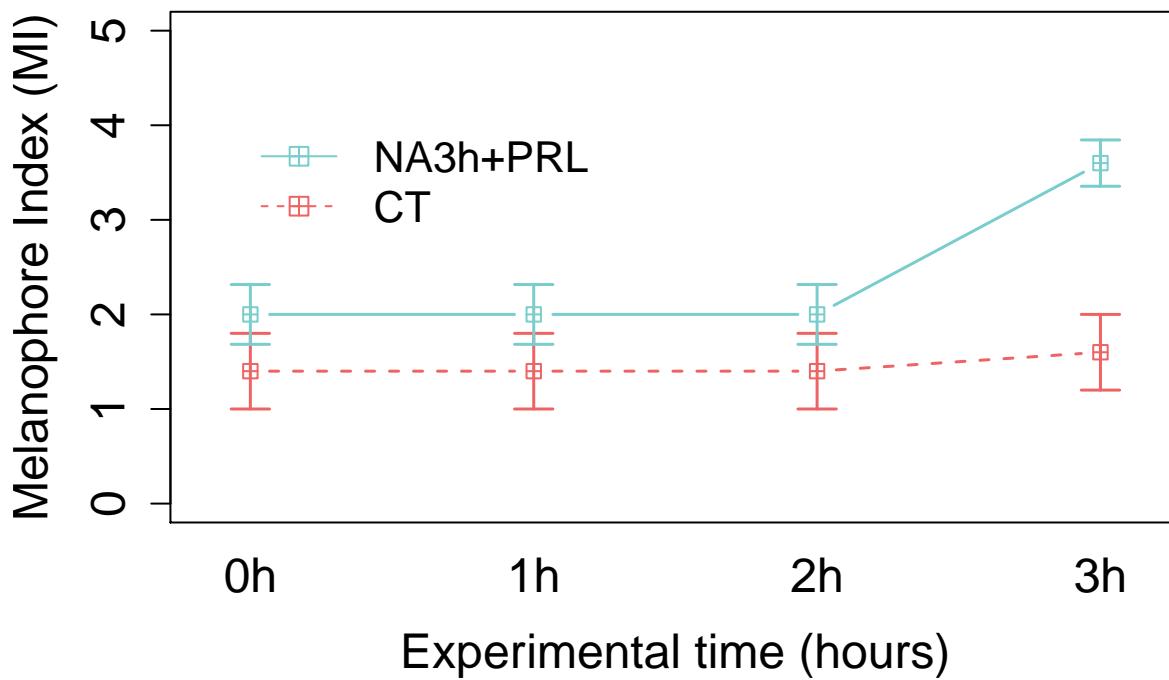
1.2.2 Black colour of Females

1.2.2.1 Noradrenaline Gráfico Black Females

```
lineplot.CI(Time, Black, Treatment, data=femaleNA, cex = 1,
           xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
           col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
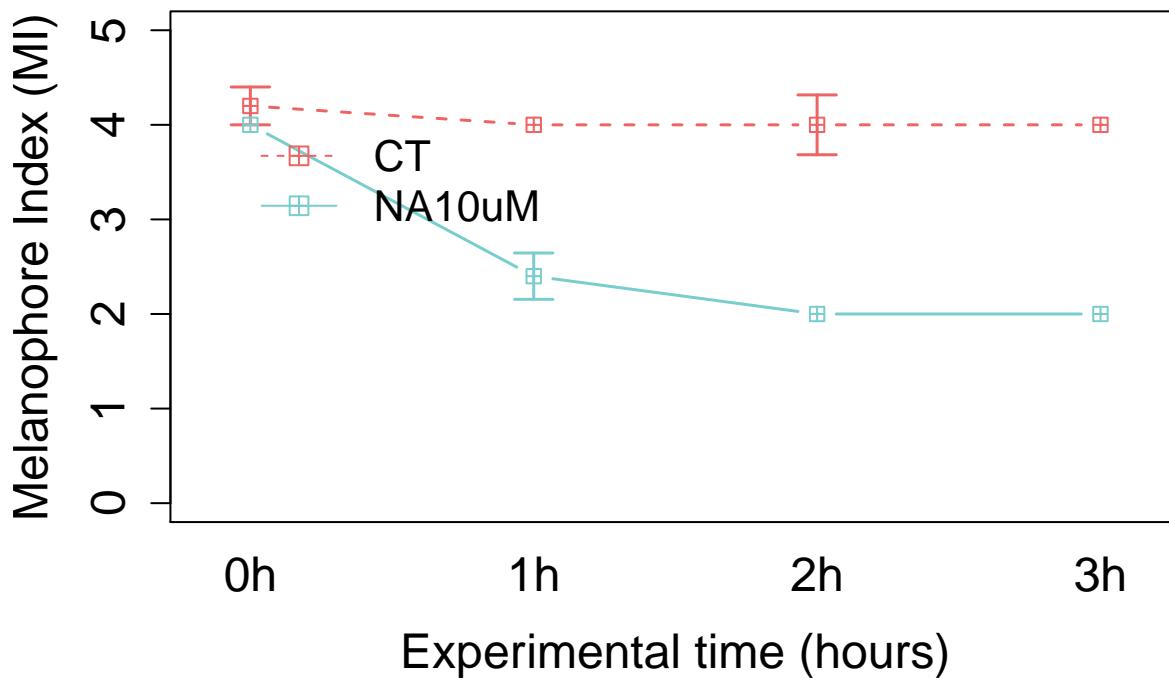

1.2.2.2 Melanocyte-Concentrating Hormone

```
lineplot.CI(Time, Black, Treatment, data=femaleMCH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
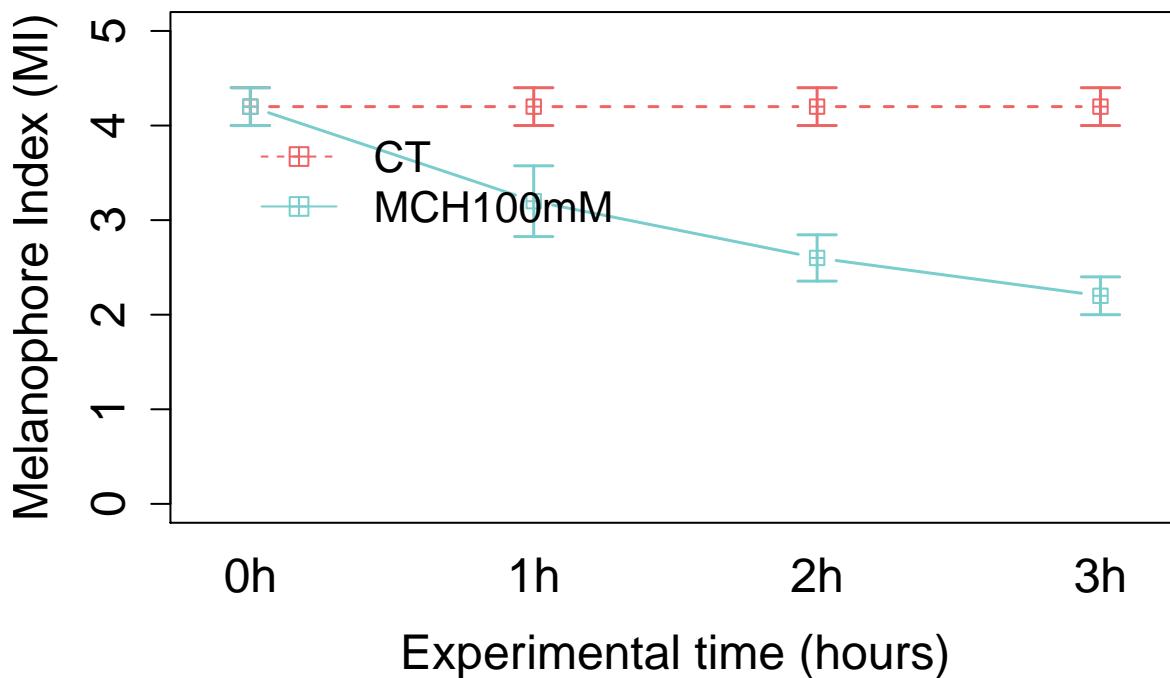

1.2.2.3 Melanotin

```
lineplot.CI(Time, Black, Treatment, data=femaleMEL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
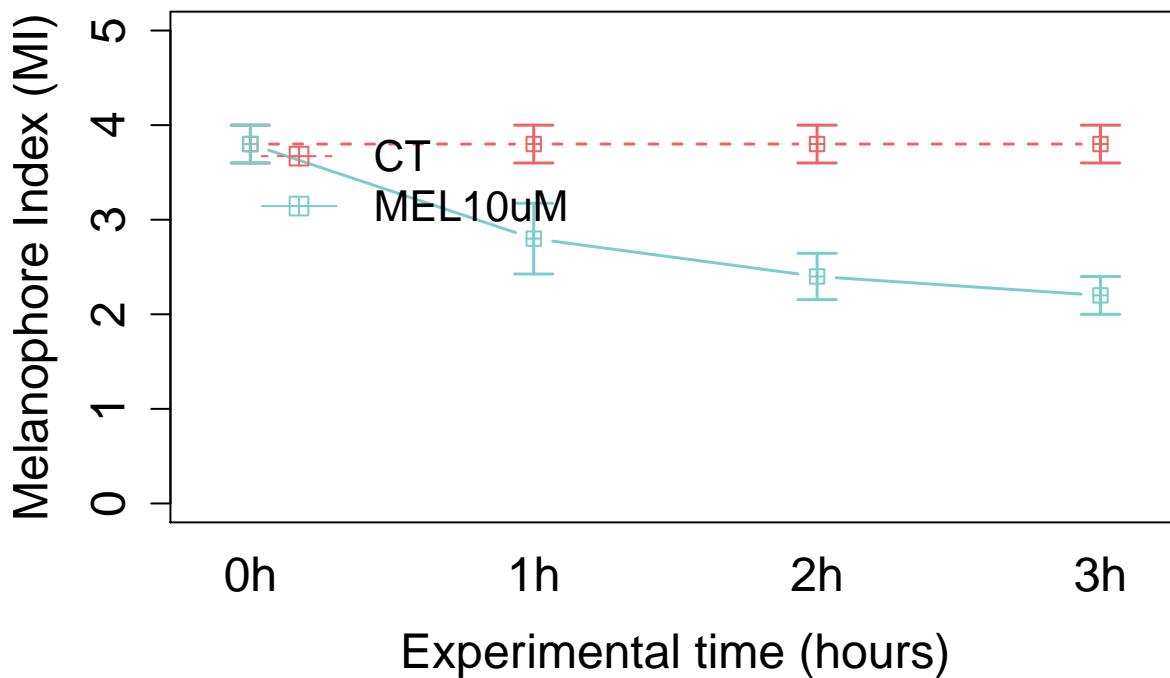
1.2.2.4 Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Black, Treatment, data=femaleMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
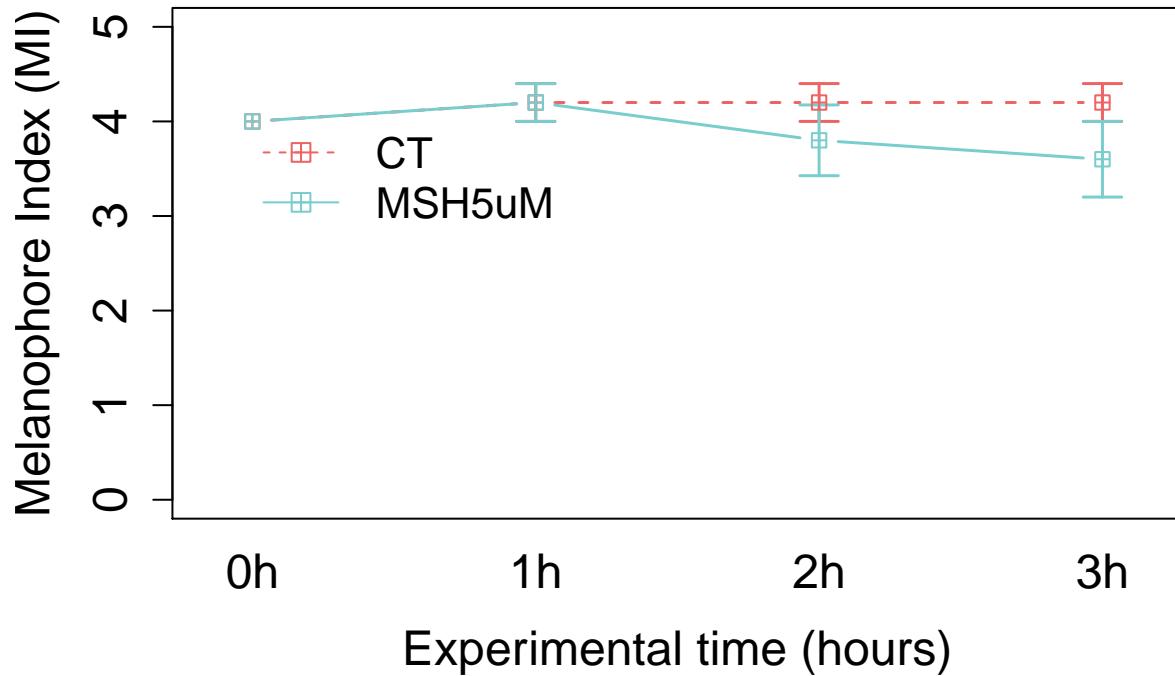

1.2.2.5 Prolactin

```
lineplot.CI(Time, Black, Treatment, data=femalePRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
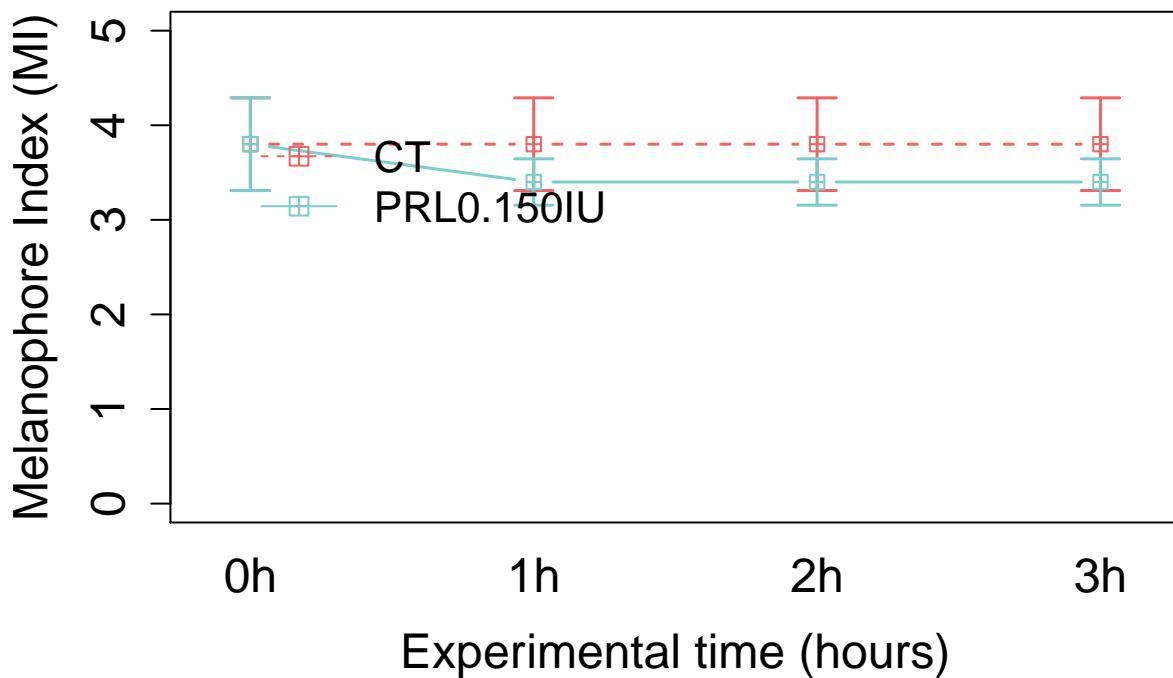
1.2.2.6 Noradrenaline and Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Black, Treatment, data=femaleNAMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
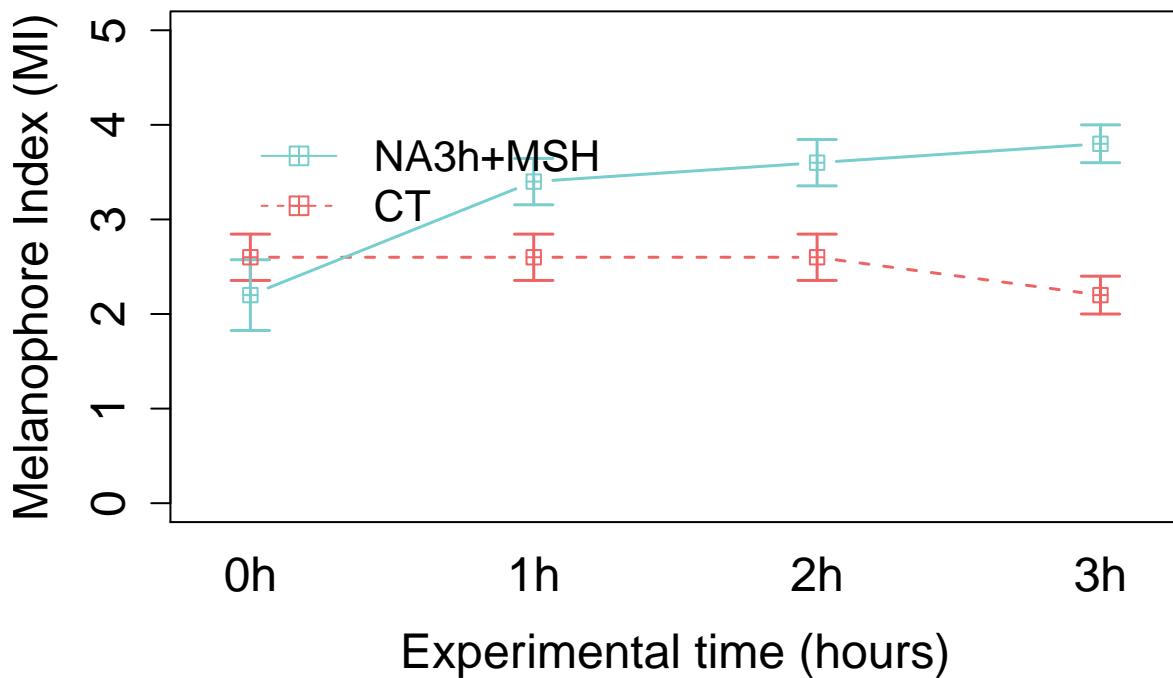

