Dehydration-induced mortality and premature hatching in gliding treefrogs with even small reductions in humidity

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Contents

INTRODUCTION

> The statistical analysis shown here are from the González et al. dehyration induced mortality and premature hatching manuscript. Analyses are shows in chronological order as reported in the manuscript. We show details on importing, manipulating, summarizing, analyzing (testing hypotheses), and visualizing the data. See manuscript for details on background, research questions, hypothesis, methods, etc.

PDF Knit settings

```
knitr::opts_chunk$set(warning=FALSE, message=FALSE)
```

Import temperature and relative humidity datasheet

```
library(readxl)
library(readxl)
dtd <- read_excel("~/Desktop/Publications/Manuscripts/2020 Dehydration induced hatching As/Dehydration 
    sheet = "Raw Temp_RH data", col_types = c("numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric"), na = "NA")
dtd
```

```
## # A tibble: 1,294 x 12
##       date experiment   day  time   age   WT2  WRH2   WT1  WRH1   BT2   BT1 block
##      <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
##  1 2.02e7          1     0  16.2 0.675  28.3  99.9  27.6 100    30.1  30.1    NA
##  2 2.02e7          1     0  16.5 0.687  27.8  95.1  27.1  99.2  27.8  28.2    NA
##  3 2.02e7          1     0  17.2 0.716  27.6  95.7  27.1  99    27.3  27.6    NA
##  4 2.02e7          1     0  17.5 0.729  27.2  96.8  26.8  99.7  27.0  27.3    NA
##  5 2.02e7          1     0  18.2 0.758  27.0  97.5  26.7 100    26.8  27.0    NA
##  6 2.02e7          1     0  18.5 0.770  26.9  97.9  26.6 100    26.7  26.8    NA
##  7 2.02e7          1     0  19.2 0.800  26.8  98.4  26.5 100    26.6  26.6    NA
##  8 2.02e7          1     0  19.5 0.812  26.6  98.4  26.3 100    26.5  26.5    NA
##  9 2.02e7          1     0  20.2 0.841  26.6  98.8  26.3 100    26.4  26.5    NA
## 10 2.02e7          1     0  20.5 0.854  26.3  98.7  26.0 100    26.2  26.2    NA
## # ... with 1,284 more rows
```

Tidy up data

```
library(tidyr)
library(dplyr)

dtd = gather(data= dtd, key ="Sensor", value = "Value",6:11) #put all values into value and sensor type
dtd
```

```
## # A tibble: 7,764 x 8
##        date experiment   day  time   age block Sensor Value
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl>
##  1 20180606          1     0  16.2 0.675    NA WT2     28.3
##  2 20180606          1     0  16.5 0.687    NA WT2     27.8
##  3 20180606          1     0  17.2 0.716    NA WT2     27.6
##  4 20180606          1     0  17.5 0.729    NA WT2     27.2
##  5 20180606          1     0  18.2 0.758    NA WT2     27.0
##  6 20180606          1     0  18.5 0.770    NA WT2     26.9
##  7 20180606          1     0  19.2 0.800    NA WT2     26.8
##  8 20180606          1     0  19.5 0.812    NA WT2     26.6
##  9 20180606          1     0  20.2 0.841    NA WT2     26.6
## 10 20180606          1     0  20.5 0.854    NA WT2     26.3
## # ... with 7,754 more rows
```

```
dtd= mutate(dtd, Type = ifelse(grepl("T", Sensor), "Temperature", ifelse(grepl("RH", Sensor), "Relative
dtd # ifelse and grepl look for matches in hte colum and give new name for the mutate... conditional mu
```

```
## # A tibble: 7,764 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WT2     28.3 Temperature
##  2 20180606          1     0  16.5 0.687    NA WT2     27.8 Temperature
##  3 20180606          1     0  17.2 0.716    NA WT2     27.6 Temperature
##  4 20180606          1     0  17.5 0.729    NA WT2     27.2 Temperature
##  5 20180606          1     0  18.2 0.758    NA WT2     27.0 Temperature
##  6 20180606          1     0  18.5 0.770    NA WT2     26.9 Temperature
##  7 20180606          1     0  19.2 0.800    NA WT2     26.8 Temperature
##  8 20180606          1     0  19.5 0.812    NA WT2     26.6 Temperature
##  9 20180606          1     0  20.2 0.841    NA WT2     26.6 Temperature
## 10 20180606          1     0  20.5 0.854    NA WT2     26.3 Temperature
## # ... with 7,754 more rows
```

Figure 2

Experiment 1

Subsetting and tidying data

```
experiment1 = dtd %>%
  filter(Sensor != "BT1") %>%
  filter(Sensor != "BT2") %>%
  filter(experiment == "1")
experiment1
```

```
## # A tibble: 1,692 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WT2     28.3 Temperature
##  2 20180606          1     0  16.5 0.687    NA WT2     27.8 Temperature
##  3 20180606          1     0  17.2 0.716    NA WT2     27.6 Temperature
##  4 20180606          1     0  17.5 0.729    NA WT2     27.2 Temperature
##  5 20180606          1     0  18.2 0.758    NA WT2     27.0 Temperature
##  6 20180606          1     0  18.5 0.770    NA WT2     26.9 Temperature
##  7 20180606          1     0  19.2 0.800    NA WT2     26.8 Temperature
##  8 20180606          1     0  19.5 0.812    NA WT2     26.6 Temperature
##  9 20180606          1     0  20.2 0.841    NA WT2     26.6 Temperature
## 10 20180606          1     0  20.5 0.854    NA WT2     26.3 Temperature
## # ... with 1,682 more rows
```

```r
unique(experiment1$Type)
```

```
## [1] "Temperature"       "Relative Humidity"
```

```r
# humid temp
experiment1_temp = experiment1 %>%
  filter(Type == "Temperature")
experiment1_temp
```

```
## # A tibble: 846 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WT2     28.3 Temperature
##  2 20180606          1     0  16.5 0.687    NA WT2     27.8 Temperature
##  3 20180606          1     0  17.2 0.716    NA WT2     27.6 Temperature
##  4 20180606          1     0  17.5 0.729    NA WT2     27.2 Temperature
##  5 20180606          1     0  18.2 0.758    NA WT2     27.0 Temperature
##  6 20180606          1     0  18.5 0.770    NA WT2     26.9 Temperature
##  7 20180606          1     0  19.2 0.800    NA WT2     26.8 Temperature
##  8 20180606          1     0  19.5 0.812    NA WT2     26.6 Temperature
##  9 20180606          1     0  20.2 0.841    NA WT2     26.6 Temperature
## 10 20180606          1     0  20.5 0.854    NA WT2     26.3 Temperature
## # ... with 836 more rows
```

```r
# humid humidity
experiment1_humidity = experiment1 %>%
  filter(Type == "Relative Humidity")
experiment1_humidity
```

```
## # A tibble: 846 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WRH2    99.9 Relative Humidity
##  2 20180606          1     0  16.5 0.687    NA WRH2    95.1 Relative Humidity
##  3 20180606          1     0  17.2 0.716    NA WRH2    95.7 Relative Humidity
##  4 20180606          1     0  17.5 0.729    NA WRH2    96.8 Relative Humidity
##  5 20180606          1     0  18.2 0.758    NA WRH2    97.5 Relative Humidity
```

```
##  6 20180606          1     0  18.5 0.770    NA WRH2    97.9 Relative Humidity
##  7 20180606          1     0  19.2 0.800    NA WRH2    98.4 Relative Humidity
##  8 20180606          1     0  19.5 0.812    NA WRH2    98.4 Relative Humidity
##  9 20180606          1     0  20.2 0.841    NA WRH2    98.8 Relative Humidity
## 10 20180606          1     0  20.5 0.854    NA WRH2    98.7 Relative Humidity
## # ... with 836 more rows
```

Temperature

Summary statistics

```
experiment1
```

```
## # A tibble: 1,692 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WT2     28.3 Temperature
##  2 20180606          1     0  16.5 0.687    NA WT2     27.8 Temperature
##  3 20180606          1     0  17.2 0.716    NA WT2     27.6 Temperature
##  4 20180606          1     0  17.5 0.729    NA WT2     27.2 Temperature
##  5 20180606          1     0  18.2 0.758    NA WT2     27.0 Temperature
##  6 20180606          1     0  18.5 0.770    NA WT2     26.9 Temperature
##  7 20180606          1     0  19.2 0.800    NA WT2     26.8 Temperature
##  8 20180606          1     0  19.5 0.812    NA WT2     26.6 Temperature
##  9 20180606          1     0  20.2 0.841    NA WT2     26.6 Temperature
## 10 20180606          1     0  20.5 0.854    NA WT2     26.3 Temperature
## # ... with 1,682 more rows
```

```
experiment1_temp_sum= experiment1_temp %>%
  group_by(Sensor) %>%
  summarise(min= min(Value), max= max(Value), mean_temp= mean(Value), SD = sd(Value), sample_size=n())
experiment1_temp_sum
```

```
## # A tibble: 2 x 6
##   Sensor   min   max mean_temp    SD sample_size
##   <chr>  <dbl> <dbl>     <dbl> <dbl>       <int>
## 1 WT1     23.4  32.9      27.1  2.06         423
## 2 WT2     23.5  34.1      27.5  2.45         423
```

High humidity:

- mean temp = 27.11052 +/- 2.059403
- range = 23.424—32.924

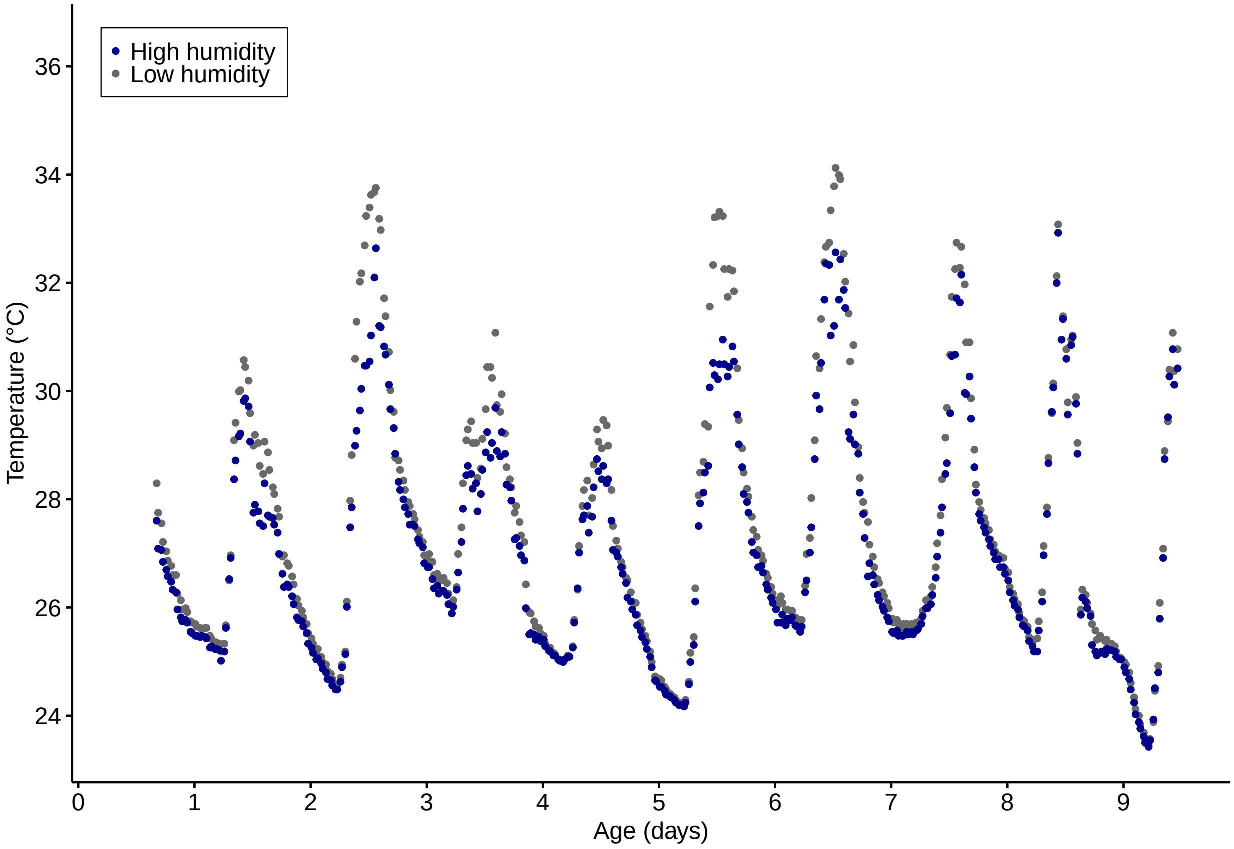
Low humidity:

- mean temp = 27.54733 +/- 2.445825
- range = 23.472—34.124
- sample size = 423

- 30min incriments, 2 WHITE sensors

Figure

```
library(ggplot2)
library(scales)
legend_title = "Hydration treatment"

temperature_1 = ggplot(experiment1_temp, aes(x=age, y=Value)) + #add the axis
  geom_point(aes(color = Sensor), size = .7) +
  labs(y="Temperature (°C)", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black"))
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background =  element_rect(color
  #theme(plot.margin = unit(c(.5, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(limits=c(NA, 36.5), breaks= pretty_breaks(n=6)) +
  scale_x_continuous(limits=c(0.4,NA), breaks= pretty_breaks(n=8)) +   # put the x axis stuff in order
  scale_color_manual(legend_title, breaks=c("WT1", "WT2"), labels=c("High humidity", "Low humidity"), va
temperature_1
```

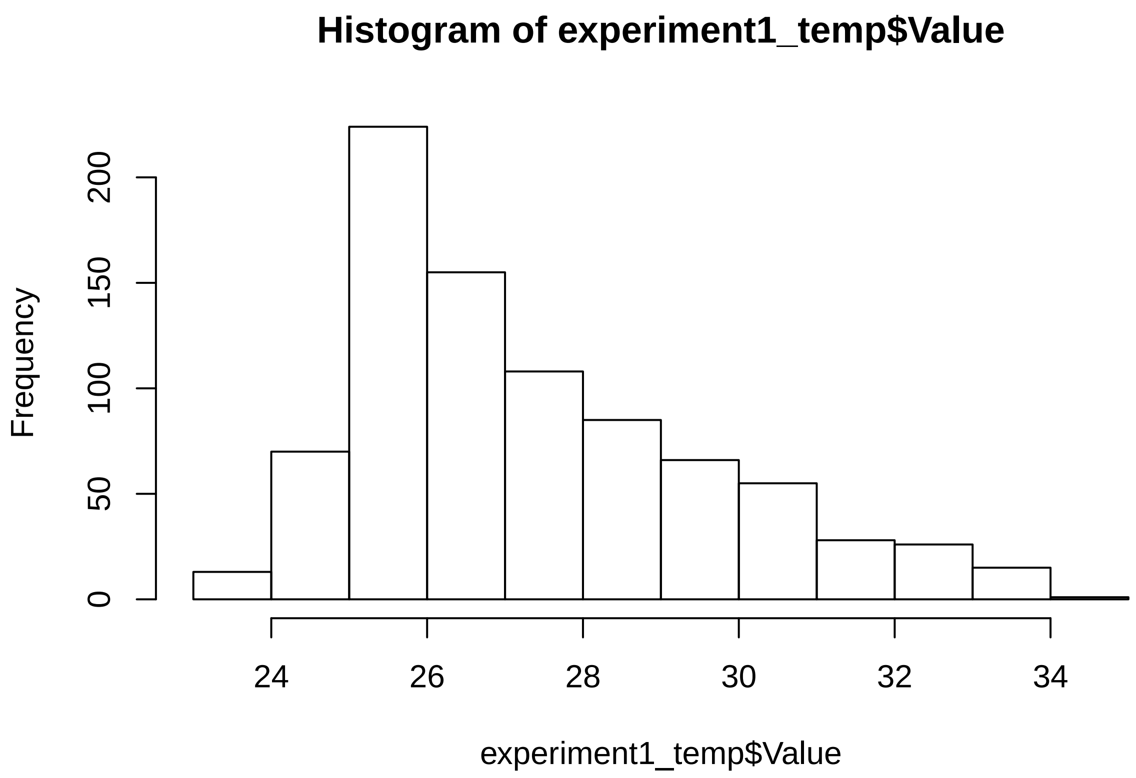


STATS

Linear model

```
library(lme4)
library(car)
```

```
library(lmerTest)

hist(experiment1_temp$Value)
```



```
#all models
temperature_1_full = lm(Value~Sensor, data = experiment1_temp)

summary(temperature_1_full)
```

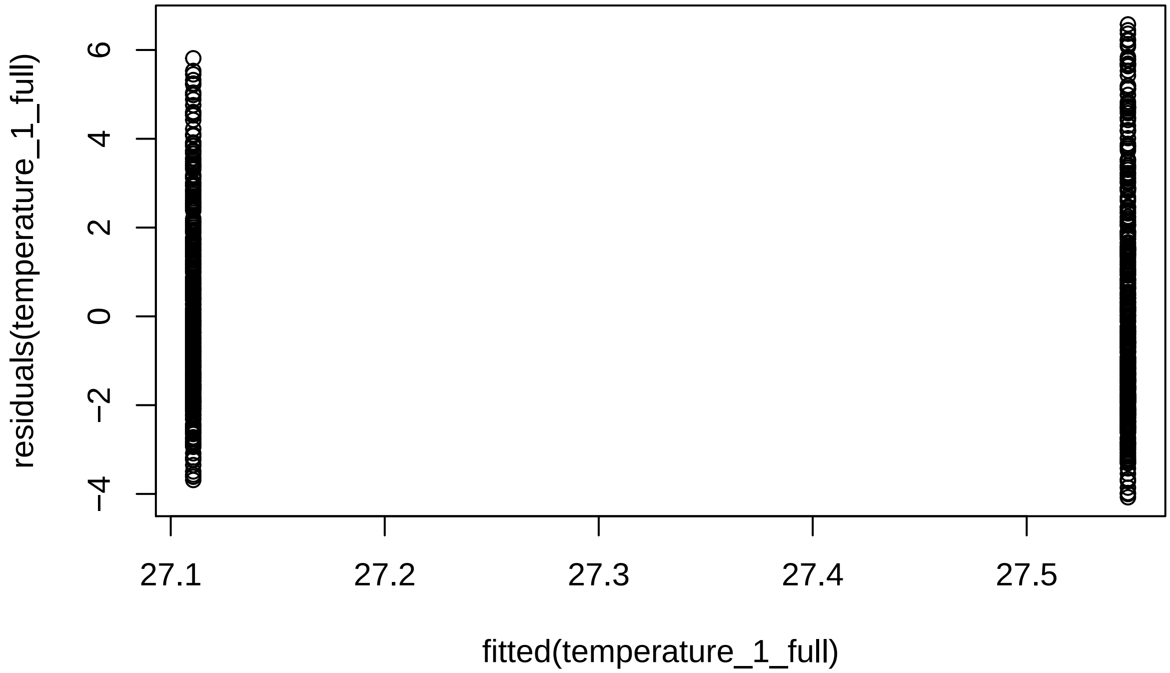
```
##
## Call:
## lm(formula = Value ~ Sensor, data = experiment1_temp)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.0753 -1.7543 -0.5844  1.4427  6.5767
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  27.1105     0.1099  246.62  < 2e-16 ***
## SensorWT2     0.4368     0.1555    2.81  0.00507 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.261 on 844 degrees of freedom
```

```
## Multiple R-squared:  0.009267,   Adjusted R-squared:  0.008093
## F-statistic: 7.895 on 1 and 844 DF,  p-value: 0.005073
```

```
Anova(temperature_1_full)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##           Sum Sq  Df F value   Pr(>F)
## Sensor      40.4   1  7.8946 0.005073 **
## Residuals 4314.2 844
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

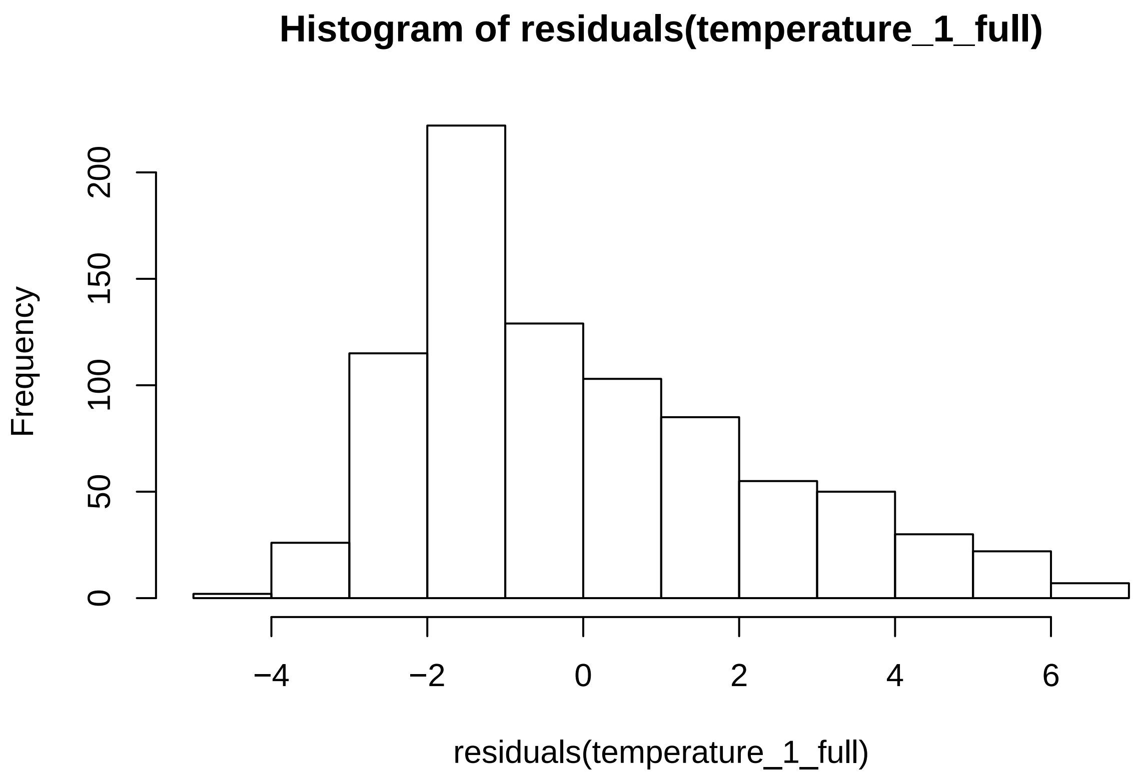
```
plot(fitted(temperature_1_full), residuals(temperature_1_full))
```



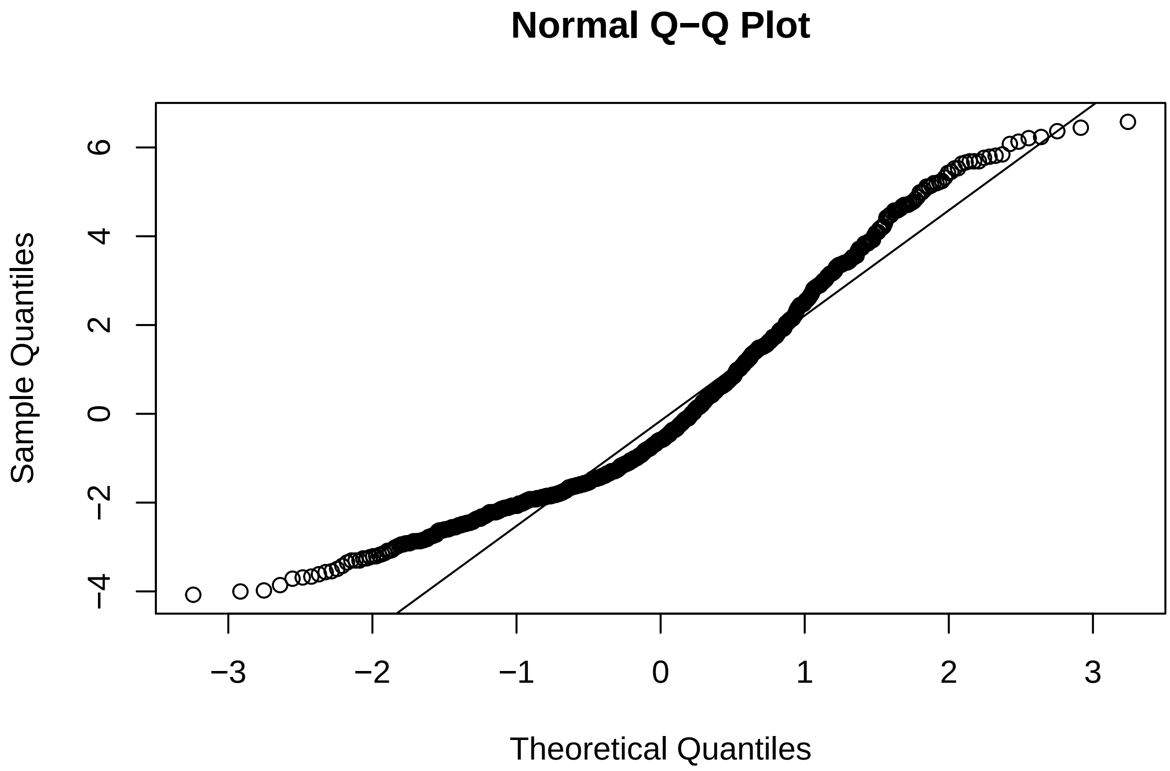
```
leveneTest(data = experiment1_temp, Value~Sensor)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value   Pr(>F)
## group   1  7.4252 0.006565 **
##       844
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(temperature_1_full))
```



```
qqnorm(residuals(temperature_1_full))
qqline(residuals(temperature_1_full))
```



```
shapiro.test(residuals(temperature_1_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(temperature_1_full)
## W = 0.93751, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(temperature_1_full) W = 0.93751, p-value < 2.2e-16

Anova Table (Type II tests)

Response: Value Sum Sq Df F value Pr(>F)
Sensor 40.4 1 7.8946 0.005073 ** Residuals 4314.2 844

Nonparametric stats:

```
wilcox.test(data=experiment1_temp, Value~Sensor)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Value by Sensor
## W = 81702, p-value = 0.02894
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 81702**
- **p-value = 0.02894**

Interpretation: Temperature differed significantly among high and low humidity treatments in Experiment I

Relative Humidity

Summary statistics

```
experiment1_humidity
```

```
## # A tibble: 846 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180606          1     0  16.2 0.675    NA WRH2    99.9 Relative Humidity
##  2 20180606          1     0  16.5 0.687    NA WRH2    95.1 Relative Humidity
##  3 20180606          1     0  17.2 0.716    NA WRH2    95.7 Relative Humidity
##  4 20180606          1     0  17.5 0.729    NA WRH2    96.8 Relative Humidity
##  5 20180606          1     0  18.2 0.758    NA WRH2    97.5 Relative Humidity
##  6 20180606          1     0  18.5 0.770    NA WRH2    97.9 Relative Humidity
##  7 20180606          1     0  19.2 0.800    NA WRH2    98.4 Relative Humidity
##  8 20180606          1     0  19.5 0.812    NA WRH2    98.4 Relative Humidity
##  9 20180606          1     0  20.2 0.841    NA WRH2    98.8 Relative Humidity
## 10 20180606          1     0  20.5 0.854    NA WRH2    98.7 Relative Humidity
## # ... with 836 more rows
```

```
experiment1_humidity_sum= experiment1_humidity %>%
  group_by(Sensor) %>%
  summarise(min= min(Value), max= max(Value), mean_humidity= mean(Value),SD = sd(Value), sample_size=n(
experiment1_humidity_sum
```

```
## # A tibble: 2 x 6
##   Sensor   min   max mean_humidity    SD sample_size
##   <chr>  <dbl> <dbl>         <dbl> <dbl>       <int>
## 1 WRH1    75.6   100          98.0  4.66         423
## 2 WRH2    66.5   100          92.2  8.01         423
```

High humidity:

- mean humidity = 97.98156 +/- 4.662164
- range = 75.6—100

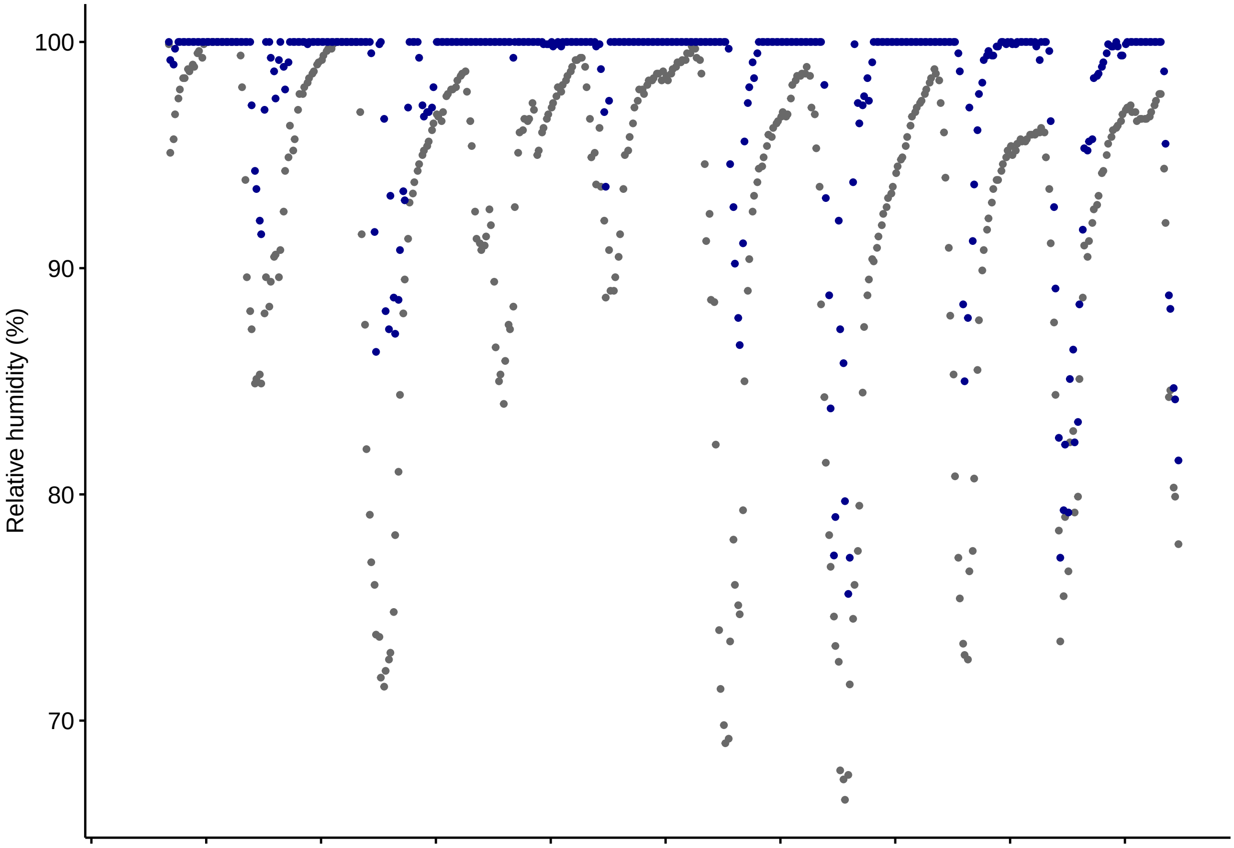
Low humidity:

- mean humidity = 92.23215 +/- 8.009737

- range = 66.5—100
- sample size = 423
- 30min incriments, 2 WHITE sensors

Figure

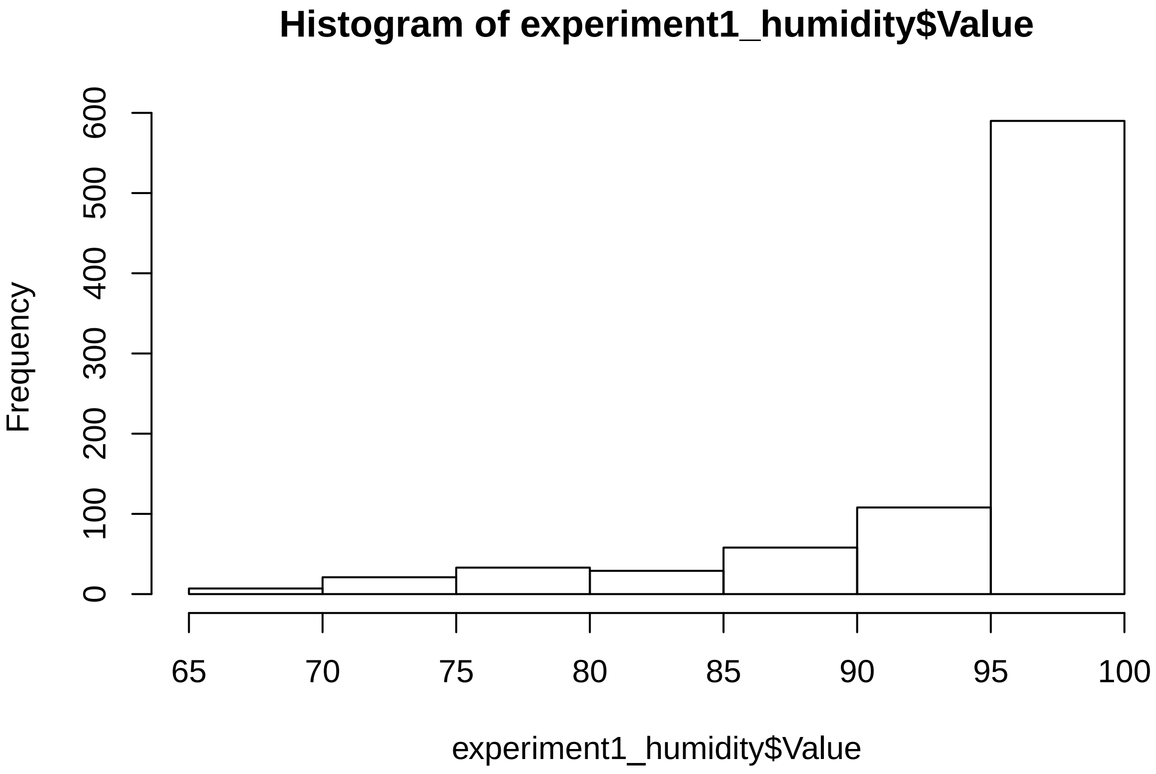
```r
library(ggplot2)
library(scales)

legend_title = "Hydration treatment"

humidity_1 = ggplot(experiment1_humidity, aes(x=age, y=Value)) + #add the axis
  geom_point(aes(color = Sensor), size = .7) +
  labs(y="Relative humidity (%)", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  theme(legend.text = element_text(size = 9), legend.title = element_blank(), legend.background = elemen
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color
  #theme(plot.margin = unit(c(1, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(oob = rescale_none, breaks= pretty_breaks(n=5)) +
  scale_x_continuous(limits=c(0.4,NA), breaks= pretty_breaks(n=8)) +   # put the x axis stuff in order
scale_color_manual(legend_title, breaks=c("WRH1", "WRH2"), labels=c("High humidity", "Low humidity"), va
humidity_1
```



STATS

Linear model

```
library(lme4)
library(car)
library(lmerTest)

hist(experiment1_humidity$Value)
```



```
#all models
humidity_1_full = lm(Value~Sensor, data = experiment1_humidity)

summary(humidity_1_full)
```

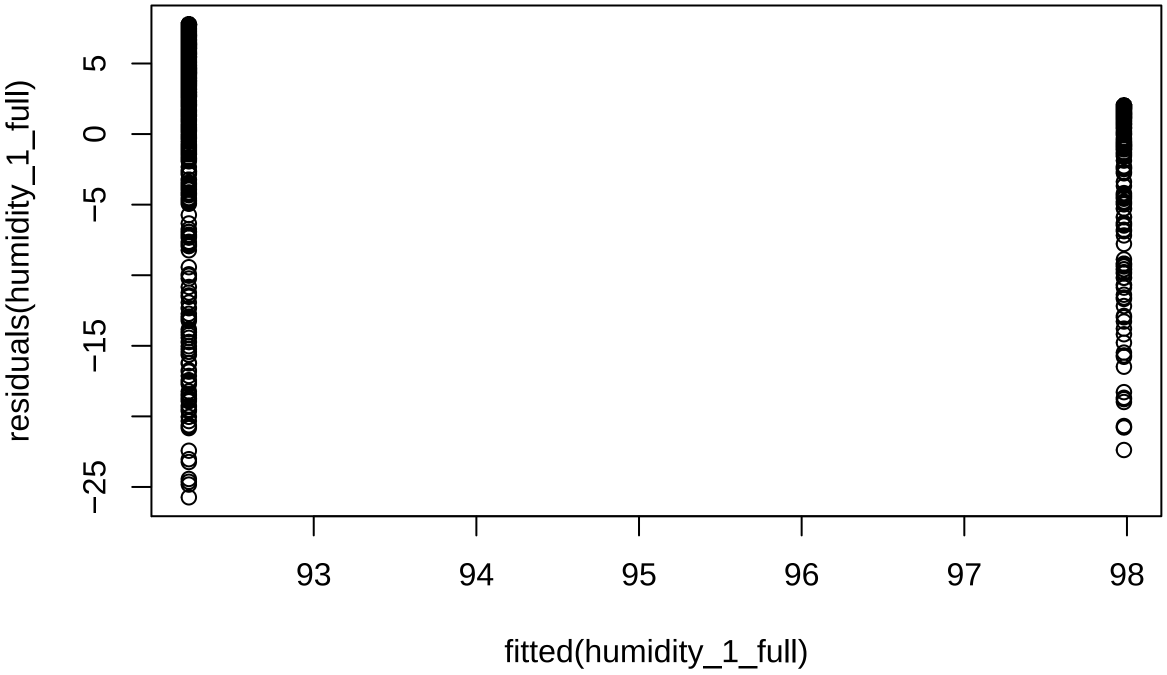
```
##
## Call:
## lm(formula = Value ~ Sensor, data = experiment1_humidity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -25.7322  -0.7816   2.0184   3.1678   7.7678
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   97.9816     0.3186  307.51   <2e-16 ***
## SensorWRH2    -5.7494     0.4506  -12.76   <2e-16 ***
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.553 on 844 degrees of freedom
## Multiple R-squared:  0.1617, Adjusted R-squared:  0.1607
## F-statistic: 162.8 on 1 and 844 DF,  p-value: < 2.2e-16
```