1.2.2.7 Noradrenaline and Prolactin

```
lineplot.CI(Time, Black, Treatment, data=femaleNAPRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Melanophore Index (MI)", cex.lab = 1.5, x.leg =
            col= c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
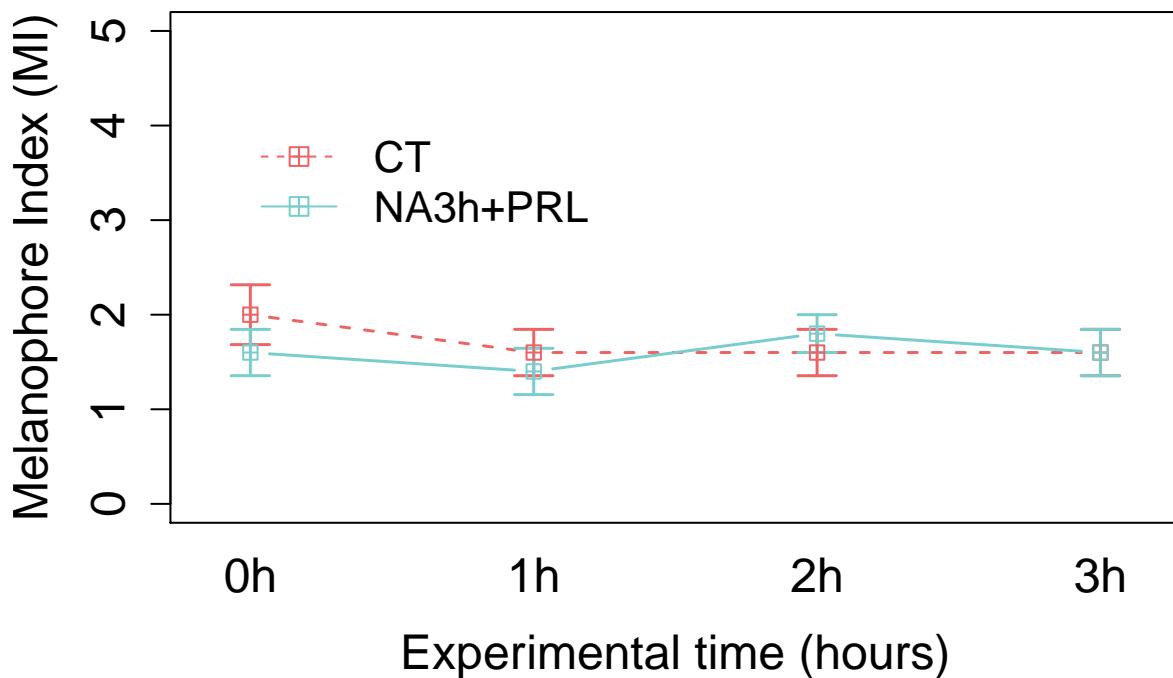

1.2.3   Red colour of Males

1.2.3.1   Noradrenaline

```
lineplot.CI(Time, Red, Treatment, data=maleNA, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
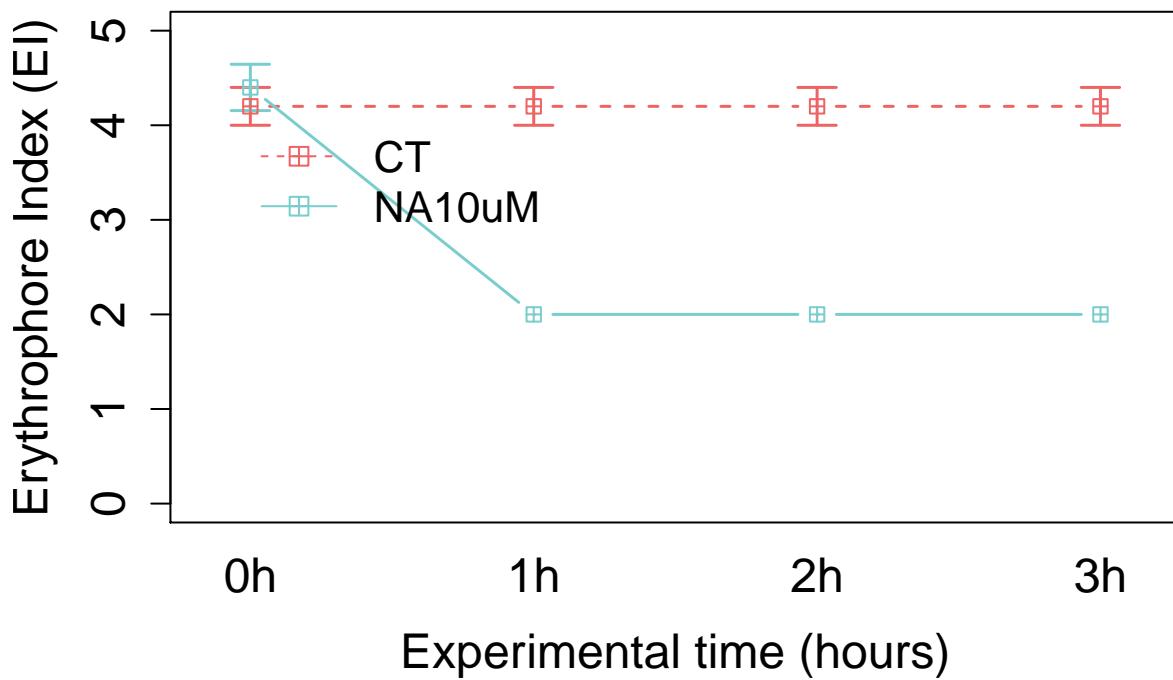
1.2.3.2 Melanocyte-Concentrating Hormone

```
lineplot.CI(Time, Red, Treatment, data=maleMCH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
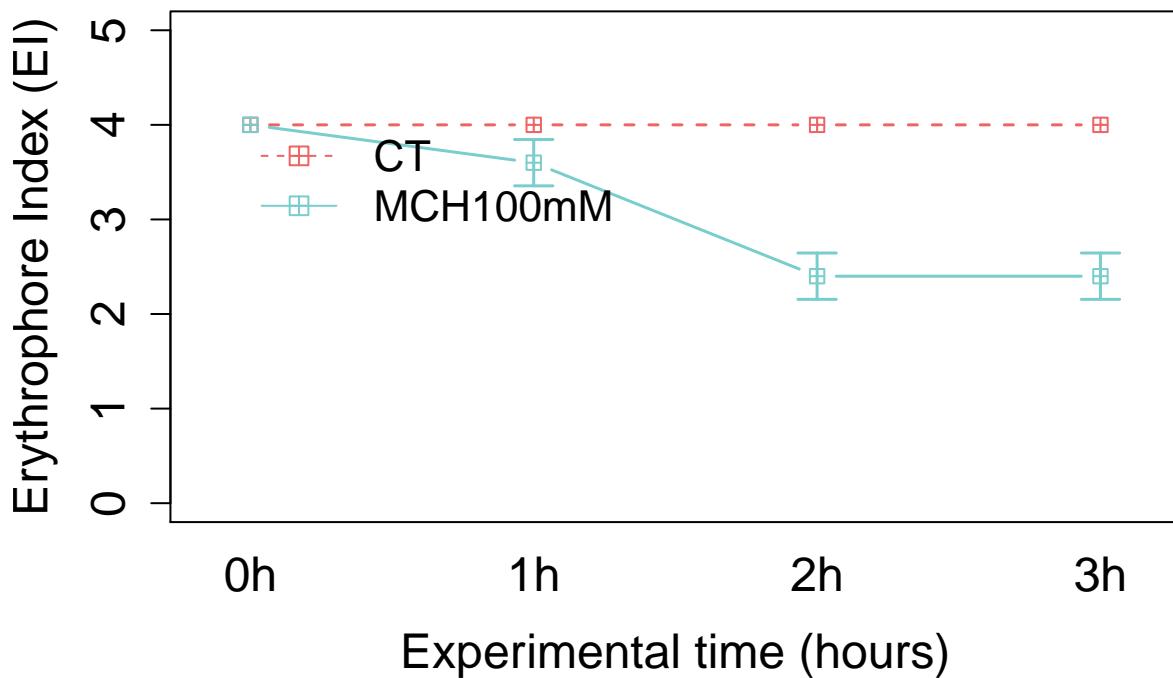

1.2.3.3 Melanotin

```
lineplot.CI(Time, Red, Treatment, data=maleMEL, cex = 1,
          xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
          col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
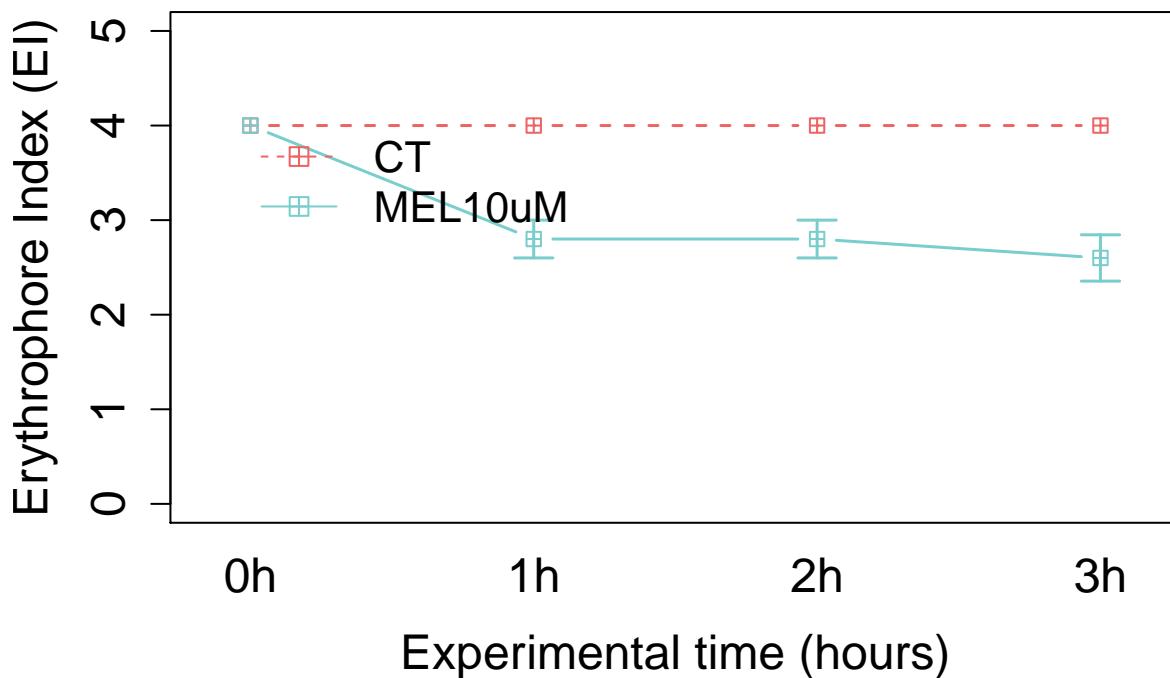

1.2.3.4   Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Red, Treatment, data=maleMSH, cex = 1,
          xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
          col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
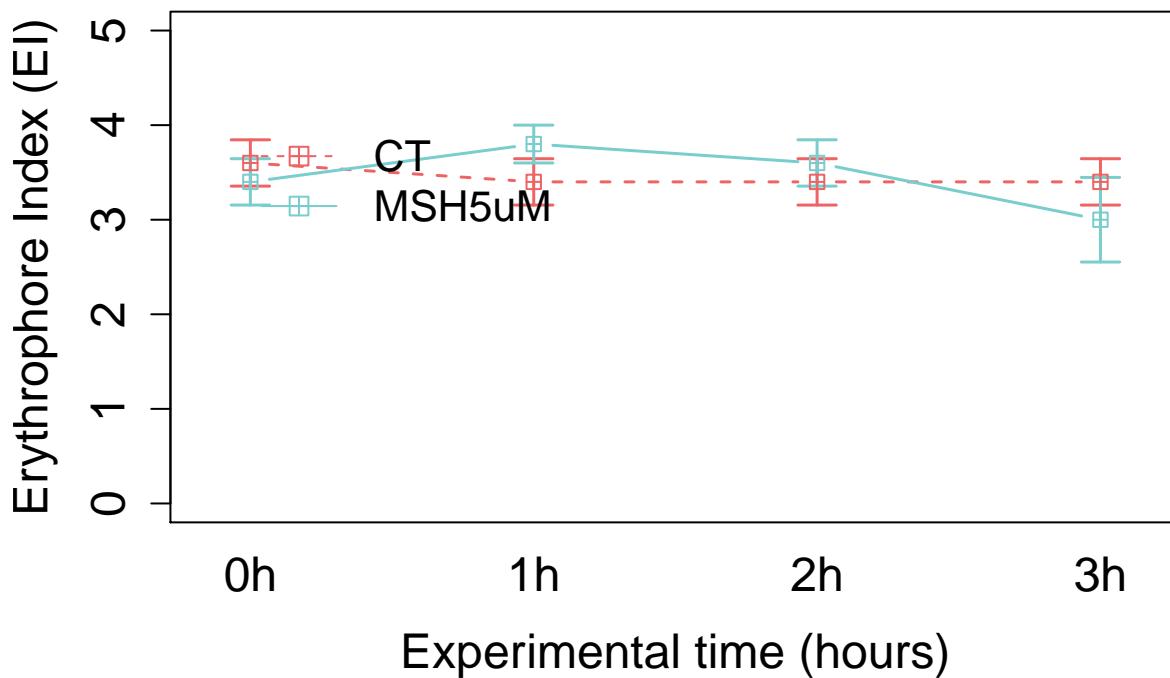
```
lineplot.CI(Time, Red, Treatment, data=malePRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
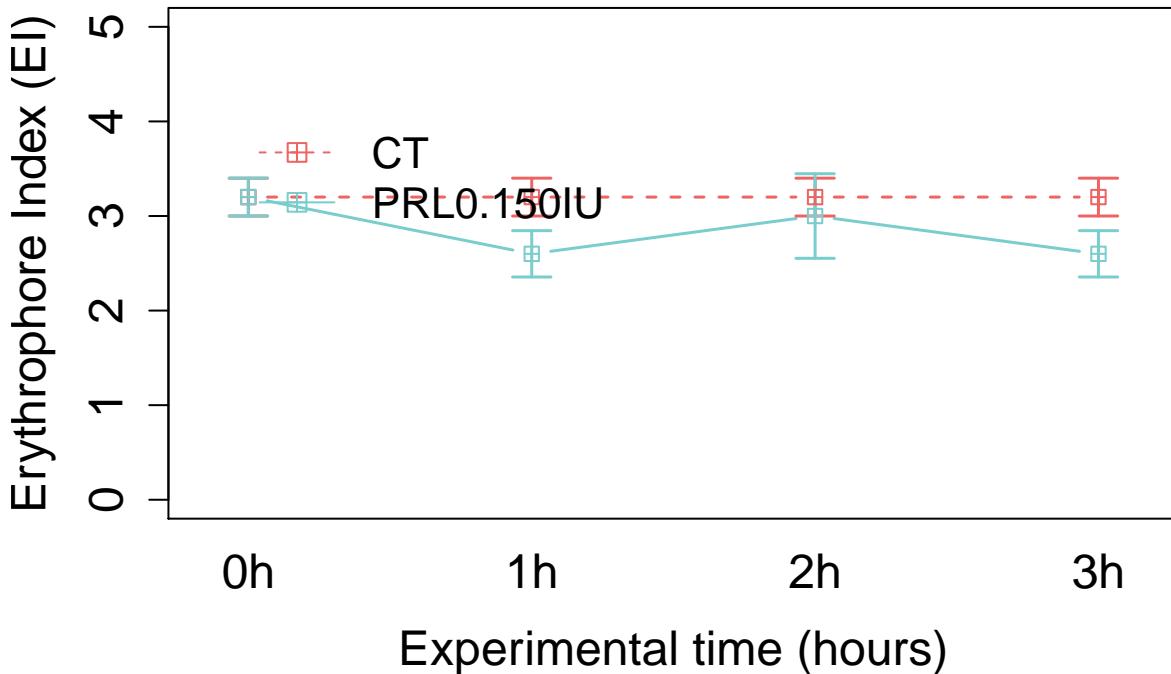

1.2.3.6 Noradrenaline and Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Red, Treatment, data=maleNAMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
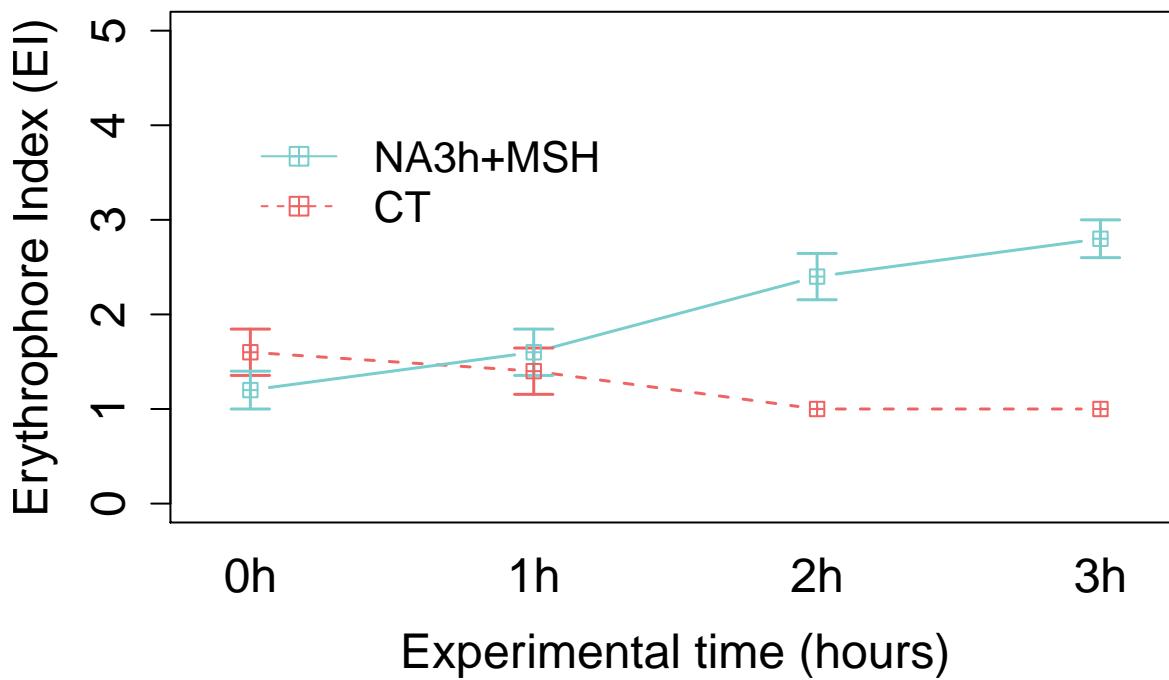
1.2.3.7 Noradrenaline and Prolactin