```r
Anova(humidity_1_full)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##           Sum Sq  Df F value    Pr(>F)
## Sensor      6991   1  162.79 < 2.2e-16 ***
## Residuals  36246 844
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
plot(fitted(humidity_1_full), residuals(humidity_1_full))
```

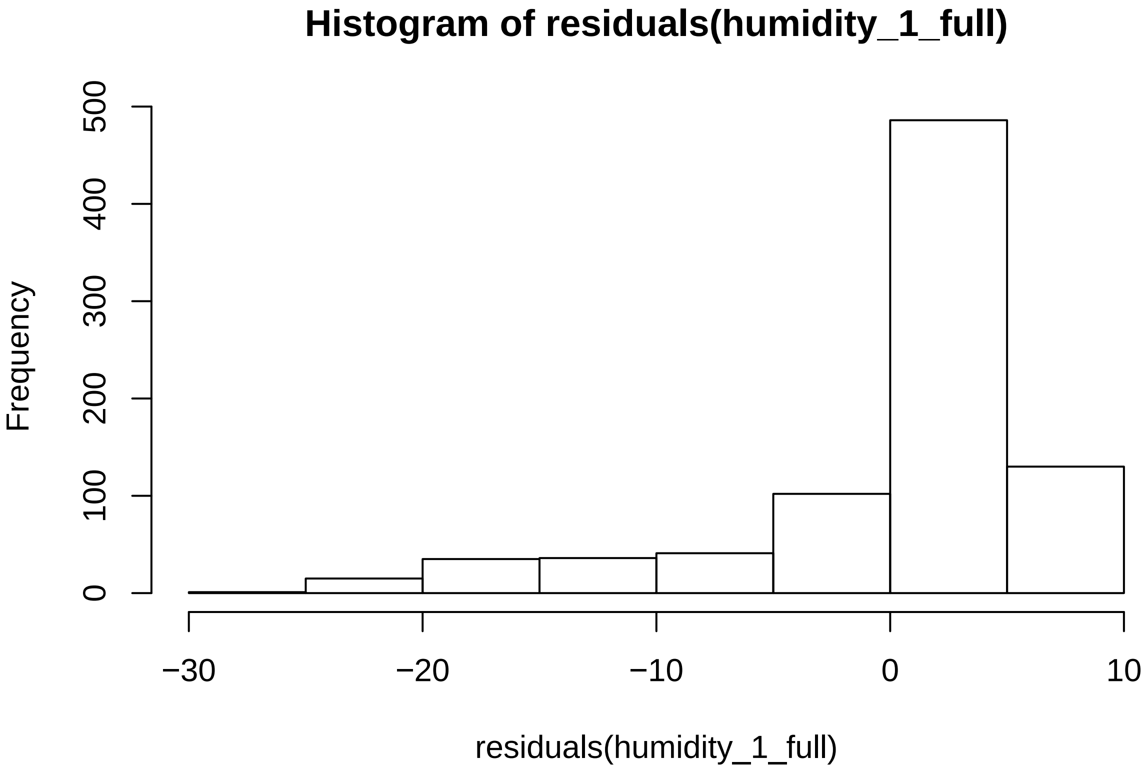


```r
leveneTest(data = experiment1_humidity, Value~Sensor)
```

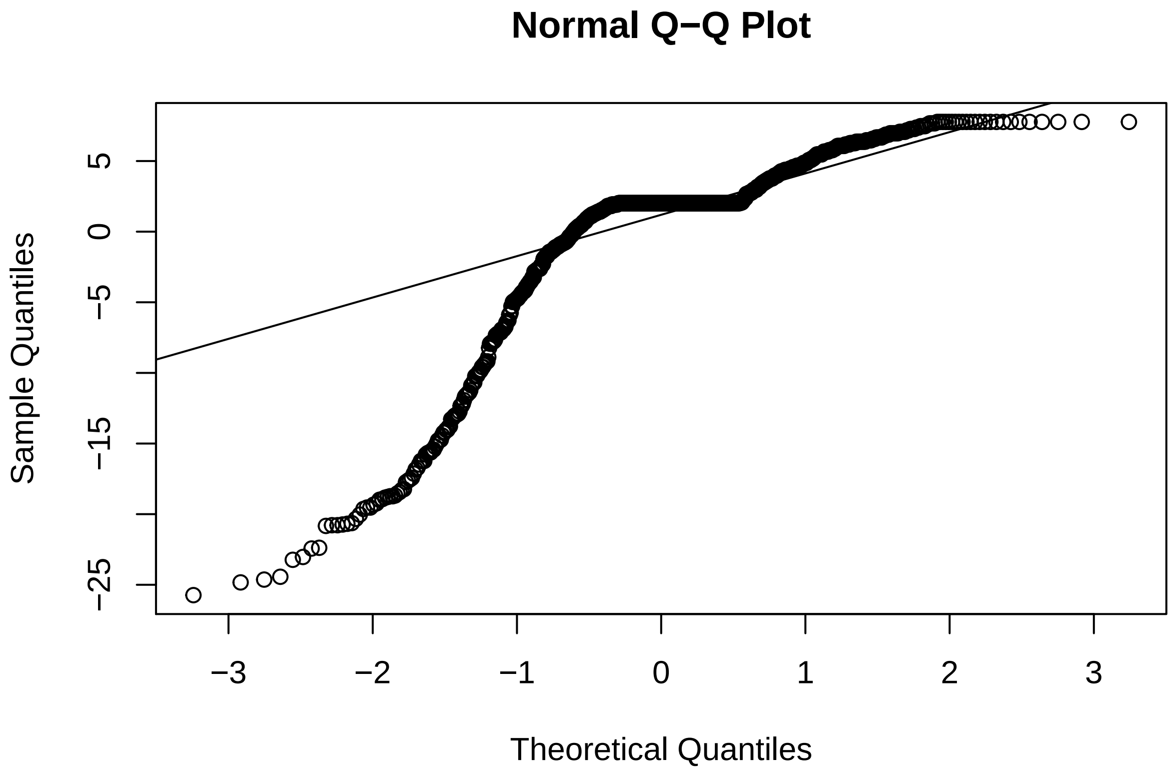
```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value    Pr(>F)
```

```
## group    1  85.955 < 2.2e-16 ***
##        844
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(humidity_1_full))
```



```
qqnorm(residuals(humidity_1_full))
qqline(residuals(humidity_1_full))
```



```
shapiro.test(residuals(humidity_1_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(humidity_1_full)
## W = 0.77878, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(temperature_1_full) W = 0.93751, p-value < 2.2e-16

Anova Table (Type II tests)

Response: Value Sum Sq Df F value Pr(>F)
Sensor 6991 1 162.79 < 2.2e-16 *** Residuals 36246 844

Nonparametric stats:

```
wilcox.test(data=experiment1_humidity, Value~Sensor)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Value by Sensor
## W = 150098, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 150098**
- **p-value < 2.2e-16**

Interpretation: Humidity differed significantly among high and low humidity treatments

---

Combined Figure 2

```
library(gridExtra)
library(cowplot)

# 4.43 x 3.43 landscape

# 1 column is 3.43 inches wide

figure2 = plot_grid(humidity_1, temperature_1, labels = "AUTO", label_x = -.01, label_y = 1, scale =1,
figure2
```

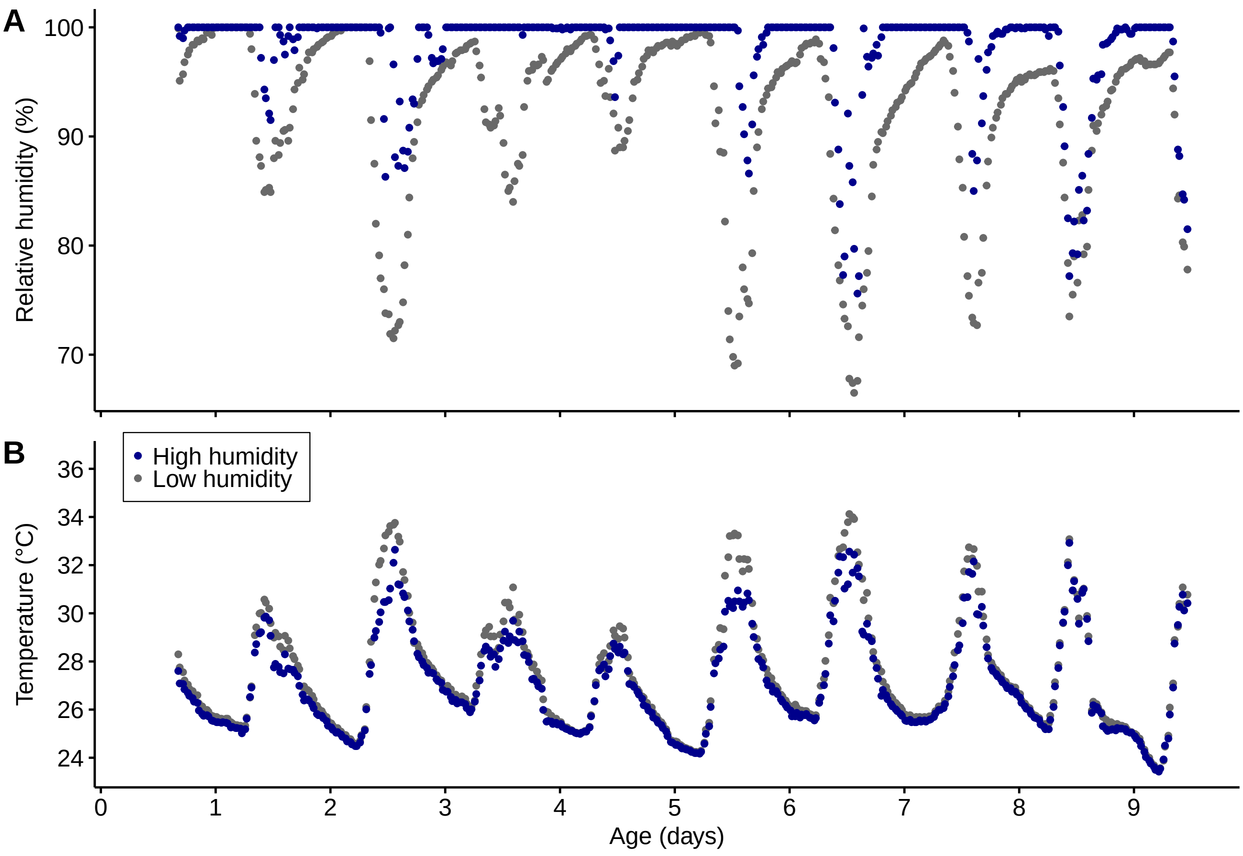


Figure 3

Experiment 2

Subsetting and tidying data

```
experiment2 = dtd %>%
  filter(Sensor != "BT1") %>%
  filter(Sensor != "BT2") %>%
  filter(experiment == "2")
experiment2
```

```
## # A tibble: 3,484 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180615          2     0  12.4 0.518     1 WT2     29.9 Temperature
##  2 20180615          2     0  13.1 0.547     1 WT2     30.4 Temperature
##  3 20180615          2     0  13.4 0.559     1 WT2     30.2 Temperature
##  4 20180615          2     0  14.1 0.588     1 WT2     30.9 Temperature
##  5 20180615          2     0  14.4 0.601     1 WT2     30.1 Temperature
##  6 20180615          2     0  15.1 0.63      1 WT2     29.8 Temperature
##  7 20180615          2     0  15.4 0.642     1 WT2     29.6 Temperature
##  8 20180615          2     0  16.1 0.672     1 WT2     28.6 Temperature
##  9 20180615          2     0  16.4 0.684     1 WT2     28.3 Temperature
## 10 20180615          2     0  17.1 0.713     1 WT2     27.9 Temperature
## # ... with 3,474 more rows
```

```
unique(experiment2$Type)
```

```
## [1] "Temperature"       "Relative Humidity"
```

```
# humid temp
experiment2_temp = experiment2 %>%
  filter(Type == "Temperature")
experiment2_temp
```

```
## # A tibble: 1,742 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180615          2     0  12.4 0.518     1 WT2     29.9 Temperature
##  2 20180615          2     0  13.1 0.547     1 WT2     30.4 Temperature
##  3 20180615          2     0  13.4 0.559     1 WT2     30.2 Temperature
##  4 20180615          2     0  14.1 0.588     1 WT2     30.9 Temperature
##  5 20180615          2     0  14.4 0.601     1 WT2     30.1 Temperature
##  6 20180615          2     0  15.1 0.63      1 WT2     29.8 Temperature
##  7 20180615          2     0  15.4 0.642     1 WT2     29.6 Temperature
##  8 20180615          2     0  16.1 0.672     1 WT2     28.6 Temperature
```

```
##  9 20180615          2     0  16.4 0.684     1 WT2     28.3 Temperature
## 10 20180615          2     0  17.1 0.713     1 WT2     27.9 Temperature
## # ... with 1,732 more rows
```

```
# humid humidity
experiment2_humidity = experiment2 %>%
  filter(Type == "Relative Humidity")
experiment2_humidity
```

```
## # A tibble: 1,742 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180615          2     0  12.4 0.518     1 WRH2    80.2 Relative Humidity
##  2 20180615          2     0  13.1 0.547     1 WRH2    84.2 Relative Humidity
##  3 20180615          2     0  13.4 0.559     1 WRH2    89.6 Relative Humidity
##  4 20180615          2     0  14.1 0.588     1 WRH2    87.6 Relative Humidity
##  5 20180615          2     0  14.4 0.601     1 WRH2    87.3 Relative Humidity
##  6 20180615          2     0  15.1 0.63      1 WRH2    88.9 Relative Humidity
##  7 20180615          2     0  15.4 0.642     1 WRH2    87.8 Relative Humidity
##  8 20180615          2     0  16.1 0.672     1 WRH2    88   Relative Humidity
##  9 20180615          2     0  16.4 0.684     1 WRH2    88.7 Relative Humidity
## 10 20180615          2     0  17.1 0.713     1 WRH2    90.6 Relative Humidity
## # ... with 1,732 more rows
```

Temperature

Summary statistics

```
experiment2_temp
```

```
## # A tibble: 1,742 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180615          2     0  12.4 0.518     1 WT2     29.9 Temperature
##  2 20180615          2     0  13.1 0.547     1 WT2     30.4 Temperature
##  3 20180615          2     0  13.4 0.559     1 WT2     30.2 Temperature
##  4 20180615          2     0  14.1 0.588     1 WT2     30.9 Temperature
##  5 20180615          2     0  14.4 0.601     1 WT2     30.1 Temperature
##  6 20180615          2     0  15.1 0.63      1 WT2     29.8 Temperature
##  7 20180615          2     0  15.4 0.642     1 WT2     29.6 Temperature
##  8 20180615          2     0  16.1 0.672     1 WT2     28.6 Temperature
##  9 20180615          2     0  16.4 0.684     1 WT2     28.3 Temperature
## 10 20180615          2     0  17.1 0.713     1 WT2     27.9 Temperature
## # ... with 1,732 more rows
```

```
experiment2_temp_sum= experiment2_temp %>%
  group_by(Sensor) %>%
  summarise(min= min(Value), max= max(Value), mean_temp= mean(Value), SD = sd(Value), sample_size=n())
experiment2_temp_sum
```

```
## # A tibble: 2 x 6
```

```
##   Sensor   min   max mean_temp    SD sample_size
##   <chr>  <dbl> <dbl>     <dbl> <dbl>       <int>
## 1 WT1     22.7  33.4      26.7  2.06         871
## 2 WT2     22.7  34.1      26.9  2.31         871
```

```
experiment2_temp_sum2 = experiment2_temp %>%
  group_by(Sensor, block) %>%
  summarise(min= min(Value), max= max(Value), mean_temp= mean(Value), SD = sd(Value), sample_size=n())
experiment2_temp_sum2
```

```
## # A tibble: 4 x 7
## # Groups:   Sensor [2]
##   Sensor block   min   max mean_temp    SD sample_size
##   <chr>  <dbl> <dbl> <dbl>     <dbl> <dbl>       <int>
## 1 WT1        1  23.7  33.4      26.9  2.12         393
## 2 WT1        2  22.7  31.6      26.5  1.98         478
## 3 WT2        1  23.6  34.1      27.2  2.40         393
## 4 WT2        2  22.7  33.9      26.7  2.21         478
```

TOTAL

High humidity:

- mean temp = 26.71280 +/- 2.055606
- range = 22.729—33.365

Medium humidity:

- mean temp = 26.92227 +/- 2.310634
- range = 22.681—34.124
- sample size = 871
- 30min incriments, 2 WHITE sensors

BLOCK 1

High humidity:

- mean temp = 26.94883 +/- 2.124349
- range = 23.665—33.365

Medium humidity:

- mean temp = 27.18472 +/- 2.400158
- range = 23.617—34.124

BLOCK 2

High humidity:

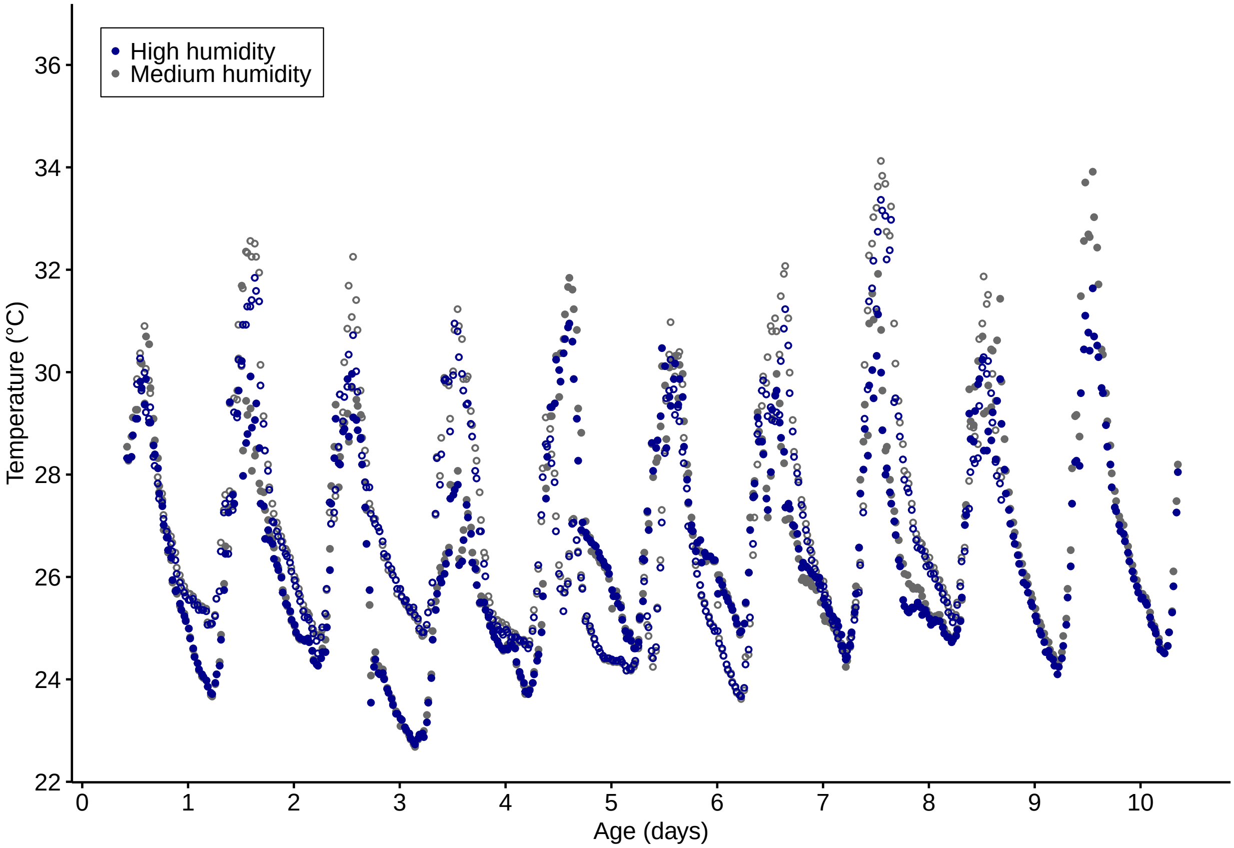
- mean temp = 26.51874 +/- 1.978533
- range = 22.729—31.637

  Medium humidity:

- mean temp = 26.70649 +/- 2.213649
- range = 22.681—33.914

Figure

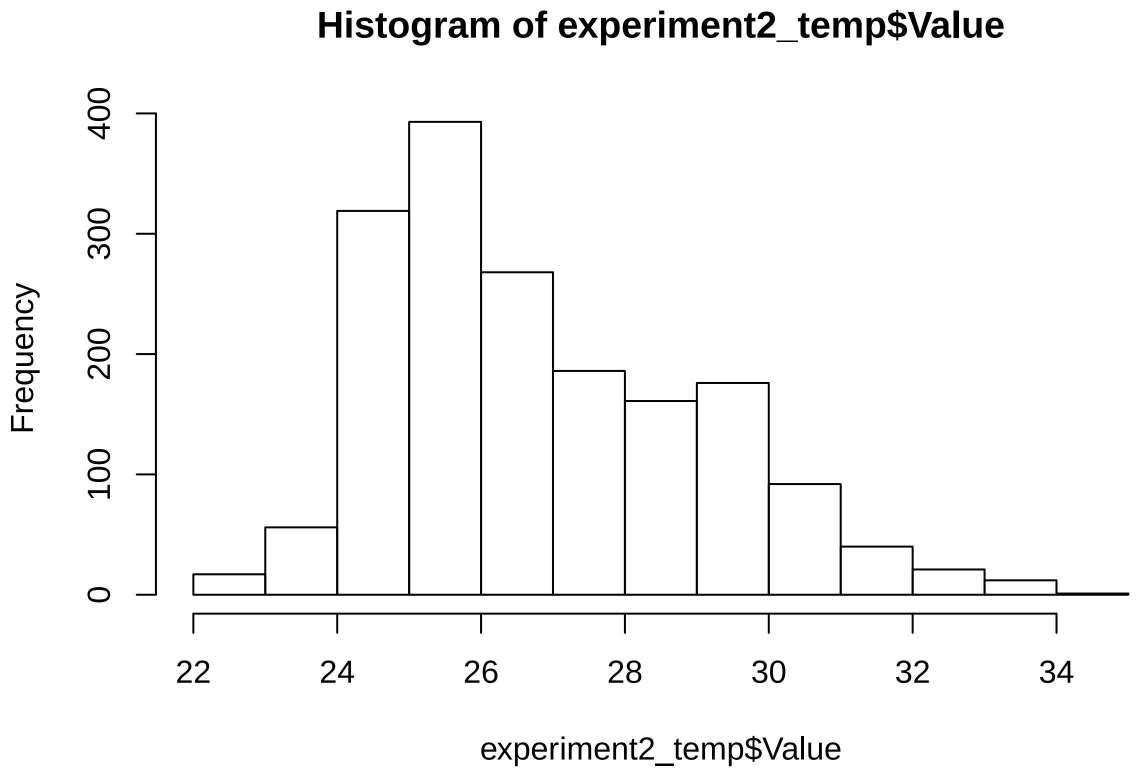
```
library(ggplot2)
library(scales)
legend_title = "Hydration treatment"

temperature_2 = ggplot(experiment2_temp, aes(x=age, y=Value)) + #add the axis
  geom_point(aes(color = Sensor, shape = as.factor(block)), size = .7) +
  labs(y="Temperature (°C)", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black")) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color =
  #theme(plot.margin = unit(c(.5, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(limits=c(NA,36.5), breaks= pretty_breaks(n=6)) +
  scale_x_continuous(limits=c(0.4,NA), breaks= pretty_breaks(n=8)) +   # put the x axis stuff in order
  scale_color_manual(legend_title, breaks=c("WT1", "WT2"), labels=c("High humidity", "Medium humidity")
  scale_shape_manual(values = c(21,19)) +
  guides(shape = FALSE)
#facet_grid(rows = vars(block), as.table = T, scales = "free", space = "free")
temperature_2
```



STATS

Linear model

```
library(lme4)
library(car)
library(lmerTest)

hist(experiment2_temp$Value)
```



```r
#all models
temperature_2_full = lm(Value~Sensor, data = experiment2_temp)

summary(temperature_2_full)
```

```
##
## Call:
## lm(formula = Value ~ Sensor, data = experiment2_temp)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.2413 -1.6968 -0.5308  1.6043  7.2017
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  26.7128     0.0741 360.505   <2e-16 ***
## SensorWT2     0.2095     0.1048   1.999   0.0458 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.187 on 1740 degrees of freedom
## Multiple R-squared:  0.002291,   Adjusted R-squared:  0.001718
## F-statistic: 3.996 on 1 and 1740 DF,  p-value: 0.04577
```

```r
Anova(temperature_2_full)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##           Sum Sq   Df F value  Pr(>F)
## Sensor      19.1    1  3.9957 0.04577 *
## Residuals 8321.2 1740
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
#block effect?
temperature_2_full_block = lm(Value~Sensor*block, data = experiment2_temp)
summary(temperature_2_full_block)
```

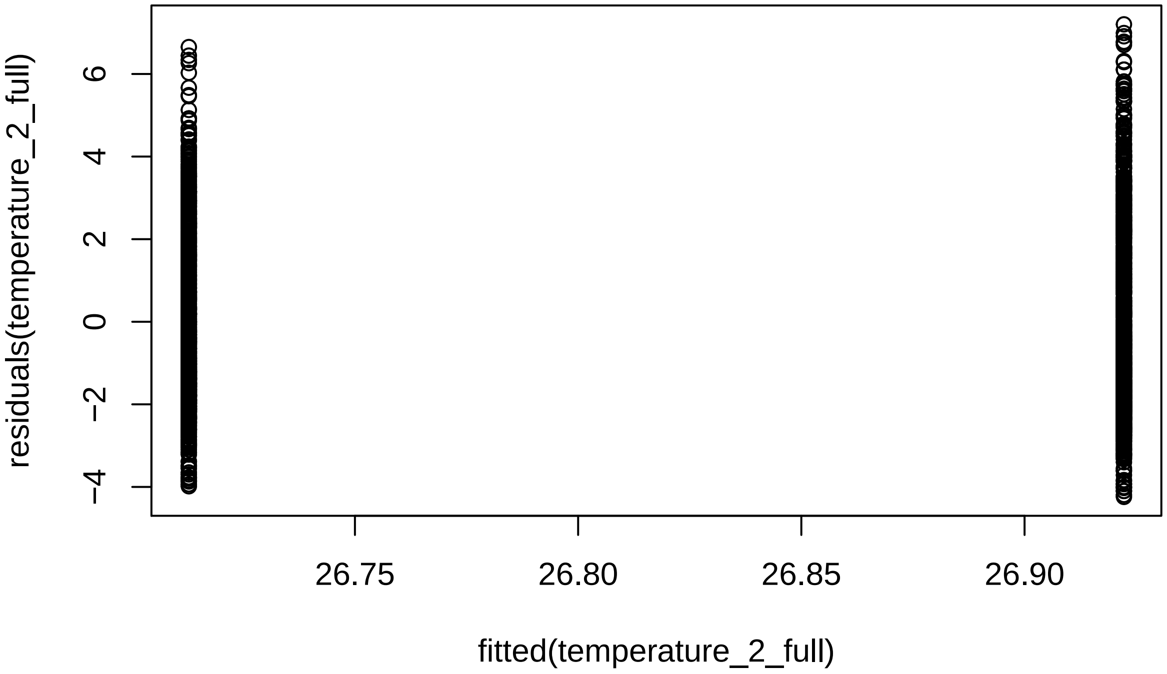
```
##
## Call:
## lm(formula = Value ~ Sensor * block, data = experiment2_temp)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.0255 -1.7207 -0.5137  1.6033  7.2075
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)      27.37893    0.24107 113.570  < 2e-16 ***
## SensorWT2         0.28402    0.34093   0.833  0.40492
## block            -0.43010    0.14819  -2.902  0.00375 **
## SensorWT2:block -0.04813    0.20958  -0.230  0.81837
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.176 on 1738 degrees of freedom
## Multiple R-squared:  0.01299,    Adjusted R-squared:  0.01129
## F-statistic: 7.624 on 3 and 1738 DF,  p-value: 4.599e-05
```

```r
Anova(temperature_2_full_block)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##              Sum Sq   Df F value    Pr(>F)
## Sensor         19.1    1  4.0344   0.04474 *
## block          89.0    1 18.7847 1.547e-05 ***
## Sensor:block    0.2    1  0.0528   0.81837
## Residuals    8231.9 1738
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
#Anova Table (Type II tests)
```

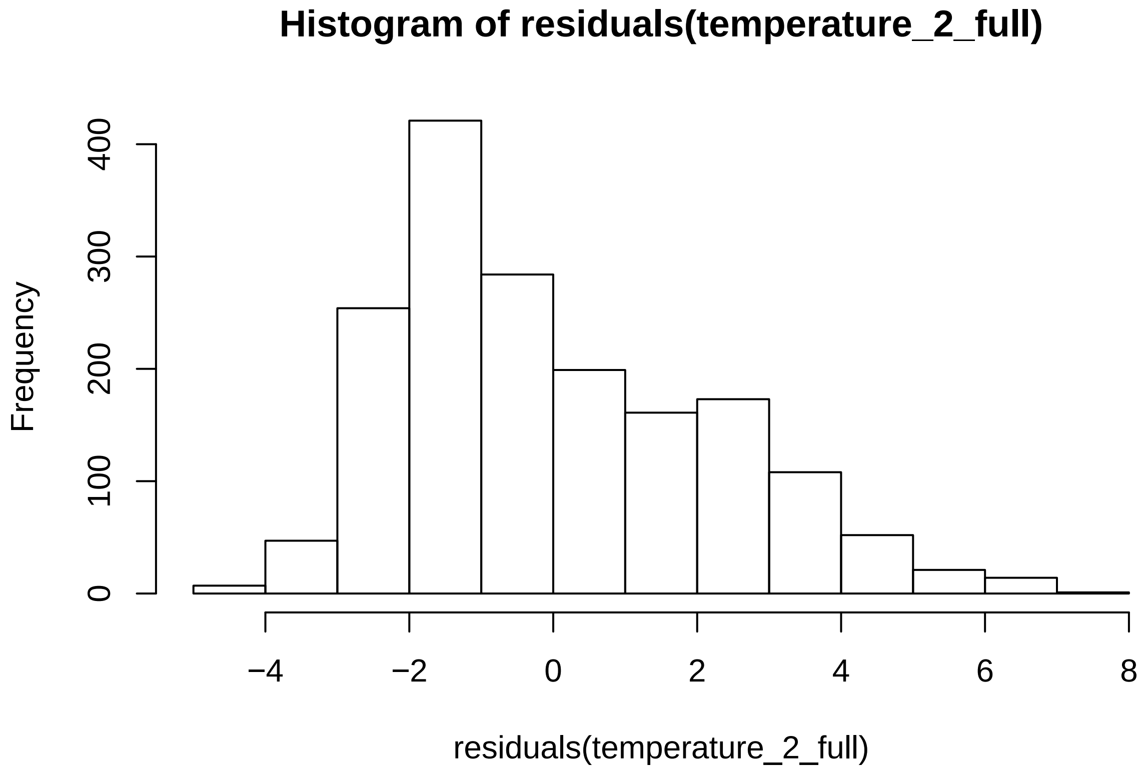
```
#Response: Value
#              Sum Sq   Df F value    Pr(>F)
#Sensor          19.1    1  4.0344   0.04474 *
#block           89.0    1 18.7847 1.547e-05 ***
#Sensor:block     0.2    1  0.0528   0.81837

plot(fitted(temperature_2_full), residuals(temperature_2_full))
```



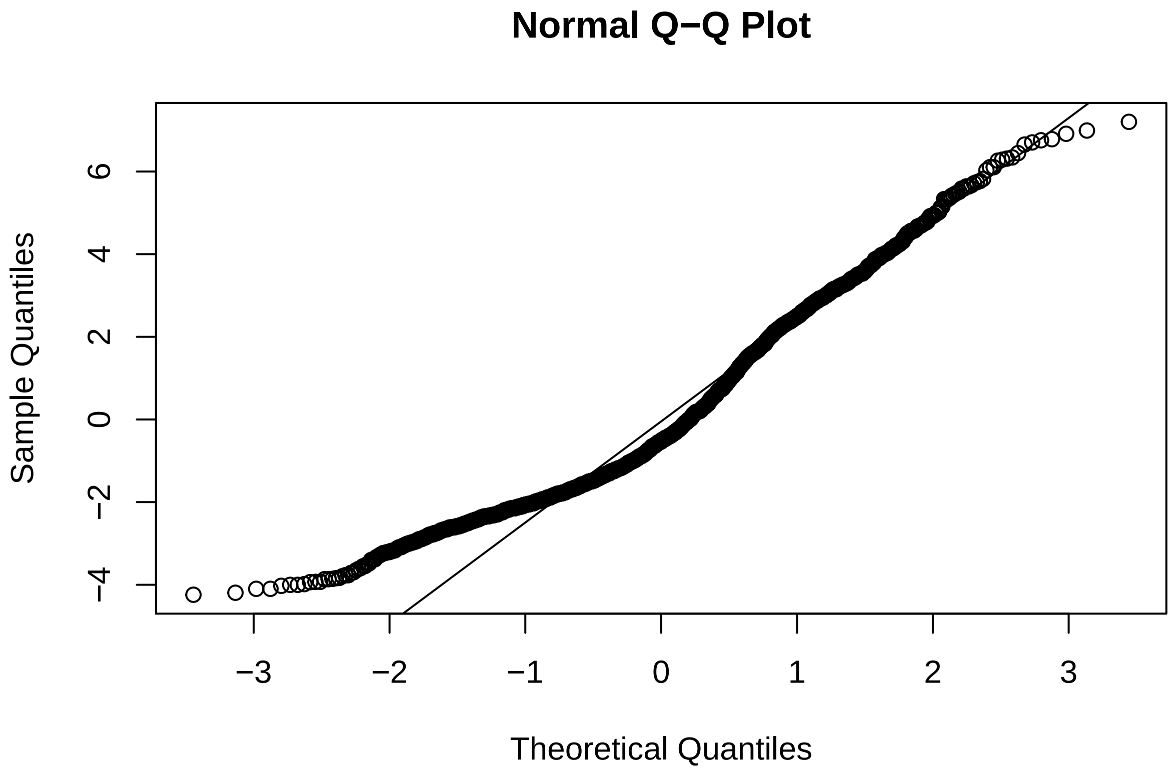
```
leveneTest(data = experiment2_temp, Value~Sensor)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##         Df F value  Pr(>F)
## group    1  6.0042 0.01437 *
##       1740
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(temperature_2_full))
```



```
qqnorm(residuals(temperature_2_full))
qqline(residuals(temperature_2_full))
```



```
shapiro.test(residuals(temperature_2_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(temperature_2_full)
## W = 0.95078, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(temperature_2_full) W = 0.95078, p-value < 2.2e-16

Anova Table (Type II tests)

Response: Value Sum Sq Df F value Pr(>F)
Sensor 19.1 1 3.9957 0.04577 * Residuals 8321.2 1740

Nonparametric stats:

```
wilcox.test(data=experiment2_temp, Value~Sensor)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Value by Sensor
## W = 365910, p-value = 0.2014
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 365910**
- **p-value = 0.2014 NS**

> Interpretation: Average temperature (across blocks) did not differ among high and medium humidity treatments

---

Relative Humidity

Summary statistics

```
experiment2_humidity
```

```
## # A tibble: 1,742 x 9
##        date experiment   day  time   age block Sensor Value Type
##       <dbl>      <dbl> <dbl> <dbl> <dbl> <dbl> <chr>  <dbl> <chr>
##  1 20180615          2     0  12.4 0.518     1 WRH2    80.2 Relative Humidity
##  2 20180615          2     0  13.1 0.547     1 WRH2    84.2 Relative Humidity
##  3 20180615          2     0  13.4 0.559     1 WRH2    89.6 Relative Humidity
##  4 20180615          2     0  14.1 0.588     1 WRH2    87.6 Relative Humidity
##  5 20180615          2     0  14.4 0.601     1 WRH2    87.3 Relative Humidity
##  6 20180615          2     0  15.1 0.63      1 WRH2    88.9 Relative Humidity
##  7 20180615          2     0  15.4 0.642     1 WRH2    87.8 Relative Humidity
##  8 20180615          2     0  16.1 0.672     1 WRH2    88   Relative Humidity
##  9 20180615          2     0  16.4 0.684     1 WRH2    88.7 Relative Humidity
## 10 20180615          2     0  17.1 0.713     1 WRH2    90.6 Relative Humidity
## # ... with 1,732 more rows
```

```
experiment2_humidity_sum = experiment2_humidity %>%
  group_by(Sensor) %>%
  summarise(min= min(Value), max= max(Value), mean_humidity= mean(Value), SD = sd(Value), sample_size=n
experiment2_humidity_sum
```

```
## # A tibble: 2 x 6
##   Sensor   min   max mean_humidity    SD sample_size
##   <chr>  <dbl> <dbl>         <dbl> <dbl>       <int>
## 1 WRH1    74.2   100          99.6  2.39         871
## 2 WRH2    67.9   100          96.3  6.43         871
```

```
experiment2_humidity_sum2 = experiment2_humidity %>%
  group_by(Sensor,block) %>%
  summarise(min= min(Value), max= max(Value), mean_humidity= mean(Value), SD = sd(Value), sample_size=n
experiment2_humidity_sum2
```

```
## # A tibble: 4 x 7
## # Groups:   Sensor [2]
##   Sensor block   min   max mean_humidity    SD sample_size
```

```
##   <chr>  <dbl> <dbl> <dbl>         <dbl> <dbl>       <int>
## 1 WRH1       1  74.2   100          99.2 3.51          393
## 2 WRH1       2  95.9   100         100.  0.305         478
## 3 WRH2       1  67.9   100          95.5 6.97          393
## 4 WRH2       2  70.4   100          97.0 5.88          478
```

TOTAL

High humidity:

- mean humidity = 99.61825 +/- 2.394476
- range = 74.2—100

Medium:

- mean humidity = 96.31814 +/- 6.431308
- range = 67.9—100
- sample size = 871
- 30min incriments, 2 WHITE sensors

BLOCK 1

High humidity:

- mean humidity = 99.20356 +/- 3.5067482
- range = 74.2—100

Medium humidity:

- mean humidity = 95.52494 +/- 6.9679177
- range = 67.9—100
- sample size = 393

BLOCK 2

High humidity:

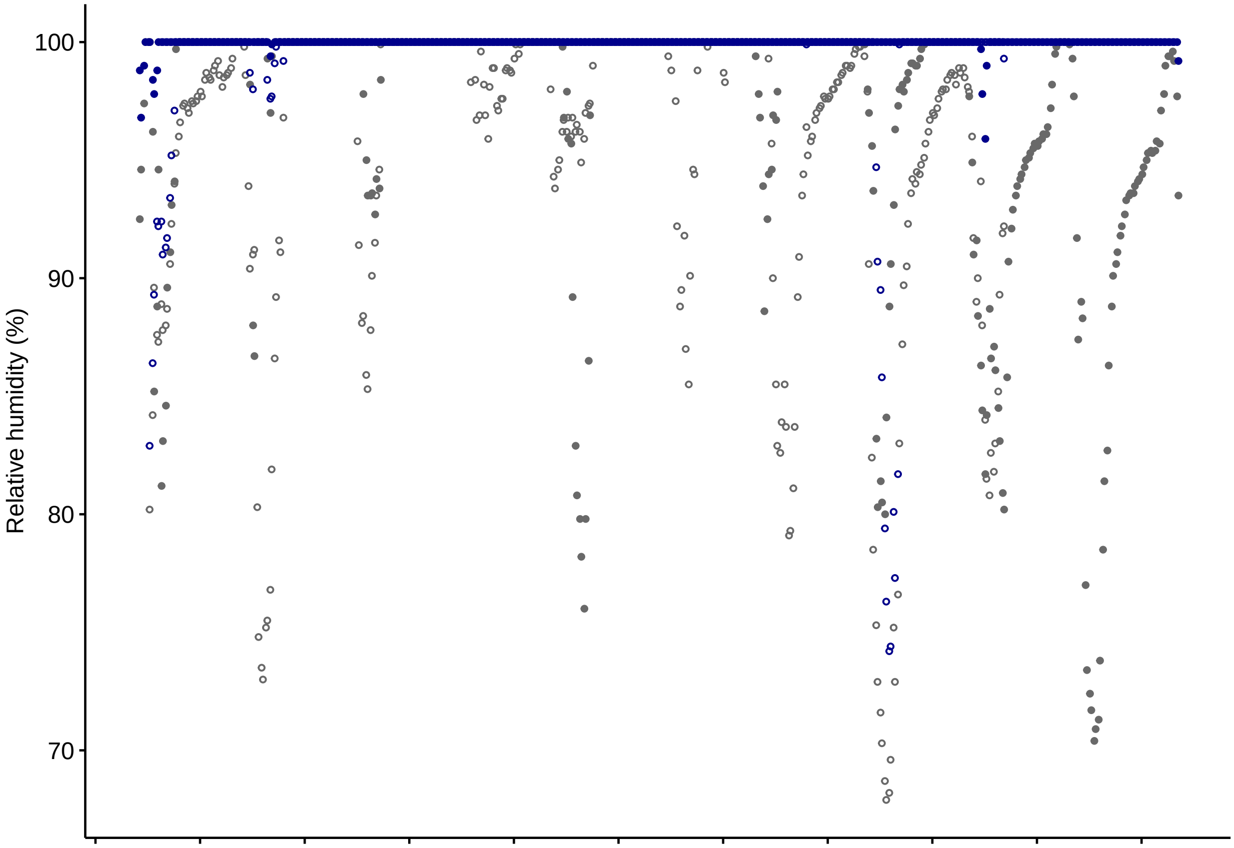
- mean humidity = 99.95921 +/- 0.3053489
- range = 95.9—100

Medium humidity:

- mean humidity = 96.97029 +/- 5.8817484
- range = 70.4—100
- sample size = 478

Figure

```
library(ggplot2)
library(scales)
legend_title = "Hydration treatment"

humidity_2 = ggplot(experiment2_humidity, aes(x=age, y=Value)) + #add the axis
  geom_point(aes(color = Sensor, shape = as.factor(block)), size = .7) +
  labs(y="Relative humidity (%)", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  theme(legend.text = element_text(size = 9), legend.title = element_blank(), legend.background = elemen
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color
  #theme(plot.margin = unit(c(1, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(oob = rescale_none, breaks= pretty_breaks(n=5)) +
  scale_x_continuous(limits=c(0.4,NA), breaks= pretty_breaks(n=8)) +   # put the x axis stuff in order
  scale_color_manual(legend_title, breaks=c("WRH1", "WRH2"), labels=c("High humidity", "Medium humidity
  scale_shape_manual(values = c(21,19))
#facet_grid(rows = vars(block), as.table = T, scales = "free", space = "free")
humidity_2
```

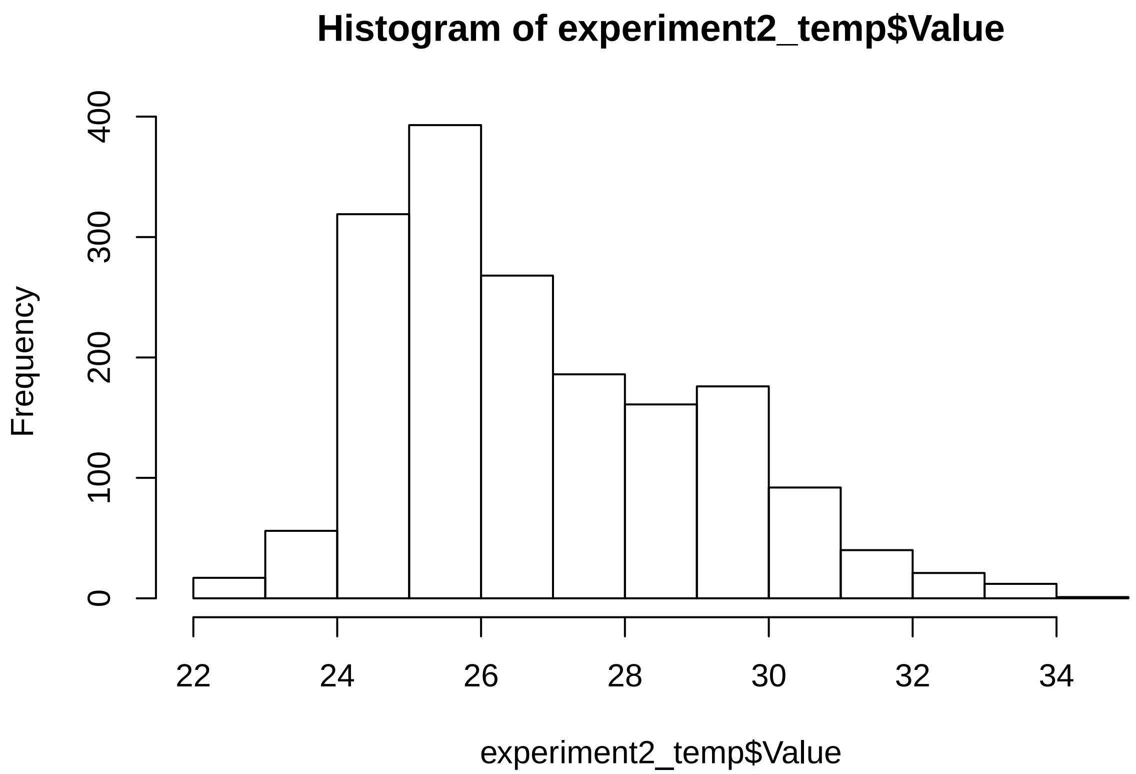


STATS

Linear model

```
library(lme4)
library(car)
library(lmerTest)
```

```r
hist(experiment2_temp$Value)
```



```r
#all models
humidity_2_full = lm(Value~Sensor, data = experiment2_humidity)

summary(humidity_2_full)
```

```
##
## Call:
## lm(formula = Value ~ Sensor, data = experiment2_humidity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -28.4181   0.3817   0.3817   3.6819   3.6819
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  99.6183     0.1644  605.86   <2e-16 ***
## SensorWRH2   -3.3001     0.2325  -14.19   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.853 on 1740 degrees of freedom
## Multiple R-squared:  0.1037, Adjusted R-squared:  0.1032
## F-statistic: 201.4 on 1 and 1740 DF,  p-value: < 2.2e-16
```

```
Anova(humidity_2_full)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##           Sum Sq   Df F value    Pr(>F)
## Sensor      4743    1  201.42 < 2.2e-16 ***
## Residuals  40973 1740
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#block effect?
humidity_2_full_block = lm(Value~Sensor*block, data = experiment2_humidity)
summary(humidity_2_full_block)
```

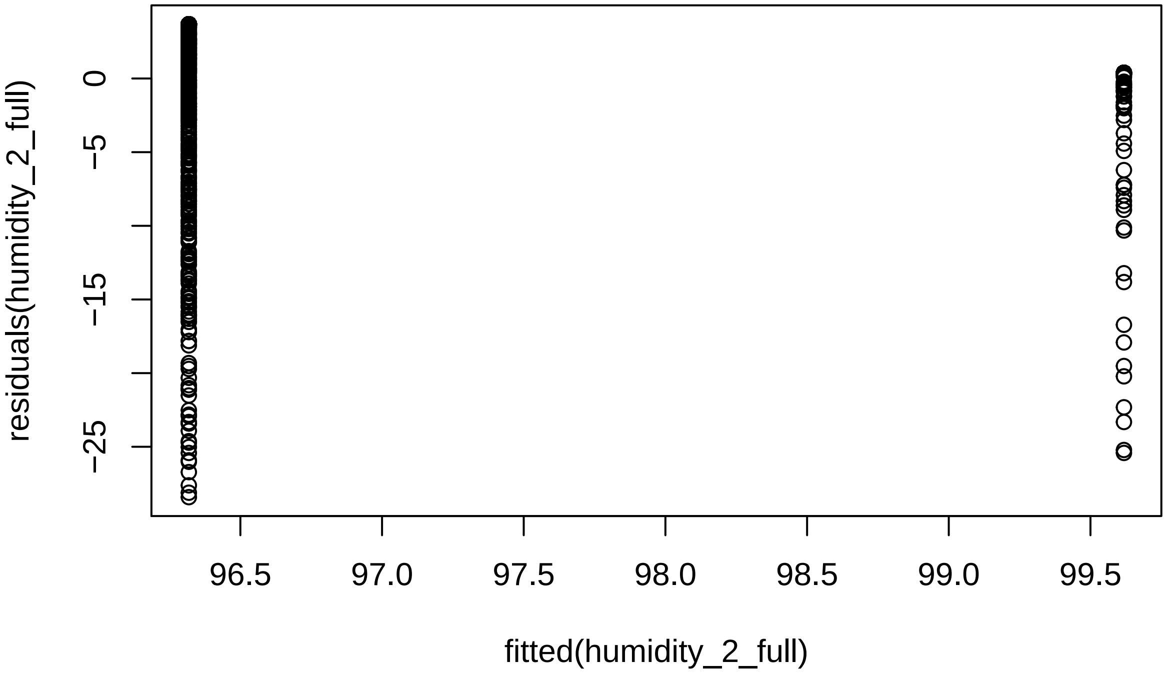
```
##
## Call:
## lm(formula = Value ~ Sensor * block, data = experiment2_humidity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -27.6249   0.0408   0.7964   3.0297   4.4751
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        98.4479     0.5341 184.340  < 2e-16 ***
## SensorWRH2         -4.3683     0.7553  -5.784 8.64e-09 ***
## block               0.7556     0.3283   2.302   0.0215 *
## SensorWRH2:block    0.6897     0.4643   1.486   0.1376
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.821 on 1738 degrees of freedom
## Multiple R-squared:  0.1163, Adjusted R-squared:  0.1148
## F-statistic: 76.24 on 3 and 1738 DF,  p-value: < 2.2e-16
```

```
Anova(humidity_2_full_block)
```

```
## Anova Table (Type II tests)
##
## Response: Value
##              Sum Sq   Df  F value    Pr(>F)
## Sensor         4743    1 204.0440 < 2.2e-16 ***
## block           522    1  22.4745 2.303e-06 ***
## Sensor:block     51    1   2.2069    0.1376
## Residuals     40399 1738
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#Anova Table (Type II tests)
```

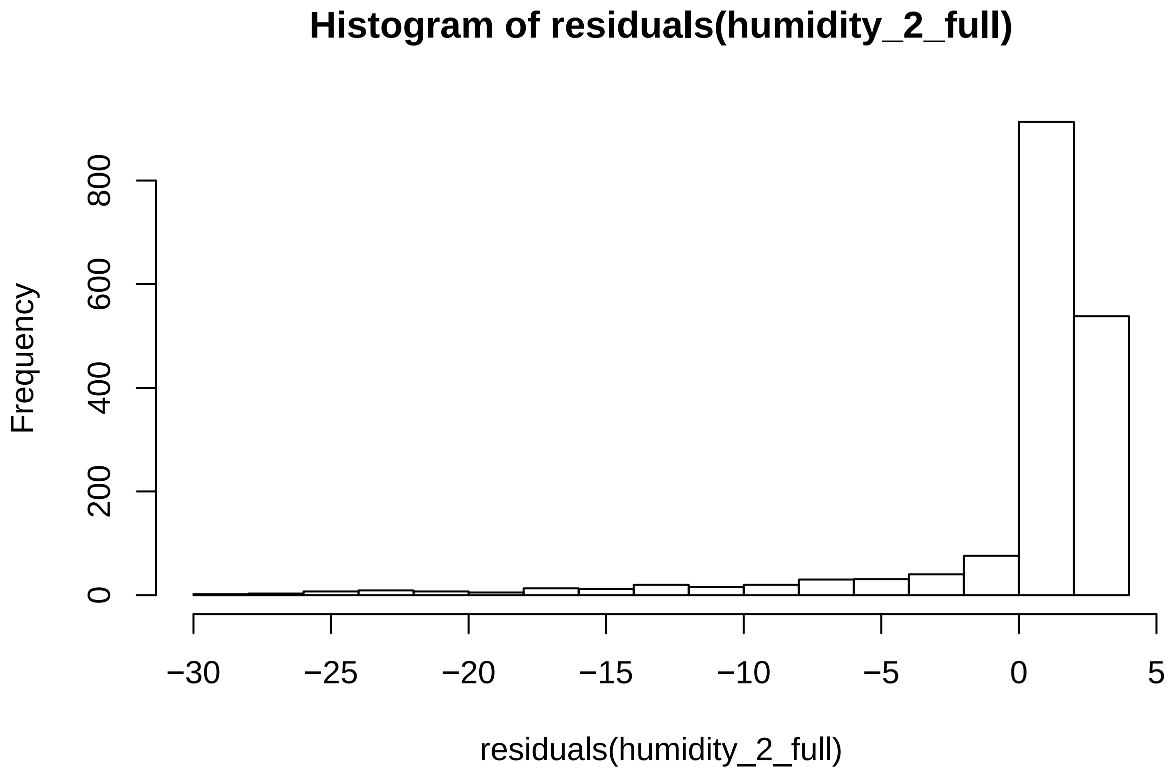
```
#Response: Value
#              Sum Sq   Df  F value    Pr(>F)
#Sensor          4743     1 204.0440 < 2.2e-16 ***
#block            522     1  22.4745 2.303e-06 ***
#Sensor:block      51     1   2.2069    0.1376
#Residuals      40399 1738

plot(fitted(humidity_2_full), residuals(humidity_2_full))
```



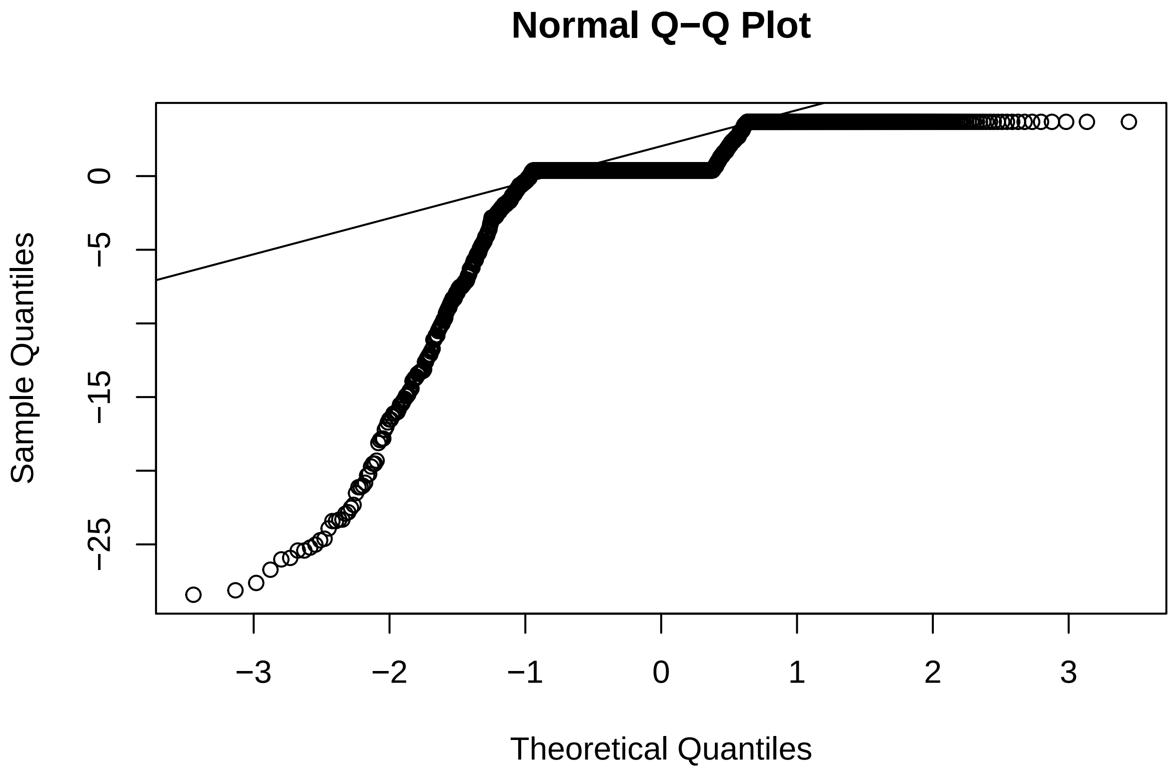
```
leveneTest(data = experiment2_humidity, Value~Sensor)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##         Df F value    Pr(>F)
## group    1  201.42 < 2.2e-16 ***
##       1740
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(humidity_2_full))
```



```
qqnorm(residuals(humidity_2_full))
qqline(residuals(humidity_2_full))
```



```
shapiro.test(residuals(humidity_2_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(humidity_2_full)
## W = 0.61067, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(humidity_2_full) W = 0.61067, p-value < 2.2e-16

Anova Table (Type II tests)

Response: Value Sum Sq Df F value Pr(>F)
Sensor 4743 1 201.42 < 2.2e-16 *** Residuals 40973 1740

Nonparametric stats:

```
wilcox.test(data=experiment2_humidity, Value~Sensor)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Value by Sensor
## W = 539527, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

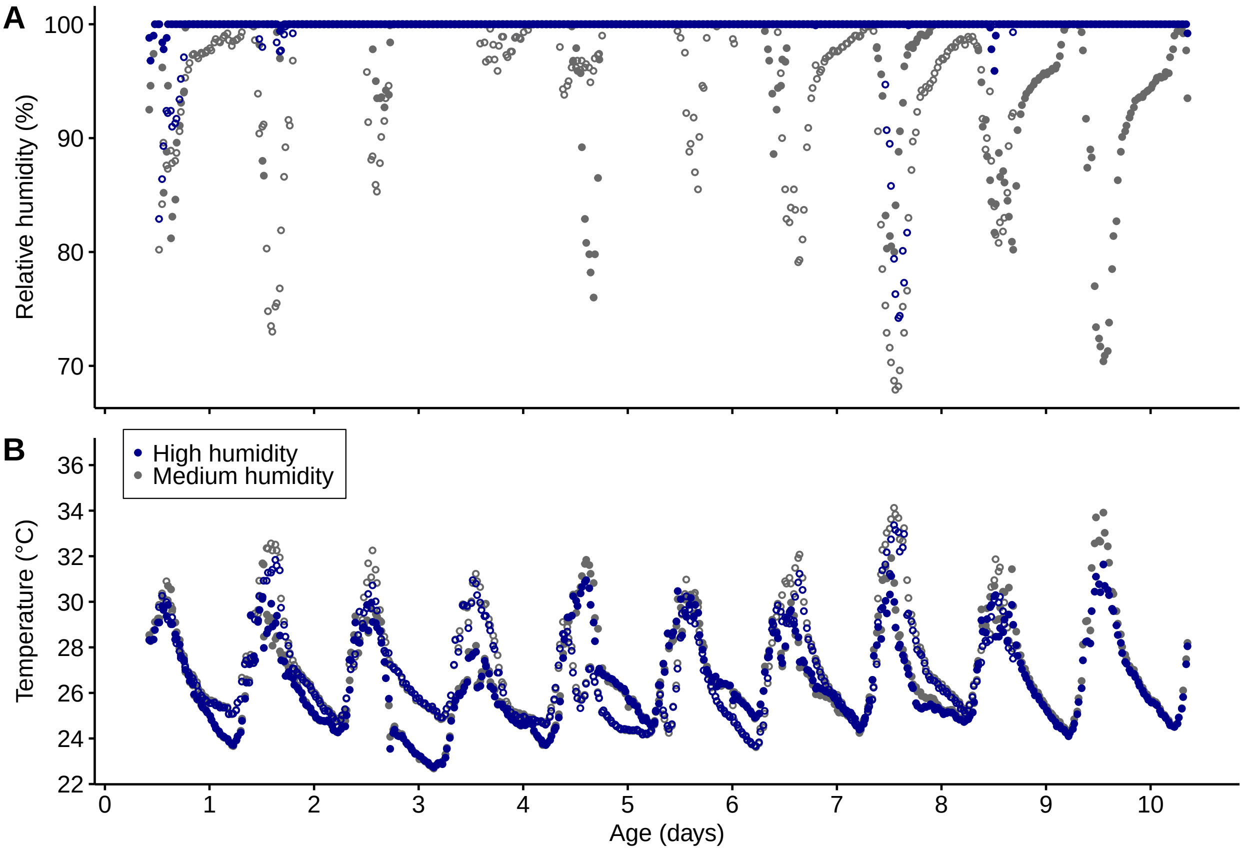
EFFECT OF TREATMENT

- **W = 539527**
- **p-value < 2.2e-16**

Interpretation: Average relative humidity (across blocks) differed significantly among humid and dry treatments.

Combined Figure 3

```
library(gridExtra)
library(cowplot)

# 1 column is 3.43 inches wide

# 4.43 x 3.43 landscape

figure3 = plot_grid(humidity_2, temperature_2, labels = "AUTO", label_x = -.01, label_y = 1, scale =1,
figure3
```



Import experimental data

```r
library(readxl)
bd = read_excel("~/Desktop/Publications/Manuscripts/2020 Dehydration induced hatching As/Dehydration Dat
    col_types = c("numeric", "numeric", "numeric",
        "numeric", "numeric", "text", "text",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "text", "numeric", "numeric",
        "numeric", "numeric", "numeric",
        "numeric", "numeric", "text"), na = "NA")
bd
```

```
## # A tibble: 2,568 x 29
##      date   age  time age_t clutch block treatment n_eggs n_eggs_excludin~
##     <dbl> <dbl> <dbl> <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>
##  1 2.02e7     0    18  0.75     53 <NA>  High hum~     25               25
##  2 2.02e7     1     0  1        53 <NA>  High hum~     25               25
##  3 2.02e7     1     6  1.25     53 <NA>  High hum~     25               25
##  4 2.02e7     1    12  1.5      53 <NA>  High hum~     25               25
##  5 2.02e7     1    18  1.75     53 <NA>  High hum~     25               25
##  6 2.02e7     2     0  2        53 <NA>  High hum~     25               25
##  7 2.02e7     2     6  2.25     53 <NA>  High hum~     25               25
##  8 2.02e7     2    12  2.5      53 <NA>  High hum~     25               25
##  9 2.02e7     2    18  2.75     53 <NA>  High hum~     25               25
## 10 2.02e7     3     0  3        53 <NA>  High hum~     25               25
## # ... with 2,558 more rows, and 20 more variables: `unhatched_including
## #   undev_including_dead_` <dbl>, `unhatched_excluding undev_ncluding
## #   dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, clutch_pic <dbl>, stage_from_clutch_pic <chr>,
## #   clutch_length <dbl>, clutch_width <dbl>, average_eggsize <dbl>,
## #   average_egg_size <dbl>, tad_pic <dbl>, average_tadlength <dbl>,
## #   average_tad_length <dbl>, notes <chr>
```

Remove all of clutch 119 (all undeveloped) and select important colums

```r
library(dplyr)
bd= bd %>%
  filter(clutch != 119) %>%
  dplyr::select(age_t, clutch, block, treatment, n_eggs:clutch_thickness, stage_from_clutch_pic:average_
  arrange(clutch)
bd
```

```
## # A tibble: 2,492 x 23
##    age_t clutch block treatment n_eggs n_eggs_excludin~ `unhatched_incl~
##    <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>            <dbl>
##  1  0.75     53 <NA>  High hum~     25               25               25
##  2  1        53 <NA>  High hum~     25               25               25
##  3  1.25     53 <NA>  High hum~     25               25               25
##  4  1.5      53 <NA>  High hum~     25               25               25
##  5  1.75     53 <NA>  High hum~     25               25               25
##  6  2        53 <NA>  High hum~     25               25               25
##  7  2.25     53 <NA>  High hum~     25               25               25
##  8  2.5      53 <NA>  High hum~     25               25               25
##  9  2.75     53 <NA>  High hum~     25               25               25
## 10  3        53 <NA>  High hum~     25               25               25
## # ... with 2,482 more rows, and 16 more variables: `unhatched_excluding
## #   undev_ncluding dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, stage_from_clutch_pic <chr>, clutch_length <dbl>,
## #   clutch_width <dbl>, average_eggsize <dbl>, average_egg_size <dbl>,
## #   average_tadlength <dbl>, average_tad_length <dbl>
```

Separate experiments

- Clutches 53-76 had both *high* and *low* treatments (12 total)
- Clutches 104-127 had both *high* and *medium* treatments (23 total (C119 no good))

Figure 4

Experiment 1

Subset data

```
verydry = bd %>%
  filter(clutch < 100)
verydry
```

```
## # A tibble: 840 x 23
##    age_t clutch block treatment n_eggs n_eggs_excludin~ `unhatched_incl~
##    <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>            <dbl>
##  1  0.75     53 <NA>  High hum~     25               25               25
##  2  1        53 <NA>  High hum~     25               25               25
##  3  1.25     53 <NA>  High hum~     25               25               25
##  4  1.5      53 <NA>  High hum~     25               25               25
##  5  1.75     53 <NA>  High hum~     25               25               25
##  6  2        53 <NA>  High hum~     25               25               25
##  7  2.25     53 <NA>  High hum~     25               25               25
##  8  2.5      53 <NA>  High hum~     25               25               25
##  9  2.75     53 <NA>  High hum~     25               25               25
## 10  3        53 <NA>  High hum~     25               25               25
## # ... with 830 more rows, and 16 more variables: `unhatched_excluding
```

```
## #   undev_ncluding dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, stage_from_clutch_pic <chr>, clutch_length <dbl>,
## #   clutch_width <dbl>, average_eggsize <dbl>, average_egg_size <dbl>,
## #   average_tadlength <dbl>, average_tad_length <dbl>
```

Experiment I – non-split clutch design

> note that WH1 = high treatment WH2 = low treatment

Experiment II

*1st block – split clutch design

> WH1 = high treatment WH2 = mediium treatment

- 2nd block – split clutch design

> WH1 = high treatment WH2 = medium treatment

Clutch thickness

> We began measurements at 2d 18:00 h. Thickness was not measured at 4d 18:00 h and was only measured in low humidity on 5d due to sensitivity of high humidity clutches to hatching; Eggs in low humidity clutches were mostly dead and therefore could be handled.

Summary statistics

```
thicknesssumm_1 = verydry %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(clutch_thickness, na.rm=T), SE= sd(clutch_thickness, na.rm=T)/sqrt(length(clutc
  na.omit() %>%
  mutate(meant = lag(mean, n=1)- mean)
thicknesssumm_1
```

```
## # A tibble: 6 x 9
## # Groups:   treatment [2]
##   treatment     age_t  mean    SE    SD   min   max sample_size  meant
##   <chr>         <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>       <int>  <dbl>
## 1 High humidity  0.75  5.5  0.337 1.17      4   7            12 NA
## 2 High humidity  2.75  7.29 0.538 1.86      4  10            12 -1.79
## 3 High humidity  3.75  7.62 0.619 2.14      4  11            12 -0.333
## 4 Low humidity   0.75  5.33 0.225 0.778     4   6            12 NA
## 5 Low humidity   2.75  3.33 0.339 1.17      2   6            12  2.00
## 6 Low humidity   3.75  2.17 0.316 1.09      1   4.5          12  1.17
```

High humidity:

- 1.7916667 + 0.3333333 / 2 == *mean 1.0625 increase per age*

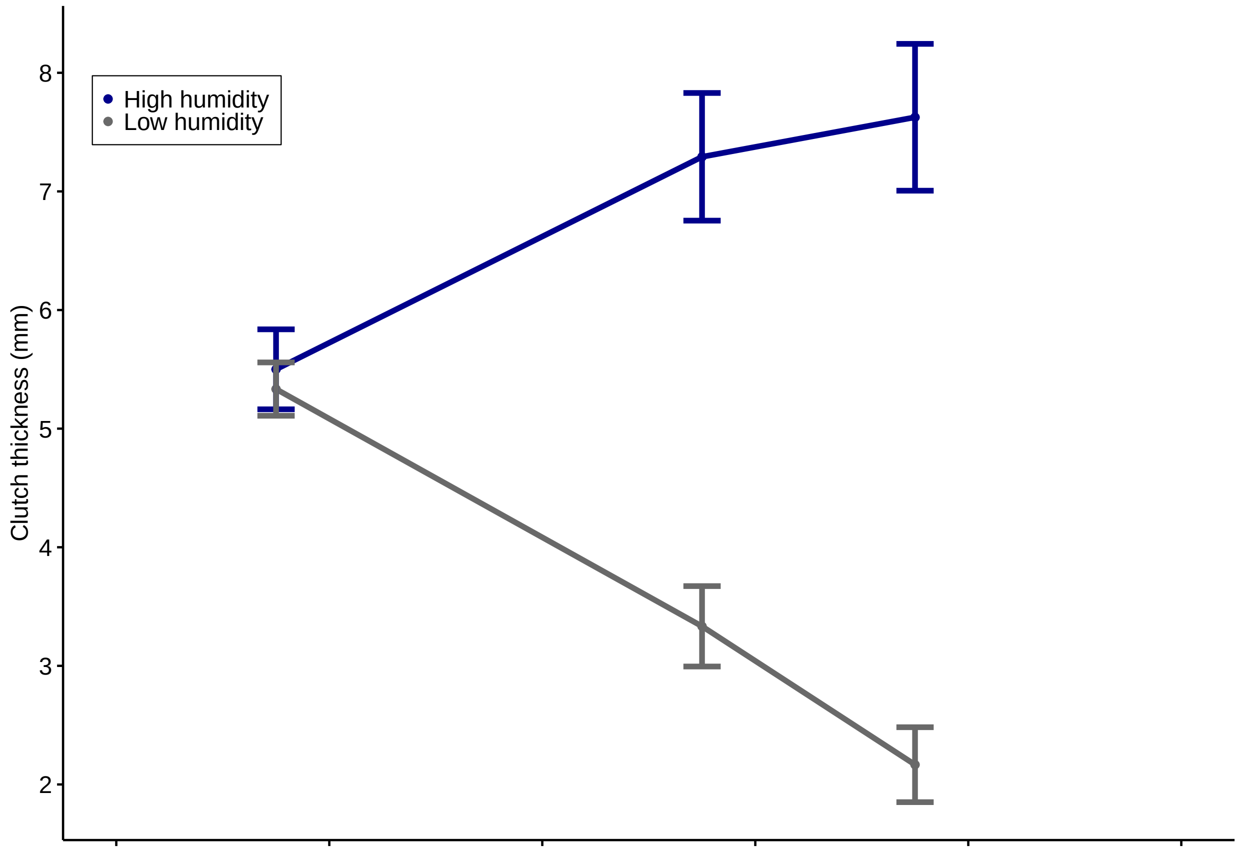
- 1-(7.625000/5.500000) = 0.3863636 increase == ~**38.6% percent increase total**

Low humidity:

- 2.0000000 + 1.1666667 / 2 == *mean 1.583333 decrease per age*
- 1- (2.166667/5.333333) = 0.5937499 decrease == ~**59.4% percent decrease total**

Figure

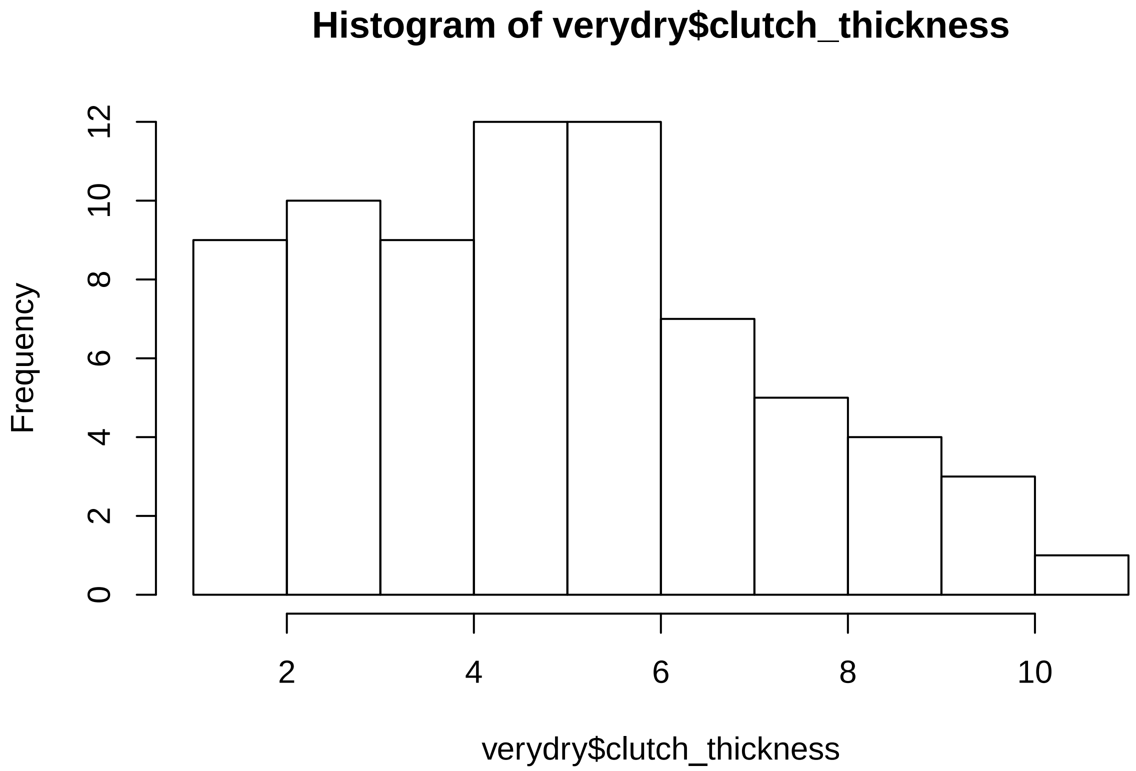
```
library(ggplot2)
library(scales)

legend_title = "Hydration treatment"

clutch_thickness_1 = ggplot(thicknesssumm_1, aes(x=age_t, y=mean)) +
  geom_line(aes(color = treatment), size = 1) +
  geom_point(aes(color=treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Clutch thickness (mm)", x="Age (days)") +
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black")) +
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color =
  scale_y_continuous(breaks= pretty_breaks(n=6)) +
  scale_x_continuous(limits=c(0,5), breaks= pretty_breaks(n=5)) +
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray"))
clutch_thickness_1
```



STATS

LMM

```
library(lme4)
library(car)
library(lmerTest)

hist(verydry$clutch_thickness)
```



```
#all models
clutch_thickness_full = lmer(clutch_thickness~age_t*treatment +  (1|clutch), data = verydry)
clutch_thickness_nointeraction = lmer(clutch_thickness~age_t+treatment +  (1|clutch),  data = verydry)
clutch_thickness_notreatment = lmer(clutch_thickness~age_t +  (1|clutch),  data = verydry)
clutch_thickness_noage = lmer(clutch_thickness~treatment +  (1|clutch), data = verydry)

#AIC comparison
library(AICcmodavg)
print(aictab(list(clutch_thickness_full,  clutch_thickness_nointeraction, clutch_thickness_notreatment,
```

```
##
## Model selection based on AICc:
##
##                                K   AICc Delta_AICc AICcWt Cum.Wt
## clutch_thickness_full          6 233.88       0.00      1      1
## clutch_thickness_noage         4 294.00      60.12      0      1
## clutch_thickness_nointeraction 5 297.19      63.31      0      1
## clutch_thickness_notreatment   4 317.71      83.83      0      1
```

```
summary(clutch_thickness_full)
```

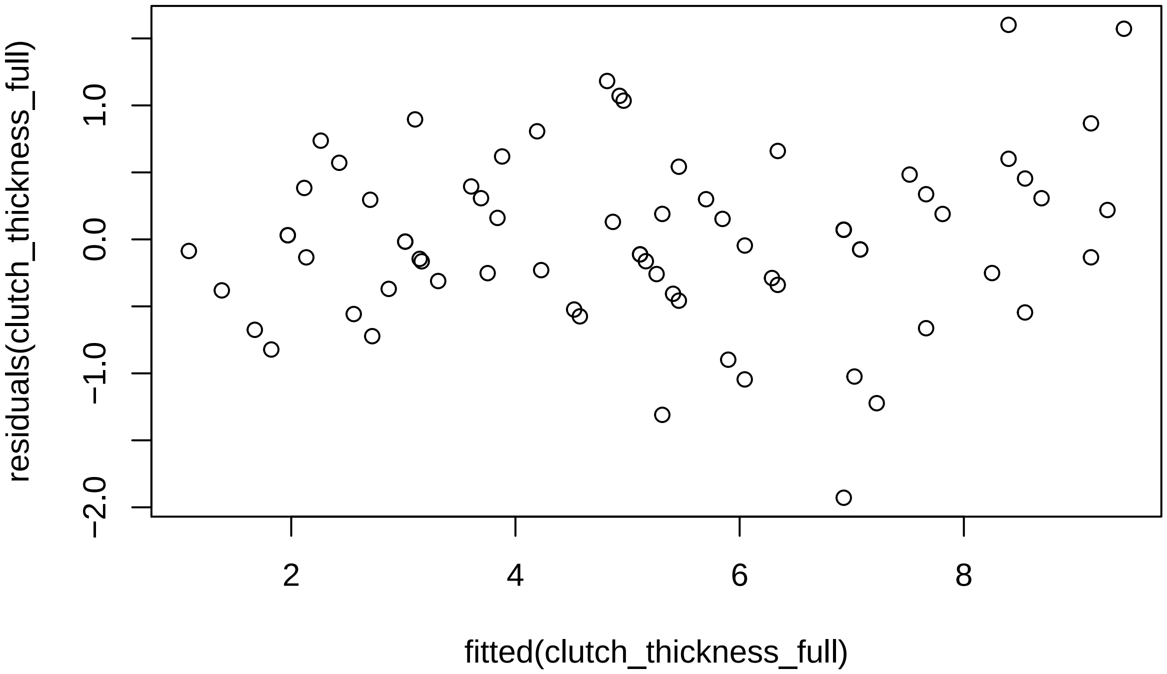
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: clutch_thickness ~ age_t * treatment + (1 | clutch)
##    Data: verydry
```

```
##
## REML criterion at convergence: 220.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.48931 -0.44816 -0.04058  0.44993  2.06671
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clutch   (Intercept) 1.5024   1.2257
##  Residual             0.6002   0.7747
## Number of obs: 72, groups:  clutch, 24
##
## Fixed effects:
##                              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)                    5.0290     0.4522 41.8051  11.122 4.55e-14 ***
## age_t                          0.7351     0.1035 46.0000   7.101 6.44e-09 ***
## treatmentLow humidity          1.1138     0.6395 41.8051   1.742   0.0889 .
## age_t:treatmentLow humidity  -1.7827     0.1464 46.0000 -12.176 5.43e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) age_t  trtmLh
## age_t       -0.553
## trtmntLwhmd -0.707  0.391
## ag_t:trtmLh  0.391 -0.707 -0.553
```