```r
lineplot.CI(Time, Red, Treatment, data=maleNAPRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Erythrophore Index (EI)", cex.lab = 1.5, x.leg =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,5))
```
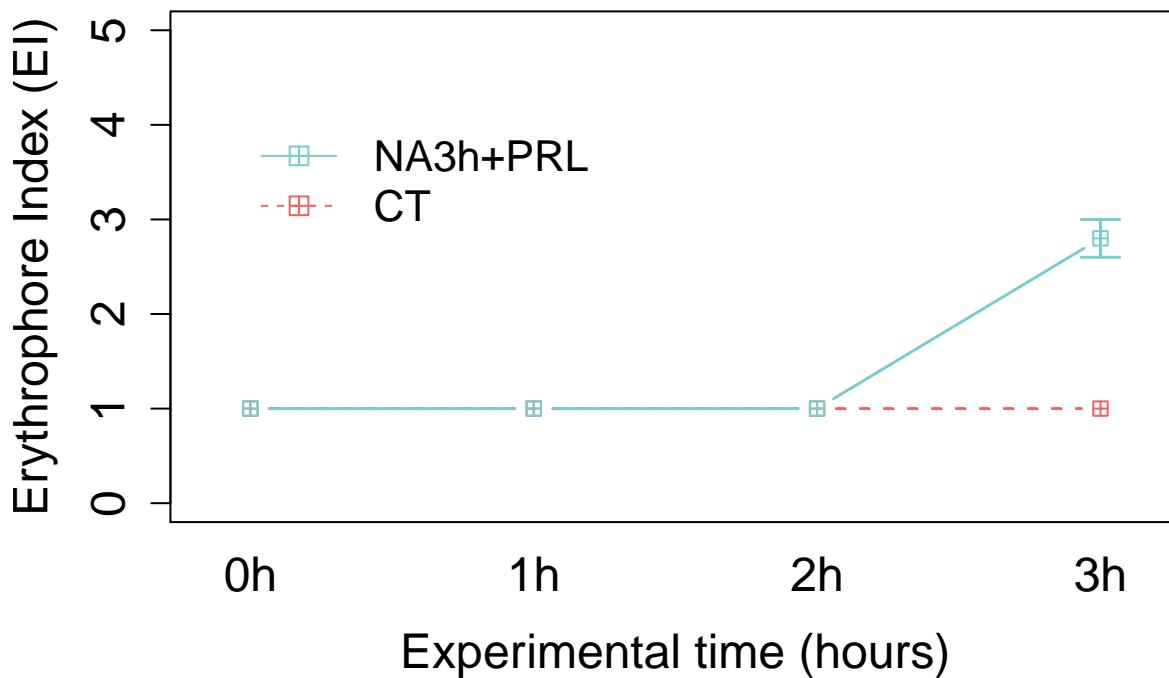

1.2.4 Yellow colour of Males

1.2.4.1 Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Yellow, Treatment, data=maleMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1))
```
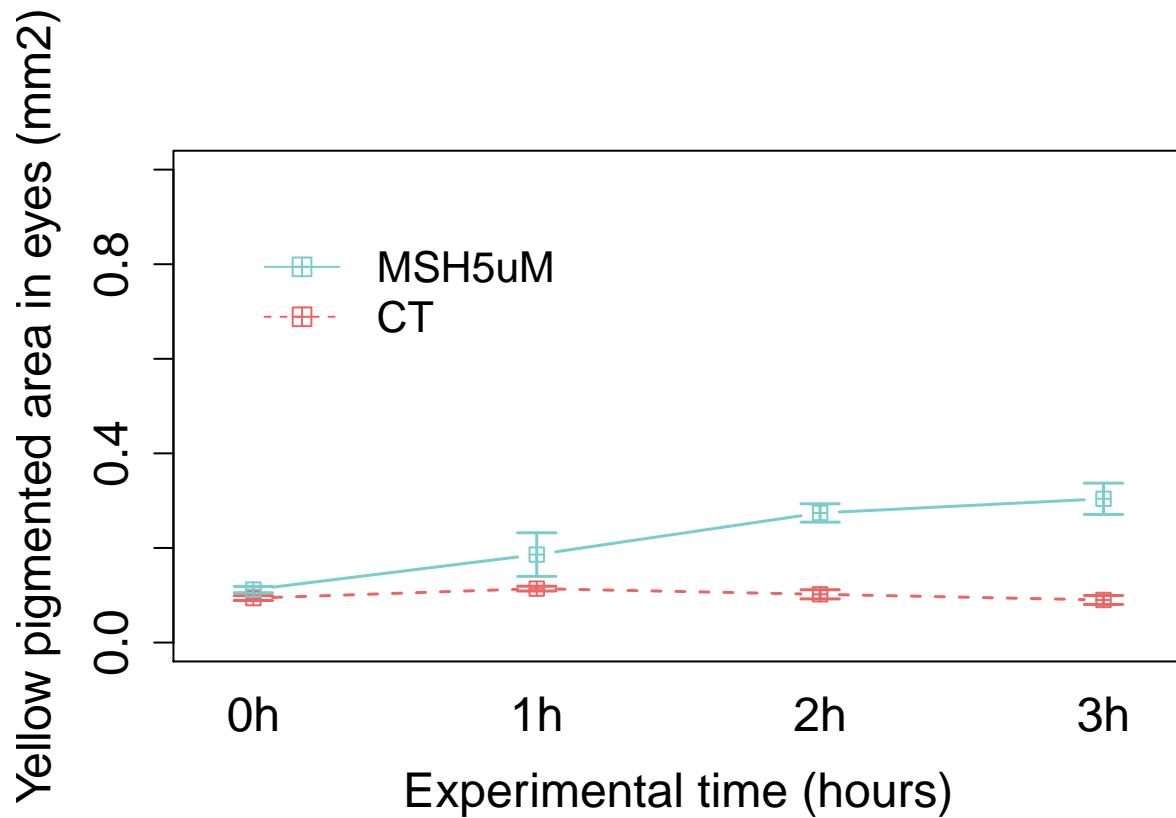

1.2.4.2   Prolactin

```
lineplot.CI(Time, Yellow, Treatment, data=malePRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1))
```
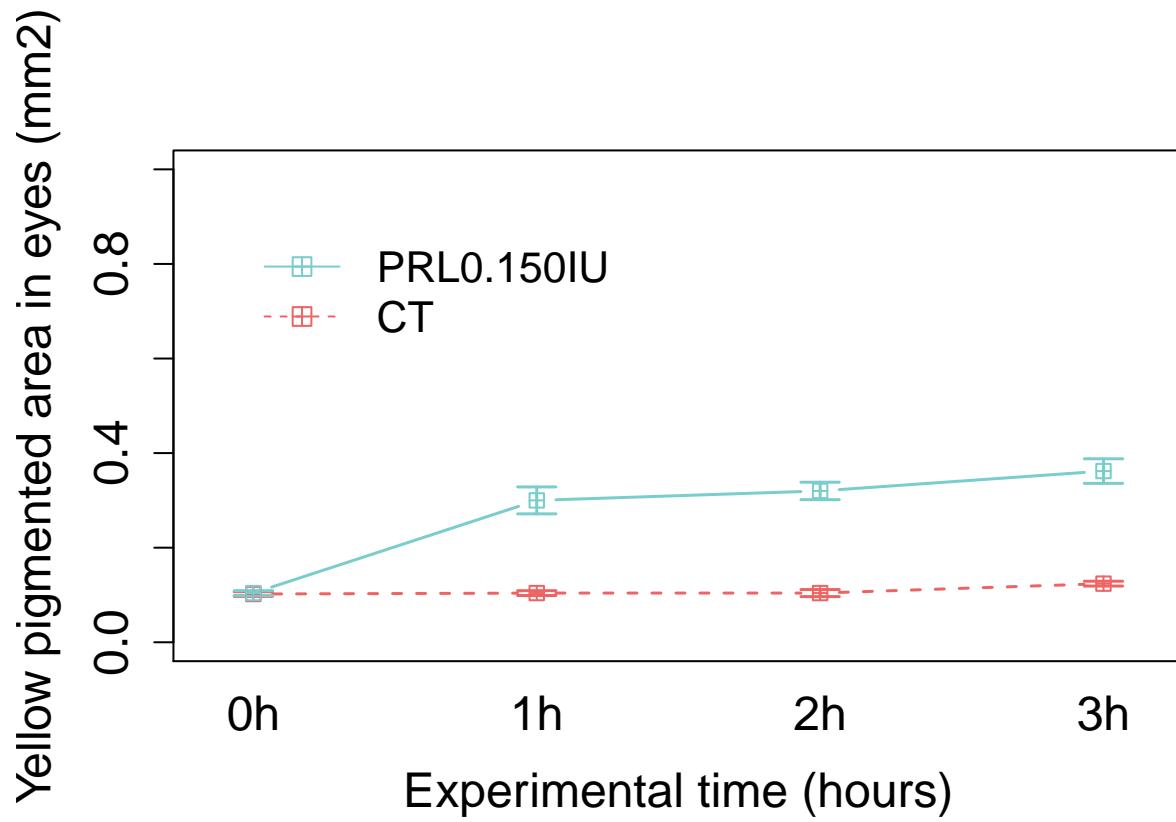
1.2.4.3 Noradrenaline and Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Yellow, Treatment, data=maleNAMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1))
```
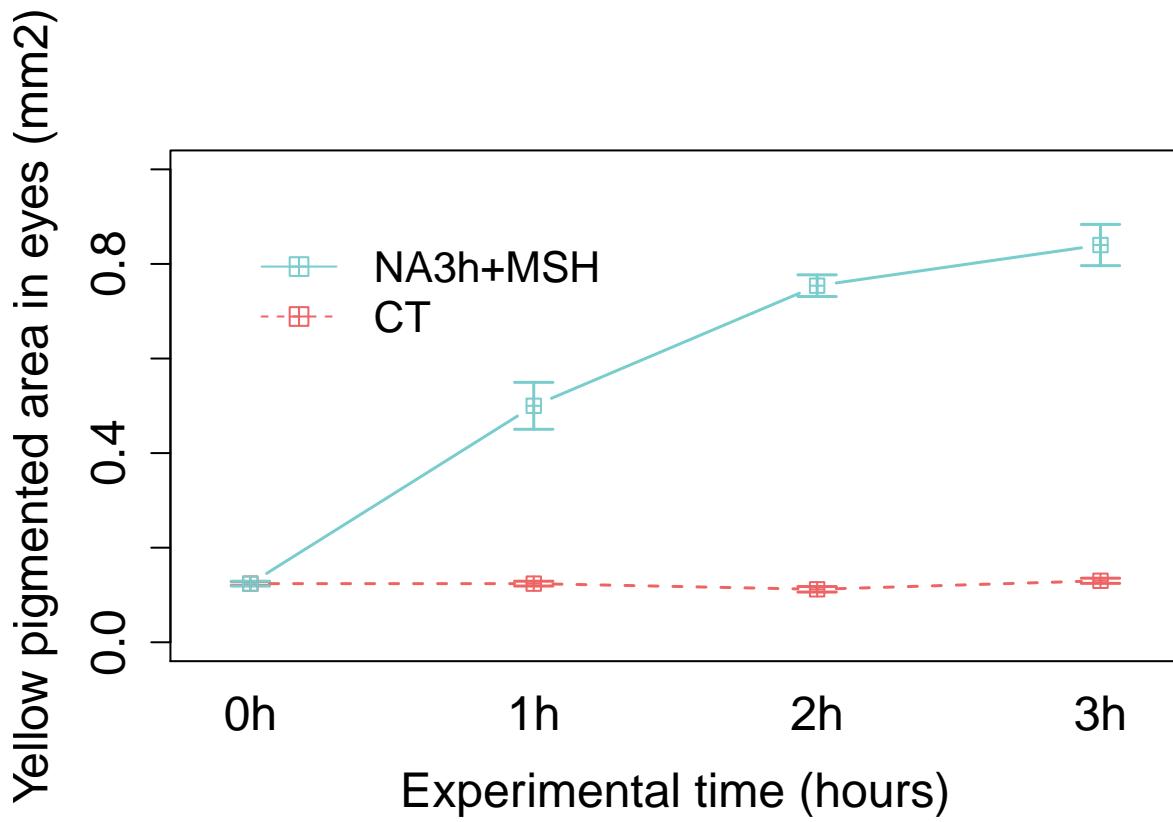
1.2.4.4 Noradrenaline and Prolactin

```
lineplot.CI(Time, Yellow, Treatment, data=maleNAPRL, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1))
```
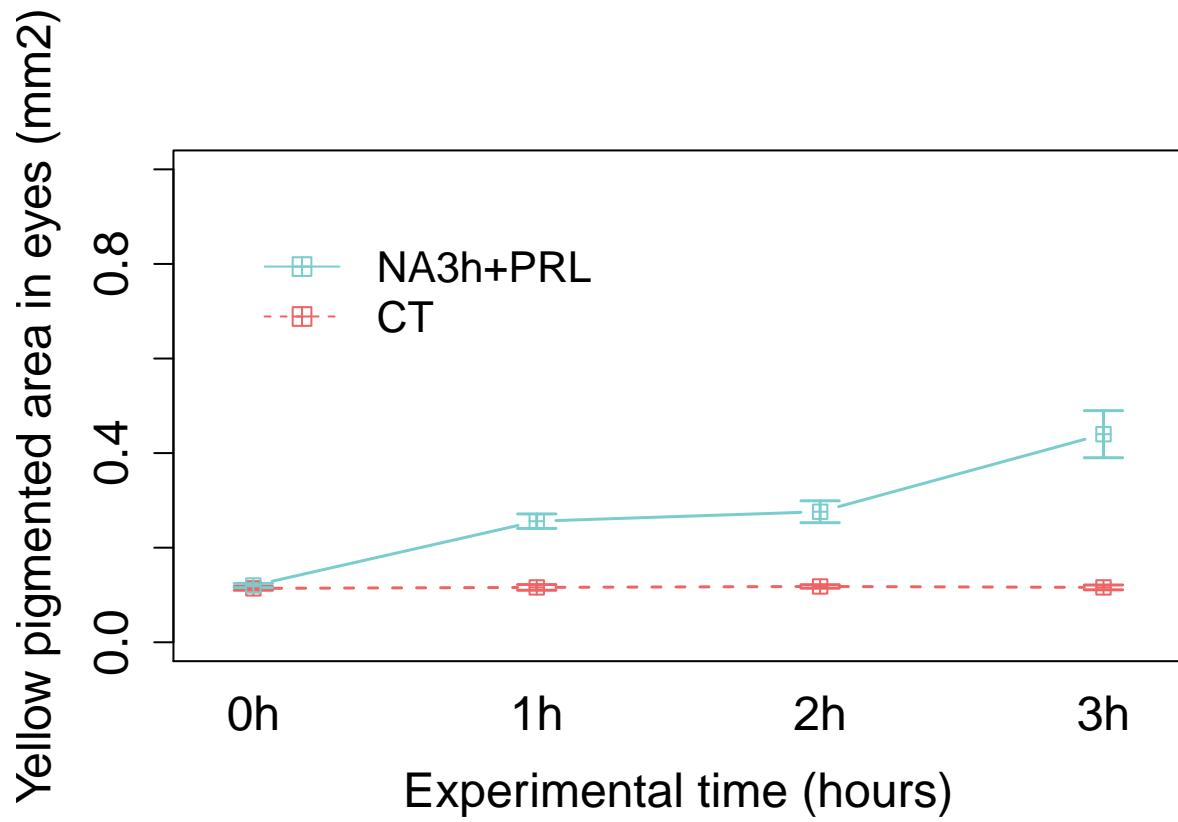
1.2.5 Yellow colour of Females

1.2.5.1 Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Yellow, Treatment, data=femaleMSH, cex = 1,
            xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
            col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1.1))
```