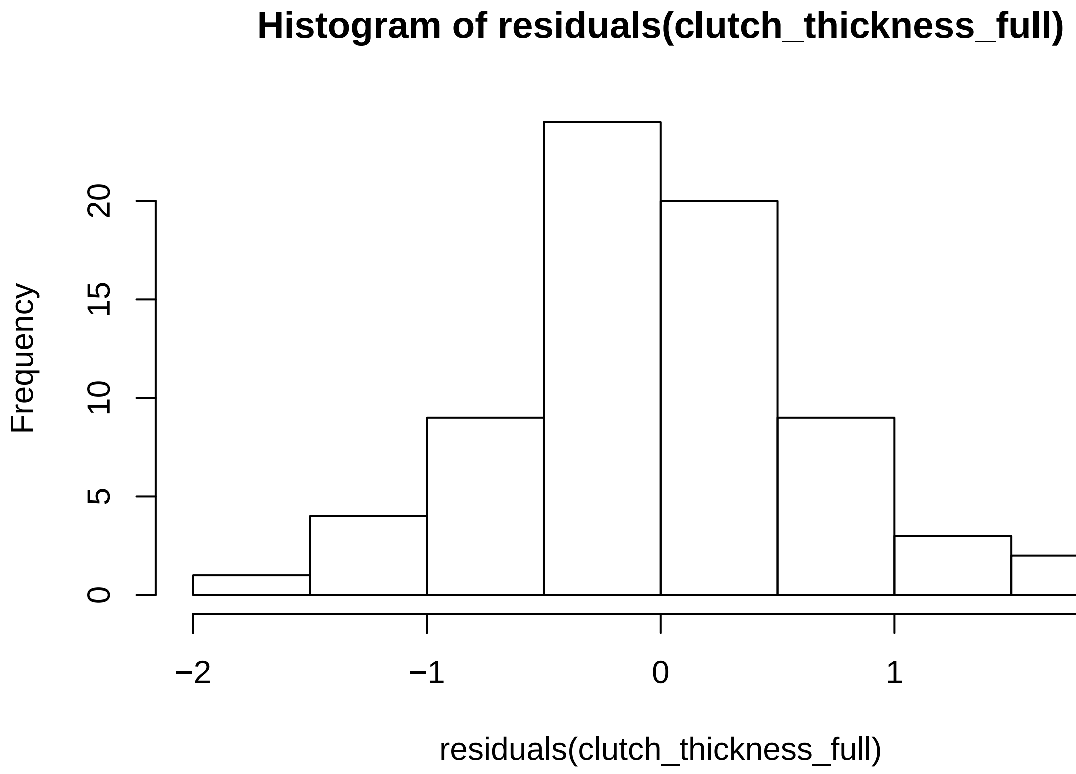
```r
Anova(clutch_thickness_full)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: clutch_thickness
##                    Chisq Df Pr(>Chisq)
## age_t             4.5555  1    0.03281 *
## treatment        35.9642  1   2.01e-09 ***
## age_t:treatment 148.2569  1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

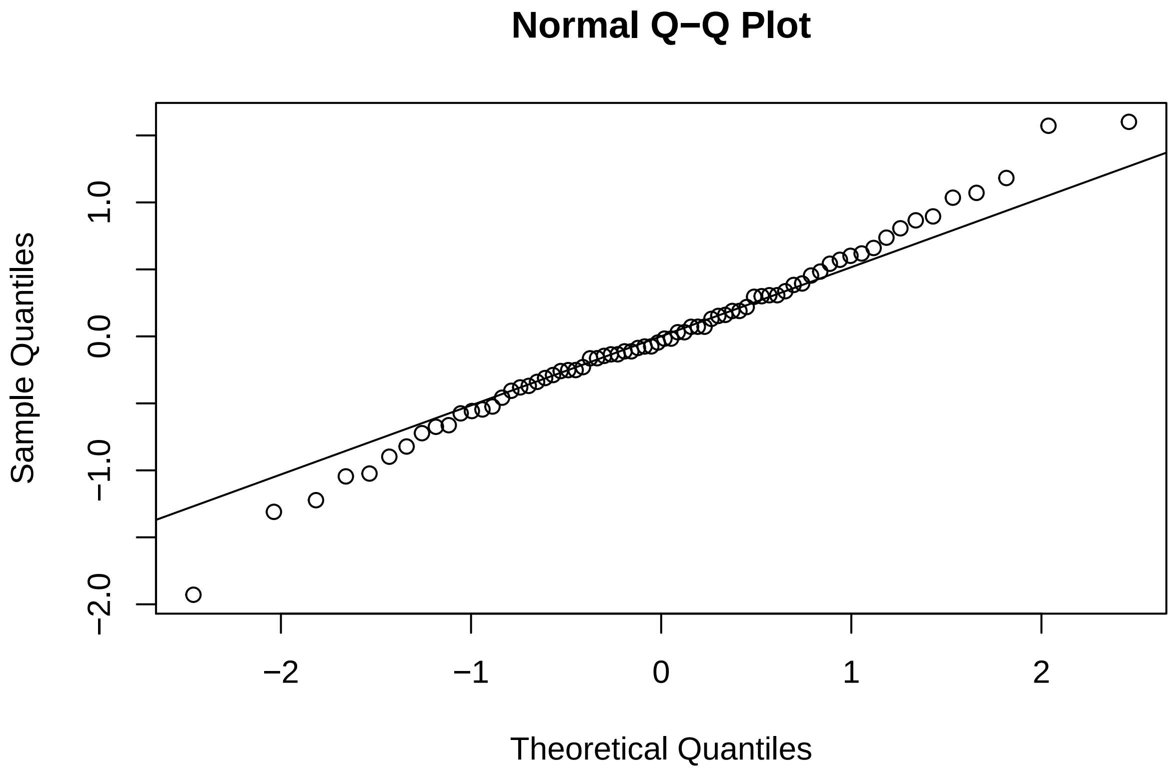
```r
plot(fitted(clutch_thickness_full), residuals(clutch_thickness_full))
```



```r
hist(residuals(clutch_thickness_full))
```



```
qqnorm(residuals(clutch_thickness_full))
qqline(residuals(clutch_thickness_full))
```



```r
shapiro.test(residuals(clutch_thickness_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(clutch_thickness_full)
## W = 0.98831, p-value = 0.7476
```

Shapiro-Wilk normality test

data: residuals(clutch_thickness_full) W = 0.98831, p-value = 0.7476

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: clutch_thickness Chisq Df Pr(>Chisq)
age_t 4.5555 1 0.03281 *
treatment 35.9641 1 2.01e-09 ***age_t:treatment 148.2569 1 < 2.2e-16***

Likelihood ratio tests

```r
anova(clutch_thickness_full, clutch_thickness_nointeraction, test = "Chisq")
```

```
## Data: verydry
## Models:
## clutch_thickness_nointeraction: clutch_thickness ~ age_t + treatment + (1 | clutch)
## clutch_thickness_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
```

```
##                                 npar    AIC    BIC  logLik deviance  Chisq Df
## clutch_thickness_nointeraction     5 293.98 305.36 -141.99   283.98
## clutch_thickness_full              6 226.83 240.49 -107.42   214.83 69.146  1
##                                 Pr(>Chisq)
## clutch_thickness_nointeraction
## clutch_thickness_full            < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(clutch_thickness_full, clutch_thickness_notreatment, test = "Chisq")
```

```
## Data: verydry
## Models:
## clutch_thickness_notreatment: clutch_thickness ~ age_t + (1 | clutch)
## clutch_thickness_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
##                               npar    AIC    BIC  logLik deviance  Chisq Df
## clutch_thickness_notreatment     4 315.23 324.33 -153.61   307.23
## clutch_thickness_full            6 226.83 240.49 -107.42   214.83 92.397  2
##                               Pr(>Chisq)
## clutch_thickness_notreatment
## clutch_thickness_full          < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(clutch_thickness_full, clutch_thickness_noage, test = "Chisq")
```

```
## Data: verydry
## Models:
## clutch_thickness_noage: clutch_thickness ~ treatment + (1 | clutch)
## clutch_thickness_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
##                        npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## clutch_thickness_noage    4 293.09 302.20 -142.54   285.09
## clutch_thickness_full     6 226.83 240.49 -107.42   214.83 70.259  2   5.54e-16
##
## clutch_thickness_noage
## clutch_thickness_full  ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X = 69.146; df = 1**
- **p-value < 2.2e-16**

EFFECT OF TREATMENT:

- **X = 92.397; df = 2**
- **p-value < 2.2e-16**

EFFECT OF AGE:

- **X = 70.259; df = 2**
- **p-value = 5.54e-16**

Egg size

> We did not take photos/measurements at 5d 18:00 h for high treatment due to sensitivity of clutches to hatching.

Summary statistics

```
eggsizesum_1 = verydry %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(average_egg_size, na.rm=T), SE= sd(average_egg_size, na.rm=T)/sqrt(length(avera
  na.omit() %>%
  mutate(meant = lag(mean, n=1)- mean)
eggsizesum_1
```

```
## # A tibble: 10 x 9
## # Groups:   treatment [2]
##    treatment     age_t  mean     SE    SD   min   max sample_size   meant
##    <chr>         <dbl> <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>   <dbl>
##  1 High humidity  0.75  3.68 0.0448 0.155  3.42  3.84          12 NA
##  2 High humidity  1.75  4.15 0.0601 0.208  3.81  4.42          12 -0.469
##  3 High humidity  2.75  4.26 0.0644 0.223  3.82  4.59          12 -0.109
##  4 High humidity  3.75  4.28 0.0716 0.248  3.71  4.57          12 -0.0192
##  5 High humidity  4.75  4.41 0.118  0.408  3.69  5.24          12 -0.138
##  6 Low humidity   0.75  3.71 0.0614 0.213  3.38  4.16          12 NA
##  7 Low humidity   1.75  3.65 0.0820 0.284  3.31  4.25          12  0.0633
##  8 Low humidity   2.75  3.21 0.0779 0.270  2.88  3.76          12  0.438
##  9 Low humidity   3.75  2.98 0.0601 0.208  2.6   3.36          12  0.231
## 10 Low humidity   4.75  2.94 0.0420 0.146  2.68  3.15          12  0.0442
```

High humidity:

- 0.46916667 + 0.10916667 + 0.01916667 + 0.13750000/ 4 == *mean 0.18375 increase per age*
- 1-(4.413333/3.678333) = 0.1998188 increase == ~**20% percent increase total**

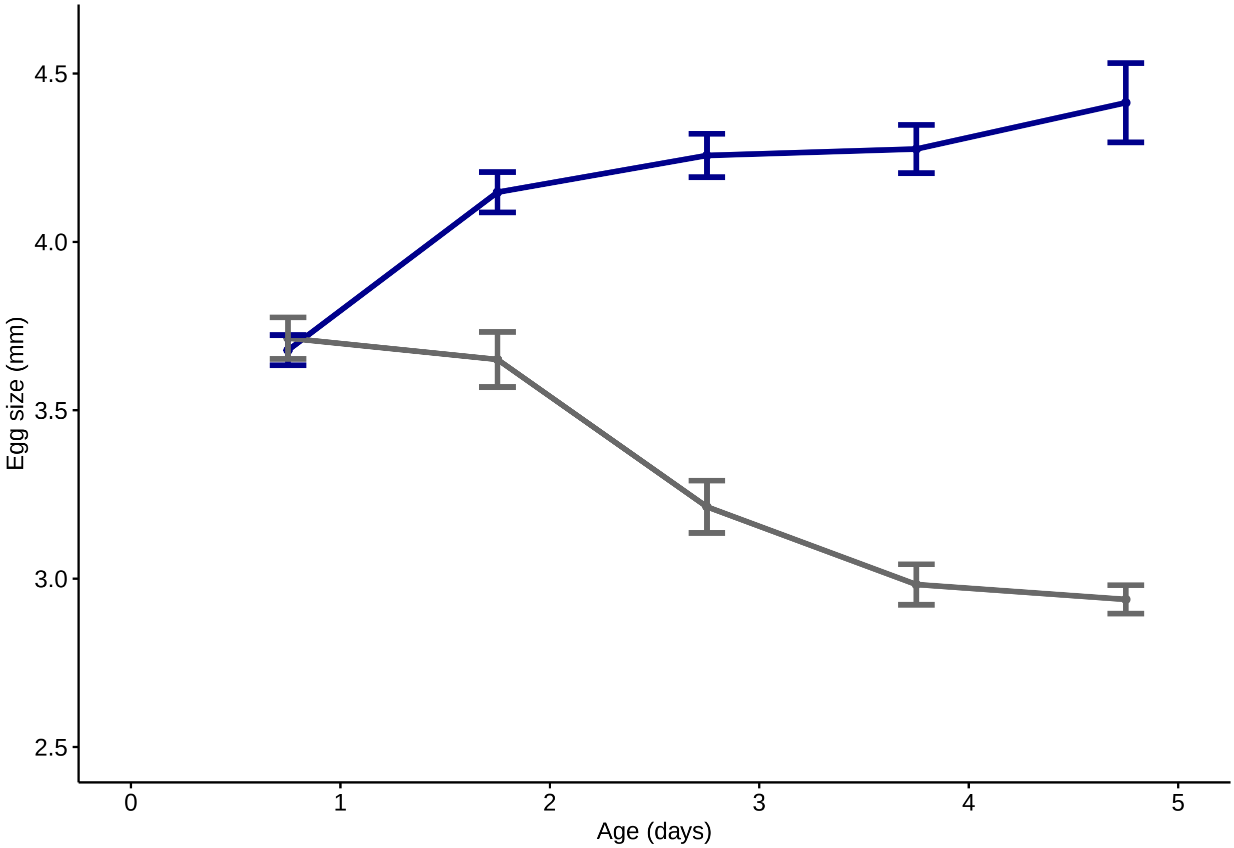
Medium humidity:

- 0.06333333 + 0.43750000 + 0.23083333 + 0.04416667/ 4 == *mean 0.1939583 decrease per age*
- 1- (2.938333/3.714167) = 0.2088851 decrease == ~**20.8% percent decrease total**

Figure

```
library(ggplot2)
library(scales)

egg_size_1 = ggplot(eggsizesum_1, aes(x=age_t, y=mean)) + #add the axis
  geom_line(aes(color = treatment), size = 1) +
  geom_point(aes(color=treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Egg size (mm)", x="Age (days)") +
  theme_classic(base_size = 9) +
  theme(legend.position = "none", legend.text = element_text(size = 9), legend.title = element_blank(),
```

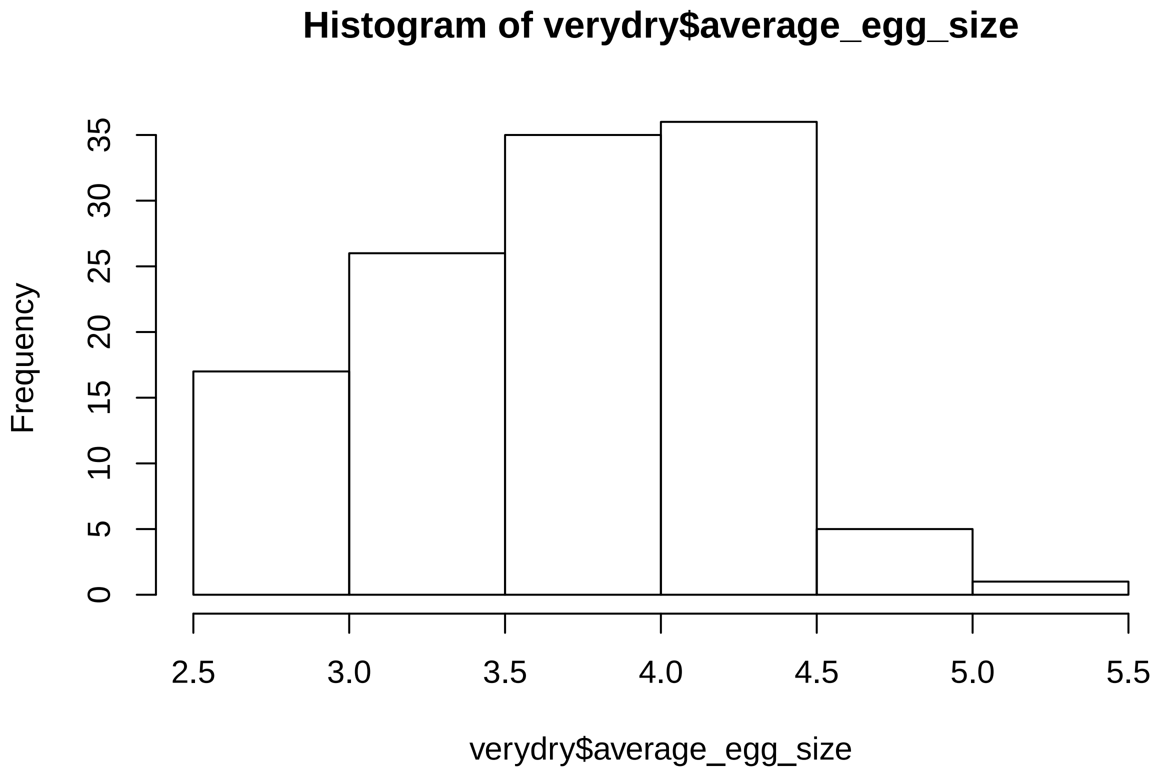
```
  #theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  scale_x_continuous(limits=c(0,5), breaks= pretty_breaks(n=5)) +
  #theme(plot.margin = unit(c(.5, .5, .1, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(limits=c(2.5,4.6), breaks=pretty_breaks(n=5)) + # allows for making "pretty" break
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray"))
egg_size_1
```



STATS

LMM

```
library(lme4)
library(car)
library(lmerTest)

hist(verydry$average_egg_size)
```



```r
#all models
egg_size_full = lmer(average_egg_size~age_t*treatment +  (1|clutch), data = verydry)
egg_size_nointeraction = lmer(average_egg_size~age_t+treatment +  (1|clutch),  data = verydry)
egg_size_notreatment = lmer(average_egg_size~age_t +  (1|clutch),  data = verydry)
egg_size_noage = lmer(average_egg_size~treatment +  (1|clutch), data = verydry)

#AIC comparison
library(AICcmodavg)
print(aictab(list(egg_size_full,  egg_size_nointeraction, egg_size_notreatment, egg_size_noage), c("egg
```

```
##
## Model selection based on AICc:
##
##                        K   AICc Delta_AICc AICcWt Cum.Wt
## egg_size_full          6   8.39       0.00      1      1
## egg_size_noage         4 119.47     111.08      0      1
## egg_size_nointeraction 5 125.53     117.14      0      1
## egg_size_notreatment   4 159.96     151.57      0      1
```

```r
summary(egg_size_full)
```

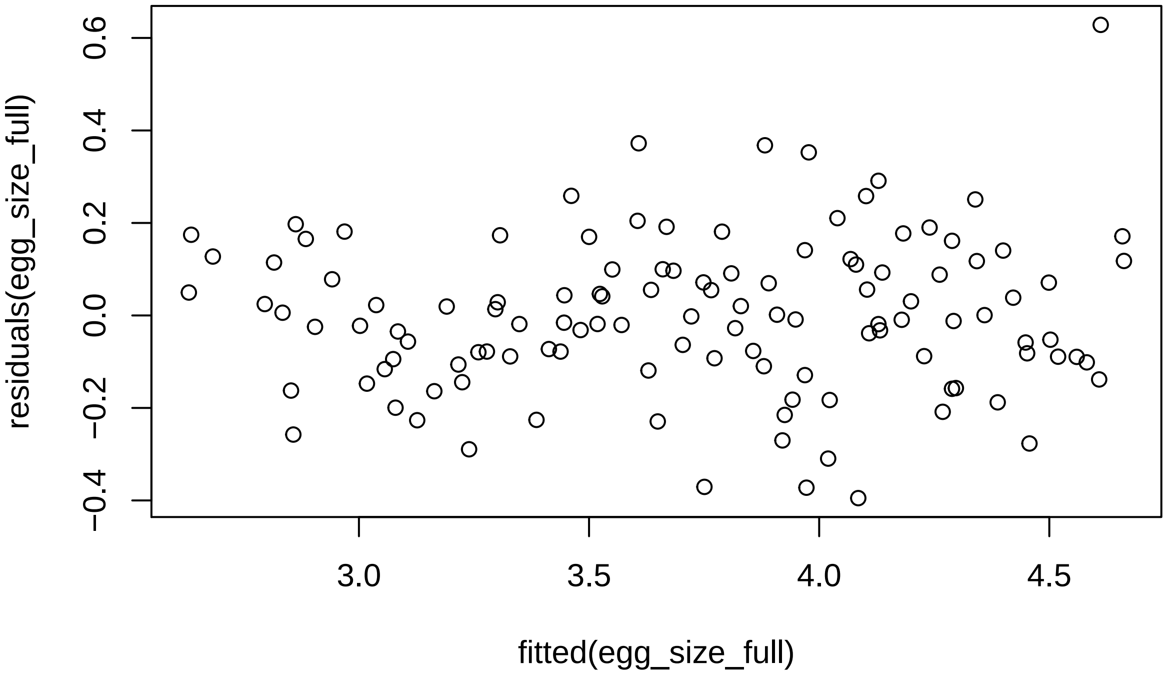
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: average_egg_size ~ age_t * treatment + (1 | clutch)
##    Data: verydry
```

```
##
## REML criterion at convergence: -4.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.1382 -0.5208 -0.0489  0.5543  3.4013
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clutch   (Intercept) 0.03622  0.1903
##  Residual             0.03412  0.1847
## Number of obs: 120, groups:  clutch, 24
##
## Fixed effects:
##                             Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)                  3.71479    0.07575 51.91332  49.042  < 2e-16 ***
## age_t                        0.15983    0.01686 94.00000   9.479 2.36e-15 ***
## treatmentLow humidity        0.19554    0.10712 51.91332   1.825   0.0737 .
## age_t:treatmentLow humidity -0.38183    0.02385 94.00000 -16.012  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) age_t  trtmLh
## age_t       -0.612
## trtmntLwhmd -0.707  0.433
## ag_t:trtmLh  0.433 -0.707 -0.612
```

```
Anova(egg_size_full)
```

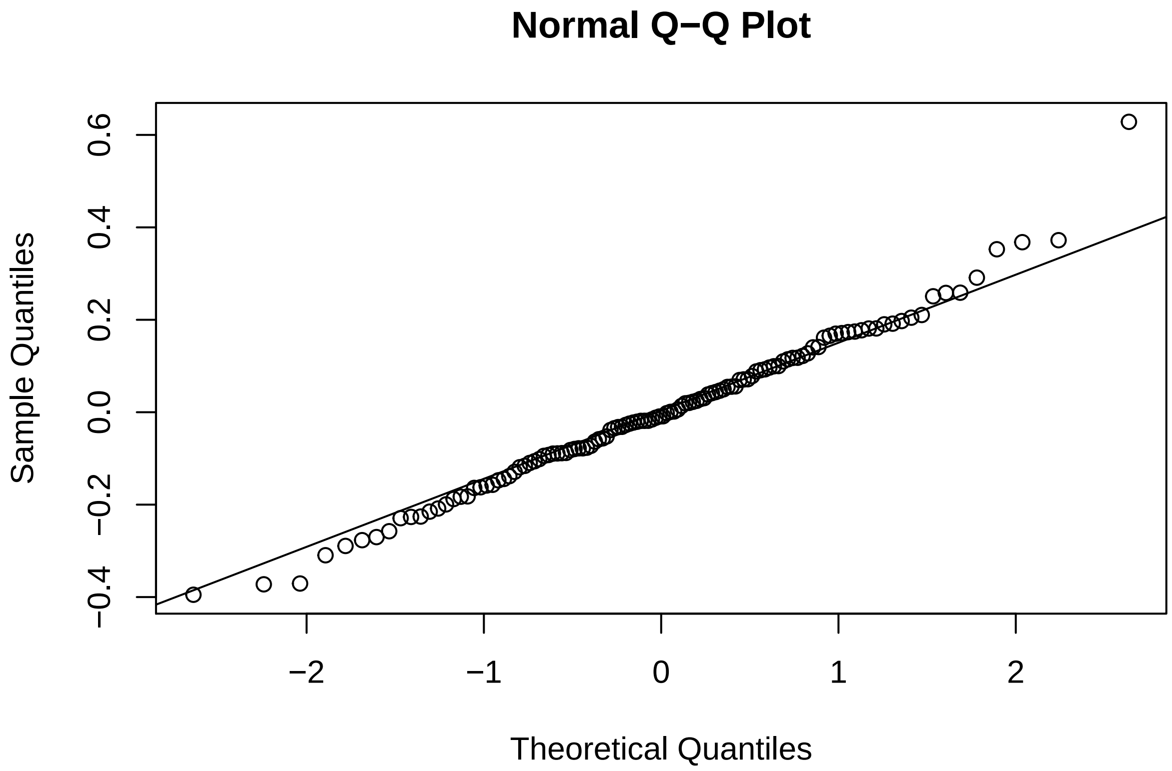
```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: average_egg_size
##                    Chisq Df Pr(>Chisq)
## age_t             6.7959  1   0.009137 **
## treatment       101.7695  1  < 2.2e-16 ***
## age_t:treatment 256.3773  1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
plot(fitted(egg_size_full), residuals(egg_size_full))
```



```
hist(residuals(egg_size_full))
```

```r
qqnorm(residuals(egg_size_full))
qqline(residuals(egg_size_full))
```



```r
shapiro.test(residuals(egg_size_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(egg_size_full)
## W = 0.98624, p-value = 0.2646
```

Shapiro-Wilk normality test

data: residuals(egg_size_full) W = 0.98624, p-value = 0.2646

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: average_egg_size Chisq Df Pr(>Chisq)
age_t 6.7959 1 0.009137 ** treatment 101.7695 1 < 2.2e-16 ***age_t:treatment 256.3773 1 < 2.2e-16***

Likelihood ratio tests

```r
anova(egg_size_full, egg_size_nointeraction, test = "Chisq")
```

```
## Data: verydry
## Models:
## egg_size_nointeraction: average_egg_size ~ age_t + treatment + (1 | clutch)
## egg_size_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                        npar     AIC     BIC  logLik deviance  Chisq Df
```

```
## egg_size_nointeraction    5 111.607 125.545 -50.804  101.607
## egg_size_full             6 -12.701   4.024  12.351  -24.701 126.31  1
##                        Pr(>Chisq)
## egg_size_nointeraction
## egg_size_full           < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(egg_size_full, egg_size_notreatment, test = "Chisq")
```

```
## Data: verydry
## Models:
## egg_size_notreatment: average_egg_size ~ age_t + (1 | clutch)
## egg_size_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                      npar     AIC     BIC  logLik deviance  Chisq Df Pr(>Chisq)
## egg_size_notreatment    4 151.064 162.214 -71.532  143.064
## egg_size_full           6 -12.701   4.024  12.351  -24.701 167.77  2  < 2.2e-16
##
## egg_size_notreatment
## egg_size_full        ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(egg_size_full, egg_size_noage, test = "Chisq")
```

```
## Data: verydry
## Models:
## egg_size_noage: average_egg_size ~ treatment + (1 | clutch)
## egg_size_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                npar     AIC     BIC  logLik deviance  Chisq Df Pr(>Chisq)
## egg_size_noage    4 111.452 122.602 -51.726  103.452
## egg_size_full     6 -12.701   4.024  12.351  -24.701 128.15  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X == 126.31; df = 1**
- **p-value < 2.2e-16**

EFFECT OF TREATMENT:

- **X = 167.77; df = 2**
- **p-value < 2.2e-16**

EFFECT OF AGE:

- **X = 128.15; df = 2**
- **p-value < 2.2e-16**

Mortality

Summary statistics

```
#add colum of total hatched and calculate mortality/survivorship
verydry = verydry %>%
    group_by(clutch,age_t,treatment) %>%
  mutate(total_hatched = max(hatched_accumalative), total_timepointhatch = hatched_time_point/total_hat
verydry
```

```
## # A tibble: 840 x 27
## # Groups:   clutch, age_t, treatment [840]
##    age_t clutch block treatment n_eggs n_eggs_excludin~ `unhatched_incl~
##    <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>            <dbl>
##  1  0.75     53 <NA>  High hum~     25               25               25
##  2  1        53 <NA>  High hum~     25               25               25
##  3  1.25     53 <NA>  High hum~     25               25               25
##  4  1.5      53 <NA>  High hum~     25               25               25
##  5  1.75     53 <NA>  High hum~     25               25               25
##  6  2        53 <NA>  High hum~     25               25               25
##  7  2.25     53 <NA>  High hum~     25               25               25
##  8  2.5      53 <NA>  High hum~     25               25               25
##  9  2.75     53 <NA>  High hum~     25               25               25
## 10  3        53 <NA>  High hum~     25               25               25
## # ... with 830 more rows, and 20 more variables: `unhatched_excluding
## #   undev_ncluding dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, stage_from_clutch_pic <chr>, clutch_length <dbl>,
## #   clutch_width <dbl>, average_eggsize <dbl>, average_egg_size <dbl>,
## #   average_tadlength <dbl>, average_tad_length <dbl>, total_hatched <dbl>,
## #   total_timepointhatch <dbl>, totalacummhatch <dbl>, survivorship <dbl>
```

```
survival= verydry %>%
  group_by(clutch,treatment) %>%
  dplyr::select(clutch, treatment, total_hatched, survivorship) %>%
  filter(survivorship == max(survivorship)) %>%
  summarise(meansurvival = mean(survivorship), SE =sd(survivorship)/sqrt(length(survivorship)), sample_
survival
```

```
## # A tibble: 24 x 5
## # Groups:   clutch [24]
##    clutch treatment     meansurvival    SE sample_size
##     <dbl> <chr>                <dbl> <dbl>       <int>
##  1     53 High humidity        1         0          14
##  2     54 High humidity        1         0          14
##  3     55 High humidity        1         0          14
##  4     56 High humidity        1         0          14
##  5     57 High humidity        0.952     0           2
##  6     58 High humidity        1         0          14
##  7     59 High humidity        0.913     0          14
##  8     60 High humidity        1         0          12
```

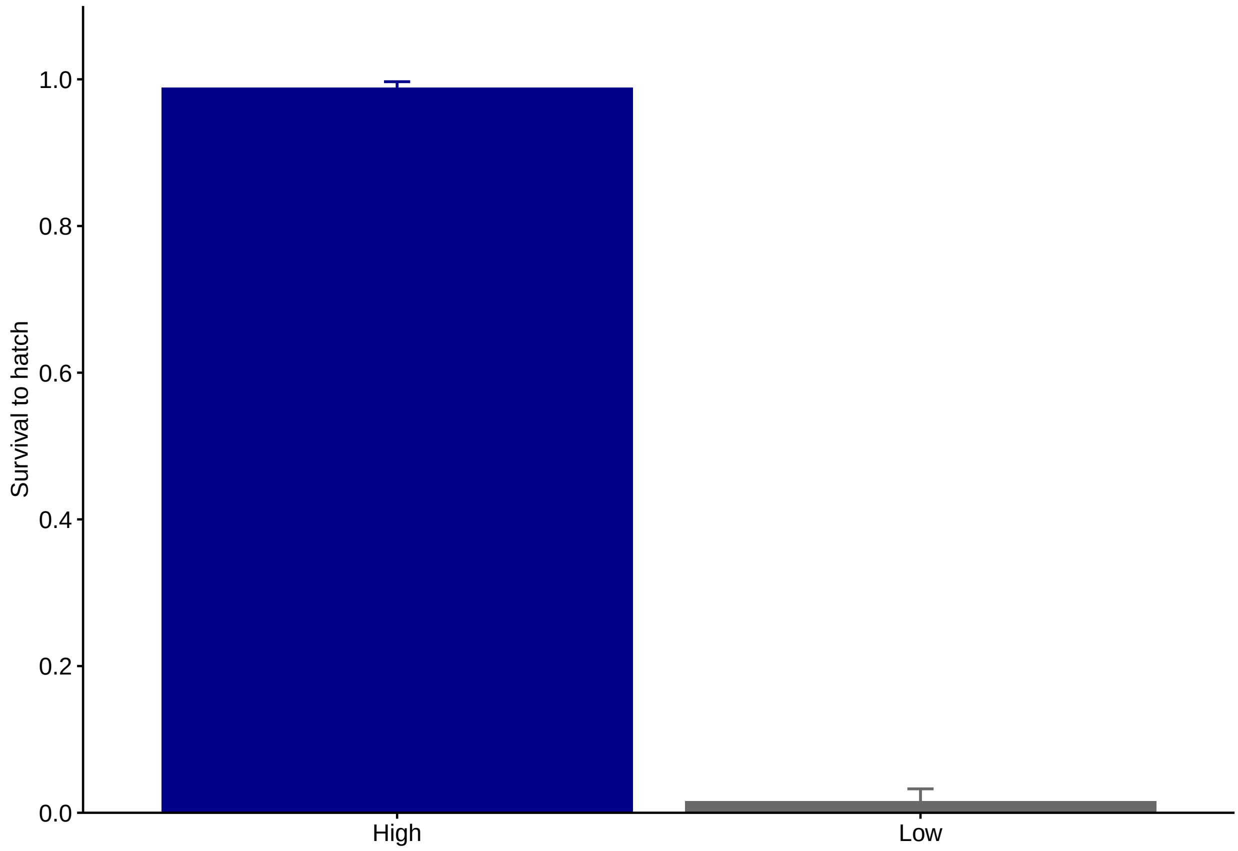
```
##  9     61 High humidity        1         0          14
## 10     62 High humidity        1         0          12
## # ... with 14 more rows
```

```
survivalsumm_1 = survival %>%
  group_by(treatment) %>%
  summarise(mean = mean(meansurvival, na.rm=T), SE= sd(meansurvival, na.rm=T)/sqrt(length(meansurvival)
  na.omit()
survivalsumm_1
```

```
## # A tibble: 2 x 7
##   treatment       mean      SE     SD   min   max sample_size
##   <chr>          <dbl>   <dbl>  <dbl> <dbl> <dbl>       <int>
## 1 High humidity 0.989  0.00794 0.0275 0.913 1              12
## 2 Low humidity  0.0163 0.0163  0.0565 0     0.196          12
```

Figure

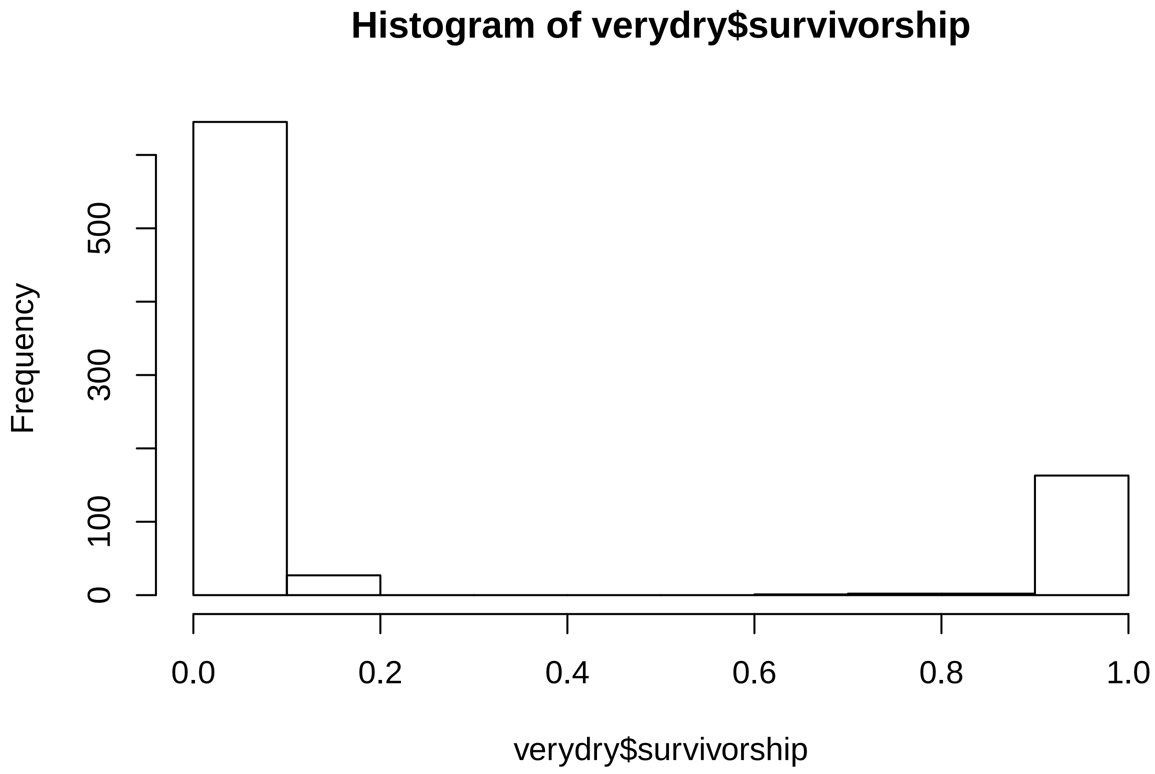
```
library(ggplot2)
library(scales)

legend_title = "Hydration Treatment"

survivorhsip_1 = ggplot(survivalsumm_1, aes(x=treatment, y=mean)) + #add the axis
  geom_errorbar(aes(color = treatment, ymin=mean-SE, ymax=mean+SE), width= .05, size= .5, position=posi
  geom_bar(stat="identity", aes(fill=treatment), position=position_dodge(.9), width = .9) + # use barpl
  labs(y="Survival to hatch") + #creat labels...
  theme_classic(base_size = 9) +
  theme(legend.position="none", axis.title.y = element_text(size = 9), axis.text = element_text(color="
  #theme(plot.margin = unit(c(.1, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(limits = c(0,1.1), expand = c(0, 0), breaks= pretty_breaks(n=6)) +
  scale_x_discrete(labels = c("High", "Low")) +
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray"))
  #geom_text(aes(y= 1.02, x = 1.5, label= "***"), color= "black", vjust = 0, size = 8) + #add significa
  #geom_segment(aes(x = 1, y = 1.02, xend = 2, yend = 1.02), size = 1) # add line segment
survivorhsip_1
```



STATS

ANOVA

```
library(lme4)
library(car)
library(lmerTest)

hist(verydry$survivorship)
```