Yellow pigmented area in eyes (mm2) / Experimental time (hours)
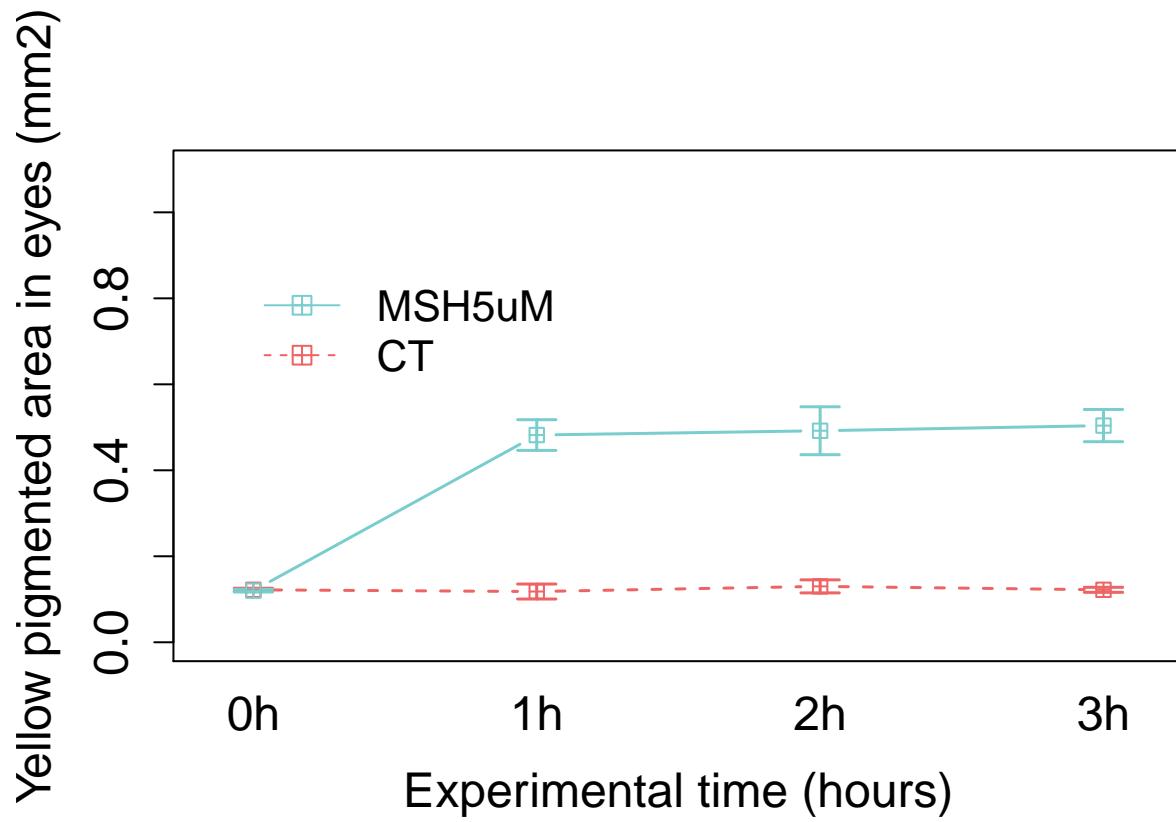
1.2.5.2 Prolactin

```
lineplot.CI(Time, Yellow, Treatment, data=femalePRL, cex = 1,
          xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
          col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1.1))
```
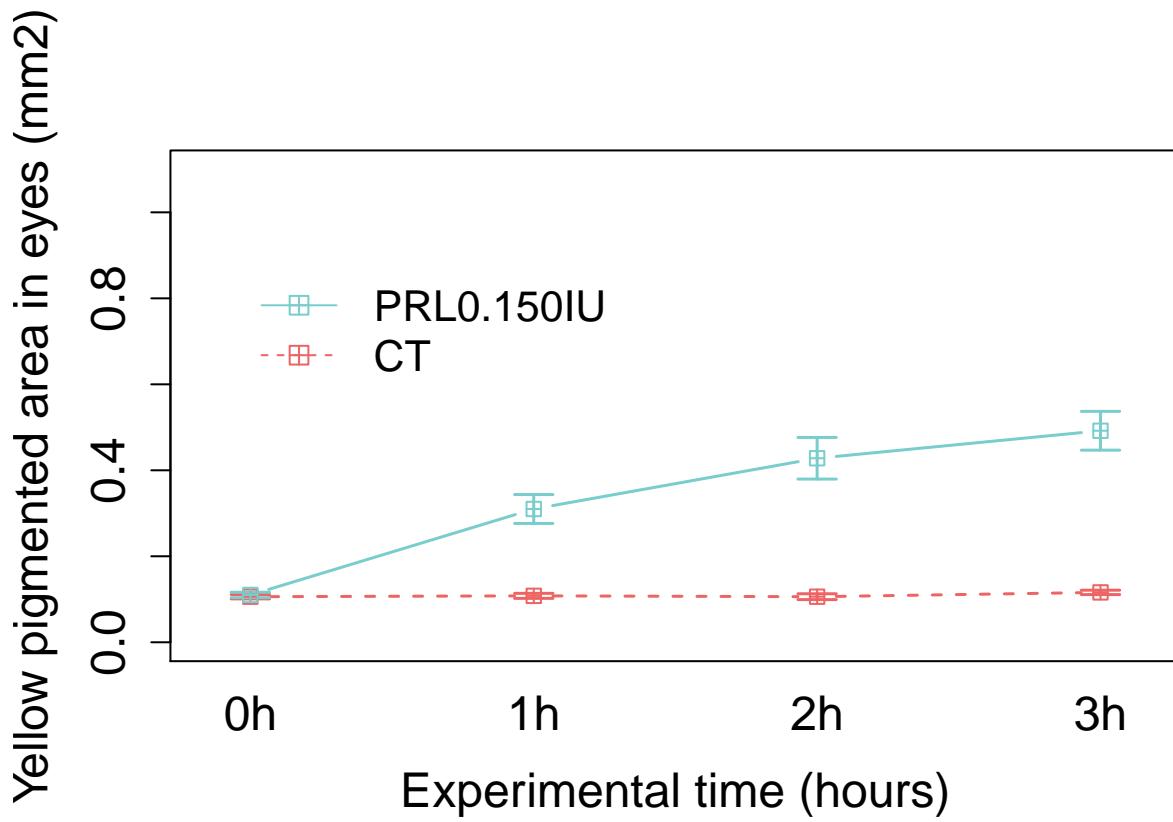
1.2.5.3 Noradrenaline and Melanocyte Stimulating Hormone

```
lineplot.CI(Time, Yellow, Treatment, data=femaleNAMSH, cex = 1,
        xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
        col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1.1))
```
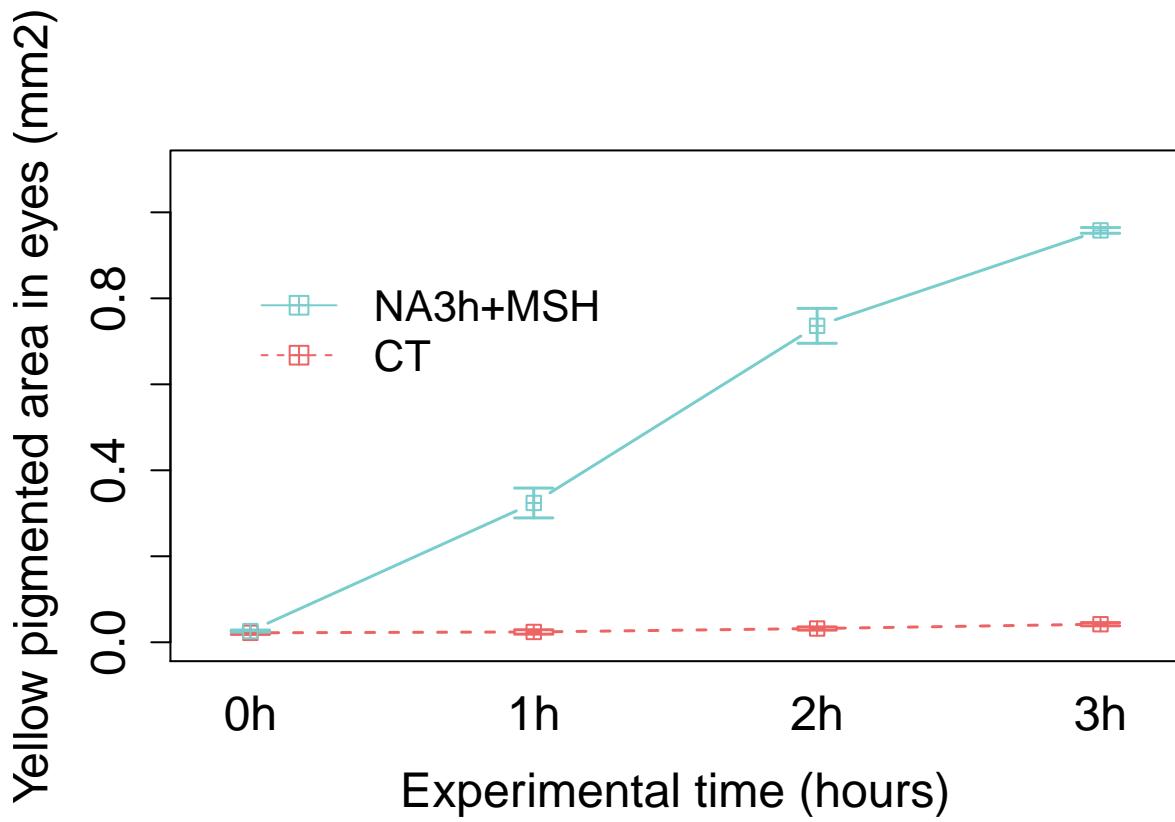
1.2.5.4 Noradrenaline and Prolactin

```
lineplot.CI(Time, Yellow, Treatment, data=femaleNAPRL, cex = 1,
          xlab = "Experimental time (hours)", ylab = "Yellow pigmented area in eyes (mm2)", cex.lab =
          col = c("#EE6363","#79CDCD"), pch = c(12,12), lwd = 1.5, ylim= c(0,1.1))
```
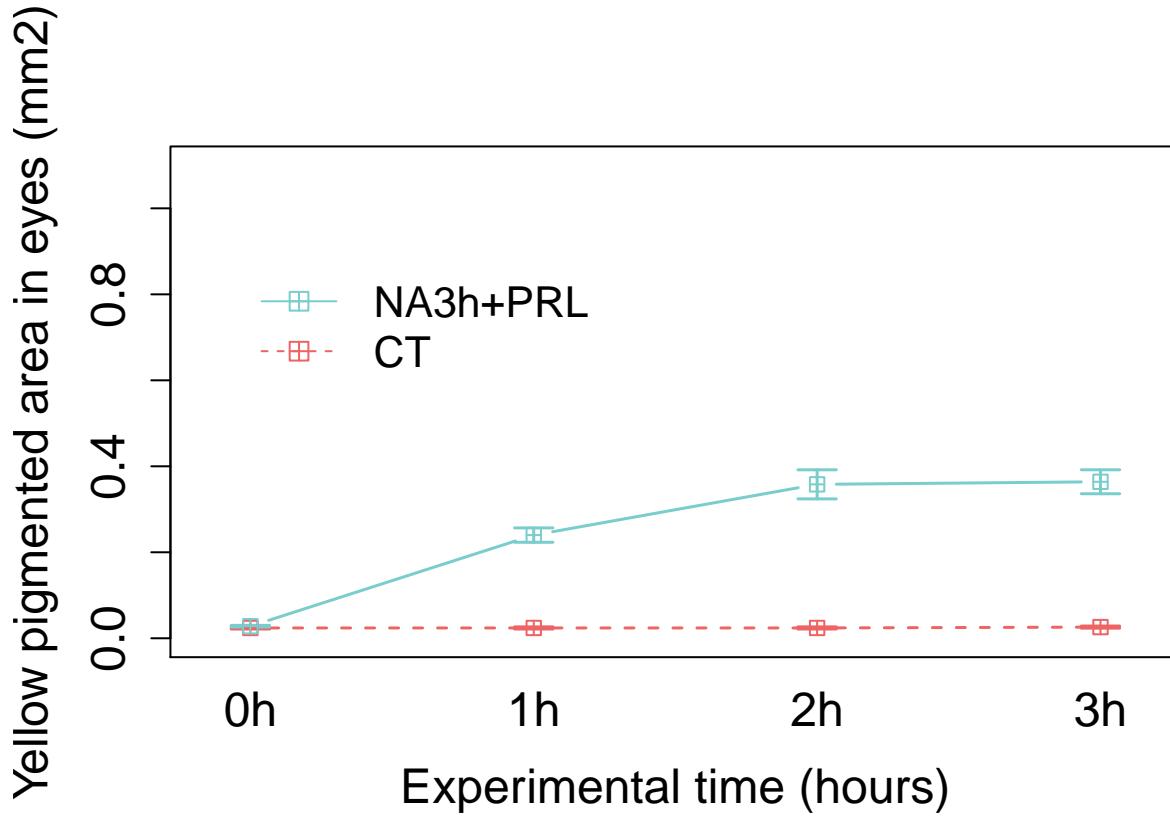
2 Converting `Animal` to factor and the colours to ordered factors to fit the GLMMs

```r
#Black Male

#NA
maleNA$Animal<-factor(maleNA$Animal)
maleNA$Black<-factor(maleNA$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)
str(maleNA)#check the conversion
```

```
## 'data.frame':    40 obs. of  6 variables:
##  $ Animal   : Factor w/ 5 levels "1","2","3","4",..: 1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","NA10uM": 1 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 2 ...
##  $ Black    : Ord.factor w/ 5 levels "1"<"2"<"3"<"4"<..: 5 5 5 5 5 5 5 5 5 4 4 ...
##  $ Red      : int  5 5 5 5 4 4 4 4 4 4 4 ...
```

```r
#MCH
maleMCH$Animal<-factor(maleMCH$Animal)
maleMCH$Black<-factor(maleMCH$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#MEL
maleMEL$Animal<-factor(maleMEL$Animal)
maleMEL$Black<-factor(maleMEL$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)
```

```r
#MSH
maleMSH$Animal<-factor(maleMSH$Animal)
maleMSH$Black<-factor(maleMSH$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)

#PRL
malePRL$Animal<-factor(malePRL$Animal)
malePRL$Black<-factor(malePRL$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)

#NA_MSH
maleNAMSH$Animal<-factor(maleNAMSH$Animal)
maleNAMSH$Black<-factor(maleNAMSH$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)

#NA_PRL
maleNAPRL$Animal<-factor(maleNAPRL$Animal)
maleNAPRL$Black<-factor(maleNAPRL$Black, levels = c( "1", "2", "3", "4", "5"), ordered = TRUE)


##Black Female

#NA
femaleNA$Animal<-factor(femaleNA$Animal)
femaleNA$Black<-factor(femaleNA$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)
str(femaleNA)
```

```
## 'data.frame':    40 obs. of  6 variables:
##  $ Animal   : Factor w/ 5 levels "6","7","8","9",..: 1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","NA10uM": 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": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Black    : Ord.factor w/ 5 levels "1"<"2"<"3"<"4"<..: 5 4 4 4 4 4 4 4 4 4 ...
##  $ Red      : int  NA NA NA NA NA NA NA NA NA NA ...
```

```r
#MCH
femaleMCH$Animal<-factor(femaleMCH$Animal)
femaleMCH$Black<-factor(femaleMCH$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#MEL
femaleMEL$Animal<-factor(femaleMEL$Animal)
femaleMEL$Black<-factor(femaleMEL$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#MSH
femaleMSH$Animal<-factor(femaleMSH$Animal)
femaleMSH$Black<-factor(femaleMSH$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#PRL
femalePRL$Animal<-factor(femalePRL$Animal)
femalePRL$Black<-factor(femalePRL$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#NA_MSH
femaleNAMSH$Animal<-factor(femaleNAMSH$Animal)
femaleNAMSH$Black<-factor(femaleNAMSH$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#NA_PRL
femaleNAPRL$Animal<-factor(femalePRL$Animal)
femaleNAPRL$Black<-factor(femaleNAPRL$Black, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)
```

```
##Red Male

#NA
maleNA$Red<-factor(maleNA$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)
str(maleNA)

## 'data.frame':     40 obs. of  6 variables:
##  $ Animal   : Factor w/ 5 levels "1","2","3","4",..: 1 1 1 1 2 2 2 2 3 3 ...
##  $ Treatment: Factor w/ 2 levels "CT","NA10uM": 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 ...
##  $ Black    : Ord.factor w/ 5 levels "1"<"2"<"3"<"4"<..: 5 5 5 5 5 5 5 5 5 4 4 ...
##  $ Red      : Ord.factor w/ 5 levels "1"<"2"<"3"<"4"<..: 5 5 5 5 4 4 4 4 4 4 4 ...
#MCH
maleMCH$Red<-factor(maleMCH$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#MEL
maleMEL$Red<-factor(maleMEL$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#MSH
maleMSH$Red<-factor(maleMSH$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#PRL
malePRL$Red<-factor(malePRL$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#NA_MSH
maleNAMSH$Red<-factor(maleNAMSH$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)

#NA_PRL
maleNAPRL$Red<-factor(maleNAPRL$Red, levels = c("1", "2", "3", "4", "5"), ordered = TRUE)
```

2.1 Statistical analysis using ordered GLMMs

Our experimental design is very similar to this tutorial. So, we'll follow it and use ordinal::clmm to build a generalized mixed-effects model separately for each sex, using a Cumulative logit model. Here, we'll use the Gaus-Hermite quadrature to fit the GLMM to the ordinal response variables. This is usually a more acurate method (see Agresti, 2010), than Laplace approximation that uses Maximum Likelihood. To accomplish this, we have to change the number of quadrature points in the argument `nAGQ` to be between 5 and 10, following the author of the package, see this thread in the R help mailing list. Since our dataset is relatively small, there will be no problem with speed. We'll build models separately for each sex and each treatment (=hormone).

2.1.1 Black colour of Males

2.1.1.1 Noradrenaline

```
modmaleNA<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleNA,
                link = "logit",
                threshold = "equidistant", nAGQ = 9) #proportional odds model

summary(modmaleNA)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNA
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40   -17.08 54.16 519(2068) 1.48e-06 2.1e+07
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 8.356    2.891
## Number of groups:  Animal 5
##
## Coefficients:
##                         Estimate Std. Error z value Pr(>|z|)
## TreatmentNA10uM          3.463e+00  2.165e+00   1.600    0.110
## Time1h                  -9.077e-07  2.043e+00   0.000    1.000
## Time2h                  -2.106e-06  2.043e+00   0.000    1.000
## Time3h                  -1.559e-06  2.043e+00   0.000    1.000
## TreatmentNA10uM:Time1h -2.496e+01  5.306e+02  -0.047    0.962
## TreatmentNA10uM:Time2h -4.300e+01  1.061e+03  -0.041    0.968
## TreatmentNA10uM:Time3h -4.890e+01  1.061e+03  -0.046    0.963
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -42.45    1061.12   -0.040
## spacing       21.50     530.56    0.041
```

Comparing levels Treatment and Time

```
Anova(modmaleNA, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##              LR Chisq Df Pr(>Chisq)
## Treatment     21.529   1  3.485e-06 ***
## Time          17.063   3  0.0006859 ***
## Treatment:Time 39.246  3  1.539e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleNA, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA10uM  0.1944734 0.1416574 NA   1.373  0.1698
##
## Time = 1h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA10uM -0.3333332 0.1577773 NA  -2.113  0.0346
##
## Time = 2h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA10uM -0.4721932 0.1416571 NA  -3.333  0.0009
```

```
##
## Time = 3h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA10uM -0.7722998 0.1438087 NA  -5.370  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.1.2 Melanocyte-Concentrating Hormone

```
modmaleMCH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleMCH, link = "logit",
          threshold = "equidistant", nAGQ = 10)
```

```
summary(modmaleMCH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleMCH
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40   -21.32 62.65 485(1932) 8.23e-06 3.3e+07
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 4.879    2.209
## Number of groups:  Animal 5
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentMCH100mM         19.8146  1369.8556   0.014    0.988
## Time1h                     1.3930     1.7412   0.800    0.424
## Time2h                     1.3930     1.7412   0.800    0.424
## Time3h                    -0.6301     1.6061  -0.392    0.695
## TreatmentMCH100mM:Time1h -19.8146  1369.8560  -0.014    0.988
## TreatmentMCH100mM:Time2h -21.2076  1369.8548  -0.015    0.988
## TreatmentMCH100mM:Time3h -19.8146  1369.8577  -0.014    0.988
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1   -4.240      1.886  -2.248
## spacing        3.052      1.010   3.022
```

Comparing levels Treatment and Time

```
Anova(modmaleMCH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##                LR Chisq Df Pr(>Chisq)
## Treatment        0.1558  1     0.6930
## Time             2.9667  3     0.3968
## Treatment:Time   4.4643  3     0.2155
```

```r
pairs(lsmeans(modmaleMCH, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast          estimate          SE df z.ratio p.value
##  CT - MCH100mM  1.239166e-01 0.1537824 NA   0.806  0.4204
##
## Time = 1h:
##  contrast          estimate          SE df z.ratio p.value
##  CT - MCH100mM -2.182558e-07 0.0646016 NA   0.000  1.0000
##
## Time = 2h:
##  contrast          estimate          SE df z.ratio p.value
##  CT - MCH100mM -8.694452e-02 0.1342493 NA  -0.648  0.5172
##
## Time = 3h:
##  contrast          estimate          SE df z.ratio p.value
##  CT - MCH100mM  2.220243e-07 0.2080277 NA   0.000  1.0000
##
## Results are averaged over the levels of: cut
```

2.1.1.3 Melatonin

```r
modmaleMEL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleMEL,
          link = "logit",
          threshold = "equidistant", nAGQ = 9)

summary(modmaleMEL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:     maleMEL
##
##  link  threshold   nobs logLik AIC    niter      max.grad cond.H
##  logit equidistant 40   -17.03 54.07 398(1197) 2.57e-07 1.2e+07
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 2.193    1.481
## Number of groups:  Animal 5
##
## Coefficients:
##                         Estimate Std. Error z value Pr(>|z|)
## TreatmentMEL10uM         20.83343  716.75358   0.029    0.977
## Time1h                   20.85528  983.37735   0.021    0.983
## Time2h                   -0.03529    1.74841  -0.020    0.984
## Time3h                   21.00637 1143.71421   0.018    0.985
## TreatmentMEL10uM:Time1h -43.56166 1128.51484  -0.039    0.969
## TreatmentMEL10uM:Time2h -21.95260  716.75484  -0.031    0.976
## TreatmentMEL10uM:Time3h -43.05882 1107.27460  -0.039    0.969
##
## Threshold coefficients:
##            Estimate Std. Error z value
```

```
## threshold.1    -4.987        2.045    -2.439
## spacing         3.007        1.186     2.536
```

Comparing levels `Treatment` and `Time`

```
Anova(modmaleMEL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##               LR Chisq Df Pr(>Chisq)
## Treatment       3.1538  1    0.07575 .
## Time            1.4744  3    0.68819
## Treatment:Time  6.9629  3    0.07309 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleMEL, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob" ))
```

```
## Time = 0h:
##  contrast        estimate          SE df z.ratio p.value
##  CT - MEL10uM  0.06403067 0.08781863 NA   0.729  0.4659
##
## Time = 1h:
##  contrast        estimate          SE df z.ratio p.value
##  CT - MEL10uM -0.25785362 0.18119507 NA  -1.423  0.1547
##
## Time = 2h:
##  contrast        estimate          SE df z.ratio p.value
##  CT - MEL10uM -0.09679797 0.14387113 NA  -0.673  0.5011
##
## Time = 3h:
##  contrast        estimate          SE df z.ratio p.value
##  CT - MEL10uM -0.17046660 0.15391361 NA  -1.108  0.2681
##
## Results are averaged over the levels of: cut
```

2.1.1.4   Melanocyte Stimulating Hormone

```
modmaleMSH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleMSH,
            link = "logit",
            threshold = "equidistant", nAGQ = 10)
```

```
summary(modmaleMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleMSH
##
##  link  threshold   nobs logLik AIC   niter    max.grad cond.H
##  logit equidistant 40   -21.63 63.26 897(712) 7.08e-06 2.3e+07
##
## Random effects:
##  Groups Name        Variance  Std.Dev.
```

```
##  Animal (Intercept) 1.798e-09 4.24e-05
## Number of groups:  Animal 5
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentMSH5uM            1.299e-06  1.517e+00    0.000    1.000
## Time1h                    -1.584e+00  1.884e+00   -0.841    0.400
## Time2h                    -1.584e+00  1.884e+00   -0.841    0.400
## Time3h                    -1.584e+00  1.884e+00   -0.841    0.400
## TreatmentMSH5uM:Time1h  2.522e+00  2.363e+00    1.067    0.286
## TreatmentMSH5uM:Time2h  1.852e+00  2.449e+00    0.756    0.450
## TreatmentMSH5uM:Time3h  2.522e+00  2.363e+00    1.067    0.286
##
## Threshold coefficients:
##              Estimate Std. Error z value
## threshold.1   -4.501      1.638   -2.748
## spacing        5.834      1.303    4.476
```

Comparing levels Treatment and Time

```
Anova(modmaleMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##              LR Chisq Df Pr(>Chisq)
## Treatment      3.7407  1     0.0531 .
## Time           0.2243  3     0.9736
## Treatment:Time  1.6293  3     0.6528
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleMSH, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - MSH5uM 1.143116e-07 0.1335067 NA   0.000  1.0000
##
## Time = 1h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - MSH5uM 1.991354e-01 0.1311429 NA   1.518  0.1289
##
## Time = 2h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - MSH5uM 1.239457e-01 0.1320559 NA   0.939  0.3479
##
## Time = 3h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - MSH5uM 1.991357e-01 0.1311429 NA   1.518  0.1289
##
## Results are averaged over the levels of: cut
```

2.1.1.5   Prolactin

```
modmalePRL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=malePRL,
          link = "logit",
```

```
            threshold = "equidistant", nAGQ = 9)
```

```
summary(modmalePRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:     malePRL
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40   -24.32 68.64 529(1590) 3.12e-06 9.8e+01
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 1.82     1.349
## Number of groups:  Animal 5
##
## Coefficients:
##                             Estimate Std. Error z value Pr(>|z|)
## TreatmentPRL0.150IU         -1.310e+00  1.683e+00  -0.779   0.436
## Time1h                      -3.428e-06  1.808e+00   0.000   1.000
## Time2h                      -2.358e+00  1.696e+00  -1.390   0.165
## Time3h                      -1.315e+00  1.684e+00  -0.781   0.435
## TreatmentPRL0.150IU:Time1h -1.053e+00  2.336e+00  -0.451   0.652
## TreatmentPRL0.150IU:Time2h -7.909e-01  2.338e+00  -0.338   0.735
## TreatmentPRL0.150IU:Time3h -7.727e-01  2.245e+00  -0.344   0.731
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -7.290     2.233   -3.265
## spacing       5.432     1.537    3.534
```

Comparing levels Treatment and Time

```
Anova(modmalePRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##                LR Chisq Df Pr(>Chisq)
## Treatment        6.1374  1    0.01324 *
## Time             7.0440  3    0.07051 .
## Treatment:Time   0.2234  3    0.97373
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmalePRL, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast         estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.1166509 0.1500528 NA  -0.777  0.4369
##
## Time = 1h:
##  contrast         estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.2475976 0.1521469 NA  -1.627  0.1037
```

```
##
## Time = 2h:
##  contrast         estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.1785211 0.1464809 NA  -1.219  0.2229
##
## Time = 3h:
##  contrast         estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.2367287 0.1552229 NA  -1.525  0.1272
##
## Results are averaged over the levels of: cut
```

2.1.1.6 Noradrenaline and Melanocyte Stimulating Hormone

```
modmaleNAMSH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleNAMSH,
            link = "logit",
            threshold = "equidistant", nAGQ = 10)
```

```
summary(modmaleNAMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNAMSH
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40    -9.42  38.84 367(1104) 9.44e-06 2.3e+08
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 2.774    1.666
## Number of groups:  Animal 10
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentNA3h+MSH         -20.4831  1184.8312  -0.017    0.986
## Time1h                      0.1172  1909.9432   0.000    1.000
## Time2h                     -0.4100  1229.5458   0.000    1.000
## Time3h                      0.2343  4034.0335   0.000    1.000
## TreatmentNA3h+MSH:Time1h   44.4955  1592.9202   0.028    0.978
## TreatmentNA3h+MSH:Time2h   85.5740  4797.7490   0.018    0.986
## TreatmentNA3h+MSH:Time3h   88.9910  2778.0673   0.032    0.974
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -22.52    1184.83  -0.019
## spacing       44.61    2290.80   0.019
```

Comparing levels Treatment and Time

```
Anova(modmaleNAMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
```

```
##                LR Chisq Df Pr(>Chisq)
## Treatment       19.252  1  1.145e-05 ***
## Time            18.167  3  0.0004063 ***
## Treatment:Time  32.140  3  4.890e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleNAMSH, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast         estimate          SE df z.ratio p.value
##  CT - NA3h+MSH -0.03853923 0.06055152 NA  -0.636  0.5245
##
## Time = 1h:
##  contrast         estimate          SE df z.ratio p.value
##  CT - NA3h+MSH  0.29479412 0.06055151 NA   4.868  <.0001
##
## Time = 2h:
##  contrast         estimate          SE df z.ratio p.value
##  CT - NA3h+MSH  0.37214408 0.06118586 NA   6.082  <.0001
##
## Time = 3h:
##  contrast         estimate          SE df z.ratio p.value
##  CT - NA3h+MSH  0.62812745 0.06055151 NA  10.373  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.1.7 Noradrenaline and Prolactin

```
modmaleNAPRL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=maleNAPRL,
            link = "logit",
            threshold = "equidistant", nAGQ = 9)
```

```
summary(modmaleNAPRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNAPRL
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40   -20.78 61.56 686(4817) 2.05e-05 9.3e+02
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 49.68    7.049
## Number of groups:  Animal 5
##
## Coefficients:
##                       Estimate Std. Error z value Pr(>|z|)
## TreatmentNA3h+PRL      5.763e+00 2.952e+00   1.952   0.0509 .
## Time1h                -2.334e-05 2.747e+00   0.000   1.0000
## Time2h                -2.334e-05 2.747e+00   0.000   1.0000
## Time3h                 2.520e+00 2.638e+00   0.955   0.3394
```
```

```
## TreatmentNA3h+PRL:Time1h -4.115e-07  3.350e+00   0.000   1.0000
## TreatmentNA3h+PRL:Time2h -4.110e-07  3.350e+00   0.000   1.0000
## TreatmentNA3h+PRL:Time3h  1.734e+01  8.749e+00   1.982   0.0475 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1   0.8634     3.7694   0.229
## spacing      10.7414     4.7367   2.268
```

Comparing levels `Treatment` and `Time`

```
Anova(modmaleNAPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##                LR Chisq Df Pr(>Chisq)
## Treatment        26.581  1  2.527e-07 ***
## Time             16.242  3  0.0010115 **
## Treatment:Time   16.769  3  0.0007885 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleNAPRL, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA3h+PRL 0.2329519 0.2533765 NA   0.919  0.3579
##
## Time = 1h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA3h+PRL 0.2329535 0.2533732 NA   0.919  0.3579
##
## Time = 2h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA3h+PRL 0.2329535 0.2533733 NA   0.919  0.3579
##
## Time = 3h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - NA3h+PRL 0.7078579 0.1281447 NA   5.524  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.2 Black colour of Females

2.1.2.1 Noradrenaline

```
modfemaleNA<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleNA,
        link = "logit",
        threshold = "equidistant", nAGQ = 9)
```

```
summary(modfemaleNA)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
```

```
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femaleNA
##
##  link  threshold   nobs logLik AIC    niter      max.grad cond.H
##  logit equidistant 40   -13.68 47.36 545(1092) 1.04e-05 1.7e+08
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 0.6126   0.7827
## Number of groups:  Animal 5
##
## Coefficients:
##                       Estimate Std. Error z value Pr(>|z|)
## TreatmentNA10uM          -1.759      2.000  -0.880   0.3791
## Time1h                   -1.759      2.000  -0.880   0.3791
## Time2h                   -1.797      2.090  -0.860   0.3900
## Time3h                   -1.759      2.000  -0.880   0.3791
## TreatmentNA10uM:Time1h   -8.507      3.491  -2.437   0.0148 *
## TreatmentNA10uM:Time2h  -29.335   3587.121  -0.008   0.9935
## TreatmentNA10uM:Time3h  -29.335   3587.122  -0.008   0.9935
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -11.559      3.658  -3.160
## spacing        6.531      1.877   3.479
```

Comparing levels Treatment and Time

```
Anova(modfemaleNA, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##               LR Chisq Df Pr(>Chisq)
## Treatment       44.054  1  3.195e-11 ***
## Time            22.353  3  5.508e-05 ***
## Treatment:Time  27.323  3  5.037e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleNA, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - NA10uM -0.05843696 0.07117852 NA  -0.821  0.4117
##
## Time = 1h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - NA10uM -0.53790204 0.08780965 NA  -6.126  <.0001
##
## Time = 2h:
##  contrast      estimate         SE df z.ratio p.value
```

```
## CT - NA10uM -0.66584612 0.03930454 NA -16.941  <.0001
##
## Time = 3h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA10uM -0.66674205 0.03664947 NA -18.192  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.2.2 Melanocyte-Concentrating Hormone

```
modfemaleMCH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleMCH,
            link = "logit",
            threshold = "equidistant", nAGQ = 9)
```

```
summary(modfemaleMCH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femaleMCH
##
##  link  threshold   nobs logLik AIC    niter       max.grad cond.H
##  logit equidistant 40    -19.58 59.16 606(2955) 1.76e-06 7.0e+02
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 7.265     2.695
## Number of groups:  Animal 5
##
## Coefficients:
##                            Estimate Std. Error z value Pr(>|z|)
## TreatmentMCH100mM         -1.768e-06  1.878e+00   0.000  1.00000
## Time1h                    -4.223e-06  1.878e+00   0.000  1.00000
## Time2h                    -4.852e-06  1.878e+00   0.000  1.00000
## Time3h                    -5.375e-06  1.878e+00   0.000  1.00000
## TreatmentMCH100mM:Time1h -8.236e+00  3.803e+00  -2.166  0.03034 *
## TreatmentMCH100mM:Time2h -1.321e+01  5.170e+00  -2.556  0.01060 *
## TreatmentMCH100mM:Time3h -1.686e+01  6.063e+00  -2.780  0.00543 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -14.023     4.942  -2.837
## spacing        8.238     2.716   3.033
```

Comparing levels Treatment and Time

```
Anova(modfemaleMCH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##               LR Chisq Df Pr(>Chisq)
```

```
## Treatment        28.787  1  8.078e-08 ***
## Time             15.162  3   0.001683 **
## Treatment:Time   28.342  3  3.078e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairs(lsmeans(modfemaleMCH, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast         estimate         SE df z.ratio p.value
##  CT - MCH100mM -4.481183e-08 0.04760461 NA   0.000  1.0000
##
## Time = 1h:
##  contrast         estimate         SE df z.ratio p.value
##  CT - MCH100mM -3.332905e-01 0.04788140 NA  -6.961  <.0001
##
## Time = 2h:
##  contrast         estimate         SE df z.ratio p.value
##  CT - MCH100mM -4.612494e-01 0.11784335 NA  -3.914  0.0001
##
## Time = 3h:
##  contrast         estimate         SE df z.ratio p.value
##  CT - MCH100mM -6.735387e-01 0.04502285 NA -14.960  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.2.3 Melatonin

```r
modfemaleMEL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleMEL,
           link = "logit",
           threshold = "equidistant", nAGQ = 8)
```

```r
summary(modfemaleMEL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 8 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femaleMEL
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40   -9.24  38.48 1314(11596) 7.82e+00 1.1e+07
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 488.9    22.11
## Number of groups:  Animal 5
##
## Coefficients:
##                         Estimate Std. Error   z value Pr(>|z|)
## TreatmentMEL10uM         -6.532121   0.001793  -3643.84   <2e-16 ***
## Time1h                   -2.363374   0.002426   -974.37   <2e-16 ***
## Time2h                   -2.183229   0.001793  -1217.45   <2e-16 ***
## Time3h                   -2.373089   0.002511   -944.95   <2e-16 ***
## TreatmentMEL10uM:Time1h -38.669845   0.002416 -16007.12   <2e-16 ***
```

```
## TreatmentMEL10uM:Time2h -49.707635    0.001794 -27704.12    <2e-16 ***
## TreatmentMEL10uM:Time3h -68.070684    4.665966     -14.59    <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##              Estimate Std. Error z value
## threshold.1 -54.753426   0.001795  -30504
## spacing      31.006311   0.002516   12322
```

Comparing levels `Treatment` and `Time`

```
Anova(modfemaleMEL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##              LR Chisq Df Pr(>Chisq)
## Treatment      35.226  1  2.936e-09 ***
## Time           20.240  3  0.0001514 ***
## Treatment:Time 19.638  3  0.0002017 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleMEL, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast        estimate           SE df      z.ratio p.value
##  CT - MEL10uM -1.667095e-08 5.776369e-11 NA -2.886060e+02  <.0001
##
## Time = 1h:
##  contrast        estimate           SE df      z.ratio p.value
##  CT - MEL10uM -5.003775e-01 1.554352e-06 NA -3.219203e+05  <.0001
##
## Time = 2h:
##  contrast        estimate           SE df      z.ratio p.value
##  CT - MEL10uM -9.875729e-01 3.477845e-05 NA -2.839612e+04  <.0001
##
## Time = 3h:
##  contrast        estimate           SE df      z.ratio p.value
##  CT - MEL10uM -1.000000e+00 5.209756e-10 NA -1.919476e+09  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.2.4   Melanocyte Stimulating Hormone

```
modfemaleMSH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleMSH,
         link = "logit",
         threshold = "equidistant", nAGQ = 9)
```

```
summary(modfemaleMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
```

45

```
## data:     femaleMSH
##
##  link  threshold   nobs logLik AIC    niter      max.grad cond.H
##  logit equidistant 40    -19.38 58.75 584(2923) 1.25e-05 2.2e+02
##
## Random effects:
##  Groups Name         Variance Std.Dev.
##  Animal (Intercept) 8.344    2.889
## Number of groups:  Animal 5
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentMSH5uM           2.464e-06  1.744e+00   0.000    1.000
## Time1h                    2.435e+00  2.513e+00   0.969    0.333
## Time2h                    2.435e+00  2.513e+00   0.969    0.333
## Time3h                    2.435e+00  2.513e+00   0.969    0.333
## TreatmentMSH5uM:Time1h   -4.125e-06  3.227e+00   0.000    1.000
## TreatmentMSH5uM:Time2h   -3.689e+00  3.031e+00  -1.217    0.224
## TreatmentMSH5uM:Time3h   -4.768e+00  3.063e+00  -1.557    0.120
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1   -3.464      1.913  -1.811
## spacing        8.383      2.407   3.483
```

Comparing levels `Treatment` and `Time`

```
Anova(modfemaleMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##               LR Chisq Df Pr(>Chisq)
## Treatment       4.6912  1    0.03032 *
## Time            3.8692  3    0.27594
## Treatment:Time  4.1388  3    0.24687
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleMSH, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate          SE df z.ratio p.value
##  CT - MSH5uM  4.511749e-08 0.03194333 NA   0.000  1.0000
##
## Time = 1h:
##  contrast      estimate          SE df z.ratio p.value
##  CT - MSH5uM -6.130008e-08 0.10019791 NA   0.000  1.0000
##
## Time = 2h:
##  contrast      estimate          SE df z.ratio p.value
##  CT - MSH5uM -8.551416e-02 0.09861147 NA  -0.867  0.3858
##
## Time = 3h:
##  contrast      estimate          SE df z.ratio p.value
##  CT - MSH5uM -1.587645e-01 0.15561202 NA  -1.020  0.3076
```

```
##
## Results are averaged over the levels of: cut
```

2.1.2.5 Prolactin

```
modfemalePRL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femalePRL,
            link = "logit",
            threshold = "equidistant", nAGQ = 10)
```

```
summary(modfemalePRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femalePRL
##
##  link  threshold   nobs logLik AIC   niter       max.grad cond.H
##  logit equidistant 40    -6.48  32.95 792(11033) 7.25e-06 7.0e+03
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 714.4    26.73
## Number of groups:  Animal 5
##
## Coefficients:
##                             Estimate Std. Error z value Pr(>|z|)
## TreatmentPRL0.150IU          7.582e-06  9.099e+00   0.000   1.000
## Time1h                      -1.705e-06  9.099e+00   0.000   1.000
## Time2h                      -2.601e-06  9.099e+00   0.000   1.000
## Time3h                      -2.269e-06  9.099e+00   0.000   1.000
## TreatmentPRL0.150IU:Time1h -1.706e+01  1.406e+01  -1.214   0.225
## TreatmentPRL0.150IU:Time2h -1.706e+01  1.406e+01  -1.214   0.225
## TreatmentPRL0.150IU:Time3h -1.706e+01  1.406e+01  -1.214   0.225
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -0.5123     9.5576  -0.054
## spacing      16.4981     8.1628   2.021
```

Comparing levels Treatment and Time

```
Anova(modfemalePRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##              LR Chisq Df Pr(>Chisq)
## Treatment     17.8579  1   2.38e-05 ***
## Time           9.9063  3    0.01938 *
## Treatment:Time  1.8385  3    0.60659
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemalePRL, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast          estimate      SE df z.ratio p.value
##  CT - PRL0.150IU -7.582379e-06  9.099024 NA   0.000  1.0000
##
## Time = 1h:
##  contrast          estimate      SE df z.ratio p.value
##  CT - PRL0.150IU  1.706369e+01 10.713409 NA   1.593  0.1112
##
## Time = 2h:
##  contrast          estimate      SE df z.ratio p.value
##  CT - PRL0.150IU  1.706369e+01 10.713539 NA   1.593  0.1112
##
## Time = 3h:
##  contrast          estimate      SE df z.ratio p.value
##  CT - PRL0.150IU  1.706369e+01 10.713467 NA   1.593  0.1112
```

2.1.2.6 Noradrenaline and Melanocyte Stimulating Hormone

```
modfemaleNAMSH<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleNAMSH,
             link = "logit",
             threshold = "equidistant", nAGQ = 10)

summary(modfemaleNAMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femaleNAMSH
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40   -24.82 69.64 531(1585) 1.63e-05 4.0e+02
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 4.941    2.223
## Number of groups:  Animal 10
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentNA3h+MSH        -2.252e+00  2.355e+00  -0.956  0.33892
## Time1h                    6.553e-06  1.674e+00   0.000  1.00000
## Time2h                    2.650e-05  1.674e+00   0.000  0.99999
## Time3h                   -2.598e+00  1.781e+00  -1.459  0.14452
## TreatmentNA3h+MSH:Time1h  7.519e+00  3.145e+00   2.391  0.01682 *
## TreatmentNA3h+MSH:Time2h  8.908e+00  3.371e+00   2.643  0.00823 **
## TreatmentNA3h+MSH:Time3h  1.315e+01  4.170e+00   3.153  0.00162 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##            Estimate Std. Error z value
## threshold.1  -7.115     2.405   -2.959
## spacing       6.485     1.682    3.857
```

Comparing levels `Treatment` and `Time`

```
Anova(modfemaleNAMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Black
##              LR Chisq Df Pr(>Chisq)
## Treatment      6.7505  1   0.009372 **
## Time           6.5701  3   0.086939 .
## Treatment:Time 22.0605  3   6.337e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleNAMSH, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast       estimate         SE df z.ratio p.value
##  CT - NA3h+MSH -0.1656375 0.14685097 NA  -1.128  0.2593
##
## Time = 1h:
##  contrast       estimate         SE df z.ratio p.value
##  CT - NA3h+MSH  0.2332466 0.17035776 NA   1.369  0.1710
##
## Time = 2h:
##  contrast       estimate         SE df z.ratio p.value
##  CT - NA3h+MSH  0.3449780 0.16512169 NA   2.089  0.0367
##
## Time = 3h:
##  contrast       estimate         SE df z.ratio p.value
##  CT - NA3h+MSH  0.6026166 0.07825383 NA   7.701  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.2.7 Noradrenalinee and Prolactin

```
modfemaleNAPRL<-clmm(Black~Treatment+Time+Treatment:Time+(1|Animal), data=femaleNAPRL,
              link = "logit",
              threshold = "equidistant", nAGQ = 9)
```

```
summary(modfemaleNAPRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Black ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    femaleNAPRL
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40    -20.64 61.27 482(1862) 5.99e-06 1.8e+02
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 5.932    2.436
## Number of groups:  Animal 5
```

```
## 
## Coefficients:
##                         Estimate Std. Error z value Pr(>|z|)
## TreatmentNA3h+PRL         -3.146      1.995  -1.577   0.1148
## Time1h                    -3.146      1.995  -1.577   0.1148
## Time2h                    -3.146      1.995  -1.577   0.1148
## Time3h                    -3.146      1.995  -1.577   0.1148
## TreatmentNA3h+PRL:Time1h   1.443      2.651   0.544   0.5863
## TreatmentNA3h+PRL:Time2h   4.878      2.941   1.659   0.0972 .
## TreatmentNA3h+PRL:Time3h   3.158      2.769   1.140   0.2542
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1   -3.779      1.891  -1.998
## spacing        7.284      1.892   3.850
```

Comparing levels Treatment and Time

```
Anova(modfemaleNAPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
## 
## Response: Black
##                LR Chisq Df Pr(>Chisq)
## Treatment        0.6992  1     0.4030
## Time             3.3806  3     0.3366
## Treatment:Time   3.7260  3     0.2926
```

```
pairs(lsmeans(modfemaleNAPRL, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -0.17613610 0.1873331 NA  -0.940  0.3471
## 
## Time = 1h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -0.19941570 0.2034444 NA  -0.980  0.3270
## 
## Time = 2h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+PRL  0.13344292 0.1824420 NA   0.731  0.4645
## 
## Time = 3h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+PRL  0.00136447 0.2190670 NA   0.006  0.9950
## 
## Results are averaged over the levels of: cut
```

2.1.3   Red Male

2.1.3.1   Noradrenaline

```
modmaleredNA<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleNA,
                link = "logit",
```

```
                    threshold = "equidistant", nAGQ = 10)
```