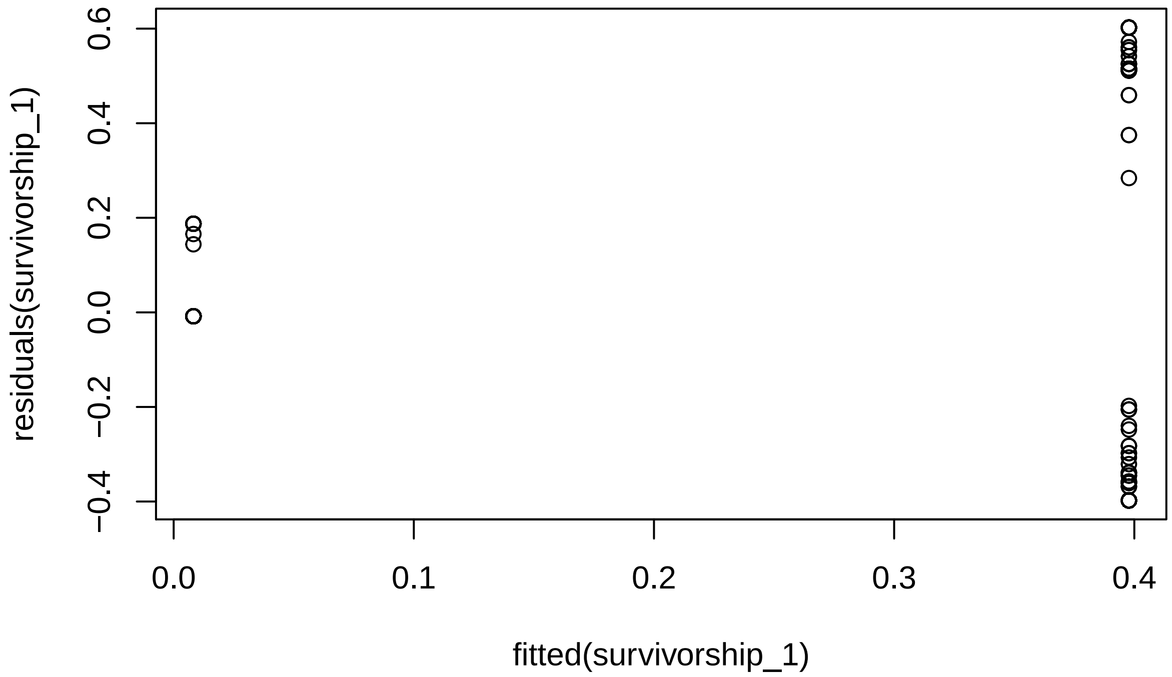
```
survivorship_1 = lm(data=verydry, survivorship~treatment)
summary(survivorship_1)
```

```
##
## Call:
## lm(formula = survivorship ~ treatment, data = verydry)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.39776 -0.39776 -0.00823 -0.00823  0.60224
##
## Coefficients:
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)              0.39776    0.01651   24.09   <2e-16 ***
## treatmentLow humidity -0.38953    0.02335  -16.68   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3384 on 838 degrees of freedom
## Multiple R-squared:  0.2493, Adjusted R-squared:  0.2484
## F-statistic: 278.3 on 1 and 838 DF,  p-value: < 2.2e-16
```

```
library(car)
Anova(survivorship_1)
```

```
## Anova Table (Type II tests)
##
## Response: survivorship
##           Sum Sq  Df F value    Pr(>F)
## treatment 31.865   1  278.26 < 2.2e-16 ***
## Residuals 95.961 838
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

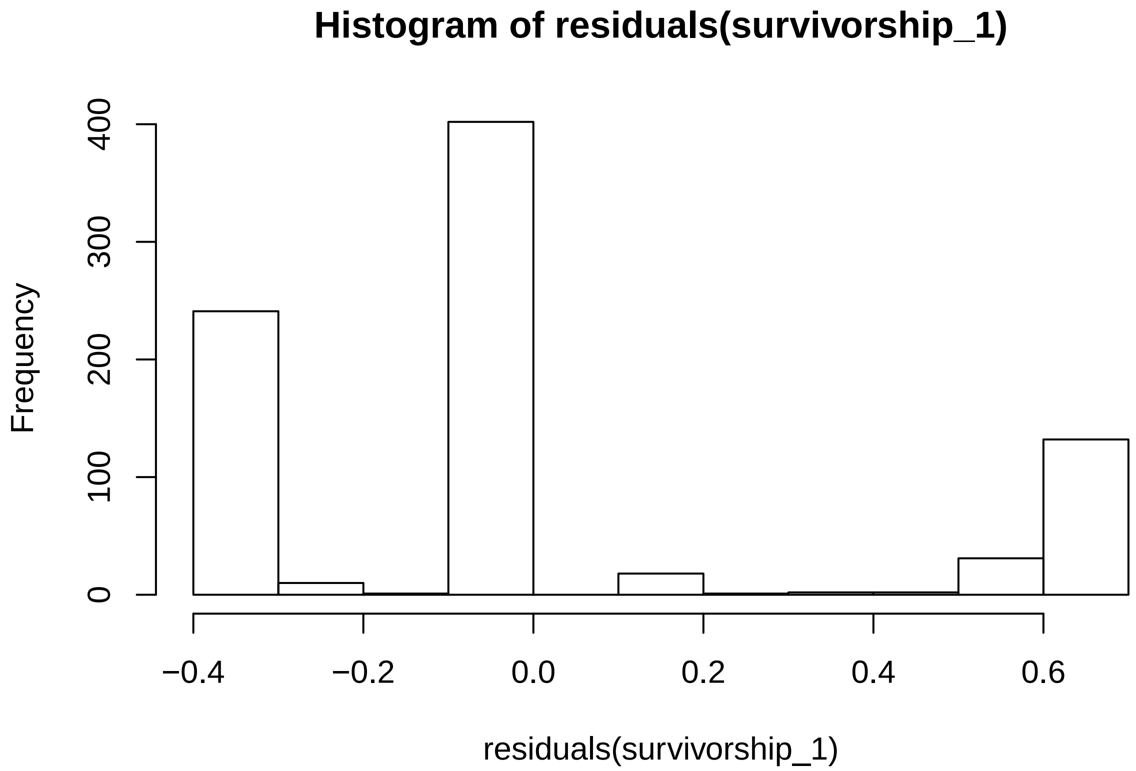
```
#Check for normality assumptions
plot(fitted(survivorship_1), residuals(survivorship_1))
```



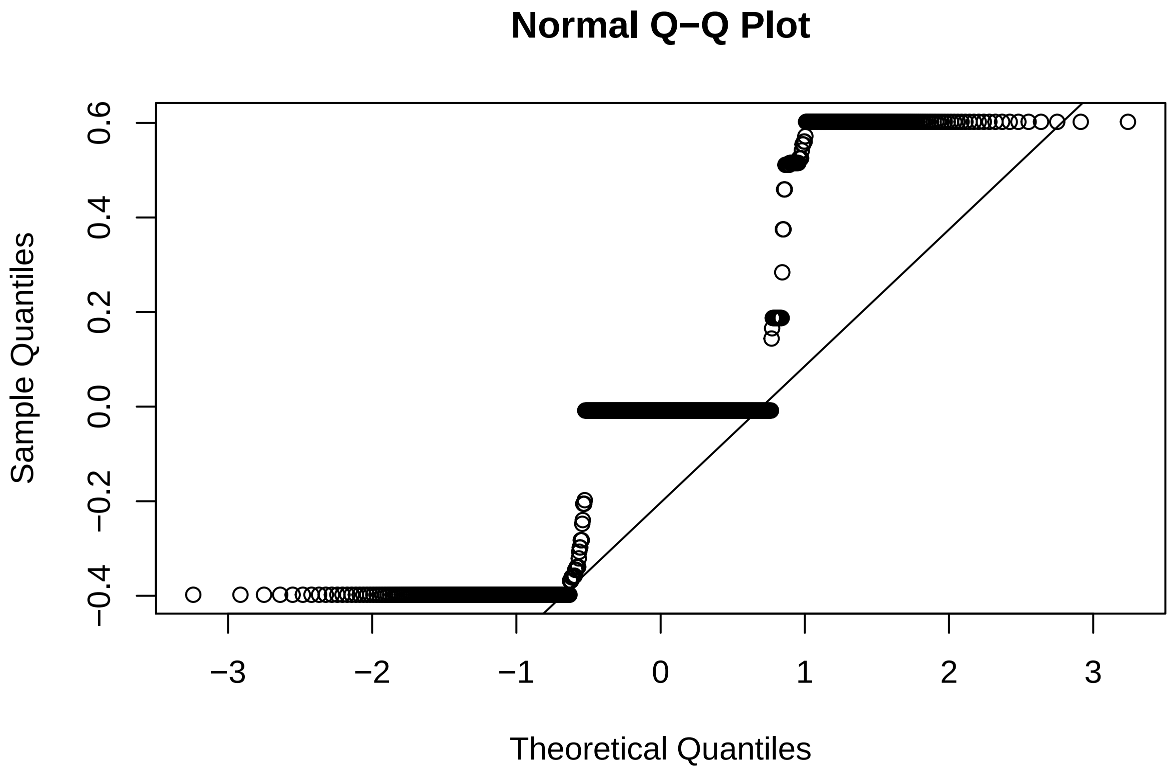
```
leveneTest(data=verydry, survivorship~treatment)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value    Pr(>F)
## group   1  278.26 < 2.2e-16 ***
##       838
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(survivorship_1))
```



```
qqnorm(residuals(survivorship_1))
qqline(residuals(survivorship_1))
```



```
shapiro.test(residuals(survivorship_1))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(survivorship_1)
## W = 0.80995, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(survivorship_1) W = 0.80995, p-value < 2.2e-16

Anova Table (Type II tests)

Response: survivorship Sum Sq Df F value Pr(>F)
treatment 31.865 1 278.26 < 2.2e-16 *** Residuals 95.961 838

Nonparametric stats here:

```
wct = wilcox.test(data=verydry, survivorship~treatment, paired = F)
wct
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  survivorship by treatment
## W = 127267, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 127270**
- **p-value = < 2.2e-16**

Combined Figure 4

```
library(gridExtra)
library(cowplot)

# 1 column is 3.43 inches wide

#  @ 3.43 x 5 portrait

figure4 = plot_grid(clutch_thickness_1, egg_size_1, survivorhsip_1, labels = "AUTO", label_x = -.01, lab
figure4
```

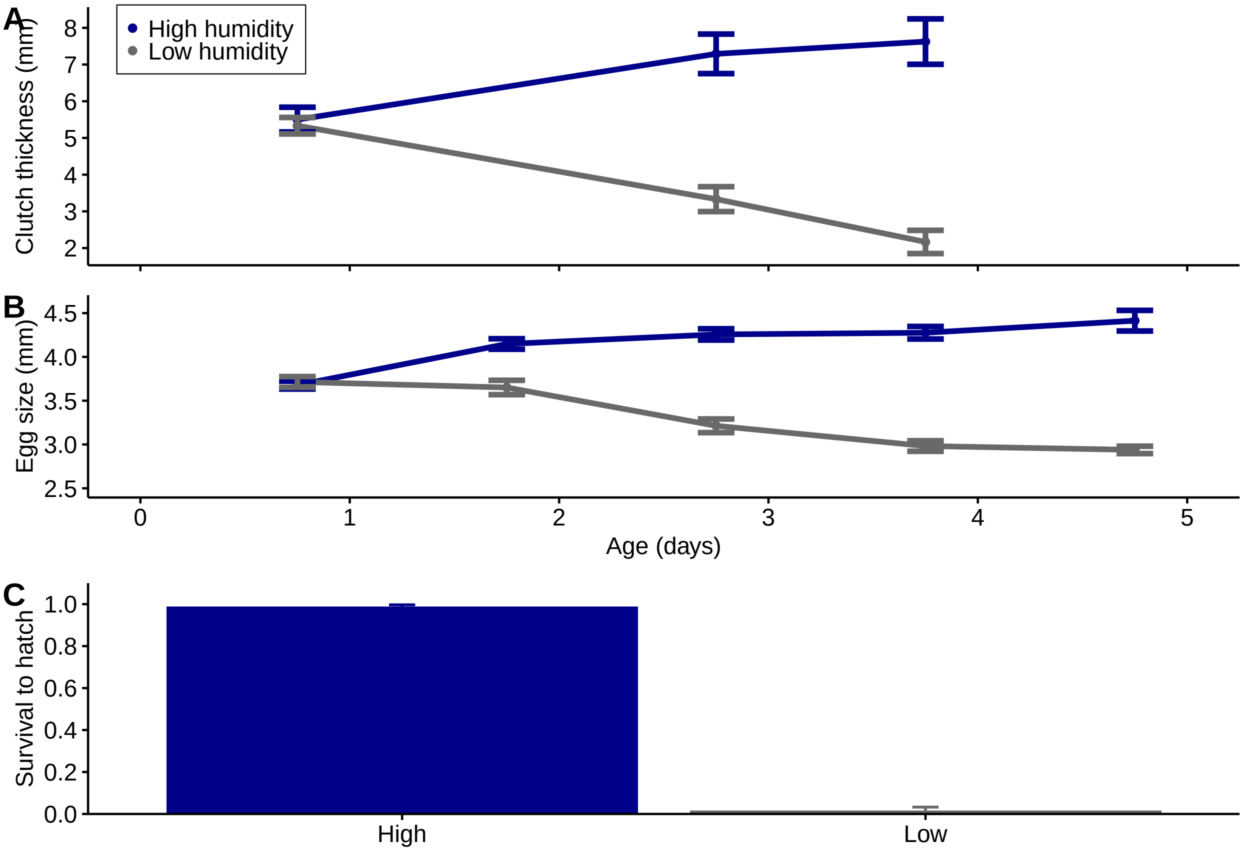


Figure 5

Experiment 2

Subset data

```
dry = bd %>%
  filter(clutch > 100) %>%
  filter(age_t > .7) #this removes first two data points of collection which we did in addition compare
dry
```

```
## # A tibble: 1,606 x 23
##    age_t clutch block treatment n_eggs n_eggs_excludin~ `unhatched_incl~
##    <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>            <dbl>
##  1  0.75     104 1     Medium h~     35               35               35
##  2  1        104 1     Medium h~     35               35               35
##  3  1.25     104 1     Medium h~     35               35               35
##  4  1.5      104 1     Medium h~     35               35               35
##  5  1.75     104 1     Medium h~     35               35               35
##  6  2        104 1     Medium h~     35               35               35
##  7  2.25     104 1     Medium h~     35               35               35
##  8  2.5      104 1     Medium h~     35               35               35
##  9  2.75     104 1     Medium h~     35               35               35
## 10  3        104 1     Medium h~     35               35               35
## # ... with 1,596 more rows, and 16 more variables: `unhatched_excluding
## #   undev_ncluding dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, stage_from_clutch_pic <chr>, clutch_length <dbl>,
## #   clutch_width <dbl>, average_eggsize <dbl>, average_egg_size <dbl>,
## #   average_tadlength <dbl>, average_tad_length <dbl>
```

```
unique(dry$treatment)
```

```
## [1] "Medium humidity" "High humidity"
```

Clutch thickness

> We performed two iterations of this experiment (clutches 104-115 and 116-127) and began measurements at 2d 18:00 h. We did not measure thickness at 4d at 18:00h and only measured it in low humimidity on 5d due to sensitivity of high humidity clutches to hatching; Eggs in low humidity clutches were mostly dead and therefore could be handled.

Summary statistics

```
thicknesssumm_2 = dry %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(clutch_thickness, na.rm=T), SE= sd(clutch_thickness, na.rm=T)/sqrt(length(clutc
  na.omit() %>%
  mutate(meant = lag(mean, n=1)- mean)
thicknesssumm_2
```

```
## # A tibble: 8 x 9
## # Groups:   treatment [2]
##   treatment       age_t  mean    SE    SD   min   max sample_size  meant
##   <chr>           <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>       <int>  <dbl>
## 1 High humidity    0.75  5.35 0.173 0.832   3     7            23 NA
## 2 High humidity    1.75  5.35 0.152 0.730   4     7            23  0
## 3 High humidity    2.75  5.83 0.174 0.834   4.5   8            23 -0.478
## 4 High humidity    3.75  6.02 0.226 1.08    5     9            23 -0.196
## 5 Medium humidity  0.75  5.02 0.176 0.846   4     7            23 NA
## 6 Medium humidity  1.75  4.39 0.183 0.878   3     7            23  0.630
## 7 Medium humidity  2.75  4.13 0.181 0.869   2.5   6            23  0.261
## 8 Medium humidity  3.75  4.39 0.160 0.768   3     6.5          23 -0.261
```

High humidity:

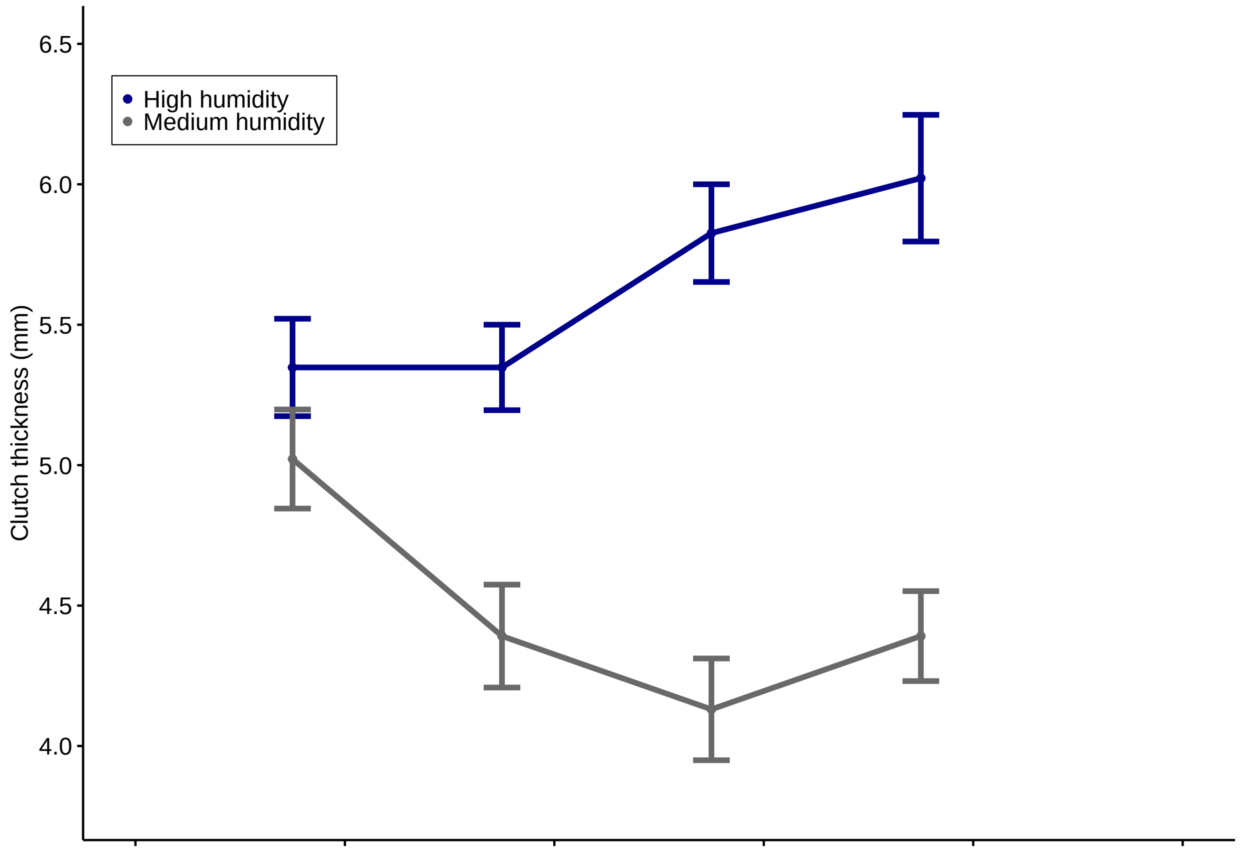
- 0.0000000 + 0.4782609 + 0.1956522 / 3 == *mean 0.2246377 increase per age*
- 1-(6.021739/5.347826) = 1.126016 increase == ~**12.6% percent increase total**

Medium humidity:

- 0.6304348 + 0.2608696 + 0.2608696 / 3 == *mean 0.384058 decrease per age*
- 1- (4.391304/5.021739) = 0.8744588 decrease == ~**12.5% percent decrease total**

Figure

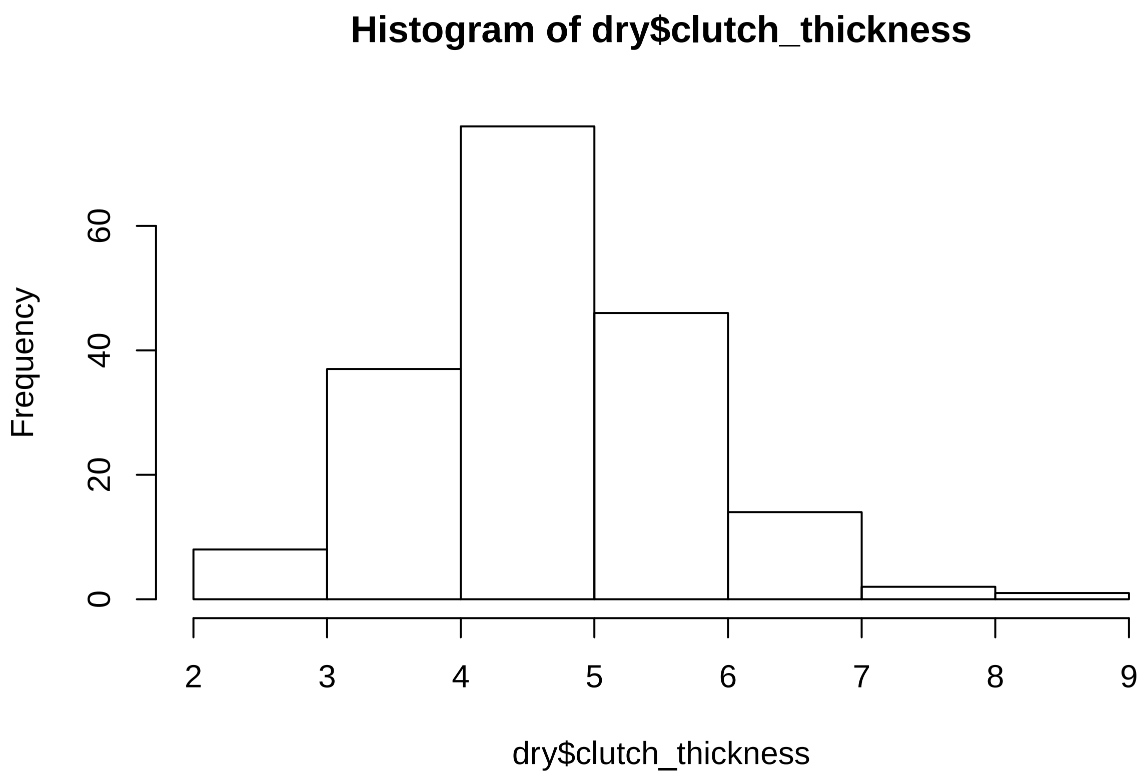
```r
library(ggplot2)
library(scales)

legend_title = "Hydration treatment"

clutch_thickness_2 = ggplot(data = thicknesssumm_2, aes(x=age_t, y=mean)) + #add the axis
  geom_line(aes(color = treatment), size = 1) +
  geom_point(aes(color=treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Clutch thickness (mm)", x="Age (days)") +
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black"))
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color
  #theme(plot.margin = unit(c(1, .5, .55, .5), "cm")) + #top, right, bottom, left
  scale_x_continuous(limits=c(0,5), breaks= pretty_breaks(n=5)) +
  scale_y_continuous(limits=c(3.8, 6.5), breaks= pretty_breaks(n=6)) +
  scale_color_manual(legend_title, values =  c("darkblue","dimgray")) +
  scale_fill_manual(guide = FALSE, values =  c("darkblue", "dimgray"))
clutch_thickness_2
```



STATS

LMM

```
library(lme4)
library(car)
library(lmerTest)

hist(dry$clutch_thickness)
```



```
#all models
clutch_thickness_2_full = lmer(clutch_thickness~age_t*treatment +  (1|clutch), data = dry)
clutch_thickness_2_nointeraction = lmer(clutch_thickness~age_t+treatment +  (1|clutch),  data = dry)
clutch_thickness_2_notreatment = lmer(clutch_thickness~age_t +  (1|clutch),  data = dry)
clutch_thickness_2_noage = lmer(clutch_thickness~treatment +  (1|clutch), data = dry)

clutch_thickness_2_full2 = lmer(clutch_thickness~age_t*treatment +  (1|clutch) + (1|block), data = dry)

#block effect?
clutch_thickness_2_block = lmer(clutch_thickness~block + (1|clutch), data = dry)
Anova(clutch_thickness_2_block)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: clutch_thickness
##        Chisq Df Pr(>Chisq)
## block 0.0306  1     0.8612
```

```
#AIC comparison
library(AICcmodavg)
print(aictab(list(clutch_thickness_full, clutch_thickness_2_full2, clutch_thickness_nointeraction, clutc
```

```
##
## Model selection based on AICc:
##
```

```
##                                K   AICc Delta_AICc AICcWt Cum.Wt
## clutch_thickness_full          6 233.88       0.00      1      1
## clutch_thickness_noage         4 294.00      60.12      0      1
## clutch_thickness_nointeraction 5 297.19      63.31      0      1
## clutch_thickness_notreatment   4 317.71      83.83      0      1
## clutch_thickness_2_full2       7 416.25     182.37      0      1
```

```
summary(clutch_thickness_2_full)
```

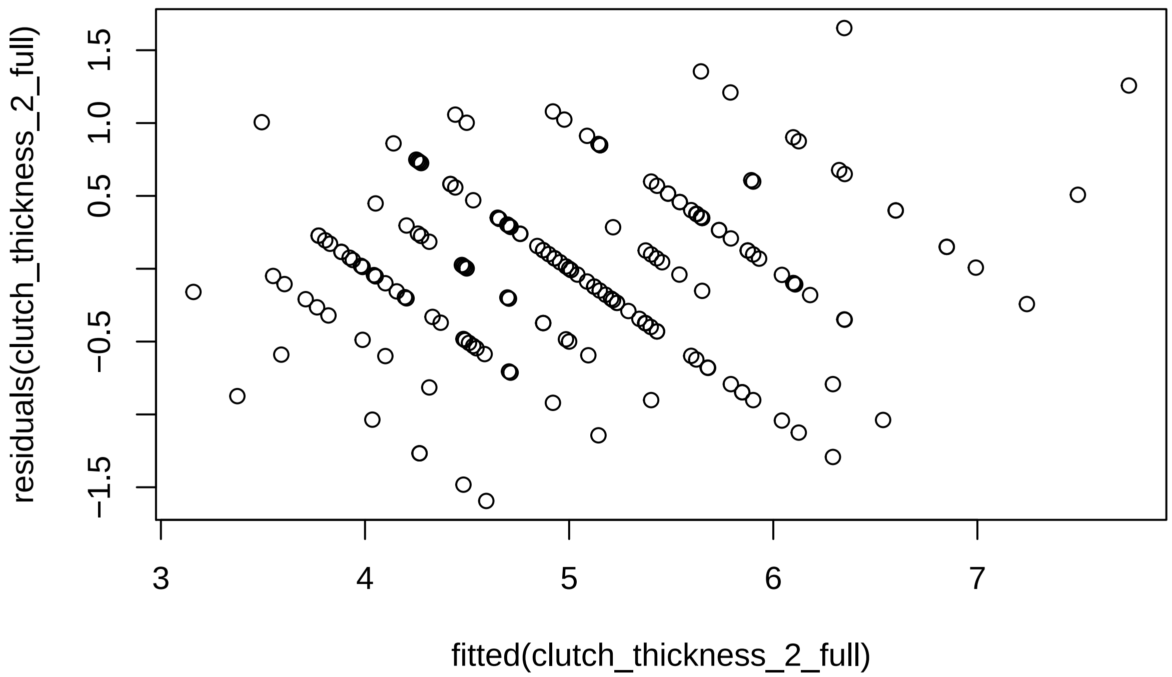
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: clutch_thickness ~ age_t * treatment + (1 | clutch)
##    Data: dry
##
## REML criterion at convergence: 401.6
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.6047 -0.5803  0.0273  0.5691  2.6996
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clutch   (Intercept) 0.3884   0.6232
##  Residual             0.3745   0.6120
## Number of obs: 184, groups:  clutch, 23
##
## Fixed effects:
##                                  Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)                       5.07337    0.19350  76.00969  26.219  < 2e-16
## age_t                             0.25000    0.05707 158.00000   4.381 2.14e-05
## treatmentMedium humidity         -0.10543    0.20276 158.00000  -0.520    0.604
## age_t:treatmentMedium humidity  -0.46522    0.08070 158.00000  -5.765 4.18e-08
##
## (Intercept)                    ***
## age_t                          ***
## treatmentMedium humidity
## age_t:treatmentMedium humidity ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) age_t  trtmMh
## age_t       -0.664
## trtmntMdmhm -0.524  0.633
## ag_t:trtmMh  0.469 -0.707 -0.896
```

```
Anova(clutch_thickness_2_full)
```

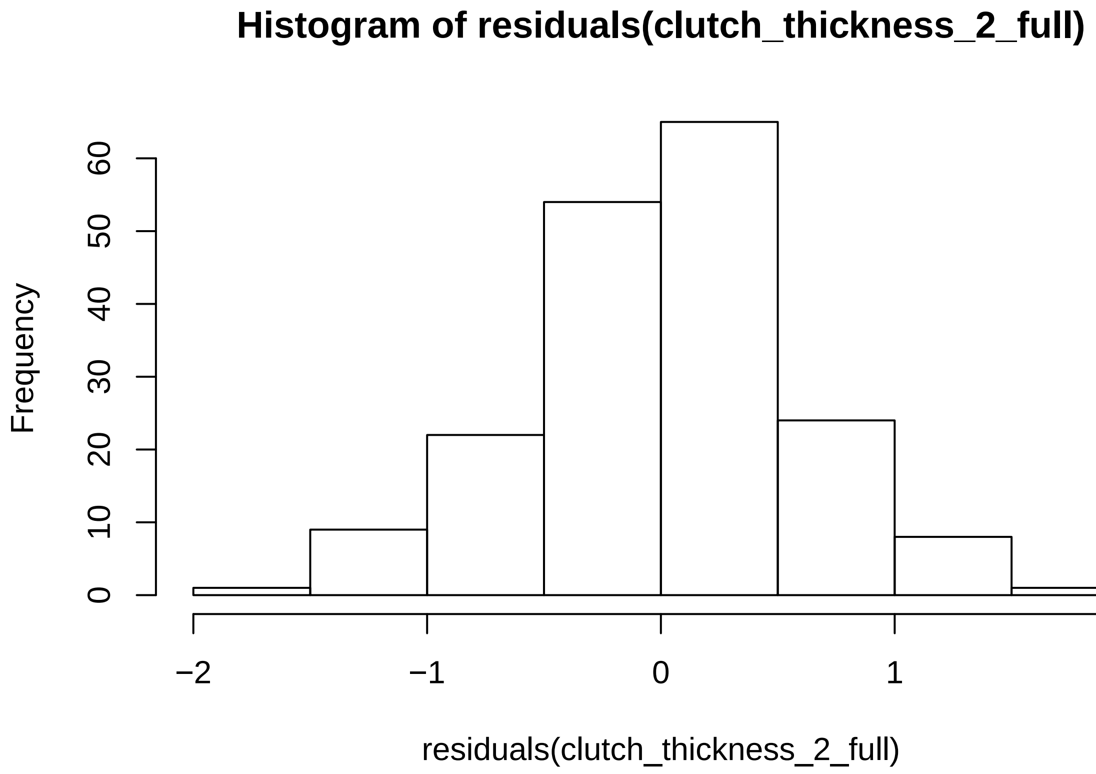
```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: clutch_thickness
##                    Chisq Df Pr(>Chisq)
## age_t             0.1858  1     0.6665
```

```
## treatment       163.0589  1  < 2.2e-16 ***
## age_t:treatment  33.2300  1  8.188e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

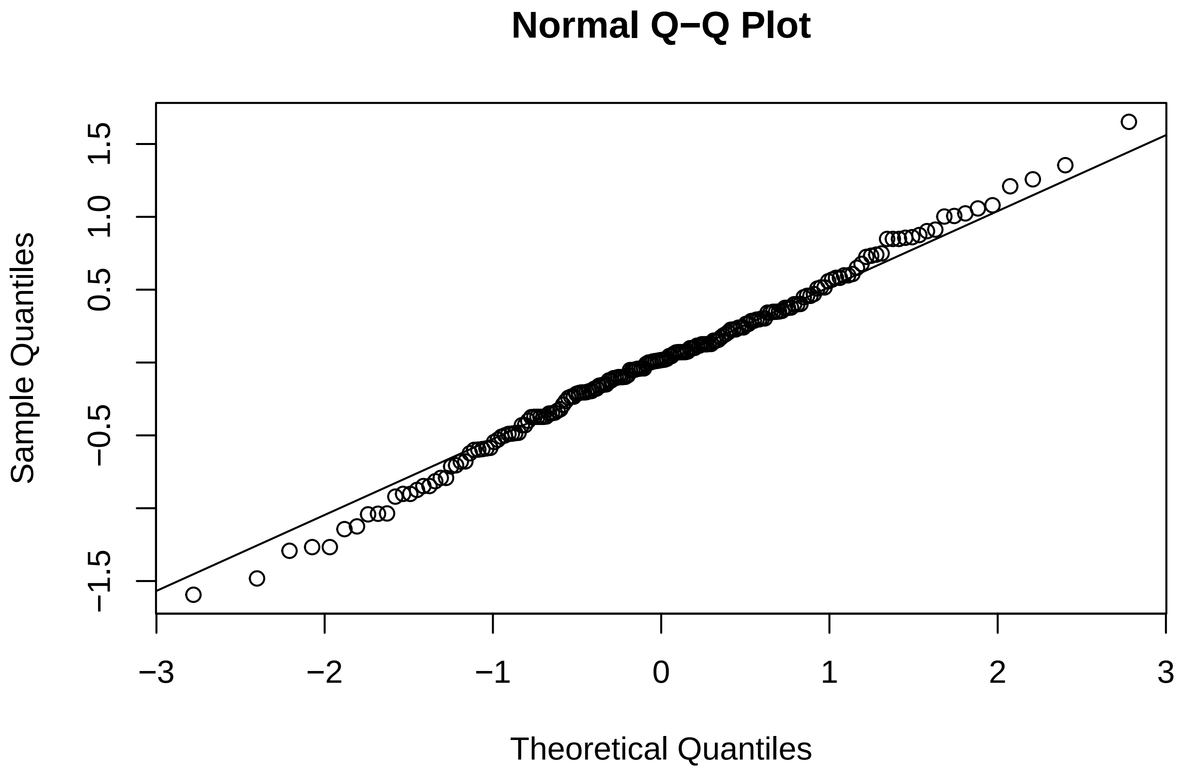
```r
plot(fitted(clutch_thickness_2_full), residuals(clutch_thickness_2_full))
```



```r
hist(residuals(clutch_thickness_2_full))
```



```
qqnorm(residuals(clutch_thickness_2_full))
qqline(residuals(clutch_thickness_2_full))
```



```r
shapiro.test(residuals(clutch_thickness_2_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(clutch_thickness_2_full)
## W = 0.99481, p-value = 0.7743
```

Shapiro-Wilk normality test

data: residuals(clutch_thickness_2_full) W = 0.99492, p-value = 0.7818

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: clutch_thickness Chisq Df Pr(>Chisq)
age_t 0.1131 1 0.7366
treatment 164.8683 1 < 2.2e-16 ***age_t:treatment 32.9758 1 9.331e-09***

Likelihood ratio tests

```r
anova(clutch_thickness_2_full, clutch_thickness_2_nointeraction, test = "Chisq")
```

```
## Data: dry
## Models:
## clutch_thickness_2_nointeraction: clutch_thickness ~ age_t + treatment + (1 | clutch)
## clutch_thickness_2_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
```

```
##                                npar    AIC    BIC  logLik deviance  Chisq Df
## clutch_thickness_2_nointeraction    5 429.41 445.49 -209.71   419.41
## clutch_thickness_2_full             6 400.68 419.97 -194.34   388.68 30.732  1
##                                Pr(>Chisq)
## clutch_thickness_2_nointeraction
## clutch_thickness_2_full           2.962e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(clutch_thickness_2_full, clutch_thickness_2_notreatment, test = "Chisq")
```

```
## Data: dry
## Models:
## clutch_thickness_2_notreatment: clutch_thickness ~ age_t + (1 | clutch)
## clutch_thickness_2_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
##                              npar    AIC    BIC  logLik deviance  Chisq Df
## clutch_thickness_2_notreatment    4 526.69 539.55 -259.35   518.69
## clutch_thickness_2_full           6 400.68 419.97 -194.34   388.68 130.01  2
##                              Pr(>Chisq)
## clutch_thickness_2_notreatment
## clutch_thickness_2_full         < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(clutch_thickness_2_full, clutch_thickness_2_noage, test = "Chisq")
```

```
## Data: dry
## Models:
## clutch_thickness_2_noage: clutch_thickness ~ treatment + (1 | clutch)
## clutch_thickness_2_full: clutch_thickness ~ age_t * treatment + (1 | clutch)
##                        npar    AIC    BIC  logLik deviance  Chisq Df
## clutch_thickness_2_noage    4 427.57 440.43 -209.78   419.57
## clutch_thickness_2_full     6 400.68 419.97 -194.34   388.68 30.888  2
##                        Pr(>Chisq)
## clutch_thickness_2_noage
## clutch_thickness_2_full   1.962e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X = 30.527; df = 1**
- **p-value 3.293e-08**

EFFECT OF TREATMENT:

- **X = 130.93; df = 2**
- **p-value < 2.2e-16**

EFFECT OF AGE:

- **X = 30.613; df = 2**
- **p-value = 2.252e-07**

Egg size

> We did not take photos/measurements at 5d 18:00 h for high treatment due to sensitivity of clutches to hatching. We took pictures from two sets at 06:00 h and 15:00 h and both at 18:00 h of 0 d.

Summary statistics

```
eggsizesum_2 = dry %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(average_egg_size, na.rm=T), SE= sd(average_egg_size, na.rm=T)/sqrt(length(averag
  na.omit() %>%
  mutate(spherical_volume = ((4/3)*(pi)*((mean/2)^3)), meant = lag(mean, n=1)- mean)
eggsizesum_2
```

```
## # A tibble: 10 x 10
## # Groups:   treatment [2]
##    treatment age_t  mean     SE    SD   min   max sample_size spherical_volume
##    <chr>     <dbl> <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>            <dbl>
##  1 High hum~  0.75  3.67 0.0550 0.264  3.06  4.09          23             25.8
##  2 High hum~  1.75  3.73 0.0495 0.237  3.12  4.12          23             27.1
##  3 High hum~  2.75  4.02 0.0539 0.259  3.55  4.35          23             33.9
##  4 High hum~  3.75  4.21 0.0665 0.319  3.65  4.73          23             39.1
##  5 High hum~  4.75  4.25 0.0732 0.351  3.76  4.84          23             40.1
##  6 Medium h~  0.75  3.52 0.0517 0.248  3.01  3.89          23             22.7
##  7 Medium h~  1.75  3.42 0.0545 0.261  2.92  3.86          23             20.9
##  8 Medium h~  2.75  3.37 0.0538 0.258  2.85  3.81          23             20.0
##  9 Medium h~  3.75  3.43 0.0527 0.253  3.02  3.8           23             21.1
## 10 Medium h~  4.75  3.28 0.0540 0.259  2.9   3.73          23             18.5
## # ... with 1 more variable: meant <dbl>
```

High humidity:

- 0.06130435 + 0.28956522 + 0.19347826 + 0.03526087/ 4 == *mean 0.1449022 increase per age*
- 1-(4.246130/3.666522) = 0.1580811 increase == ~**15.8% percent increase total**

Medium humidity:

- 0.09778261 + 0.05175652 + 0.06175652 + 0.14434783/ 4 == *mean 0.08891087 decrease per age*
- 1- (3.283043/3.515174) = 0.06603684 decrease == ~**6.6% percent decrease total**

Spherical Volume

High humidity:

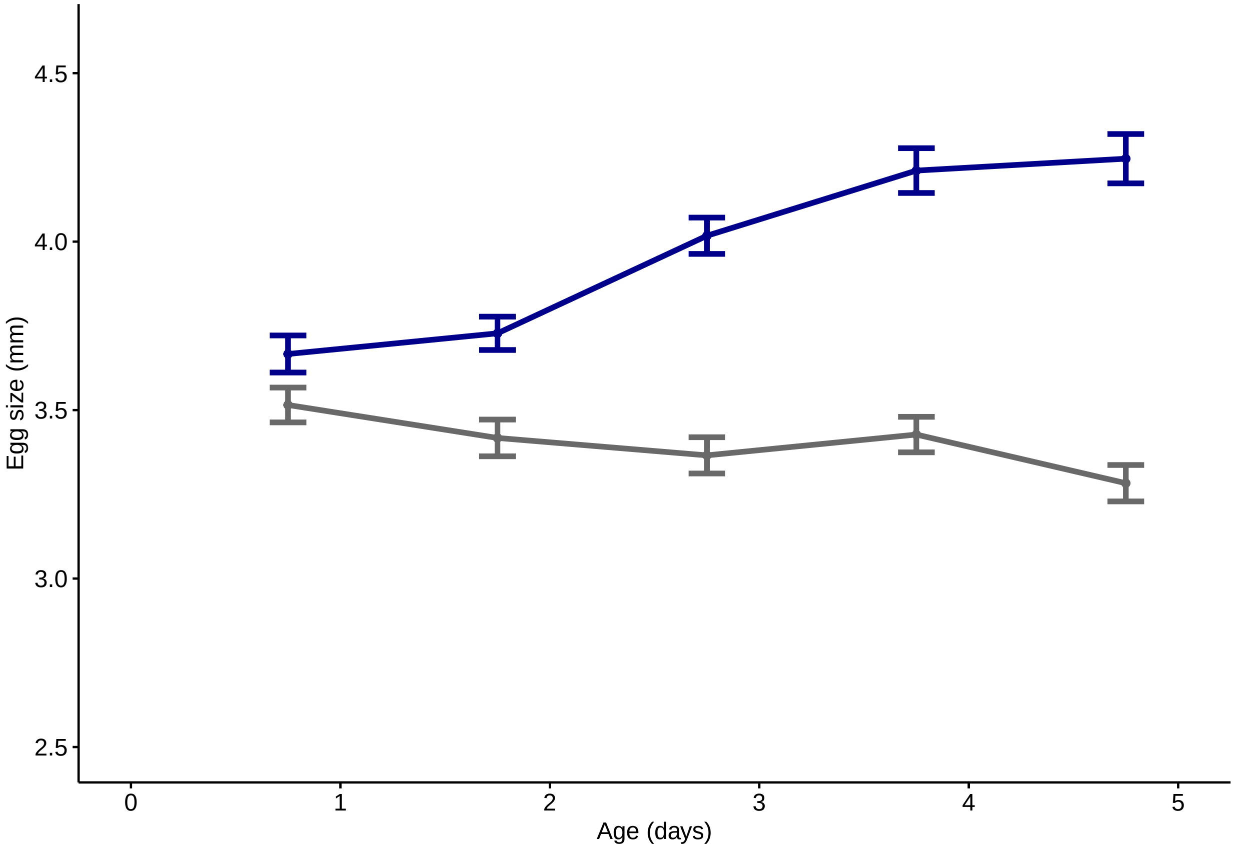
- 1-(40.08470/25.80842) = 0.5531637 increase == ~**55.3% percent increase total**

Medium humidity:

- 1- (18.52800/22.74255) = 0.1853156 decrease == ~**18.5% percent decrease total**

Figure

```
library(ggplot2)
library(scales)

legend_title = "Treatment"

egg_size_2 = ggplot(eggsizesum_2, aes(x=age_t, y=mean)) + #add the axis
  geom_line(aes(color = treatment), size = 1) +
  geom_point(aes(color=treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Egg size (mm)", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  #theme(plot.margin = unit(c(.5, .5, .1, .5), "cm")) + #top, right, bottom, left
  theme(legend.position = "none", legend.text = element_text(size = 9), legend.title = element_blank(),
  #theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  scale_x_continuous(limits=c(0,5), breaks= pretty_breaks(n=5)) +
  scale_y_continuous(limits=c(2.5,4.6), breaks=pretty_breaks(n=5)) +
  scale_color_manual(legend_title, values = c("darkblue","dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray"))
egg_size_2
```

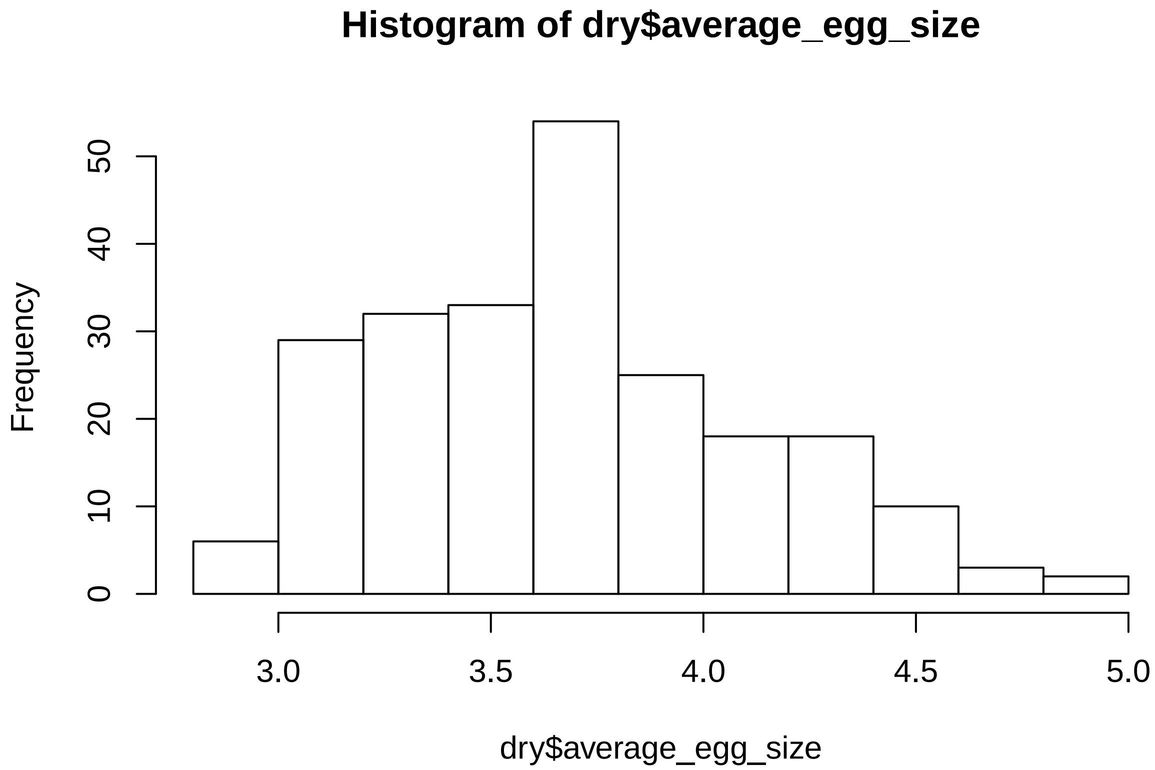


STATS

LMM

```
library(lme4)
library(car)
```

```r
library(lmerTest)

hist(dry$average_egg_size)
```



```r
#all models
egg_size_2_full = lmer(average_egg_size~age_t*treatment +  (1|clutch), data = dry)
egg_size_2_nointeraction = lmer(average_egg_size~age_t+treatment +  (1|clutch),  data = dry)
egg_size_2_notreatment = lmer(average_egg_size~age_t +  (1|clutch),  data = dry)
egg_size_2_noage = lmer(average_egg_size~treatment +  (1|clutch), data = dry)

egg_size_2_full2 = lmer(average_egg_size~age_t*treatment +  (1|clutch) + (1|block), data = dry)

#block effect?
egg_size_2_block = lmer(average_egg_size~block + (1|clutch), data = dry)
Anova(egg_size_2_block)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: average_egg_size
##        Chisq Df Pr(>Chisq)
## block 3.9174  1    0.04779 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#AIC comparison
library(AICcmodavg)
print(aictab(list(egg_size_2_full,  egg_size_2_full2, egg_size_2_nointeraction, egg_size_2_notreatment,
```

```
##
## Model selection based on AICc:
##
##                          K   AICc Delta_AICc AICcWt Cum.Wt
## egg_size_2_full          6 -49.24       0.00   0.59   0.59
## egg_size_2_full2         7 -48.49       0.75   0.41   1.00
## egg_size_2_nointeraction 5  62.09     111.34   0.00   1.00
## egg_size_2_noage         4  80.20     129.44   0.00   1.00
## egg_size_2_notreatment   4 255.46     304.71   0.00   1.00
```

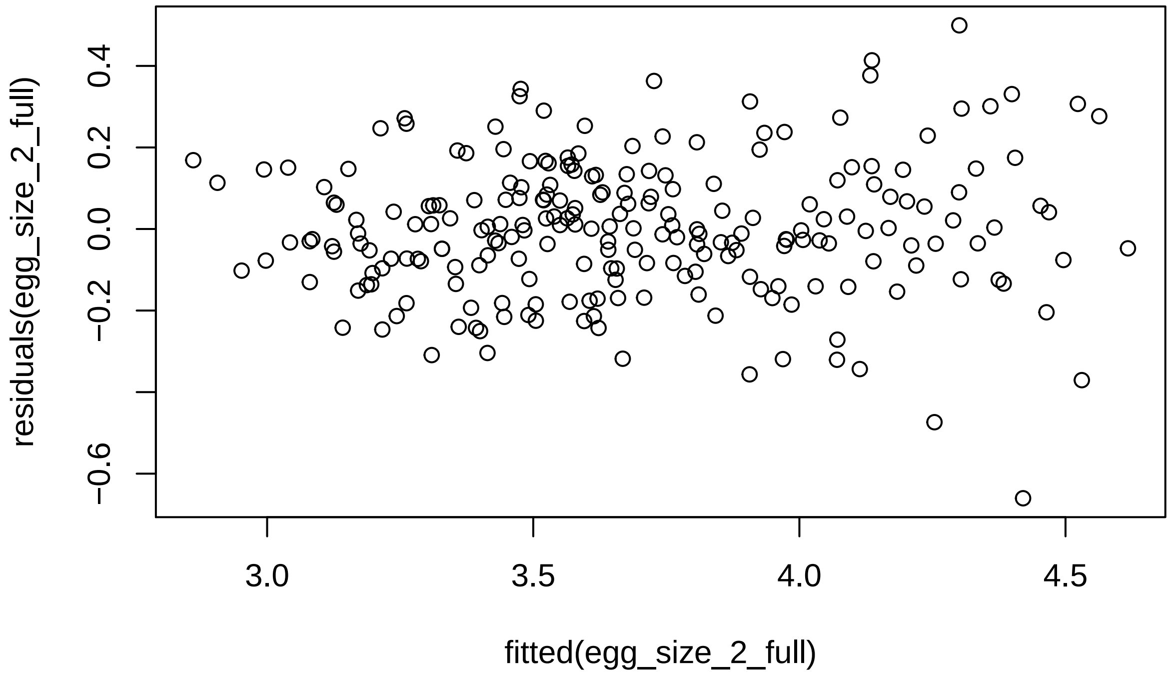
```
summary(egg_size_2_full)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: average_egg_size ~ age_t * treatment + (1 | clutch)
##    Data: dry
##
## REML criterion at convergence: -61.6
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.7380 -0.5480 -0.0177  0.5813  2.8275
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clutch   (Intercept) 0.04514  0.2125
##  Residual             0.03120  0.1766
## Number of obs: 230, groups:  clutch, 23
##
## Fixed effects:
##                                 Estimate Std. Error         df t value
## (Intercept)                     3.522126   0.057095  51.403634  61.689
## age_t                           0.164226   0.011648 204.000000  14.100
## treatmentMedium humidity        0.004523   0.050937 204.000000   0.089
## age_t:treatmentMedium humidity -0.209652   0.016472 204.000000 -12.728
##                               Pr(>|t|)
## (Intercept)                     <2e-16 ***
## age_t                           <2e-16 ***
## treatmentMedium humidity         0.929
## age_t:treatmentMedium humidity  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) age_t  trtmMh
## age_t       -0.561
## trtmntMdmhm -0.446  0.629
## ag_t:trtmMh  0.397 -0.707 -0.889
```

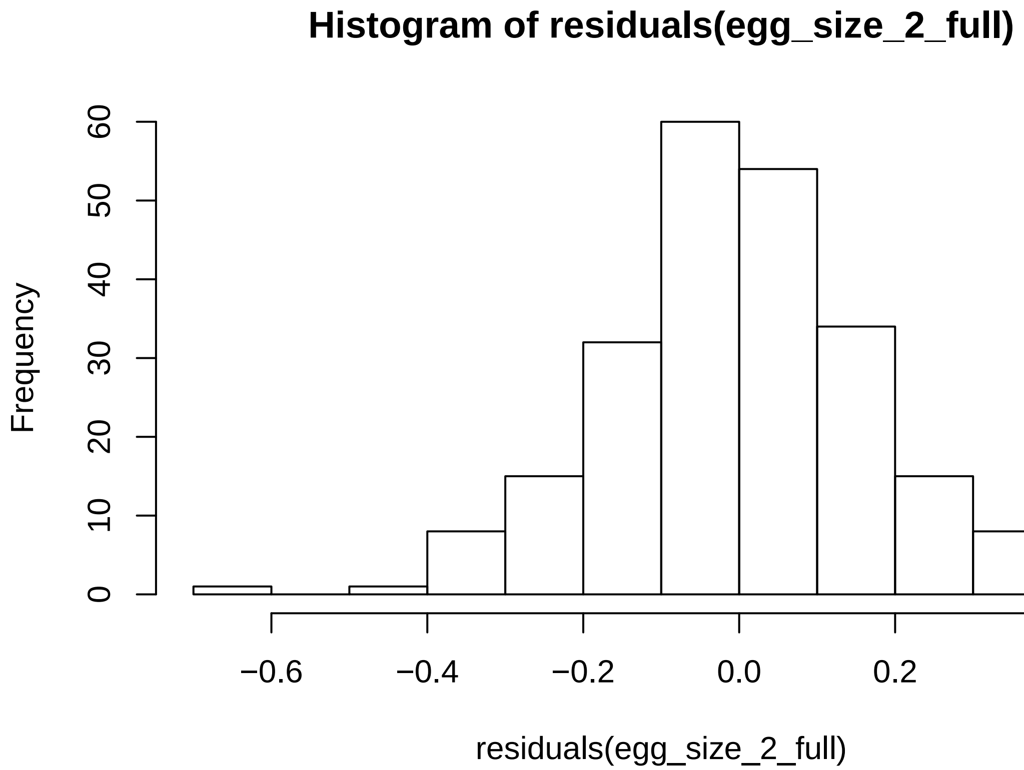
```
Anova(egg_size_2_full)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: average_egg_size
##                    Chisq Df Pr(>Chisq)
## age_t             52.015  1  5.507e-13 ***
## treatment        602.966  1  < 2.2e-16 ***
## age_t:treatment  161.994  1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

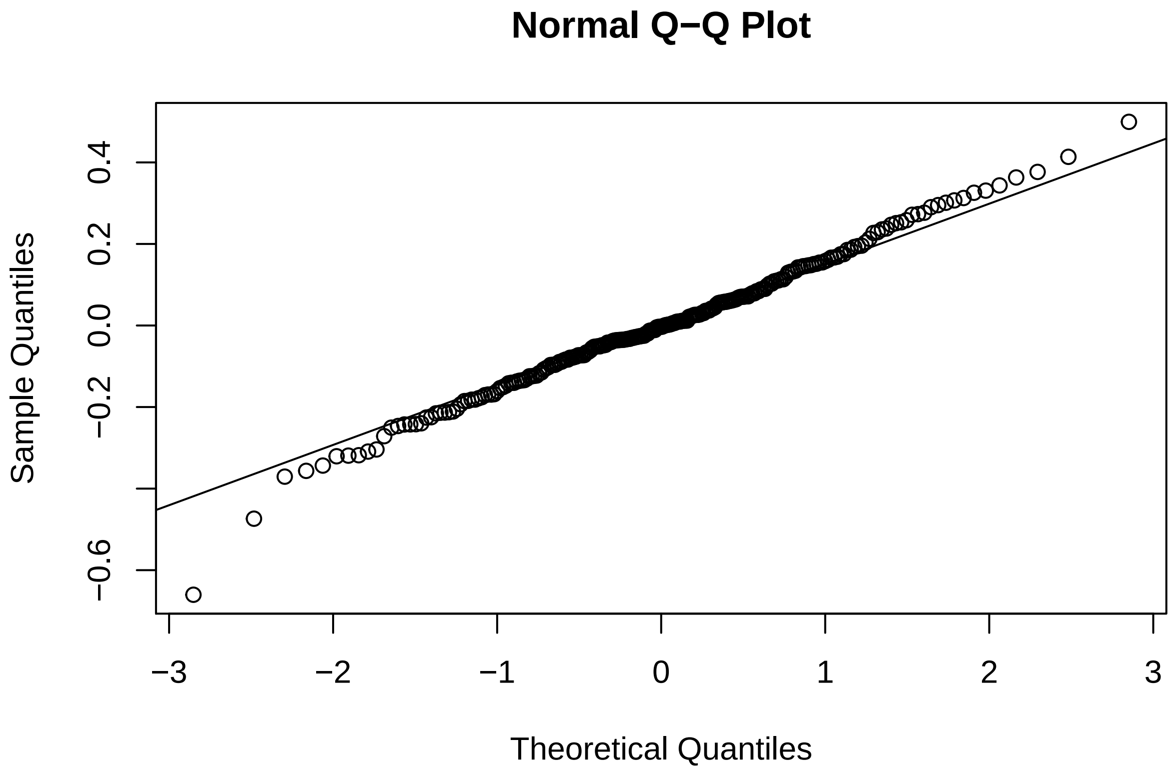
```
plot(fitted(egg_size_2_full), residuals(egg_size_2_full))
```



```
hist(residuals(egg_size_2_full))
```



```
qqnorm(residuals(egg_size_2_full))
qqline(residuals(egg_size_2_full))
```



```
shapiro.test(residuals(egg_size_2_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(egg_size_2_full)
## W = 0.99234, p-value = 0.2769
```

Shapiro-Wilk normality test

data: residuals(egg_size_2_full) W = 0.99262, p-value = 0.3006

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: average_egg_size Chisq Df Pr(>Chisq)
age_t 50.785 1 1.03e-12 ***treatment 604.233 1 < 2.2e-16*** age_t:treatment 160.208 1 < 2.2e-16 ***

Likelihood ratio tests

```
anova(egg_size_2_full, egg_size_2_nointeraction, test = "Chisq")
```

```
## Data: dry
## Models:
## egg_size_2_nointeraction: average_egg_size ~ age_t + treatment + (1 | clutch)
## egg_size_2_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                          npar     AIC     BIC  logLik deviance  Chisq Df
```

```
## egg_size_2_nointeraction    5  45.183  62.373 -17.591   35.183
## egg_size_2_full             6 -73.808 -53.179  42.904  -85.808 120.99  1
##                          Pr(>Chisq)
## egg_size_2_nointeraction
## egg_size_2_full           < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(egg_size_2_full, egg_size_2_notreatment, test = "Chisq")
```

```
## Data: dry
## Models:
## egg_size_2_notreatment: average_egg_size ~ age_t + (1 | clutch)
## egg_size_2_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                        npar     AIC     BIC   logLik deviance  Chisq Df
## egg_size_2_notreatment    4 244.720 258.472 -118.360  236.720
## egg_size_2_full           6 -73.808 -53.179   42.904  -85.808 322.53  2
##                        Pr(>Chisq)
## egg_size_2_notreatment
## egg_size_2_full         < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(egg_size_2_full, egg_size_2_noage, test = "Chisq")
```

```
## Data: dry
## Models:
## egg_size_2_noage: average_egg_size ~ treatment + (1 | clutch)
## egg_size_2_full: average_egg_size ~ age_t * treatment + (1 | clutch)
##                  npar     AIC     BIC  logLik deviance Chisq Df Pr(>Chisq)
## egg_size_2_noage    4  70.691  84.443 -31.345   62.691
## egg_size_2_full     6 -73.808 -53.179  42.904  -85.808 148.5  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X = 120.07; df = 1**
- **p-value < 2.2e-16**

EFFECT OF TREATMENT:

- **X = 323.02; df = 2**
- **p-value < 2.2e-16**

EFFECT OF AGE:

- **X = 146.74; df = 2**
- **p-value < 2.2e-16**

Mortality

Summary statistics

```
# calculate total that hatched and survivorship from total tested
dry2 = dry %>%
    group_by(clutch,age_t,treatment) %>%
  mutate(total_hatched = max(hatched_accumalative), total_timepointhatch = hatched_time_point/total_hat
dry2
```

```
## # A tibble: 1,606 x 27
## # Groups:   clutch, age_t, treatment [1,606]
##    age_t clutch block treatment n_eggs n_eggs_excludin~ `unhatched_incl~
##    <dbl>  <dbl> <chr> <chr>      <dbl>            <dbl>            <dbl>
##  1  0.75    104 1     Medium h~     35               35               35
##  2  1       104 1     Medium h~     35               35               35
##  3  1.25    104 1     Medium h~     35               35               35
##  4  1.5     104 1     Medium h~     35               35               35
##  5  1.75    104 1     Medium h~     35               35               35
##  6  2       104 1     Medium h~     35               35               35
##  7  2.25    104 1     Medium h~     35               35               35
##  8  2.5     104 1     Medium h~     35               35               35
##  9  2.75    104 1     Medium h~     35               35               35
## 10  3       104 1     Medium h~     35               35               35
## # ... with 1,596 more rows, and 20 more variables: `unhatched_excluding
## #   undev_ncluding dead` <dbl>, `available_to_hatch/time point` <dbl>,
## #   hatched_accumalative <dbl>, hatched_time_point <dbl>,
## #   dead_dessication <dbl>, `un/less developed` <dbl>,
## #   accumulative_prop_hatched <dbl>, timepoint_prop_hatched <dbl>,
## #   clutch_thickness <dbl>, stage_from_clutch_pic <chr>, clutch_length <dbl>,
## #   clutch_width <dbl>, average_eggsize <dbl>, average_egg_size <dbl>,
## #   average_tadlength <dbl>, average_tad_length <dbl>, total_hatched <dbl>,
## #   total_timepointhatch <dbl>, totalacummhatch <dbl>, survivorship <dbl>
```

```
survival2 = dry2 %>%
  group_by(clutch,treatment) %>%
  dplyr::select(clutch, treatment, total_hatched, survivorship) %>%
  filter(survivorship == max(survivorship)) %>%
  summarise(meansurvival = mean(survivorship), SE =sd(survivorship)/sqrt(length(survivorship)), sample_
survival2
```

```
## # A tibble: 46 x 5
## # Groups:   clutch [23]
##    clutch treatment       meansurvival    SE sample_size
##     <dbl> <chr>                  <dbl> <dbl>       <int>
##  1    104 High humidity          1        NA           1
##  2    104 Medium humidity        0.914     0          12
##  3    105 High humidity          1         0           3
##  4    105 Medium humidity        0         0          33
##  5    106 High humidity          1         0          12
##  6    106 Medium humidity        0.292     0          12
##  7    107 High humidity          1         0          11
##  8    107 Medium humidity        0.792     0          12
```

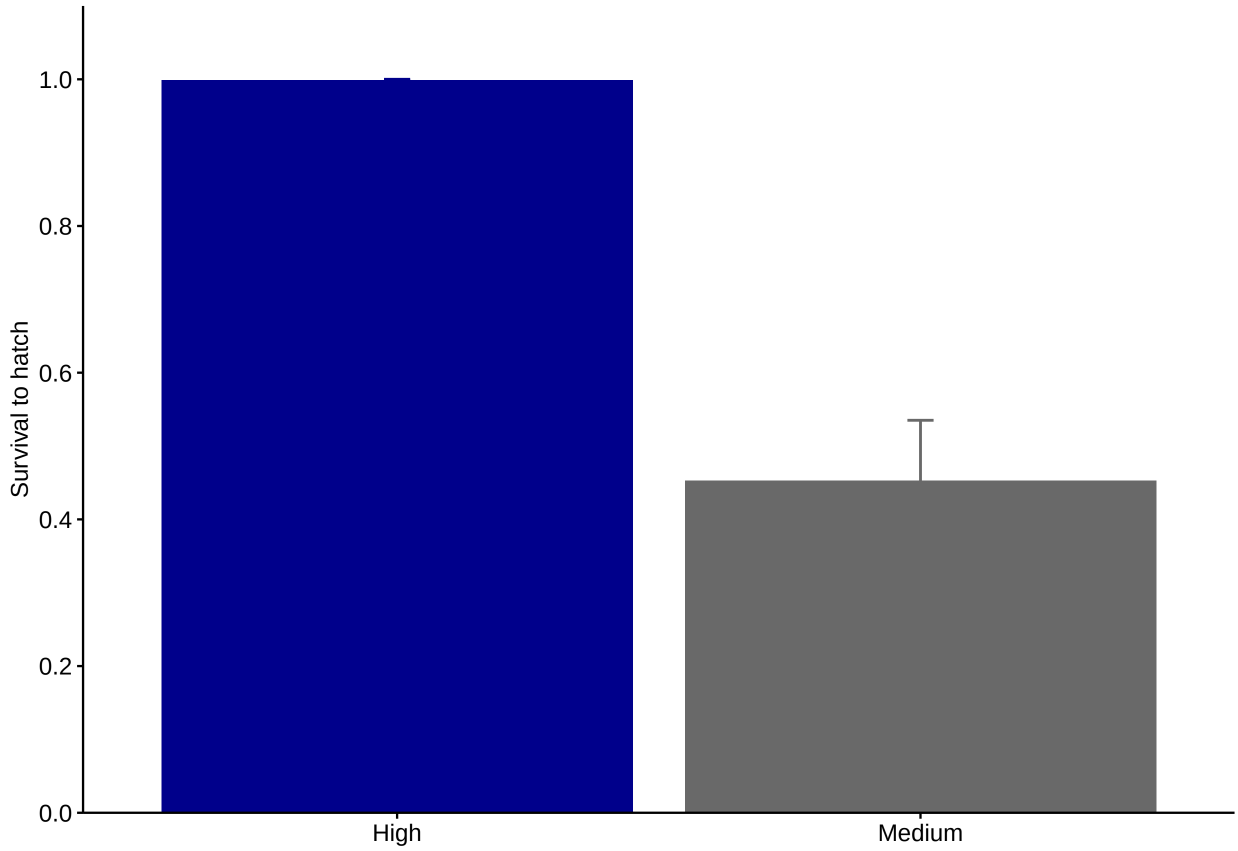
```
##  9    108 High humidity          1         0          12
## 10    108 Medium humidity        0.655     0          11
## # ... with 36 more rows
```

```
survivalsumm_2 = survival2 %>%
  group_by(treatment) %>%
  summarise(mean = mean(meansurvival, na.rm=T), SE= sd(meansurvival, na.rm=T)/sqrt(length(meansurvival)
  na.omit()
survivalsumm_2
```

```
## # A tibble: 2 x 7
##   treatment        mean       SE      SD   min   max sample_size
##   <chr>           <dbl>    <dbl>   <dbl> <dbl> <dbl>       <int>
## 1 High humidity   0.999 0.000988 0.00474 0.977     1          23
## 2 Medium humidity 0.453 0.0823   0.395   0         1          23
```

Figure

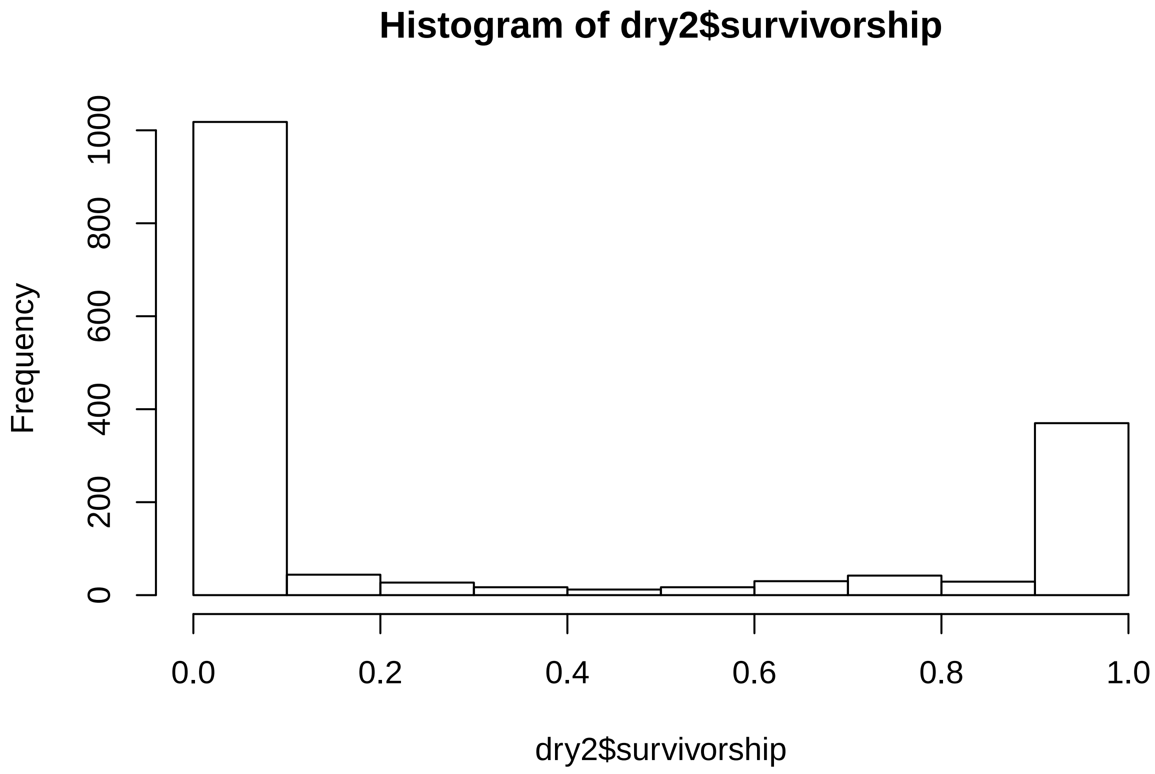
```
library(ggplot2)
library(scales)

legend_title = "Hydration Treatment"

survivorhsip_2 = ggplot(survivalsumm_2, aes(x=treatment, y=mean)) + #add the axis
  geom_errorbar(aes(color = treatment, ymin=mean-SE, ymax=mean+SE), width= .05, size= .5, position=posi
  geom_bar(stat="identity", aes(fill=treatment), position=position_dodge(.9), width = .9) + # use barpl
  labs(y="Survival to hatch") + #creat labels...
  theme_classic(base_size = 9) +
  theme(legend.position="none", axis.title.y = element_text(size = 9), axis.text = element_text(color="
  #theme(plot.margin = unit(c(.1, .5, .5, .5), "cm")) + #top, right, bottom, left
  scale_y_continuous(limits = c(0,1.1), expand = c(0, 0), breaks= pretty_breaks(n=6)) +
  scale_x_discrete(labels = c("High", "Medium")) +
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray"))
  #geom_text(aes(y= 1.02, x = 1.5, label= "***"), color= "black", vjust = 0, size = 8) + #add significa
  #geom_segment(aes(x = 1, y = 1.02, xend = 2, yend = 1.02), size = 1) # add line segment
survivorhsip_2
```



STATS

ANOVA

```r
library(lme4)
library(car)
library(lmerTest)

hist(dry2$survivorship)
```



```
survivorship_2 = lm(data=dry2, survivorship~treatment)
summary(survivorship_2)
```

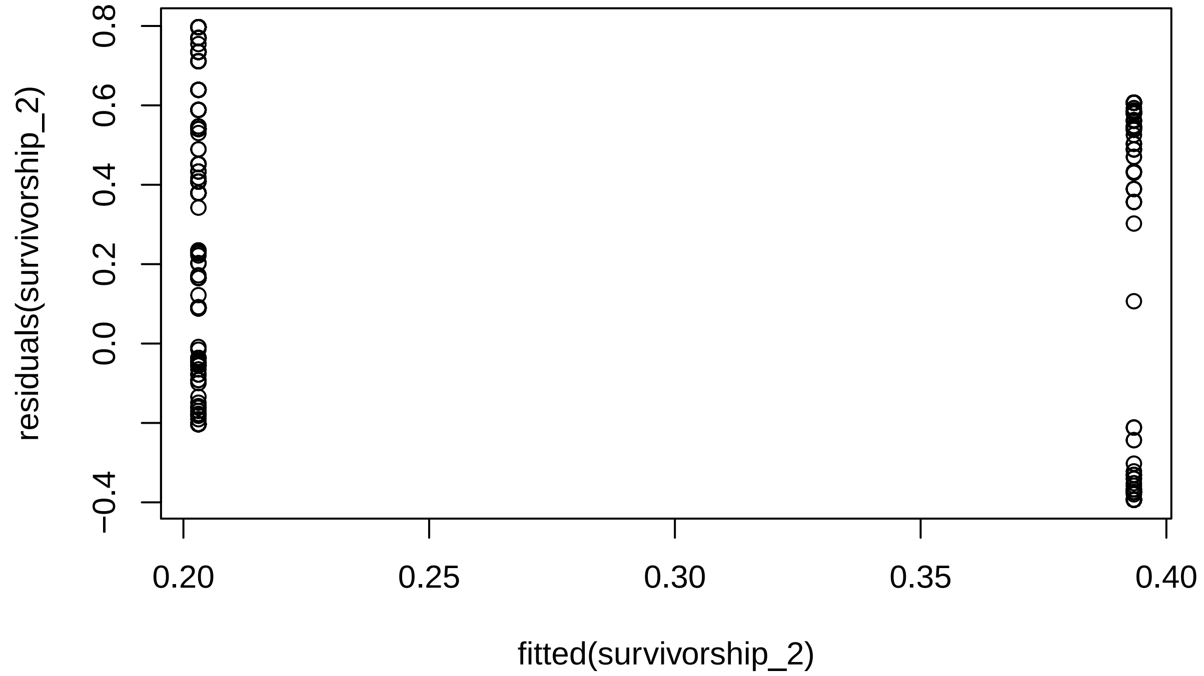
```
##
## Call:
## lm(formula = survivorship ~ treatment, data = dry2)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.3934 -0.3934 -0.2031  0.5405  0.7969
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)               0.39340    0.01464  26.874   <2e-16 ***
## treatmentMedium humidity -0.19034    0.02070  -9.194   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4148 on 1604 degrees of freedom
## Multiple R-squared:  0.05006,    Adjusted R-squared:  0.04947
## F-statistic: 84.54 on 1 and 1604 DF,  p-value: < 2.2e-16
```

```
Anova(survivorship_2)
```

```
## Anova Table (Type II tests)
```

```
##
## Response: survivorship
##             Sum Sq   Df F value    Pr(>F)
## treatment  14.546    1  84.536 < 2.2e-16 ***
## Residuals 275.999 1604
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

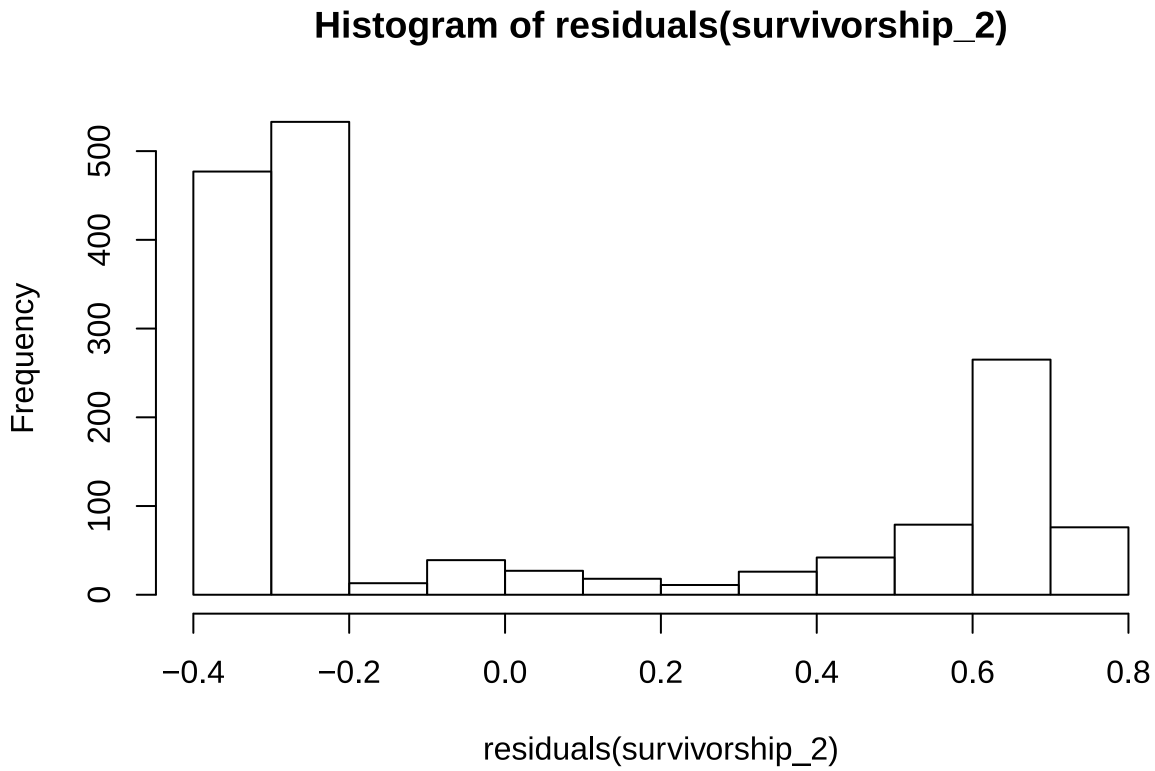
```
#Check for normality assumptions
plot(fitted(survivorship_2), residuals(survivorship_2))
```



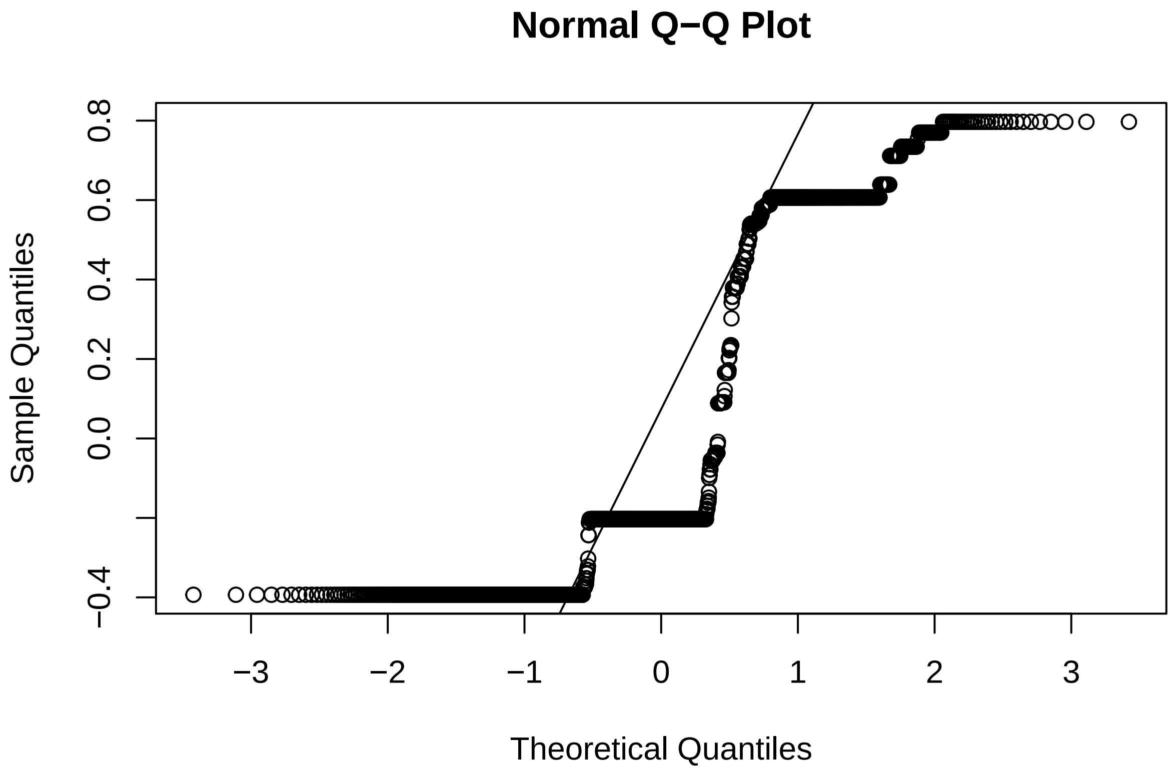
```
leveneTest(data=dry2, survivorship~treatment)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##         Df F value    Pr(>F)
## group    1  84.536 < 2.2e-16 ***
##       1604
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hist(residuals(survivorship_2))
```