`summary`(modmaleredNA)

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNA
##
##  link  threshold   nobs logLik AIC    niter        max.grad cond.H
##  logit equidistant 40   -5.07  30.14 1493(12171) 1.48e+01 9.1e+09
##
## Random effects:
##  Groups Name         Variance Std.Dev.
##  Animal (Intercept) 269.4     16.41
## Number of groups:  Animal 5
##
## Coefficients:
##                         Estimate Std. Error  z value Pr(>|z|)
## TreatmentNA10uM          1.013e+01  1.816e-03 5579.487   <2e-16 ***
## Time1h                  -7.313e-02  2.013e-03  -36.331   <2e-16 ***
## Time2h                  -7.313e-02  2.013e-03  -36.331   <2e-16 ***
## Time3h                  -7.313e-02  2.013e-03  -36.331   <2e-16 ***
## TreatmentNA10uM:Time1h -1.007e+02  1.544e+02   -0.652    0.515
## TreatmentNA10uM:Time2h -1.007e+02  1.544e+02   -0.652    0.515
## TreatmentNA10uM:Time3h -1.007e+02  1.544e+02   -0.652    0.515
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1 -47.00703    0.00188  -25005
## spacing      60.35719    0.00188   32107
```

Comparing levels Treatment and Time

`Anova`(modmaleredNA, type="II")

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##               LR Chisq Df Pr(>Chisq)
## Treatment       26.316  1  2.899e-07 ***
## Time            13.362  3    0.003916 **
## Treatment:Time  35.677  3  8.763e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

`pairs`(`lsmeans`(modmaleredNA, ~ Treatment|Time, adjust= "tukey", mode = "cum.prob"))

```
## Time = 0h:
##  contrast      estimate          SE df      z.ratio p.value
##  CT - NA10uM 0.01920914 5.364965e-05 NA  3.580480e+02  <.0001
##
## Time = 1h:
```

```
## contrast       estimate        SE df     z.ratio p.value
## CT - NA10uM -0.50000074 2.259276e-09 NA -2.213101e+08  <.0001
##
## Time = 2h:
## contrast       estimate        SE df     z.ratio p.value
## CT - NA10uM -0.50000074 2.259278e-09 NA -2.213099e+08  <.0001
##
## Time = 3h:
## contrast       estimate        SE df     z.ratio p.value
## CT - NA10uM -0.50000074 2.259277e-09 NA -2.213100e+08  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.3.2 Melanocyte-Concentrating Hormone

```
modmaleredMCH<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleMCH,
                    link = "logit",
                    threshold = "equidistant", nAGQ = 9)
```

```
summary(modmaleredMCH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleMCH
##
##  link  threshold   nobs logLik AIC   niter      max.grad cond.H
##  logit equidistant 40    -5.83  31.66 1251(5283) 5.73e+00 2.3e+00
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 70.15    8.375
## Number of groups:  Animal 5
##
## Coefficients:
##                          Estimate Std. Error  z value Pr(>|z|)
## TreatmentMCH100mM          -1.131175   0.003051   -370.7   <2e-16 ***
## Time1h                      0.908648   0.003115    291.7   <2e-16 ***
## Time2h                     -0.553411   0.003159   -175.2   <2e-16 ***
## Time3h                     -0.553413   0.003159   -175.2   <2e-16 ***
## TreatmentMCH100mM:Time1h  -21.317856   0.003115  -6844.5   <2e-16 ***
## TreatmentMCH100mM:Time2h  -46.054819   0.003159 -14580.4   <2e-16 ***
## TreatmentMCH100mM:Time3h  -46.054814   0.003159 -14580.4   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1 -45.383867   0.003052  -14873
## spacing      21.135166   0.003115    6786
```

Comparing levels Treatment and Time

```r
Anova(modmaleredMCH, type="II")
```

```
## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met
## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##               LR Chisq Df Pr(>Chisq)
## Treatment       41.998  1  9.136e-11 ***
## Time            32.081  3  5.032e-07 ***
## Treatment:Time  -0.185  3          1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairs(lsmeans(modmaleredMCH, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast        estimate           SE df   z.ratio p.value
##  CT - MCH100mM -3.090062e-11 1.759816e-13 NA  -175.590  <.0001
##
## Time = 1h:
##  contrast        estimate           SE df   z.ratio p.value
##  CT - MCH100mM -3.124220e-02 1.642048e-04 NA  -190.264  <.0001
##
## Time = 2h:
##  contrast        estimate           SE df   z.ratio p.value
##  CT - MCH100mM -9.566868e-01 2.188158e-04 NA -4372.110  <.0001
##
## Time = 3h:
##  contrast        estimate           SE df   z.ratio p.value
##  CT - MCH100mM -9.566866e-01 2.188165e-04 NA -4372.096  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.3.3 Melatonin

```r
modmaleredMEL<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleMEL,
                    link = "logit",
                    threshold = "equidistant", nAGQ = 8)

summary(modmaleredMEL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 8 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleMEL
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40   -5.18  30.35 972(4776) 3.92e+00 1.7e+00
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 51.94    7.207
## Number of groups:  Animal 5
##
## Coefficients:
##                          Estimate Std. Error  z value Pr(>|z|)
## TreatmentMEL10uM          1.370280   0.003648     375.6  <2e-16 ***
## Time1h                    1.054897   0.003695     285.5  <2e-16 ***
## Time2h                    1.054897   0.003695     285.5  <2e-16 ***
## Time3h                   -0.732646   0.003644    -201.0  <2e-16 ***
## TreatmentMEL10uM:Time1h -42.832476   0.003696 -11590.4  <2e-16 ***
## TreatmentMEL10uM:Time2h -42.832476   0.003696 -11590.4  <2e-16 ***
## TreatmentMEL10uM:Time3h -45.605649   0.003647 -12504.4  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1 -46.653446   0.003643  -12805
## spacing      23.049934   0.003583    6434
```

Comparing levels Treatment and Time

```
Anova(modmaleredMEL, type="II")
```

```
## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##           LR Chisq Df Pr(>Chisq)
## Treatment   48.978  1  2.589e-12 ***
## Time        29.945  3  1.417e-06 ***
```

```
## Treatment:Time   -0.409  3           1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairs(lsmeans(modmaleredMEL, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast          estimate         SE df    z.ratio p.value
##  CT - MEL10uM  2.093210e-11 1.096891e-13 NA    190.831  <.0001
##
## Time = 1h:
##  contrast          estimate         SE df    z.ratio p.value
##  CT - MEL10uM -5.009671e-01 7.119502e-06 NA -70365.458  <.0001
##
## Time = 2h:
##  contrast          estimate         SE df    z.ratio p.value
##  CT - MEL10uM -5.009671e-01 7.119502e-06 NA -70365.458  <.0001
##
## Time = 3h:
##  contrast          estimate         SE df    z.ratio p.value
##  CT - MEL10uM -5.781889e-01 4.943378e-04 NA  -1169.623  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.3.4 Melanocyte Stimulating Hormone

```r
modmaleredMSH<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleMSH,
                    link = "logit",
                    threshold = "equidistant", nAGQ = 9)
```

```r
summary(modmaleredMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:     maleMSH
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40    -31.52 83.04 497(1008) 7.93e-06 1.5e+02
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 0.2736   0.5231
## Number of groups:  Animal 5
##
## Coefficients:
##                    Estimate Std. Error z value Pr(>|z|)
## TreatmentMSH5uM     -0.7594     1.2701  -0.598    0.550
## Time1h              -0.7594     1.2701  -0.598    0.550
## Time2h              -0.7594     1.2701  -0.598    0.550
## Time3h              -0.7594     1.2701  -0.598    0.550
## TreatmentMSH5uM:Time1h  2.5108  1.9298   1.301    0.193
## TreatmentMSH5uM:Time2h  1.4947  1.8038   0.829    0.407
## TreatmentMSH5uM:Time3h -0.6931  1.9173  -0.362    0.718
```

```
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -3.8211     1.2528  -3.050
## spacing       3.3346     0.8307   4.014
```

Comparing levels `Treatment` and `Time`

```
Anova(modmaleredMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##               LR Chisq Df Pr(>Chisq)
## Treatment       0.0221  1     0.8818
## Time            1.9013  3     0.5931
## Treatment:Time  3.3030  3     0.3472
```

```
pairs(lsmeans(modmaleredMSH, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - MSH5uM -0.1051896 0.1724189 NA  -0.610  0.5418
##
## Time = 1h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - MSH5uM  0.2094027 0.1554877 NA   1.347  0.1781
##
## Time = 2h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - MSH5uM  0.1020781 0.1736547 NA   0.588  0.5567
##
## Time = 3h:
##  contrast      estimate        SE df z.ratio p.value
##  CT - MSH5uM -0.2015115 0.1951733 NA  -1.032  0.3018
##
## Results are averaged over the levels of: cut
```

2.1.3.5 Prolactin

```
modmaleredPRL<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=malePRL,
                    link = "logit",
                    threshold = "equidistant", nAGQ = 9)
```

```
summary(modmaleredPRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 9 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    malePRL
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40   -22.04 64.07 512(2483) 4.86e-06 1.7e+02
##
## Random effects:
```

```
##   Groups Name        Variance Std.Dev.
##   Animal (Intercept) 8.724    2.954
## Number of groups:  Animal 5
##
## Coefficients:
##                              Estimate Std. Error z value Pr(>|z|)
## TreatmentPRL0.150IU         2.638e-06  1.740e+00   0.000    1.000
## Time1h                      1.137e-05  1.740e+00   0.000    1.000
## Time2h                      1.143e-05  1.740e+00   0.000    1.000
## Time3h                      1.137e-05  1.740e+00   0.000    1.000
## TreatmentPRL0.150IU:Time1h -4.641e+00  2.889e+00  -1.606    0.108
## TreatmentPRL0.150IU:Time2h -1.459e+00  2.467e+00  -0.591    0.554
## TreatmentPRL0.150IU:Time3h -4.641e+00  2.889e+00  -1.606    0.108
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1   -5.503      2.421  -2.273
## spacing        8.074      2.540   3.179
```

Comparing levels `Treatment` and `Time`

```
Anova(modmaleredPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##               LR Chisq Df Pr(>Chisq)
## Treatment       7.4189  1   0.006454 **
## Time            4.1164  3   0.249171
## Treatment:Time  4.4903  3   0.213156
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleredPRL, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast            estimate          SE df z.ratio p.value
##  CT - PRL0.150IU  9.238110e-08 0.06093720 NA   0.000  1.0000
##
## Time = 1h:
##  contrast            estimate          SE df z.ratio p.value
##  CT - PRL0.150IU -1.814994e-01 0.19163175 NA  -0.947  0.3436
##
## Time = 2h:
##  contrast            estimate          SE df z.ratio p.value
##  CT - PRL0.150IU -3.336189e-02 0.06135338 NA  -0.544  0.5866
##
## Time = 3h:
##  contrast            estimate          SE df z.ratio p.value
##  CT - PRL0.150IU -1.814994e-01 0.19163172 NA  -0.947  0.3436
##
## Results are averaged over the levels of: cut
```

2.1.3.6   Noradrenaline and Melanocyte Stimulating Hormone

```
modmaleredNAMSH<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleNAMSH,
                      link = "logit",
                      threshold = "equidistant", nAGQ = 10)

summary(modmaleredNAMSH)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 10 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNAMSH
##
##  link  threshold   nobs logLik AIC   niter     max.grad cond.H
##  logit equidistant 40   -17.33 54.67 425(1266) 2.47e-06 1.4e+08
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 3.254    1.804
## Number of groups:  Animal 10
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentNA3h+MSH          -2.678      2.254  -1.188    0.235
## Time1h                     -1.327      1.760  -0.754    0.451
## Time2h                    -22.886   2739.710  -0.008    0.993
## Time3h                    -22.442   3063.089  -0.007    0.994
## TreatmentNA3h+MSH:Time1h    3.852      2.639   1.460    0.144
## TreatmentNA3h+MSH:Time2h   45.995   1369.855   0.034    0.973
## TreatmentNA3h+MSH:Time3h   48.231   4745.318   0.010    0.992
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1  -0.6634     1.4629  -0.453
## spacing      21.7746  3063.0895   0.007
```

Comparing levels Treatment and Time

```
Anova(modmaleredNAMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##               LR Chisq Df Pr(>Chisq)
## Treatment       9.2290  1   0.002382 **
## Time            2.3991  3   0.493809
## Treatment:Time 31.1798  3   7.791e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleredNAMSH, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - NA3h+MSH -0.2711520 0.18918086 NA  -1.433  0.1518
##
## Time = 1h:
```

```
## contrast           estimate           SE df z.ratio p.value
## CT - NA3h+MSH  0.1425309 0.23120223 NA   0.616  0.5376
##
## Time = 2h:
## contrast           estimate           SE df z.ratio p.value
## CT - NA3h+MSH  0.6681741 0.15646032 NA   4.271  <.0001
##
## Time = 3h:
## contrast           estimate           SE df z.ratio p.value
## CT - NA3h+MSH  0.9403657 0.08911502 NA  10.552  <.0001
##
## Results are averaged over the levels of: cut
```

2.1.3.7 Noradrenaline and Prolactin

```
modmaleredNAPRL<-clmm(Red~Treatment+Time+Treatment:Time+(1|Animal), data=maleNAPRL,
                      link = "logit",
                      threshold = "equidistant", nAGQ = 6)
```

```
summary(modmaleredNAPRL)
```

```
## Cumulative Link Mixed Model fitted with the adaptive Gauss-Hermite
## quadrature approximation with 6 quadrature points
##
## formula: Red ~ Treatment + Time + Treatment:Time + (1 | Animal)
## data:    maleNAPRL
##
##  link  threshold   nobs logLik AIC    niter      max.grad cond.H
##  logit equidistant 40    -2.19  24.38 1274(6958) 4.36e+00 1.4e+11
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Animal (Intercept) 115.9    10.77
## Number of groups:  Animal 5
##
## Coefficients:
##                           Estimate Std. Error   z value Pr(>|z|)
## TreatmentNA3h+PRL         -0.743615   0.003621  -205.364   <2e-16 ***
## Time1h                    -2.708943 253.098530    -0.011    0.991
## Time2h                    -2.708943 253.098530    -0.011    0.991
## Time3h                     3.044457   0.003621   840.787   <2e-16 ***
## TreatmentNA3h+PRL:Time1h  -1.981007 956.121858    -0.002    0.998
## TreatmentNA3h+PRL:Time2h  -1.981007 956.121858    -0.002    0.998
## TreatmentNA3h+PRL:Time3h  50.642764   0.003621 13985.997   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Threshold coefficients:
##             Estimate Std. Error z value
## threshold.1 27.683347   0.003621    7645
## spacing     16.175435   0.003387    4776
```

Comparing levels `Treatment` and `Time`
```

```
Anova(modmaleredNAPRL, type="II")
```

```
## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning in update.uC(rho): Non finite negative log-likelihood
##   at iteration 17

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
## In addition: Absolute convergence criterion was met, but relative criterion was not met

## Analysis of Deviance Table (Type II tests)
##
## Response: Red
##              LR Chisq Df Pr(>Chisq)
## Treatment      14.4055  1  0.0001474 ***
## Time           23.0360  3  3.969e-05 ***
## Treatment:Time  0.0814  3  0.9939693
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleredNAPRL, ~ Treatment|Time, adjust= "tukey",  mode = "cum.prob"))
```

```
## Time = 0h:
##  contrast          estimate           SE df    z.ratio p.value
##  CT - NA3h+PRL -2.489120e-13 1.313561e-15 NA   -189.494  <.0001
##
## Time = 1h:
##  contrast          estimate           SE df    z.ratio p.value
##  CT - NA3h+PRL  0.000000e+00 2.123017e-13 NA      0.000  1.0000
##
## Time = 2h:
##  contrast          estimate           SE df    z.ratio p.value
##  CT - NA3h+PRL  0.000000e+00 2.123017e-13 NA      0.000  1.0000
##
## Time = 3h:
##  contrast          estimate           SE df    z.ratio p.value
##  CT - NA3h+PRL  9.999433e-01 3.479338e-07 NA 2873947.464  <.0001
##
## Results are averaged over the levels of: cut
```

Diagnose

```
assumption1 <- clm(Black~Treatment+Time, data=maleMCH, threshold = "equidistant")

scale_test(assumption1)
```

```
## Warning: (1) Hessian is numerically singular: parameters are not uniquely determined
```

```
## In addition: Absolute and relative convergence criteria were met

## Tests of scale effects
##
## formula: Black ~ Treatment + Time
##           Df  logLik    AIC     LRT Pr(>Chi)
## <none>        -27.660 67.319
## Treatment  1 -27.640 69.281 0.0380  0.84534
## Time       3 -23.986 65.973 7.3465  0.06164 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
nominal_test(assumption1)
```

```
## Tests of nominal effects
##
## formula: Black ~ Treatment + Time
##           Df  logLik    AIC     LRT Pr(>Chi)
## <none>        -27.660 67.319
## Treatment  1 -27.646 69.292 0.0270   0.8695
## Time       3 -25.556 69.111 4.2077   0.2399
```

2.1.4   Yellow colour in Males

For the yellow colour, we'll use a GLMM with beta distribution, since it's a proportion of the yellow colour in the total area. To do this, we'll use the glmmmADMB package. We'll model each sex separately, begining with males.

2.1.4.1   Melanocyte Stimulating Hormone

Before, modelling we'll look at the data to see its distribution using a histogram.