```r
qqnorm(residuals(survivorship_2))
qqline(residuals(survivorship_2))
```



```
shapiro.test(residuals(survivorship_2))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(survivorship_2)
## W = 0.7797, p-value < 2.2e-16
```

Shapiro-Wilk normality test

data: residuals(survivorship_2) W = 0.77905, p-value $<$ 2.2e-16

Anova Table (Type II tests)

Response: survivorship Sum Sq Df F value Pr(>F)
treatment 14.51 1 84.416 $<$ 2.2e-16 *** Residuals 276.39 1608

Nonparametric stats here:

```
wct = wilcox.test(data=dry2, survivorship~treatment, paired = F)
wct
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  survivorship by treatment
## W = 384077, p-value = 3.261e-14
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 385758**
- **p-value = 3.636e-14**

Combined Figure 5

```
library(gridExtra)
library(cowplot)

# 1 column is 3.43 inches wide

#  @ 3.43 x 5 portrait

figure5 = plot_grid(clutch_thickness_2, egg_size_2, survivorhsip_2, labels = "AUTO", label_x = -.01, la
figure5
```

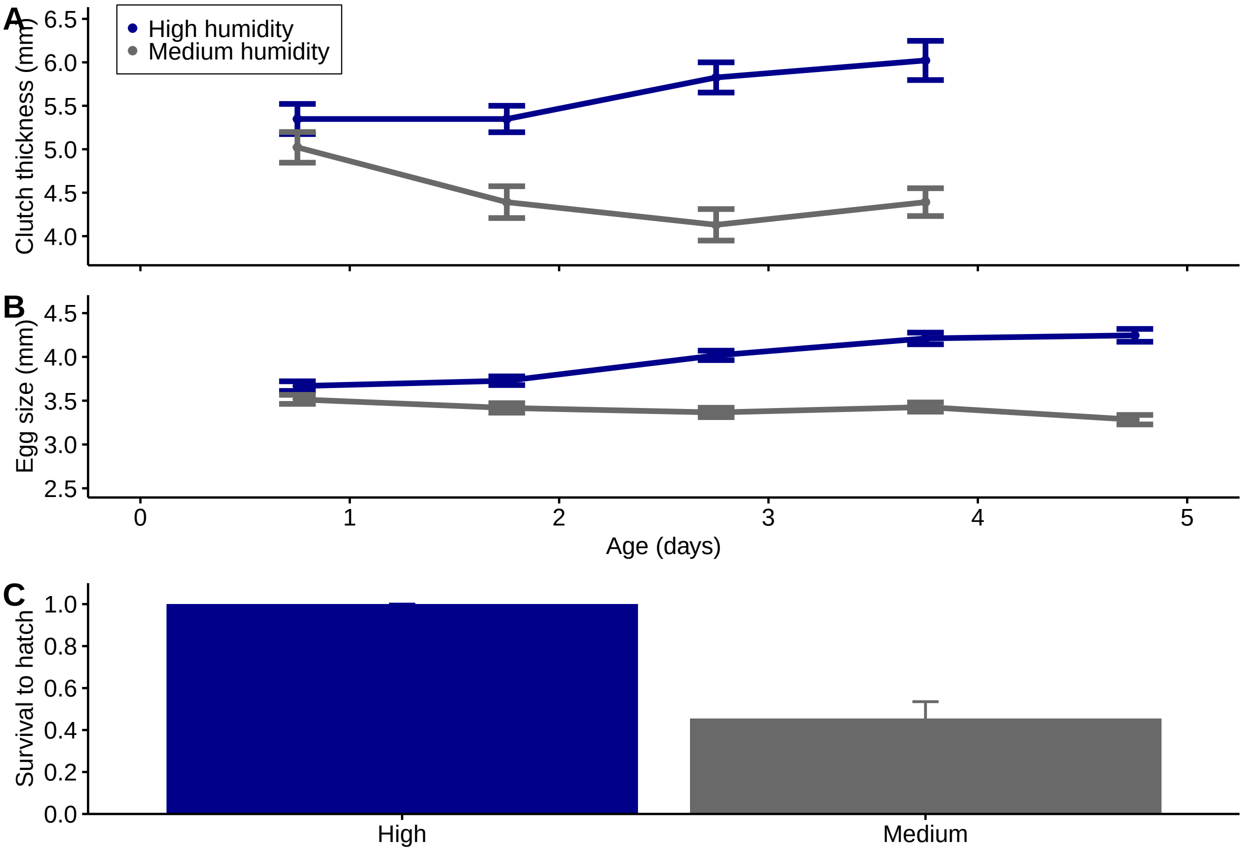


Figure 6

Hatching timing

> Here we only analyzed cluthces which had animals that hatched. Thus, hatching timing here is "out of the total that hatched" so that the y axis will be proportion hatched. We also included data starting at 4d to avoid adding 0 values with no meaning before hatching competence.

Summary statistics

```
# need to take out data with lots of 0s from 0d-4d or else stats dont hold up and get error... too 0 he
ht = dry %>%
    group_by(clutch,treatment) %>%
  mutate(total_hatched = max(hatched_accumalative), totalprop_timepointhatch = hatched_time_point/total_
  filter(total_hatched != 0, age_t > 3.9) %>%
  dplyr::select(age_t, block, clutch, treatment, accumulative_prop_hatched, hatched_time_point, hatched_
  arrange(treatment, age_t) %>%
  filter(age_t< 8.6)
ht
```

```
## # A tibble: 741 x 10
## # Groups:   clutch, treatment [39]
##    age_t block clutch treatment accumulative_pr~ hatched_time_po~
##    <dbl> <chr>  <dbl> <chr>                <dbl>            <dbl>
##  1     4 1        104 High hum~                0                0
##  2     4 1        105 High hum~                0                0
##  3     4 1        106 High hum~                0                0
##  4     4 1        107 High hum~                0                0
##  5     4 1        108 High hum~                0                0
##  6     4 1        109 High hum~                0                0
##  7     4 1        110 High hum~                0                0
##  8     4 1        111 High hum~                0                0
##  9     4 1        112 High hum~                0                0
## 10     4 1        113 High hum~                0                0
## # ... with 731 more rows, and 4 more variables: hatched_accumalative <dbl>,
## #   n_eggs <dbl>, total_hatched <dbl>, totalprop_acummhatch <dbl>
```

```
ht2 = dry %>%
    group_by(clutch,treatment,block) %>%
  mutate(total_hatched = max(hatched_accumalative), totalprop_timepointhatch = hatched_time_point/total_
  filter(total_hatched != 0, age_t > 3.9) %>%
  dplyr::select(age_t, block, clutch, treatment, accumulative_prop_hatched, hatched_time_point, hatched_
  arrange(treatment, age_t, block) %>%
  filter(age_t< 8.6)
ht2
```

```
## # A tibble: 741 x 10
## # Groups:   clutch, treatment, block [39]
##    age_t block clutch treatment accumulative_pr~ hatched_time_po~
##    <dbl> <chr>  <dbl> <chr>                <dbl>            <dbl>
##  1     4 1        104 High hum~                0                0
##  2     4 1        105 High hum~                0                0
```

```
##  3     4 1        106 High hum~                0                0
##  4     4 1        107 High hum~                0                0
##  5     4 1        108 High hum~                0                0
##  6     4 1        109 High hum~                0                0
##  7     4 1        110 High hum~                0                0
##  8     4 1        111 High hum~                0                0
##  9     4 1        112 High hum~                0                0
## 10     4 1        113 High hum~                0                0
## # ... with 731 more rows, and 4 more variables: hatched_accumalative <dbl>,
## #   n_eggs <dbl>, total_hatched <dbl>, totalprop_acummhatch <dbl>
```

```
dmeanprophatch = ht %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(totalprop_acummhatch, na.rm=T), SE= sd(totalprop_acummhatch, na.rm=T)/sqrt(leng
  na.omit()
dmeanprophatch
```

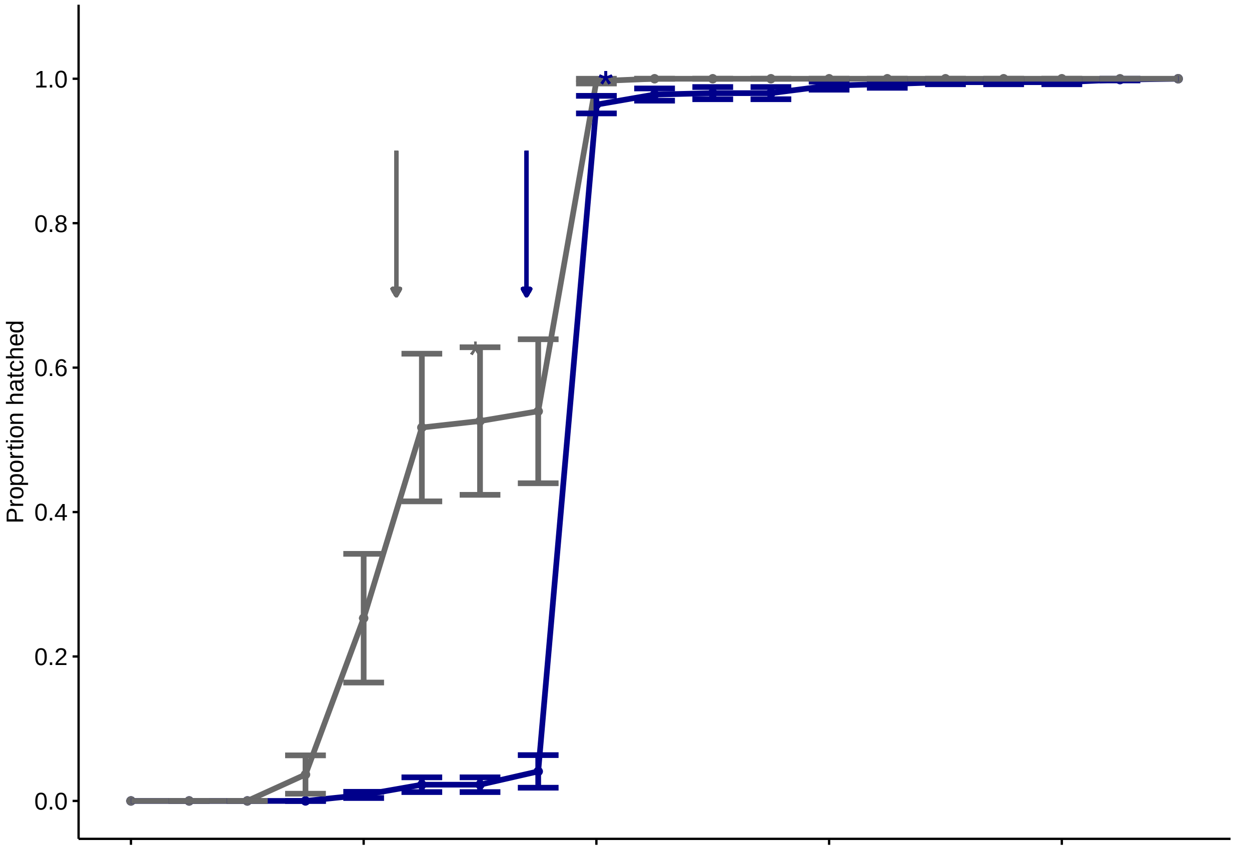
```
## # A tibble: 38 x 8
## # Groups:   treatment [2]
##    treatment     age_t    mean      SE     SD   min    max sample_size
##    <chr>         <dbl>   <dbl>   <dbl>  <dbl> <dbl>  <dbl>       <int>
##  1 High humidity  4    0       0       0      0     0                23
##  2 High humidity  4.25 0       0       0      0     0                23
##  3 High humidity  4.5  0       0       0      0     0                23
##  4 High humidity  4.75 0       0       0      0     0                23
##  5 High humidity  5    0.00832 0.00431 0.0206 0     0.0909           23
##  6 High humidity  5.25 0.0225  0.0102  0.0489 0     0.182            23
##  7 High humidity  5.5  0.0225  0.0102  0.0489 0     0.182            23
##  8 High humidity  5.75 0.0409  0.0226  0.108  0     0.5              23
##  9 High humidity  6    0.964   0.0122  0.0583 0.824 1                23
## 10 High humidity  6.25 0.978   0.00844 0.0405 0.857 1                23
## # ... with 28 more rows
```

```
dmeanprophatch2 = ht %>%
  group_by(treatment, age_t, block) %>%
  summarise(mean = mean(totalprop_acummhatch, na.rm=T), SE= sd(totalprop_acummhatch, na.rm=T)/sqrt(leng
  na.omit()
dmeanprophatch2
```

```
## # A tibble: 76 x 9
## # Groups:   treatment, age_t [38]
##    treatment     age_t block  mean      SE     SD   min    max sample_size
##    <chr>         <dbl> <chr>  <dbl>   <dbl>  <dbl> <dbl>  <dbl>       <int>
##  1 High humidity  4    1     0      0       0          0 0               12
##  2 High humidity  4    2     0      0       0          0 0               11
##  3 High humidity  4.25 1     0      0       0          0 0               12
##  4 High humidity  4.25 2     0      0       0          0 0               11
##  5 High humidity  4.5  1     0      0       0          0 0               12
##  6 High humidity  4.5  2     0      0       0          0 0               11
##  7 High humidity  4.75 1     0      0       0          0 0               12
##  8 High humidity  4.75 2     0      0       0          0 0               11
##  9 High humidity  5    1     0      0       0          0 0               12
## 10 High humidity  5    2     0.0174 0.00833 0.0276     0 0.0909          11
## # ... with 66 more rows
```

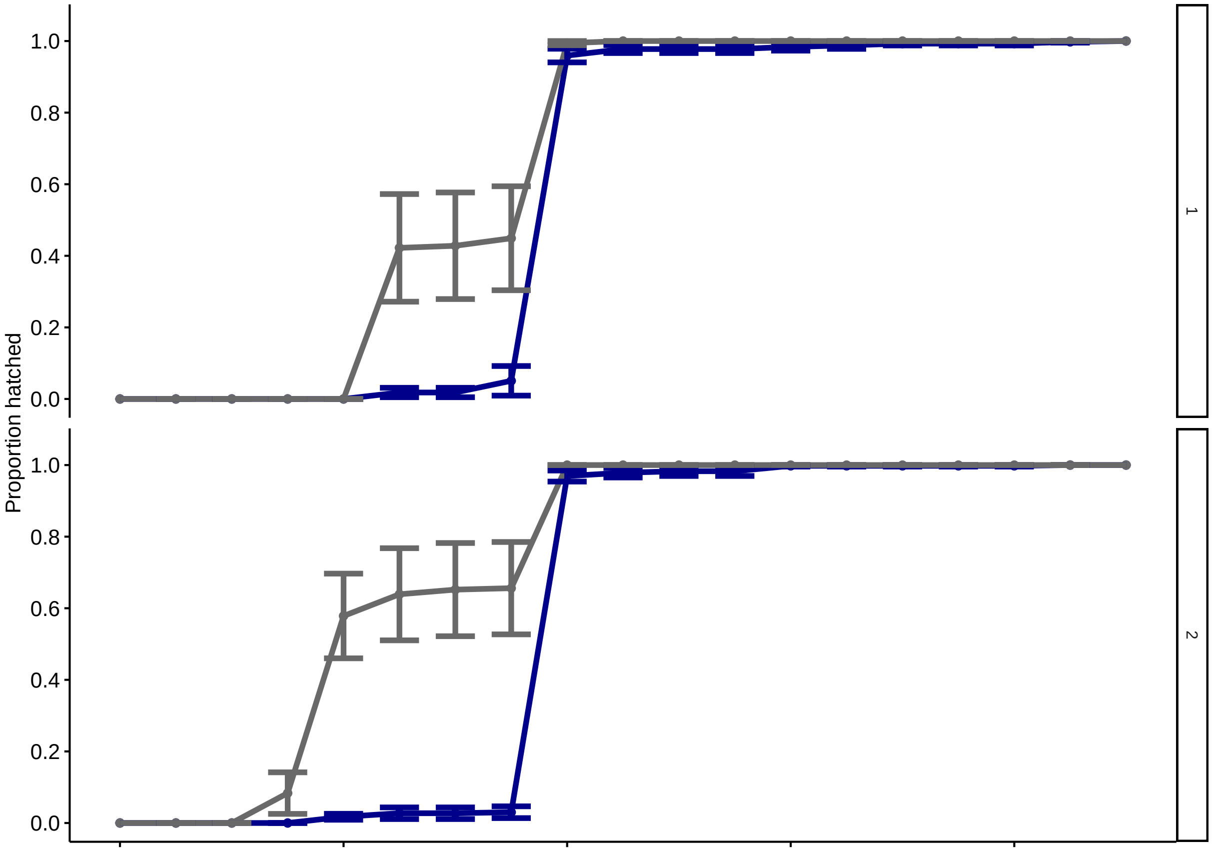
Figure

```
library(ggplot2)
library(scales)

hatching_timing = ggplot(dmeanprophatch, aes(x=age_t, y=mean)) + #add the axis
  geom_point(aes(color = treatment), size = 1) +
  geom_line(aes(color = treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Proportion hatched", x="Age (days)") + #creat labels...
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black"))
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.position= "none") +
  scale_x_continuous(limits=c(4,8.5),breaks= pretty_breaks(n=6)) + # allows for making "pretty" breaks
  scale_y_continuous(limits=c(0,1.05), breaks= pretty_breaks(n=6)) + # allows for making "pretty" break
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray")) +
  geom_segment(aes(x = 5.7, y = .9, xend = 5.7, yend = 0.7),
               arrow = arrow(length = unit(0.1, "cm"), type = "closed"), color = "darkblue", size=.7
  geom_segment(aes(x = 5.14, y = .9, xend = 5.14, yend = .7),
               arrow = arrow(length = unit(0.1, "cm"), type = "closed"), color = "dimgray", size=.7)
  geom_text(aes(y= 0.6, x = 5.48, label= "*"), color= "dimgray", vjust = 0, size = 5) +  #add significa
  geom_text(aes(y= 0.975, x = 6.04, label= "*"), color= "darkblue", vjust = 0, size = 5)
hatching_timing
```



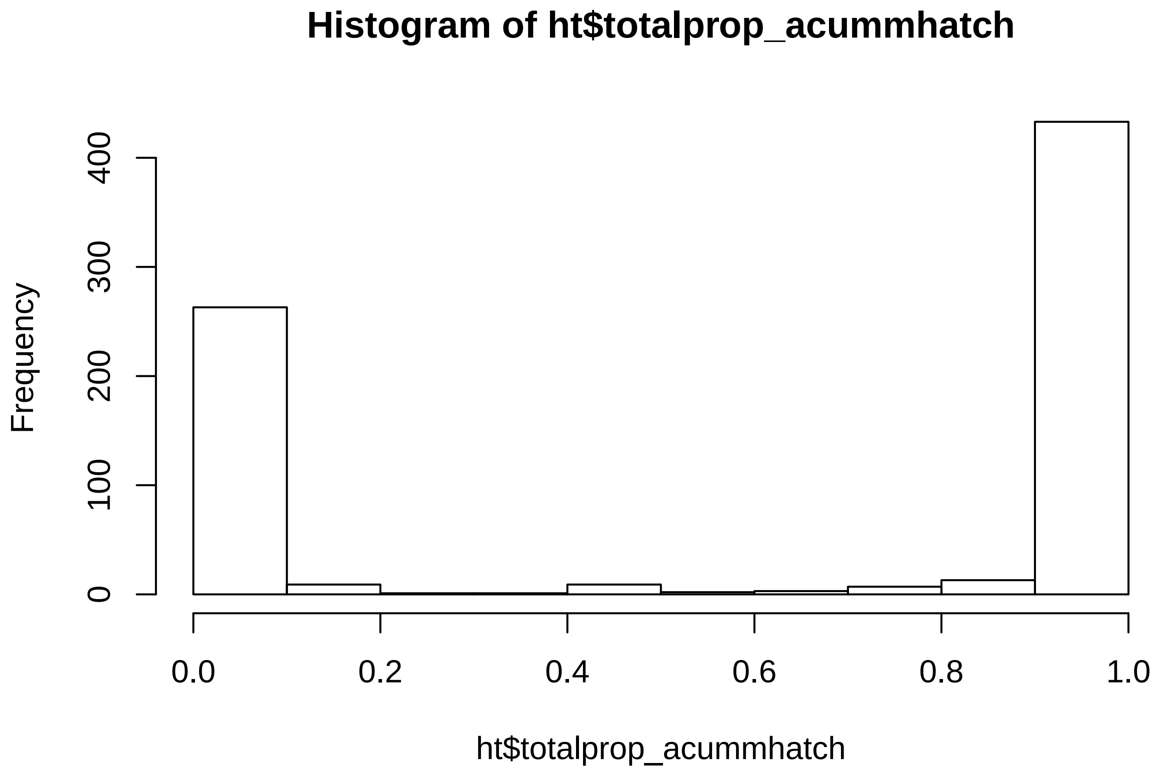
Hatching timing visualized by test block

```r
hatching_timing2 = ggplot(dmeanprophatch2, aes(x=age_t, y=mean)) + #add the axis
  geom_point(aes(color = treatment), size = 1) +
  geom_line(aes(color = treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="\nProportion hatched", x="Age (days)") + #creat labels...
  theme_classic(base_size = 8) +
  theme(legend.text = element_text(size = 8), legend.title = element_blank(), legend.background = elemen
  theme(axis.title.x = element_blank(), axis.text.x = element_blank()) +
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color
  scale_x_continuous(limits=c(4,8.5),breaks= pretty_breaks(n=6)) + # allows for making "pretty" breaks
  scale_y_continuous(limits=c(0,1.05), breaks= pretty_breaks(n=6)) + # allows for making "pretty" break
  scale_color_manual(legend_title, values = c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values = c("darkblue", "dimgray")) +
  facet_grid(rows = vars(block), scales = "free")
hatching_timing2
```



Binomial GLMM

```r
library(lme4)
library(car)
library(lmerTest)

hist(ht$totalprop_acummhatch)
```



```
#all models
hatchingtiming_full = glmer(totalprop_acummhatch ~ treatment*age_t + (1|clutch), family = "binomial", d
hatchingtiming_nointeraction = glmer(totalprop_acummhatch ~ treatment + age_t + (1|clutch), family = "b
hatchingtiming_notreatment = glmer(totalprop_acummhatch ~ age_t + (1|clutch), family = "binomial", data
hatchingtiming_noage = glmer(totalprop_acummhatch ~ treatment + (1|clutch), family = "binomial", data =

hatchingtiming_full2 = glmer(totalprop_acummhatch ~ treatment*age_t + (1|clutch) + (1|block), family =

#AIC comparison
library(AICcmodavg)
print(aictab(list(hatchingtiming_full, hatchingtiming_full2, hatchingtiming_nointeraction, hatchingtimi
```

```
##
## Model selection based on AICc:
##
##                              K     AICc Delta_AICc AICcWt Cum.Wt
## hatchingtiming_full          5  2529.22       0.00   0.63   0.63
## hatchingtiming_full2         6  2530.24       1.03   0.37   1.00
## hatchingtiming_nointeraction 4  2890.27     361.06   0.00   1.00
## hatchingtiming_notreatment   3  3845.65    1316.44   0.00   1.00
## hatchingtiming_noage         3 24967.12   22437.91   0.00   1.00
```

Model without block random effect is best model!!!

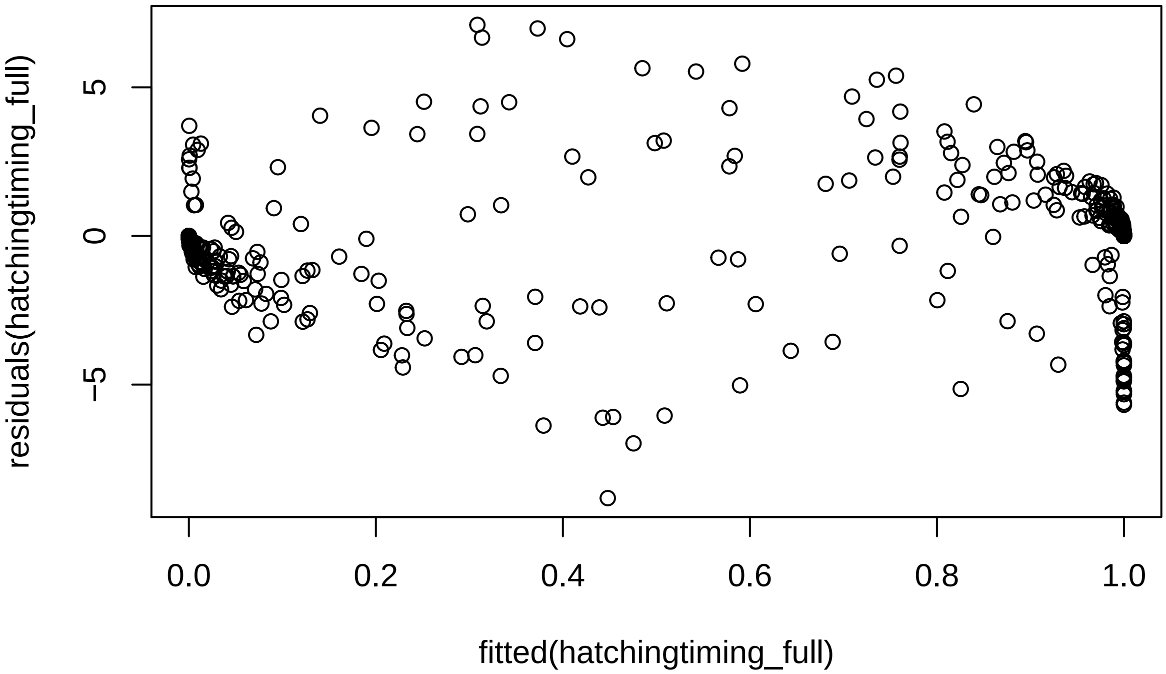
```r
summary(hatchingtiming_full)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: totalprop_acummhatch ~ treatment * age_t + (1 | clutch)
##    Data: ht
## Weights: total_hatched
##
##      AIC      BIC   logLik deviance df.resid
##   2529.1   2552.2  -1259.6   2519.1      736
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -5225.1    -0.1     0.0     0.1    12.6
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  clutch (Intercept) 1.045    1.022
## Number of obs: 741, groups:  clutch, 23
##
## Fixed effects:
##                                 Estimate Std. Error z value Pr(>|z|)
## (Intercept)                    -55.27663    0.18358  -301.1   <2e-16 ***
## treatmentMedium humidity        30.93242    0.19397   159.5   <2e-16 ***
## age_t                            9.38654    0.04450   210.9   <2e-16 ***
## treatmentMedium humidity:age_t  -4.89814    0.03782  -129.5   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) trtmMh age_t
## trtmntMdmhm -0.242
## age_t       -0.601  0.241
## trtmntMhm:_  0.171 -0.906 -0.191
```

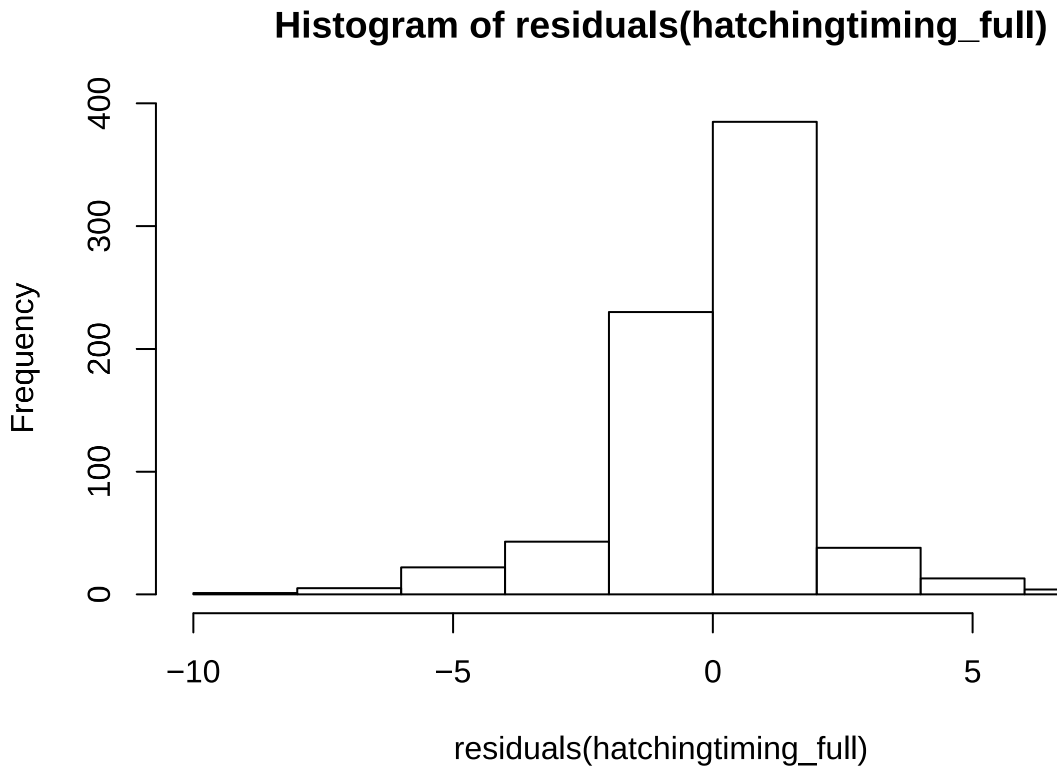
```r
Anova(hatchingtiming_full)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: totalprop_acummhatch
##                 Chisq Df Pr(>Chisq)
## treatment        9926  1  < 2.2e-16 ***
## age_t           35972  1  < 2.2e-16 ***
## treatment:age_t 16772  1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

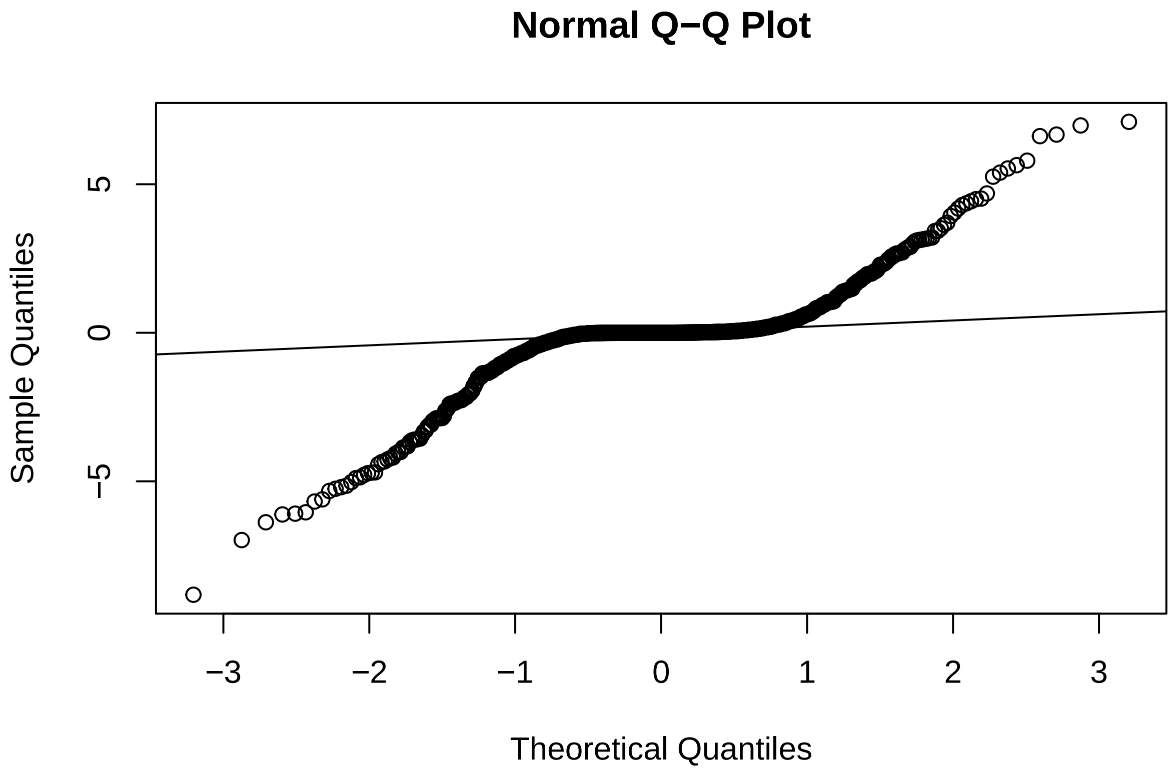
```r
plot(fitted(hatchingtiming_full), residuals(hatchingtiming_full))
```



```r
hist(residuals(hatchingtiming_full))
```



```
qqnorm(residuals(hatchingtiming_full))
qqline(residuals(hatchingtiming_full))
```



```
shapiro.test(residuals(hatchingtiming_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(hatchingtiming_full)
## W = 0.7996, p-value < 2.2e-16
```

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: totalprop_acummhatch Chisq Df Pr(>Chisq)
treatment 9926 1 < 2.2e-16 ***age_t 35972 1 < 2.2e-16*** treatment:age_t 16772 1 < 2.2e-16 ***

Likelihood ratio tests

```
anova(hatchingtiming_full, hatchingtiming_nointeraction, test = "Chisq")
```

```
## Data: ht
## Models:
## hatchingtiming_nointeraction: totalprop_acummhatch ~ treatment + age_t + (1 | clutch)
## hatchingtiming_full: totalprop_acummhatch ~ treatment * age_t + (1 | clutch)
##                              npar    AIC    BIC  logLik deviance  Chisq Df
## hatchingtiming_nointeraction    4 2890.2 2908.7 -1441.1   2882.2
## hatchingtiming_full             5 2529.1 2552.2 -1259.6   2519.1 363.08  1
##                              Pr(>Chisq)
```

```
## hatchingtiming_nointeraction
## hatchingtiming_full           < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(hatchingtiming_full, hatchingtiming_notreatment, test = "Chisq")
```

```
## Data: ht
## Models:
## hatchingtiming_notreatment: totalprop_acummhatch ~ age_t + (1 | clutch)
## hatchingtiming_full: totalprop_acummhatch ~ treatment * age_t + (1 | clutch)
##                            npar    AIC    BIC  logLik deviance  Chisq Df
## hatchingtiming_notreatment    3 3845.6 3859.4 -1919.8   3839.6
## hatchingtiming_full           5 2529.1 2552.2 -1259.6   2519.1 1320.5  2
##                            Pr(>Chisq)
## hatchingtiming_notreatment
## hatchingtiming_full         < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(hatchingtiming_full, hatchingtiming_noage, test = "Chisq")
```

```
## Data: ht
## Models:
## hatchingtiming_noage: totalprop_acummhatch ~ treatment + (1 | clutch)
## hatchingtiming_full: totalprop_acummhatch ~ treatment * age_t + (1 | clutch)
##                      npar     AIC     BIC   logLik deviance Chisq Df Pr(>Chisq)
## hatchingtiming_noage    3 24967.1 24980.9 -12480.5  24961.1
## hatchingtiming_full     5  2529.1  2552.2  -1259.6   2519.1 22442  2  < 2.2e-16
##
## hatchingtiming_noage
## hatchingtiming_full  ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X = 363.08; df = 1**
- **p-value < 2.2e-16**

EFFECT OF TREATMENT:

- **X = 1320.5; df = 2**
- **p-value < 2.2e-16**

EFFECT OF AGE:

- **X = 22442; df = 2**
- **p-value < 2.2e-16**

Mean hatching age

```
meanhatchingagesum = ht %>%
  group_by(treatment, clutch) %>%
  filter(hatched_time_point != 0) %>%
  summarise(meanhatchingage = mean(age_t))
meanhatchingagesum
```

```
## # A tibble: 39 x 3
## # Groups:   treatment [2]
##    treatment     clutch meanhatchingage
##    <chr>          <dbl>           <dbl>
##  1 High humidity    104            6.5
##  2 High humidity    105            7
##  3 High humidity    106            5.88
##  4 High humidity    107            6
##  5 High humidity    108            6
##  6 High humidity    109            5.67
##  7 High humidity    110            6.92
##  8 High humidity    111            6
##  9 High humidity    112            6
## 10 High humidity    113            6.5
## # ... with 29 more rows
```

```
meanhatchingagesumsum = meanhatchingagesum %>%
  group_by(treatment) %>%
  summarise(mean = mean(meanhatchingage, na.rm=T), SE= sd(meanhatchingage, na.rm=T)/sqrt(length(meanhat
  na.omit()
meanhatchingagesumsum
```

```
## # A tibble: 2 x 7
##   treatment        mean     SE    SD   min   max sample_size
##   <chr>           <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>
## 1 High humidity    6.04 0.0975 0.468  5.42     7          23
## 2 Medium humidity  5.48 0.0745 0.298  5        6          16
```

```
meanhatchingagesum2 = ht %>%
  group_by(treatment, clutch, block) %>%
  filter(hatched_time_point != 0) %>%
  summarise(meanhatchingage = mean(age_t))
meanhatchingagesum2
```

```
## # A tibble: 39 x 4
## # Groups:   treatment, clutch [39]
##    treatment     clutch block meanhatchingage
##    <chr>          <dbl> <chr>           <dbl>
##  1 High humidity    104 1                6.5
##  2 High humidity    105 1                7
##  3 High humidity    106 1                5.88
##  4 High humidity    107 1                6
##  5 High humidity    108 1                6
```

```
##   6 High humidity    109 1                   5.67
##   7 High humidity    110 1                   6.92
##   8 High humidity    111 1                   6
##   9 High humidity    112 1                   6
##  10 High humidity    113 1                   6.5
## # ... with 29 more rows
```

```
meanhatchingagesumsum2 = meanhatchingagesum2 %>%
  group_by(treatment, block) %>%
  summarise(mean = mean(meanhatchingage, na.rm=T), SE= sd(meanhatchingage, na.rm=T)/sqrt(length(meanhat
  na.omit()
meanhatchingagesumsum2
```

```
## # A tibble: 4 x 8
## # Groups:   treatment [2]
##   treatment       block  mean     SE    SD   min   max sample_size
##   <chr>           <chr> <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>
## 1 High humidity   1      6.17 0.130  0.452  5.62  7             12
## 2 High humidity   2      5.90 0.139  0.463  5.42  6.88          11
## 3 Medium humidity 1      5.62 0.0944 0.283  5.25  6              9
## 4 Medium humidity 2      5.30 0.0856 0.227  5     5.75           7
```