```r
ggplot(maleMSH, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
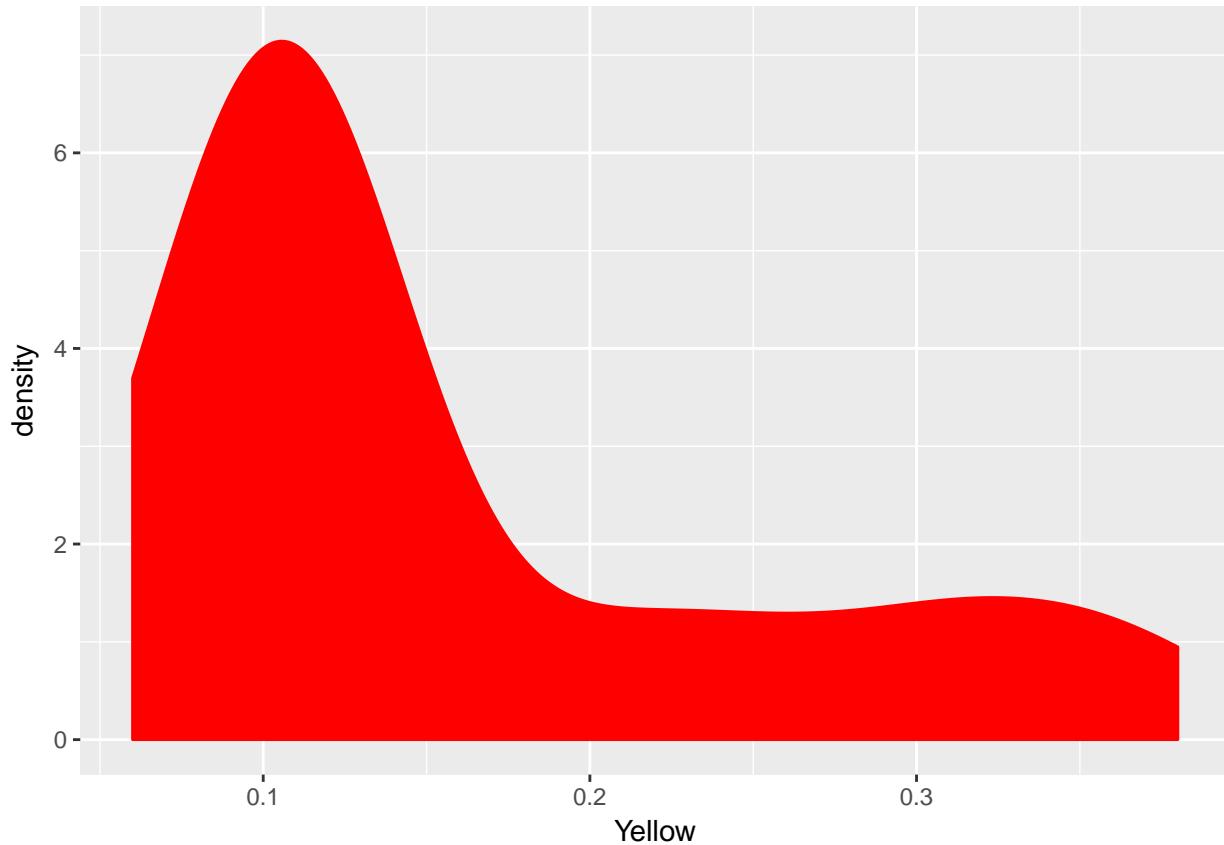
2.1.4.1.1 Statistical model

```
modmaleyellowMSH<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=maleMS

summary(modmaleyellowMSH)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = maleMSH, family = "beta")
##
## AIC: -138.2
##
## Coefficients:
##                         Estimate Std. Error z value Pr(>|z|)
## (Intercept)              -2.2483     0.1520  -14.80  < 2e-16 ***
## TreatmentMSH5uM           0.1798     0.1839    0.98     0.33
## Time1h                    0.2019     0.1832    1.10     0.27
## Time2h                    0.0849     0.1868    0.45     0.65
## Time3h                   -0.0500     0.1916   -0.26     0.79
## TreatmentMSH5uM:Time1h    0.3410     0.2457    1.39     0.17
## TreatmentMSH5uM:Time2h    1.0087     0.2425    4.16  3.2e-05 ***
## TreatmentMSH5uM:Time3h    1.2812     0.2452    5.22  1.7e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##              Variance StdDev
## (Intercept)  0.02439 0.1562
##
## Beta dispersion parameter: 123.34 (std. err.: 29.546)
##
## Log-likelihood: 79.0927
```

Comparing levels

```
Anova(modmaleyellowMSH, type="II")#Analysis of Deviance table
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##              Df   Chisq Pr(>Chisq)
## Treatment     1  2.9714     0.08475 .
## Time          3  2.1839     0.53513
## Treatment:Time 3 42.3556  3.372e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleyellowMSH, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast     estimate       SE df z.ratio p.value
##  CT - MSH5uM -0.1797656 0.1839200 NA  -0.977  0.3284
##
## Time = 1h:
##  contrast     estimate       SE df z.ratio p.value
##  CT - MSH5uM -0.5207340 0.3028887 NA  -1.719  0.0856
##
## Time = 2h:
##  contrast     estimate       SE df z.ratio p.value
##  CT - MSH5uM -1.1884540 0.2931451 NA  -4.054  0.0001
##
## Time = 3h:
##  contrast     estimate       SE df z.ratio p.value
##  CT - MSH5uM -1.4609898 0.2933409 NA  -4.981  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.4.2 Prolactin

```
ggplot(malePRL, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
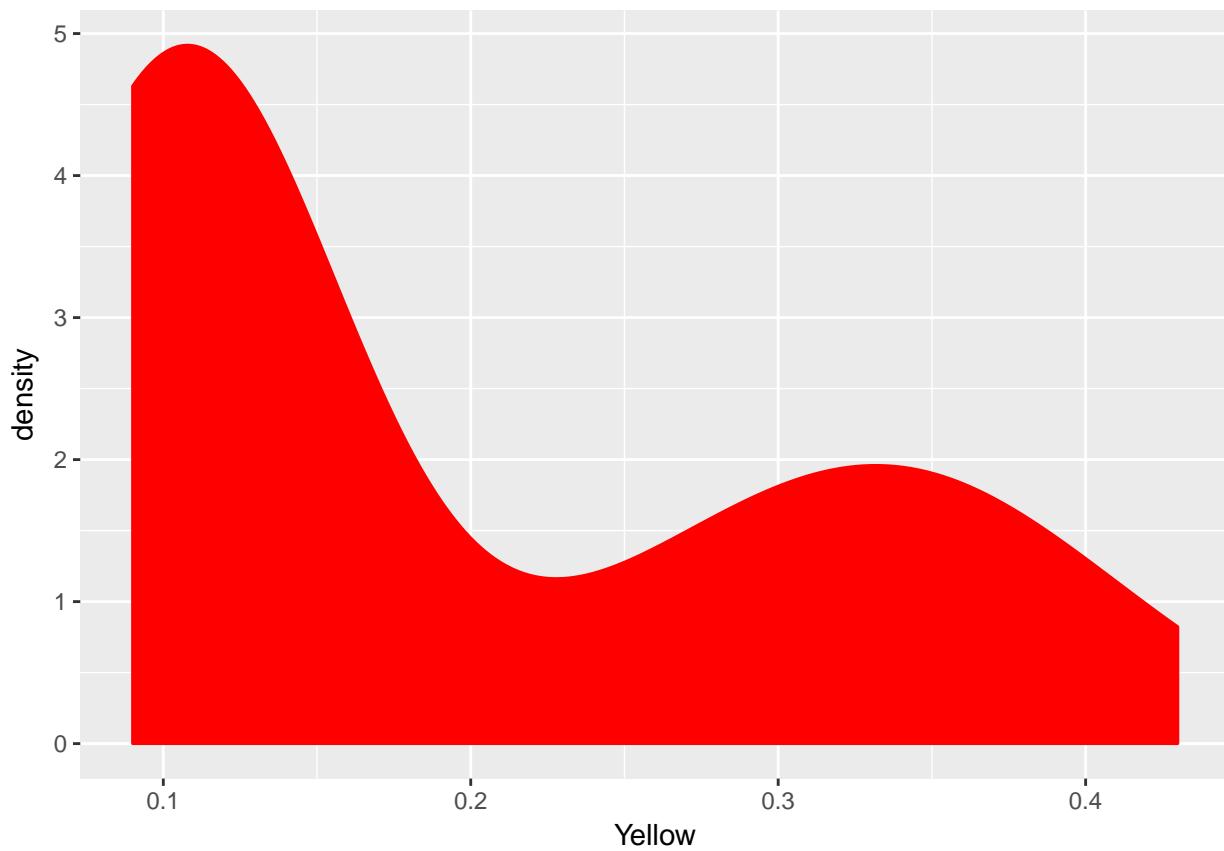
2.1.4.2.1 Statistical model

```
modmaleyellowPRL<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=malePR
```

```
summary(modmaleyellowPRL)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = malePRL, family = "beta")
##
## AIC: -159.7
##
## Coefficients:
##                           Estimate Std. Error z value Pr(>|z|)
## (Intercept)                -2.1580     0.1036  -20.83  < 2e-16 ***
## TreatmentPRL0.150IU         0.0212     0.1458    0.15     0.88
## Time1h                      0.0212     0.1458    0.15     0.88
## Time2h                      0.0163     0.1459    0.11     0.91
## Time3h                      0.2171     0.1407    1.54     0.12
## TreatmentPRL0.150IU:Time1h  1.2586     0.1914    6.58  4.8e-11 ***
## TreatmentPRL0.150IU:Time2h  1.3650     0.1910    7.15  8.9e-13 ***
## TreatmentPRL0.150IU:Time3h  1.3485     0.1864    7.24  4.6e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##             Variance    StdDev
## (Intercept) 1.125e-07 0.0003355
##
## Beta dispersion parameter: 196.68 (std. err.: 43.948)
##
## Log-likelihood: 89.8296
```

Comapring levels

```
Anova(modmaleyellowPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##               Df    Chisq Pr(>Chisq)
## Treatment      1   2.7103     0.0997 .
## Time           3   5.0645     0.1671
## Treatment:Time 3 124.6561     <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleyellowPRL, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.02117305 0.1457500 NA  -0.145  0.8845
##
## Time = 1h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.27973687 0.2365224 NA  -5.411  <.0001
##
## Time = 2h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.38620669 0.2339695 NA  -5.925  <.0001
##
## Time = 3h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.36969889 0.2171676 NA  -6.307  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.4.3 Noradrenaline and Melanocyte Stimulating Hormone

```
ggplot(maleNAMSH, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
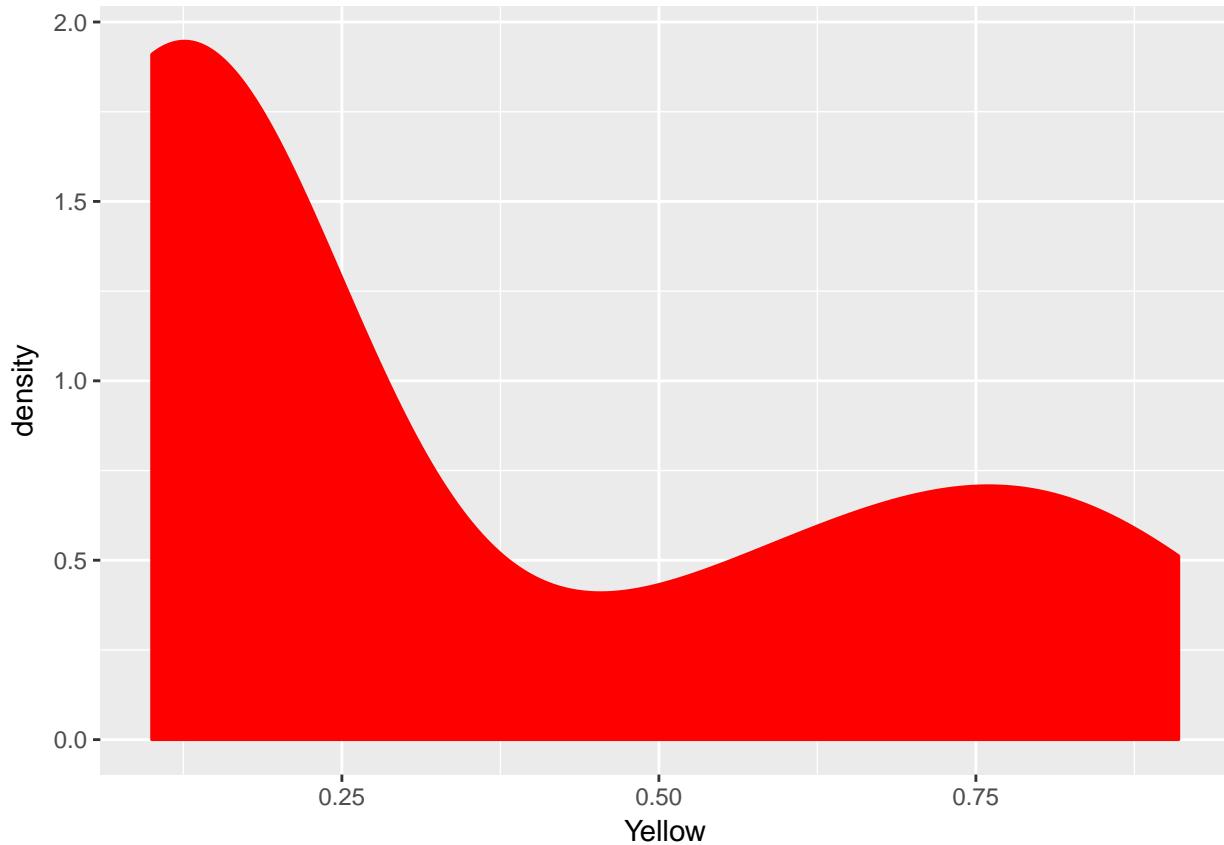
2.1.4.3.1 Statistical model

```
modmaleyellowNAMSH<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=male
```

```
summary(modmaleyellowNAMSH)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = maleNAMSH, family = "beta")
##
## AIC: -123.1
##
## Coefficients:
##                        Estimate Std. Error z value Pr(>|z|)
## (Intercept)            -1.91370    0.14855  -12.88  < 2e-16 ***
## TreatmentNA3h+MSH      -0.00111    0.20969   -0.01     1.00
## Time1h                 -0.00111    0.20969   -0.01     1.00
## Time2h                 -0.11300    0.21398   -0.53     0.60
## Time3h                  0.05066    0.20783    0.24     0.81
## TreatmentNA3h+MSH:Time1h  1.91258    0.27609    6.93  4.3e-12 ***
## TreatmentNA3h+MSH:Time2h  3.14640    0.28560   11.02  < 2e-16 ***
## TreatmentNA3h+MSH:Time3h  3.59622    0.29136   12.34  < 2e-16 ***
## ---
## 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: 77.65 (std. err.: 17.329)
##
## Log-likelihood: 71.5275
```

Comparing levels

```
Anova(modmaleyellowNAMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##                 Df    Chisq Pr(>Chisq)
## Treatment        1   2.0723     0.1500
## Time             3   1.9429     0.5843
## Treatment:Time   3 307.7679     <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleyellowNAMSH, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - NA3h+MSH  0.001109552 0.2096900 NA   0.005  0.9958
##
## Time = 1h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -1.911467727 0.3293600 NA  -5.804  <.0001
##
## Time = 2h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -3.145289135 0.3459657 NA  -9.091  <.0001
##
## Time = 3h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -3.595107535 0.3550182 NA -10.127  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.4.4 Noradrenalinee and Prolactin

```
ggplot(maleNAPRL, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
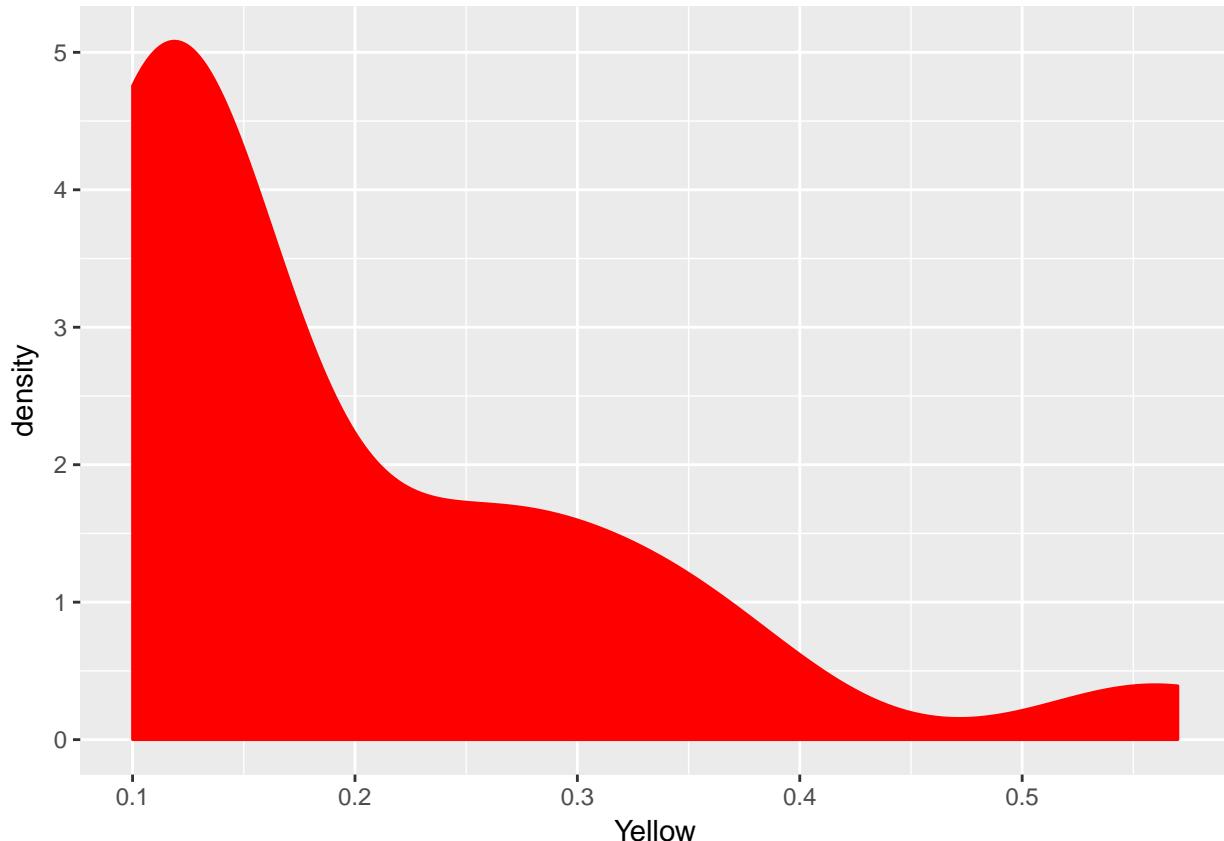
2.1.4.4.1   Statistical model

```
modmaleyellowNAPRL<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=male
```

```
summary(modmaleyellowNAPRL)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = maleNAPRL, family = "beta")
##
## AIC: -142.3
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## (Intercept)               -2.0244     0.1200  -16.87  < 2e-16 ***
## TreatmentNA3h+PRL          0.0562     0.1677    0.34     0.74
## Time1h                     0.0164     0.1690    0.10     0.92
## Time2h                     0.0385     0.1683    0.23     0.82
## Time3h                     0.0178     0.1689    0.11     0.92
## TreatmentNA3h+PRL:Time1h   0.8879     0.2241    3.96  7.4e-05 ***
## TreatmentNA3h+PRL:Time2h   0.9620     0.2228    4.32  1.6e-05 ***
## TreatmentNA3h+PRL:Time3h   1.7028     0.2202    7.73  1.0e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##             Variance    StdDev
## (Intercept) 1.125e-07 0.0003355
##
## Beta dispersion parameter: 131.23 (std. err.: 29.306)
##
## Log-likelihood: 81.1582
```

Comparing levels

```
Anova(modmaleyellowNAPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##                Df   Chisq Pr(>Chisq)
## Treatment       1  2.0319     0.1540
## Time            3  0.9383     0.8162
## Treatment:Time  3 83.9493     <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modmaleyellowNAPRL, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -0.05624498 0.1677400 NA  -0.335  0.7374
##
## Time = 1h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -0.94416948 0.2767583 NA  -3.412  0.0006
##
## Time = 2h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -1.01825788 0.2723391 NA  -3.739  0.0002
##
## Time = 3h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -1.75902176 0.2608874 NA  -6.742  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.5 Yellow colour of Females

2.1.5.1 Melanocyte Stimulating Hormone

```
ggplot(femaleMSH, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
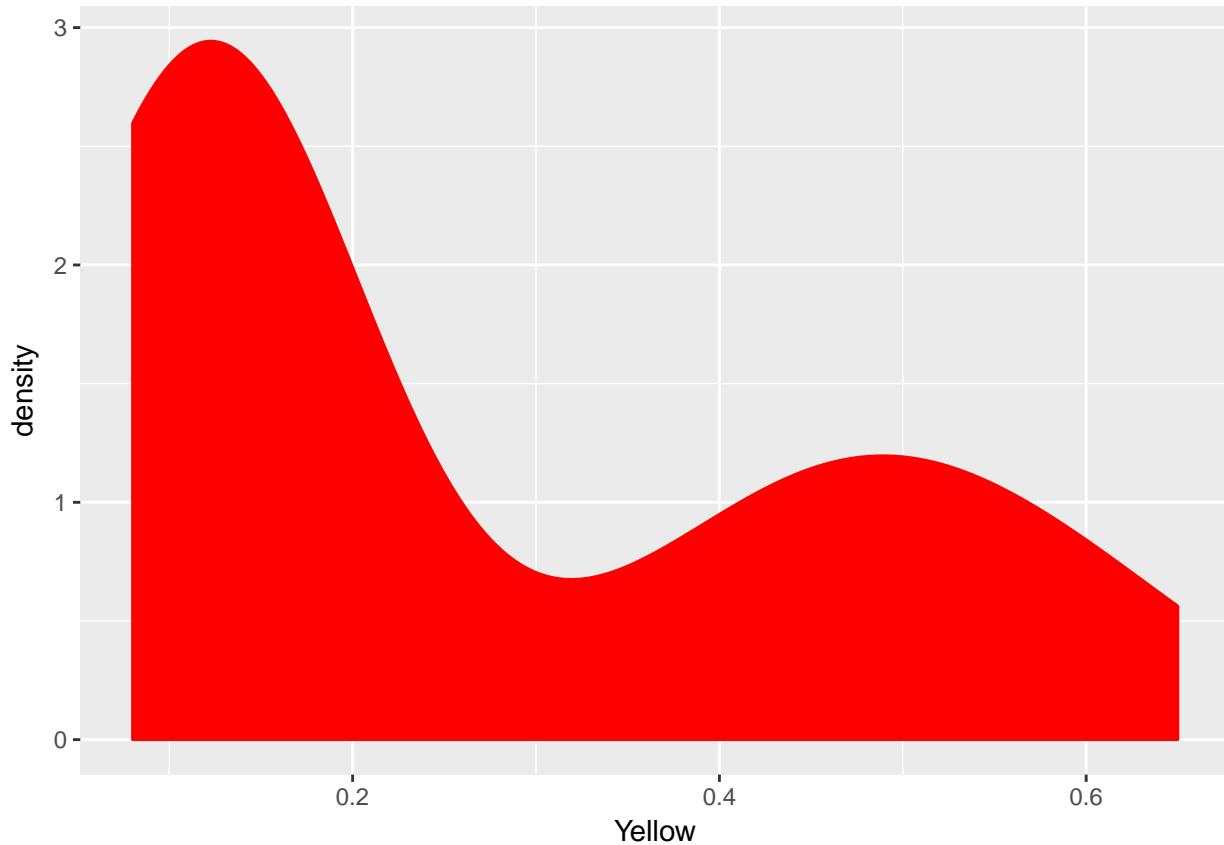
2.1.5.1.1 Statistical model

```
modfemaleyellowMSH<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=femal
```

```
summary(modfemaleyellowMSH)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = femaleMSH, family = "beta")
##
## AIC: -125.7
##
## Coefficients:
##                      Estimate Std. Error z value Pr(>|z|)
## (Intercept)           -1.9762     0.1516  -13.04   <2e-16 ***
## TreatmentMSH5uM       -0.0165     0.1743   -0.09     0.92
## Time1h                -0.0393     0.1747   -0.22     0.82
## Time2h                 0.0644     0.1718    0.38     0.71
## Time3h                -0.0055     0.1740   -0.03     0.97
## TreatmentMSH5uM:Time1h  1.9593     0.2290    8.56   <2e-16 ***
## TreatmentMSH5uM:Time2h  1.8937     0.2267    8.35   <2e-16 ***
## TreatmentMSH5uM:Time3h  2.0163     0.2289    8.81   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##               Variance StdDev
## (Intercept)   0.03844 0.1961
##
## Beta dispersion parameter: 120.44 (std. err.: 28.831)
##
## Log-likelihood: 72.8388
```

Comparing levels

```r
Anova(modfemaleyellowMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##              Df    Chisq Pr(>Chisq)
## Treatment     1   3.1170    0.07748 .
## Time          3   5.0052    0.17142
## Treatment:Time 3 191.6689    < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairs(lsmeans(modfemaleyellowMSH, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - MSH5uM  0.0164774 0.1742700 NA   0.095  0.9247
##
## Time = 1h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - MSH5uM -1.9428037 0.2815979 NA  -6.899  <.0001
##
## Time = 2h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - MSH5uM -1.8772535 0.2732448 NA  -6.870  <.0001
##
## Time = 3h:
##  contrast      estimate         SE df z.ratio p.value
##  CT - MSH5uM -1.9998268 0.2712966 NA  -7.371  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.5.2 Prolactin

```r
ggplot(femalePRL, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
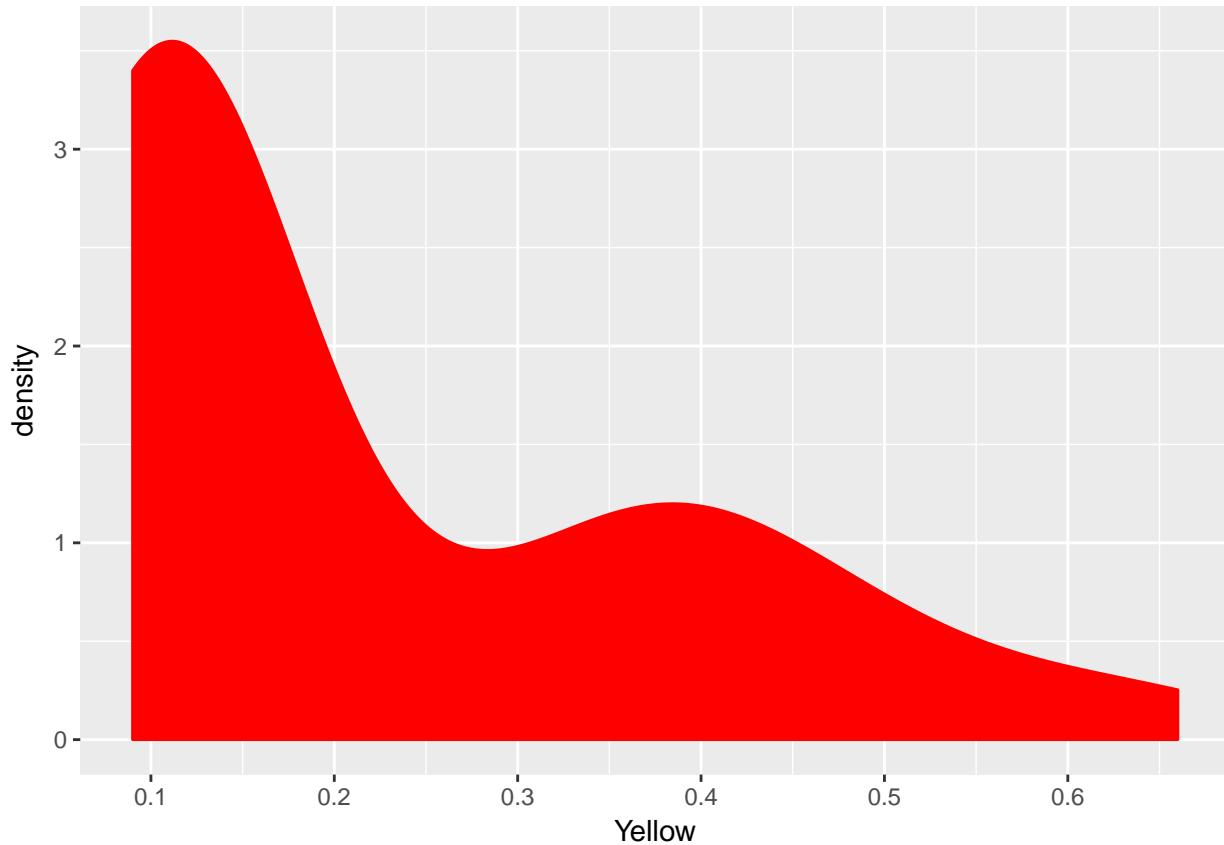
2.1.5.2.1 Statistical model

```
modfemaleyellowPRL<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=femal

summary(modfemaleyellowPRL)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = femalePRL, family = "beta")
##
## AIC: -128.4
##
## Coefficients:
##                           Estimate Std. Error z value Pr(>|z|)
## (Intercept)              -2.110683   0.150770  -14.00  < 2e-16 ***
## TreatmentPRL0.150IU       0.039244   0.187790    0.21     0.83
## Time1h                    0.000351   0.189470    0.00     1.00
## Time2h                   -0.005394   0.189400   -0.03     0.98
## Time3h                    0.093664   0.186090    0.50     0.61
## TreatmentPRL0.150IU:Time1h  1.259632   0.248560    5.07  4.0e-07 ***
## TreatmentPRL0.150IU:Time2h  1.784348   0.246170    7.25  4.2e-13 ***
## TreatmentPRL0.150IU:Time3h  1.949182   0.243350    8.01  1.1e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##             Variance StdDev
## (Intercept)  0.02346 0.1532
##
## Beta dispersion parameter: 111.48 (std. err.: 26.707)
##
## Log-likelihood: 74.2075
```

Comparing levels

```
Anova(modfemaleyellowPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##               Df    Chisq Pr(>Chisq)
## Treatment      1   2.6926     0.1008
## Time           3   2.2326     0.5256
## Treatment:Time 3 122.1706     <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleyellowPRL, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -0.03924428 0.1877900 NA  -0.209  0.8345
##
## Time = 1h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.29887613 0.3079069 NA  -4.218  <.0001
##
## Time = 2h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.82359213 0.3003592 NA  -6.071  <.0001
##
## Time = 3h:
##  contrast          estimate        SE df z.ratio p.value
##  CT - PRL0.150IU -1.98842653 0.2866425 NA  -6.937  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.5.3 Noradrenalinee and Melanocyte Stimulating Hormone

```
ggplot(femaleNAMSH, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
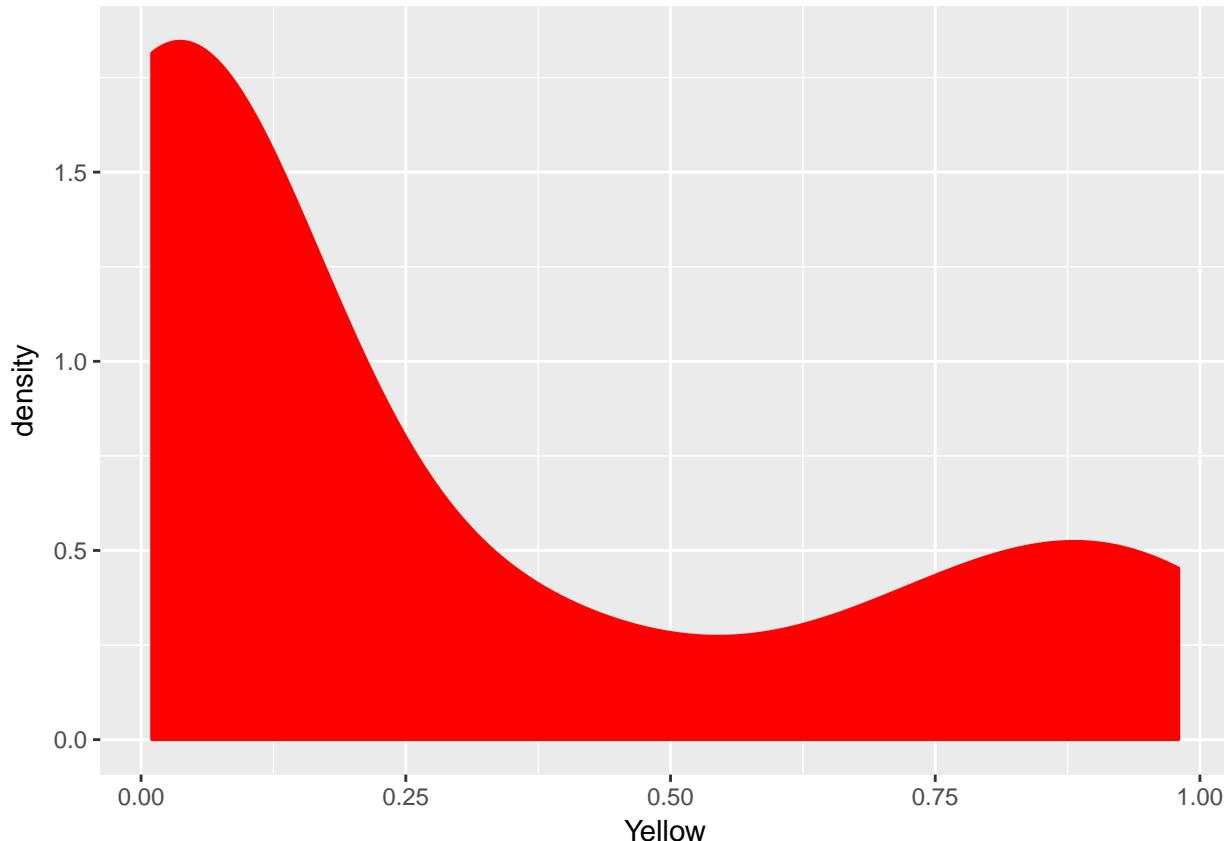
2.1.5.3.1 Statistical model

```
modfemaleyellowNAMSH<-glmmadmb(Yellow/10~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=

summary(modfemaleyellowNAMSH)
```

```
##
## Call:
## glmmadmb(formula = Yellow/10 ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = femaleNAMSH, family = "beta")
##
## AIC: -331.7
##
## Coefficients:
##                         Estimate Std. Error z value Pr(>|z|)
## (Intercept)             -5.7507      0.3253  -17.68  < 2e-16 ***
## TreatmentNA3h+MSH        0.1527      0.4493    0.34  0.73401
## Time1h                   0.0392      0.4573    0.09  0.93161
## Time2h                   0.2960      0.4392    0.67  0.50035
## Time3h                   0.5157      0.4243    1.22  0.22423
## TreatmentNA3h+MSH:Time1h  2.1755     0.5659    3.84  0.00012 ***
## TreatmentNA3h+MSH:Time2h  2.7783     0.5441    5.11  3.3e-07 ***
## TreatmentNA3h+MSH:Time3h  2.8490     0.5307    5.37  8.0e-08 ***
## ---
## 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) 1.654e-07 0.0004067
##
## Beta dispersion parameter: 403.43 (std. err.: 0.00036004)
##
## Log-likelihood: 175.844
```

Comparing levels

```
Anova(modfemaleyellowNAMSH, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow/10
##                Df   Chisq Pr(>Chisq)
## Treatment       1  5.7568    0.01642 *
## Time            3 10.7156    0.01337 *
## Treatment:Time  3 50.1200  7.533e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleyellowNAMSH, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -0.1526621 0.4492800 NA  -0.340  0.7340
##
## Time = 1h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -2.3281198 0.7138221 NA  -3.261  0.0011
##
## Time = 2h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -2.9310003 0.6464468 NA  -4.534  <.0001
##
## Time = 3h:
##  contrast        estimate        SE df z.ratio p.value
##  CT - NA3h+MSH -3.0016203 0.5723002 NA  -5.245  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```

2.1.5.4 Noradrenalinee and Prolactin

```
ggplot(femaleNAPRL, aes(Yellow)) +
  geom_density(colour="red", fill="red") +
  theme(legend.position="none")
```
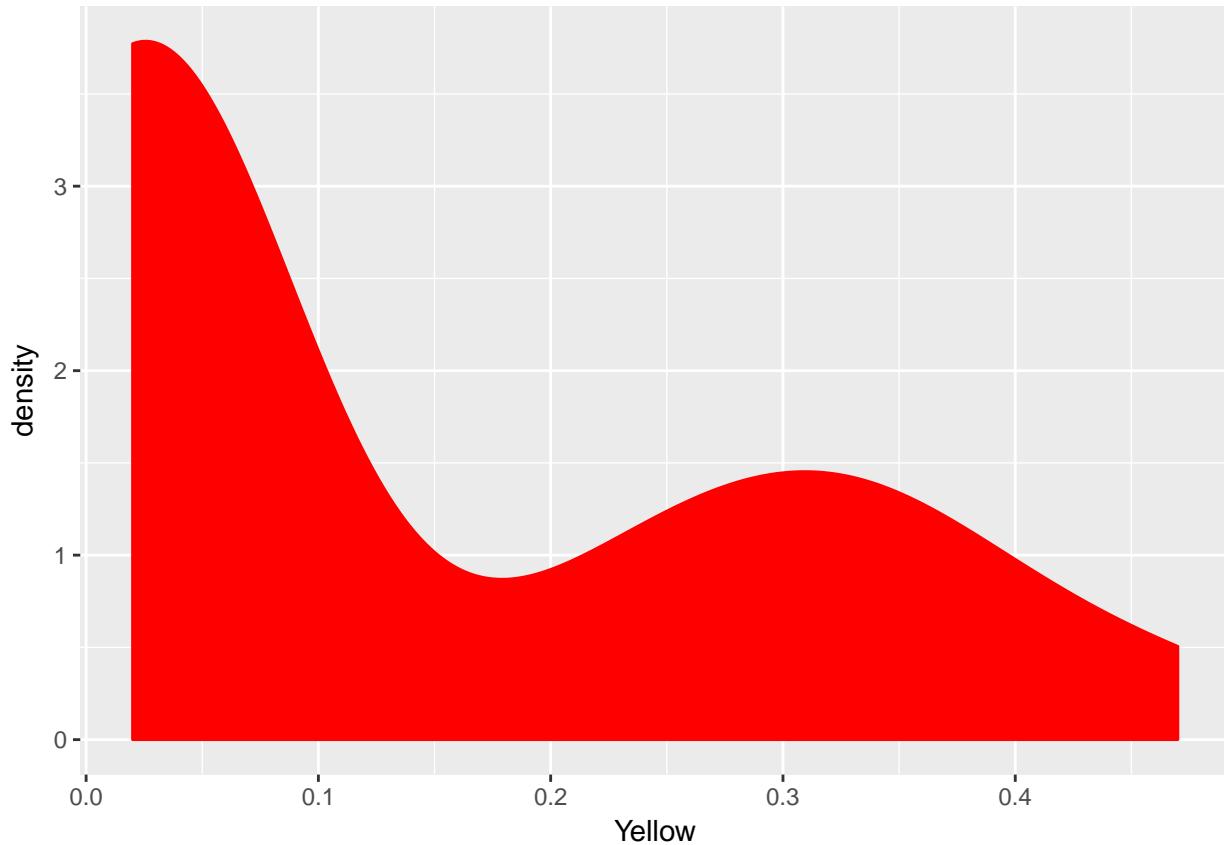
2.1.5.4.1 Statistical model

```
modfemaleyellowNAPRL<-glmmadmb(Yellow~Treatment+Time+Treatment:Time+(1|Animal), family= "beta", data=fer

summary(modfemaleyellowNAPRL)
```

```
##
## Call:
## glmmadmb(formula = Yellow ~ Treatment + Time + Treatment:Time +
##     (1 | Animal), data = femaleNAPRL, family = "beta")
##
## AIC: -192.2
##
## Coefficients:
##                          Estimate Std. Error z value Pr(>|z|)
## (Intercept)              -3.62e+00   1.99e-01  -18.23  < 2e-16 ***
## TreatmentNA3h+PRL         1.51e-01   2.71e-01    0.56     0.58
## Time1h                    1.99e-07   2.79e-01    0.00     1.00
## Time2h                    8.96e-08   2.79e-01    0.00     1.00
## Time3h                    7.52e-02   2.75e-01    0.27     0.78
## TreatmentNA3h+PRL:Time1h  2.32e+00   3.44e-01    6.73  1.7e-11 ***
## TreatmentNA3h+PRL:Time2h  2.88e+00   3.42e-01    8.40  < 2e-16 ***
## TreatmentNA3h+PRL:Time3h  2.83e+00   3.39e-01    8.36  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Number of observations: total=40, Animal=5
```

```
## Random effect variance(s):
## Group=Animal
##             Variance    StdDev
## (Intercept) 1.125e-07 0.0003355
##
## Beta dispersion parameter: 182.22 (std. err.: 41.083)
##
## Log-likelihood: 106.075
```

Comparing levels

```
Anova(modfemaleyellowNAPRL, type="II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: Yellow
##               Df    Chisq Pr(>Chisq)
## Treatment      1   8.9981   0.002703 **
## Time           3  21.7382  7.395e-05 ***
## Treatment:Time 3 148.4720  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairs(lsmeans(modfemaleyellowNAPRL, ~ Treatment|Time, adjust= "tukey"))
```

```
## Time = 0h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -0.150997 0.2705200 NA  -0.558  0.5767
##
## Time = 1h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -2.466481 0.4235145 NA  -5.824  <.0001
##
## Time = 2h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -3.027132 0.4133840 NA  -7.323  <.0001
##
## Time = 3h:
##  contrast       estimate        SE df z.ratio p.value
##  CT - NA3h+PRL -2.983070 0.3888851 NA  -7.671  <.0001
##
## Results are given on the log odds ratio (not the response) scale.
```