TOTAL

- *mean hatching age high* = 6.043478 +/- 0.09750533 N = 23
- *mean hatching age medium* = 5.479167 +/- 0.07453560 N =16

  1 - 5.479167/6.043478 = 0.09337521 = ~9.3%

BLOCK 1

- *mean hatching age high* = 6.173611 +/- 0.4518597
- range=5.625000—7.000, N = 12
- *mean hatching age medium* = 5.615741 +/- 0.2831937
- range=5.250000—6.000, N = 9

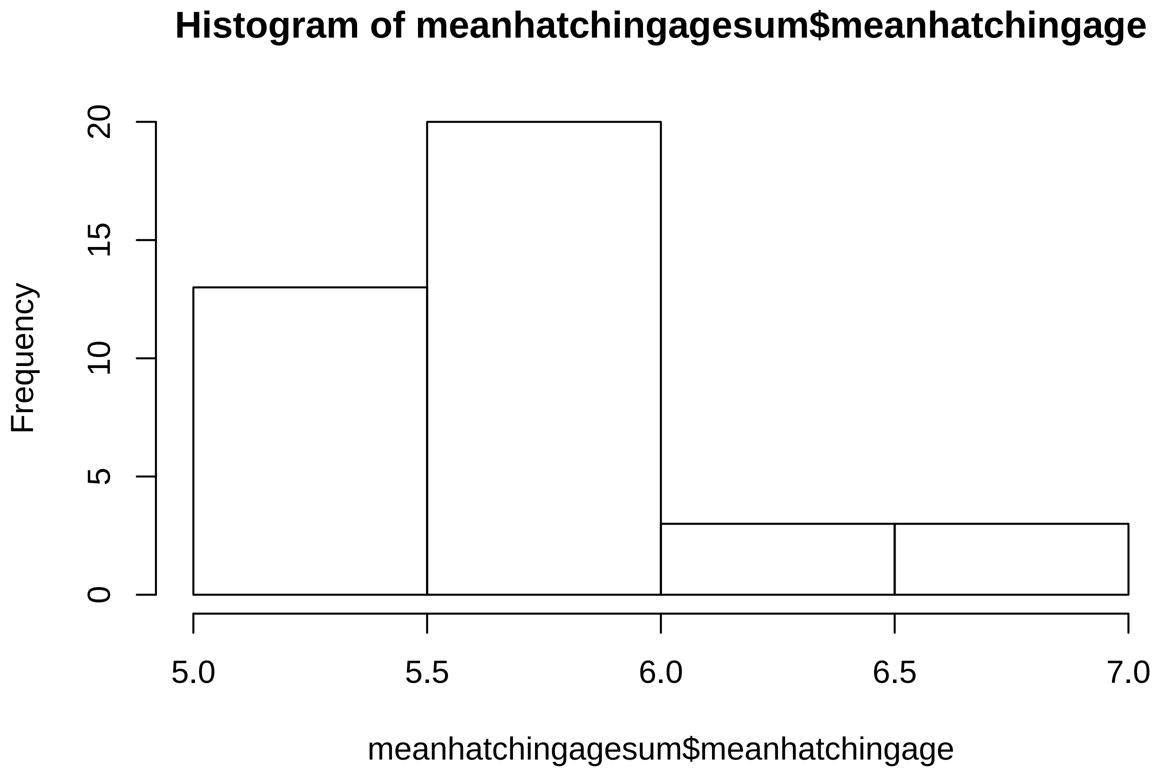
BLOCK 2

- *mean hatching age high* = 5.901515 +/- 0.4625836
- range=5.416667—6.875, N = 11
- *mean hatching age medium* = 5.303571 +/- 0.2265817
- range=5.000000—5.750, N = 7

STATS

ANOVA

```
library(lme4)
library(car)
library(lmerTest)

hist(meanhatchingagesum$meanhatchingage)
```



```
mean_hatching_age = lm(data=meanhatchingagesum, meanhatchingage~treatment)
summary(mean_hatching_age)
```

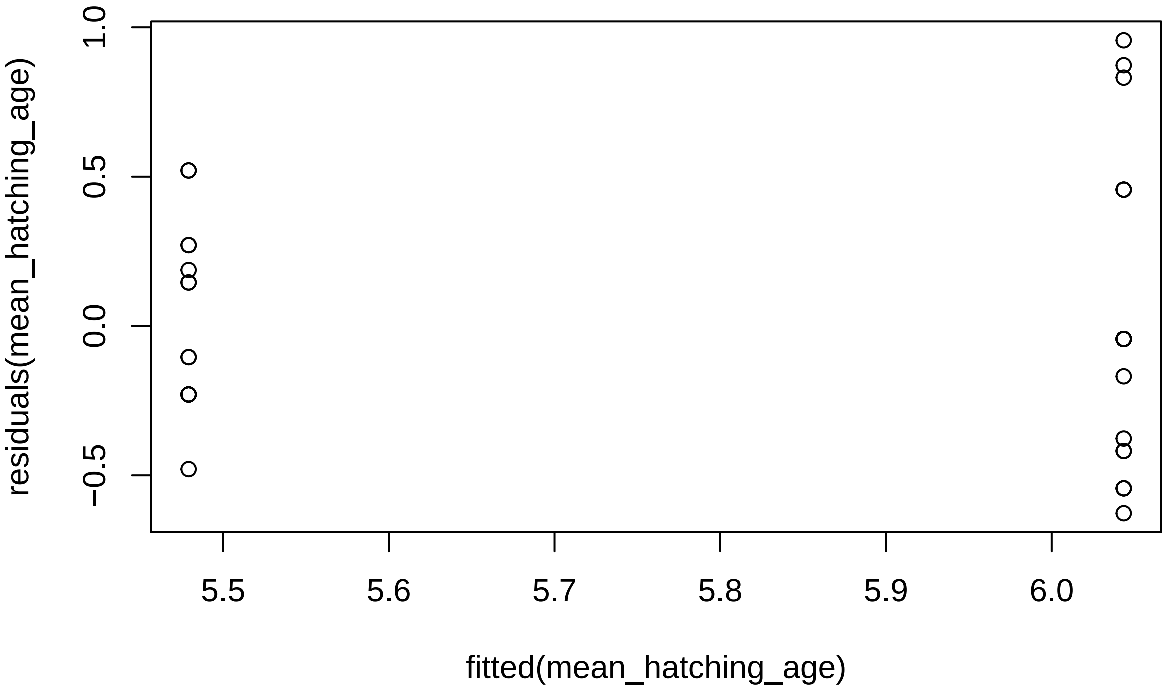
```
##
## Call:
## lm(formula = meanhatchingage ~ treatment, data = meanhatchingagesum)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.62681 -0.22917 -0.04348  0.22917  0.95652
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)               6.04348    0.08497  71.126  < 2e-16 ***
## treatmentMedium humidity -0.56431    0.13266  -4.254 0.000137 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4075 on 37 degrees of freedom
## Multiple R-squared:  0.3284, Adjusted R-squared:  0.3103
## F-statistic:  18.1 on 1 and 37 DF,  p-value: 0.0001372
```

```
Anova(mean_hatching_age)
```

```
## Anova Table (Type II tests)
```

```
##
## Response: meanhatchingage
##           Sum Sq Df F value    Pr(>F)
## treatment 3.0048  1  18.096 0.0001372 ***
## Residuals 6.1440 37
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

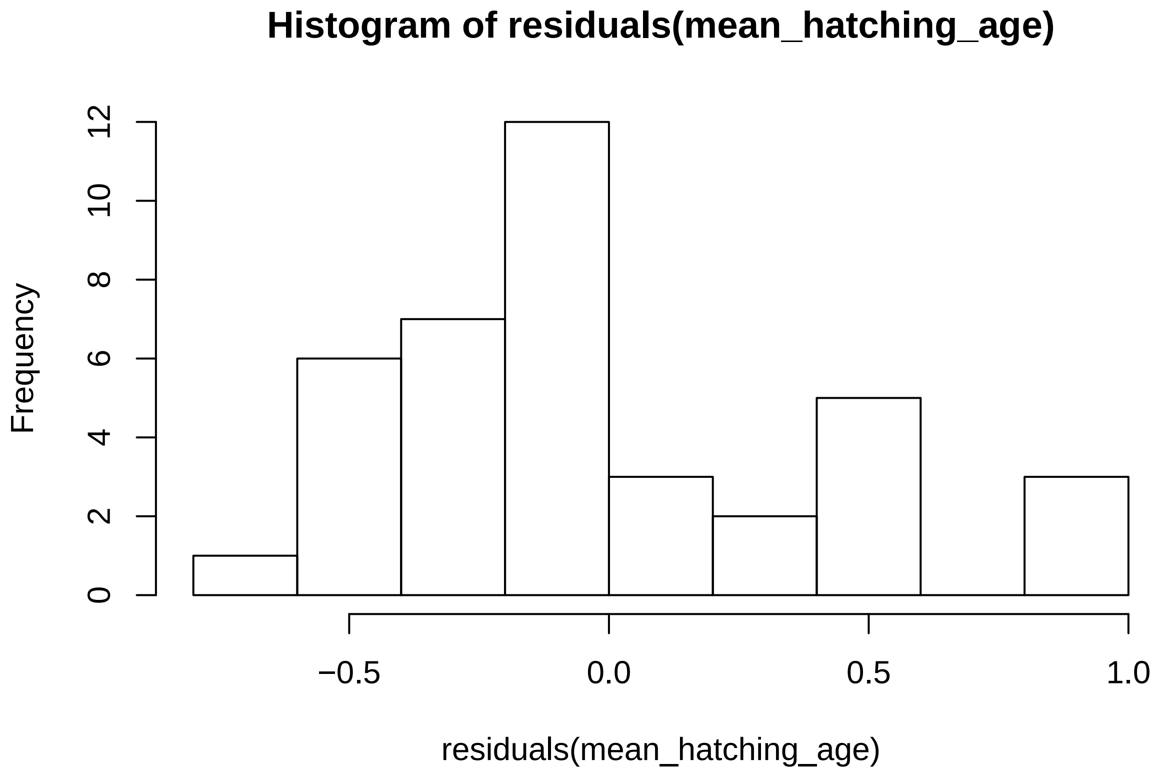
```
#Check for normality assumptions:
#homogeneity of variance assumption
plot(fitted(mean_hatching_age), residuals(mean_hatching_age))
```



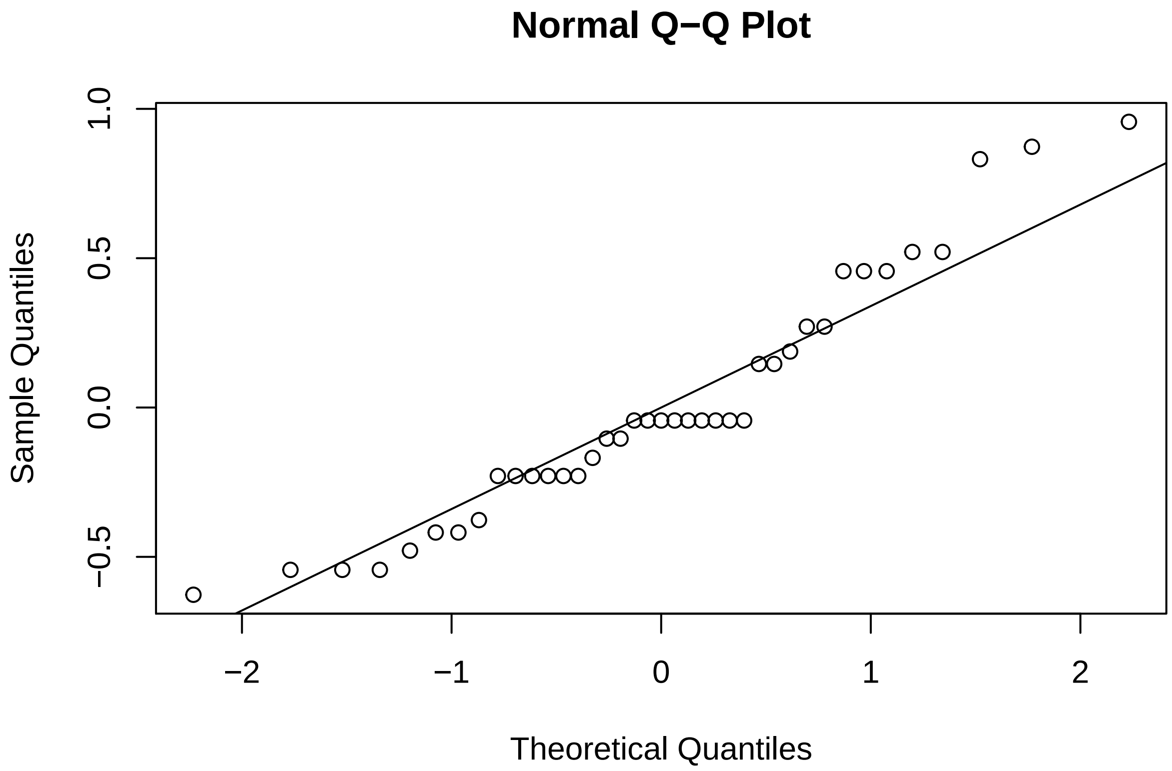
```
leveneTest(data=meanhatchingagesum, meanhatchingage~treatment)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.8675 0.3577
##       37
```

```
#normality assumption
hist(residuals(mean_hatching_age))
```



```r
qqnorm(residuals(mean_hatching_age))
qqline(residuals(mean_hatching_age))
```



```r
shapiro.test(residuals(mean_hatching_age))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(mean_hatching_age)
## W = 0.93767, p-value = 0.0319
```

Shapiro-Wilk normality test

data: residuals(mean_hatching_age) W = 0.93767, p-value = 0.0319

Anova Table (Type II tests)

Response: meanhatchingage Sum Sq Df F value Pr(>F)
treatment 3.0048 1 18.096 0.0001372 *** Residuals 6.1440 37

Nonparametric stats here:

```r
wct = wilcox.test(data=meanhatchingagesum, meanhatchingage~treatment, paired = F)
wct
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  meanhatchingage by treatment
## W = 312.5, p-value = 0.0002099
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 312.5**
- **p-value = 0.0002099**

Mean age at hatching onset

```
hatchingageatonset = ht %>%
  group_by(treatment, clutch) %>%
  filter(hatched_time_point != 0) %>% # get only data with hatching positive
  filter(age_t == min(age_t)) # get only first age with hatching per clutch
hatchingageatonset
```

```
## # A tibble: 39 x 10
## # Groups:   treatment, clutch [39]
##    age_t block clutch treatment accumulative_pr~ hatched_time_po~
##    <dbl> <chr>  <dbl> <chr>                <dbl>            <dbl>
##  1  5    2        117 High hum~           0.0192                1
##  2  5    2        122 High hum~           0.0909                1
##  3  5    2        124 High hum~           0.025                 1
##  4  5    2        125 High hum~           0.0192                1
##  5  5    2        127 High hum~           0.0351                2
##  6  5.25 1        109 High hum~           0.15                  3
##  7  5.25 1        114 High hum~           0.0625                1
##  8  5.75 1        106 High hum~           0.0417                1
##  9  5.75 2        126 High hum~           0.0132                1
## 10  6    1        104 High hum~           0.696                16
## # ... with 29 more rows, and 4 more variables: hatched_accumalative <dbl>,
## #   n_eggs <dbl>, total_hatched <dbl>, totalprop_acummhatch <dbl>
```

```
hatchingageatonsetsum = hatchingageatonset %>%
  group_by(treatment) %>%
  summarise(mean = mean(age_t, na.rm=T), SE= sd(age_t, na.rm=T)/sqrt(length(age_t)), SD = sd(age_t, na.
  na.omit()
hatchingageatonsetsum
```

```
## # A tibble: 2 x 7
##   treatment        mean     SE    SD   min   max sample_size
##   <chr>           <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>
## 1 High humidity    5.70 0.0902 0.433  5        6          23
## 2 Medium humidity  5.14 0.0853 0.341  4.75     6          16
```

```r
hatchingageatonset2 = ht %>%
  group_by(treatment, block, clutch) %>%
  filter(hatched_time_point != 0) %>% # get only data with hatching positive
  filter(age_t == min(age_t)) # get only first age with hatching per clutch
hatchingageatonset2
```

```
## # A tibble: 39 x 10
## # Groups:   treatment, block, clutch [39]
##    age_t block clutch treatment accumulative_pr~ hatched_time_po~
##    <dbl> <chr>  <dbl> <chr>                <dbl>            <dbl>
##  1  5    2        117 High hum~           0.0192                1
##  2  5    2        122 High hum~           0.0909                1
##  3  5    2        124 High hum~           0.025                 1
##  4  5    2        125 High hum~           0.0192                1
##  5  5    2        127 High hum~           0.0351                2
##  6  5.25 1        109 High hum~           0.15                  3
##  7  5.25 1        114 High hum~           0.0625                1
##  8  5.75 1        106 High hum~           0.0417                1
##  9  5.75 2        126 High hum~           0.0132                1
## 10  6    1        104 High hum~           0.696                16
## # ... with 29 more rows, and 4 more variables: hatched_accumalative <dbl>,
## #   n_eggs <dbl>, total_hatched <dbl>, totalprop_acummhatch <dbl>
```

```r
hatchingageatonsetsum2 = hatchingageatonset2 %>%
  group_by(treatment, block) %>%
  summarise(mean = mean(age_t, na.rm=T), SE= sd(age_t, na.rm=T)/sqrt(length(age_t)), SD = sd(age_t, na.
  na.omit()
hatchingageatonsetsum2
```

```
## # A tibble: 4 x 8
## # Groups:   treatment [2]
##   treatment       block  mean     SE    SD   min   max sample_size
##   <chr>           <chr> <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>
## 1 High humidity   1      5.85 0.0840 0.291  5.25   6             12
## 2 High humidity   2      5.52 0.152  0.506  5      6             11
## 3 Medium humidity 1      5.33 0.0833 0.25   5.25   6              9
## 4 Medium humidity 2      4.89 0.107  0.283  4.75   5.5            7
```

TOTAL

- *mean hatching onset age high* = 5.70 +/- 0.4327 N = 23
- *mean hatching onset age medium* = 5.14 +/- 0.3411 N =16

BLOCK 1

- *mean hatching onset age high* = 5.854167 +/- 0.2911250
- range=5.25—6.0, N = 12
- *mean hatching onset age medium* = 5.333333 +/- 0.2500000
- range=5.25—6.0, N = 9

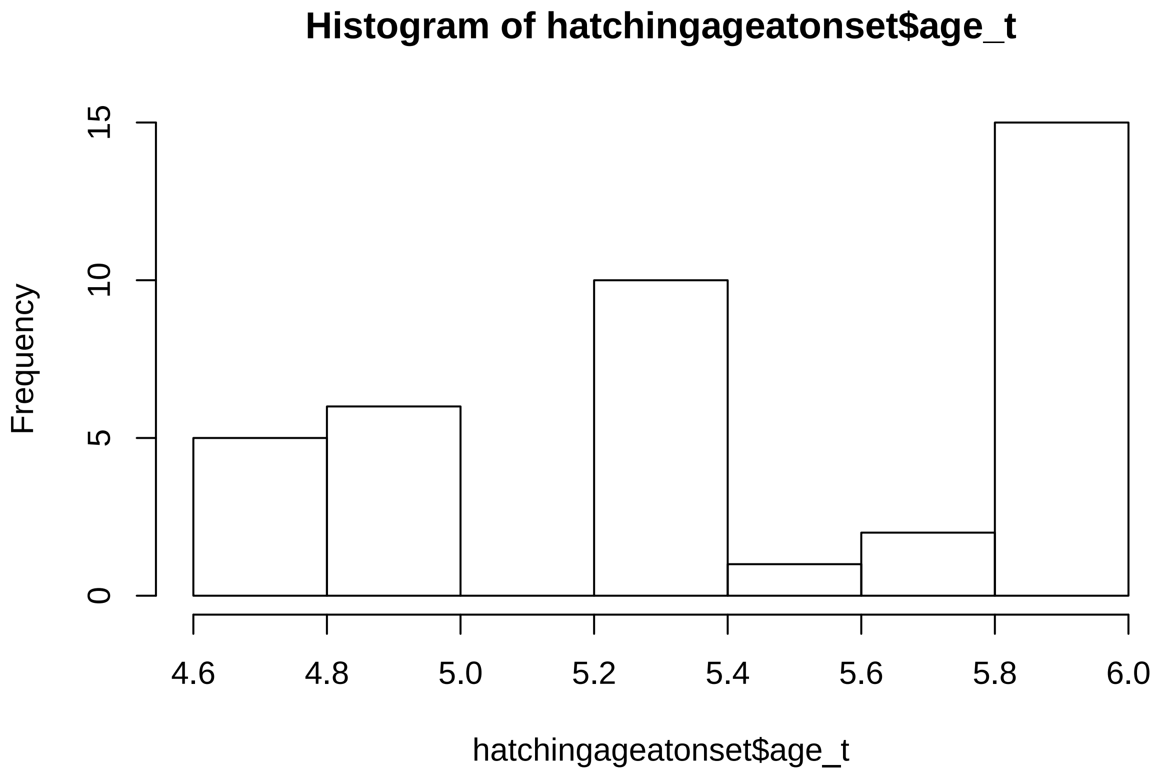
BLOCK 2

- *mean hatching onset age high* = 5.522727 +/- 0.5056499
- range=5.00—6.0, N = 11
- *mean hatching onset age medium* = 4.892857 +/- 0.2834734
- range=4.75—5.5, N = 7

STATS

ANOVA

```
library(lme4)
library(car)
library(lmerTest)

hist(hatchingageatonset$age_t)
```



```
mean_hatching_age = lm(data=hatchingageatonset, age_t~treatment)
summary(mean_hatching_age)
```

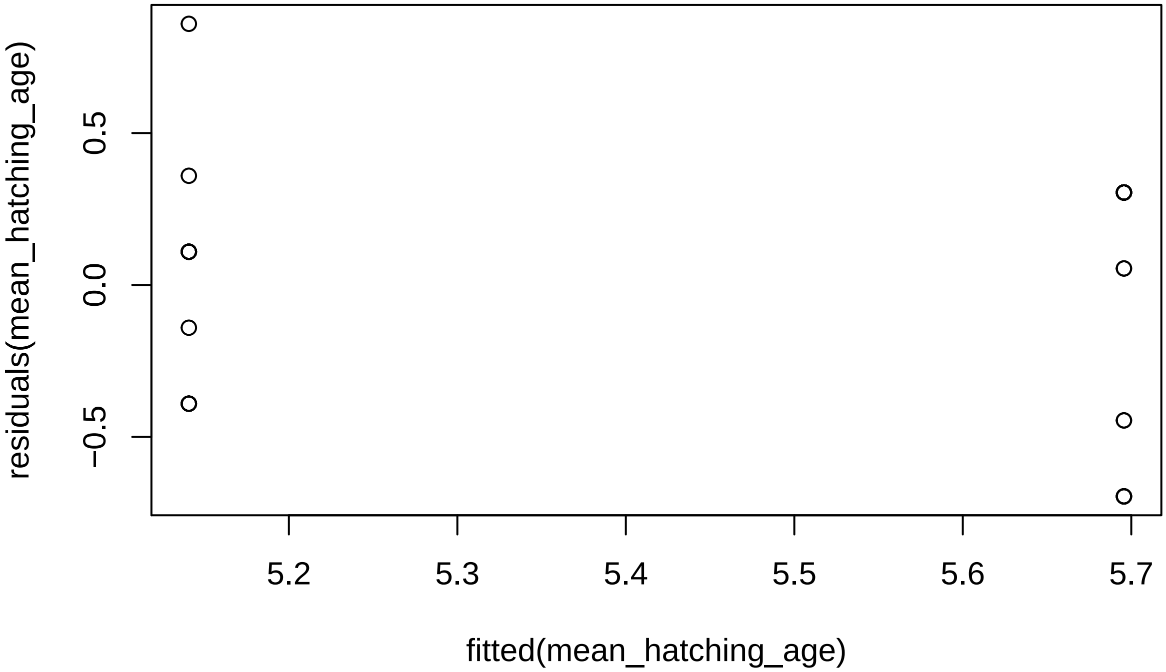
```
##
## Call:
## lm(formula = age_t ~ treatment, data = hatchingageatonset)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.6956 -0.3906  0.1094  0.3044  0.8594
```

```
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)               5.69565    0.08302  68.604  < 2e-16 ***
## treatmentMedium humidity -0.55503    0.12962  -4.282 0.000126 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3982 on 37 degrees of freedom
## Multiple R-squared:  0.3314, Adjusted R-squared:  0.3133
## F-statistic: 18.34 on 1 and 37 DF,  p-value: 0.0001261
```

```
Anova(mean_hatching_age)
```

```
## Anova Table (Type II tests)
##
## Response: age_t
##           Sum Sq Df F value    Pr(>F)
## treatment 2.9068  1  18.336 0.0001261 ***
## Residuals 5.8657 37
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

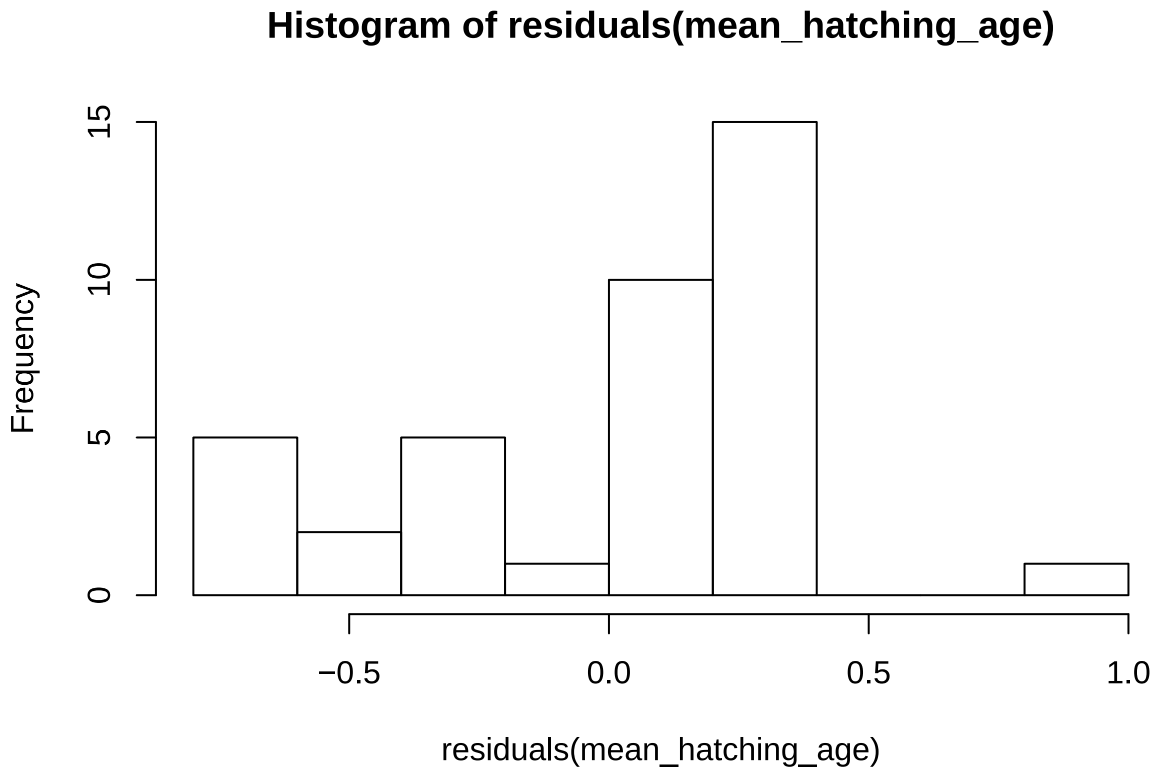
```
#Check for normality assumptions
plot(fitted(mean_hatching_age), residuals(mean_hatching_age))
```



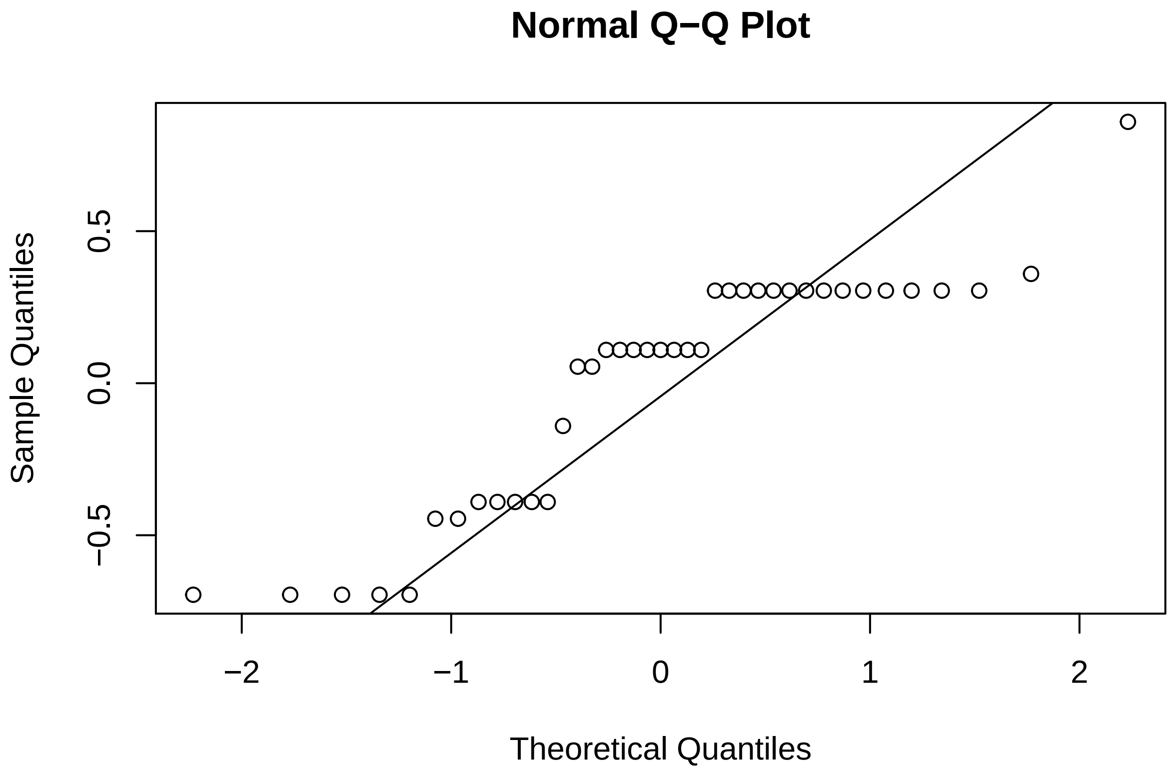
```
leveneTest(data=hatchingageatonset, age_t~treatment)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.3301 0.5691
##       37
```

```
hist(residuals(mean_hatching_age))
```



```
qqnorm(residuals(mean_hatching_age))
qqline(residuals(mean_hatching_age))
```



```
shapiro.test(residuals(mean_hatching_age))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(mean_hatching_age)
## W = 0.85777, p-value = 0.0001671
```

Shapiro-Wilk normality test

data: residuals(mean_hatching_age) W = 0.85777, p-value = 0.0001671

Anova Table (Type II tests)

Response: age_t Sum Sq Df F value Pr(>F)
treatment 2.9068 1 18.336 0.0001261 *** Residuals 5.8657 37

Nonparametric stats here:

```
wct = wilcox.test(data=hatchingageatonset, age_t~treatment, paired = F)
wct
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  age_t by treatment
## W = 294.5, p-value = 0.001065
## alternative hypothesis: true location shift is not equal to 0
```

EFFECT OF TREATMENT

- **W = 294.5**
- **p-value = 0.001065**

Hatchling size

Summary statistics

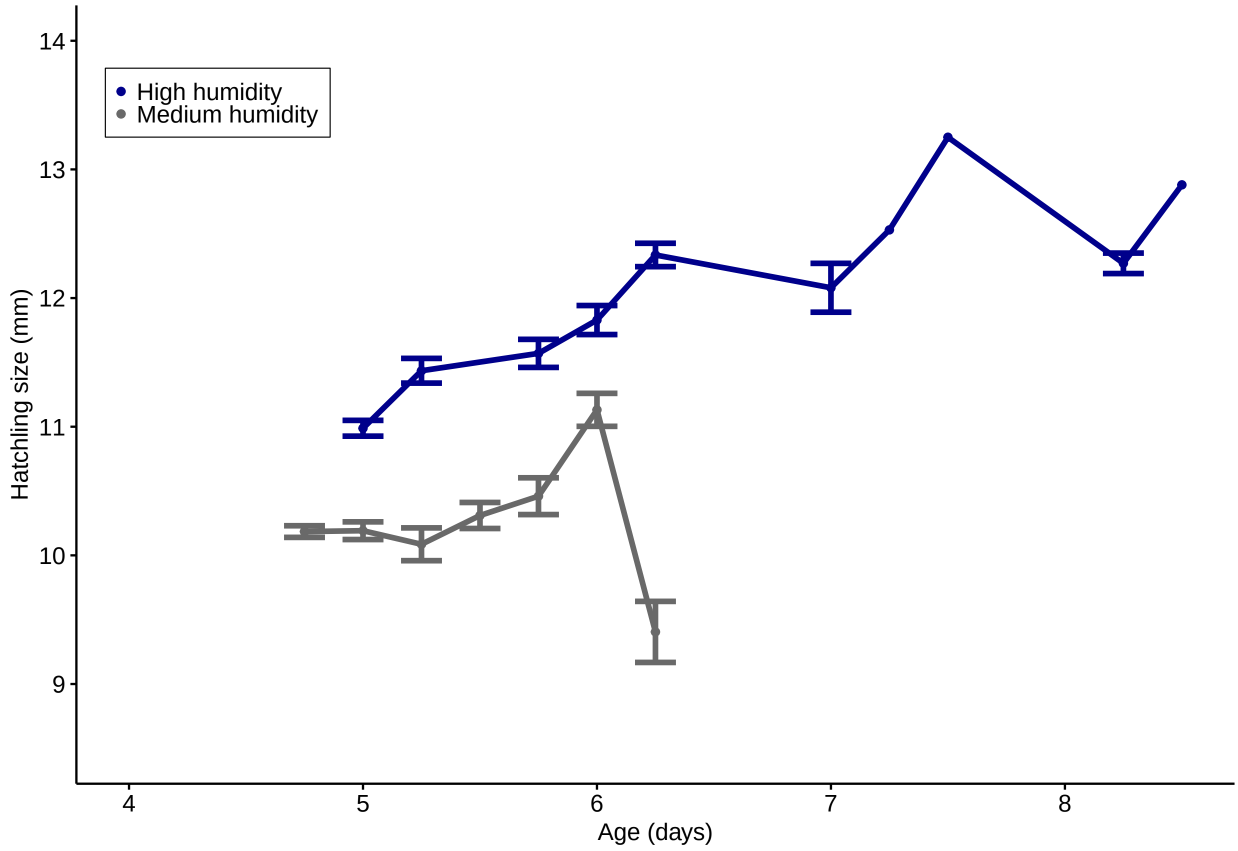
```
tadpolelengthsum = dry %>%
  group_by(treatment, age_t) %>%
  summarise(mean = mean(average_tad_length, na.rm=T), SE= sd(average_tad_length, na.rm=T)/sqrt(length(a
  filter(mean > 0) %>%
  mutate(meant = lag(mean, n=1)- mean)
tadpolelengthsum
```

```
## # A tibble: 17 x 9
## # Groups:   treatment [2]
##    treatment       age_t  mean     SE    SD   min   max sample_size    meant
##    <chr>           <dbl> <dbl>  <dbl> <dbl> <dbl> <dbl>       <int>    <dbl>
##  1 High humidity    5    11.0  0.0613 0.294 10.5   11.2          23 NA
##  2 High humidity    5.25 11.4  0.0961 0.461 11.1   12.1          23 -0.447
##  3 High humidity    5.75 11.6  0.109  0.523 11.2   12.3          23 -0.135
##  4 High humidity    6    11.8  0.113  0.541 10.2   12.6          23 -0.259
##  5 High humidity    6.25 12.3  0.0908 0.436 11.6   12.8          23 -0.506
##  6 High humidity    7    12.1  0.190  0.911 11.1   12.9          23  0.255
##  7 High humidity    7.25 12.5 NA     NA     12.5   12.5          23 -0.450
##  8 High humidity    7.5  13.2 NA     NA     13.2   13.2          23 -0.72
##  9 High humidity    8.25 12.3  0.0796 0.382 12     12.5          23  0.98
## 10 High humidity    8.5  12.9 NA     NA     12.9   12.9          23 -0.61
## 11 Medium humidity  4.75 10.2  0.0451 0.216 10     10.5          23 NA
## 12 Medium humidity  5    10.2  0.0687 0.330  9.76  10.8          23 -0.00667
## 13 Medium humidity  5.25 10.1  0.128  0.612  8.96  10.8          23  0.106
## 14 Medium humidity  5.5  10.3  0.101  0.485 10.0   10.9          23 -0.224
## 15 Medium humidity  5.75 10.5  0.143  0.685  9.83  11.2          23 -0.150
## 16 Medium humidity  6    11.1  0.128  0.615 10.3   12.2          23 -0.671
## 17 Medium humidity  6.25  9.41 0.237  1.14   8.6   10.2          23  1.73
```

Figure

```
tadpole_length = ggplot(data = tadpolelengthsum, aes(x=age_t, y=mean)) + #add the axis
  geom_line(aes(color = treatment), size = 1) +
  geom_point(aes(color=treatment), size = 1) +
  geom_errorbar(aes(color = treatment, group = age_t, ymin=mean-SE, ymax=mean+SE), width= .175, size= 1
  labs(y="Hatchling size (mm)", x="Age (days)") + #creat labels...
```

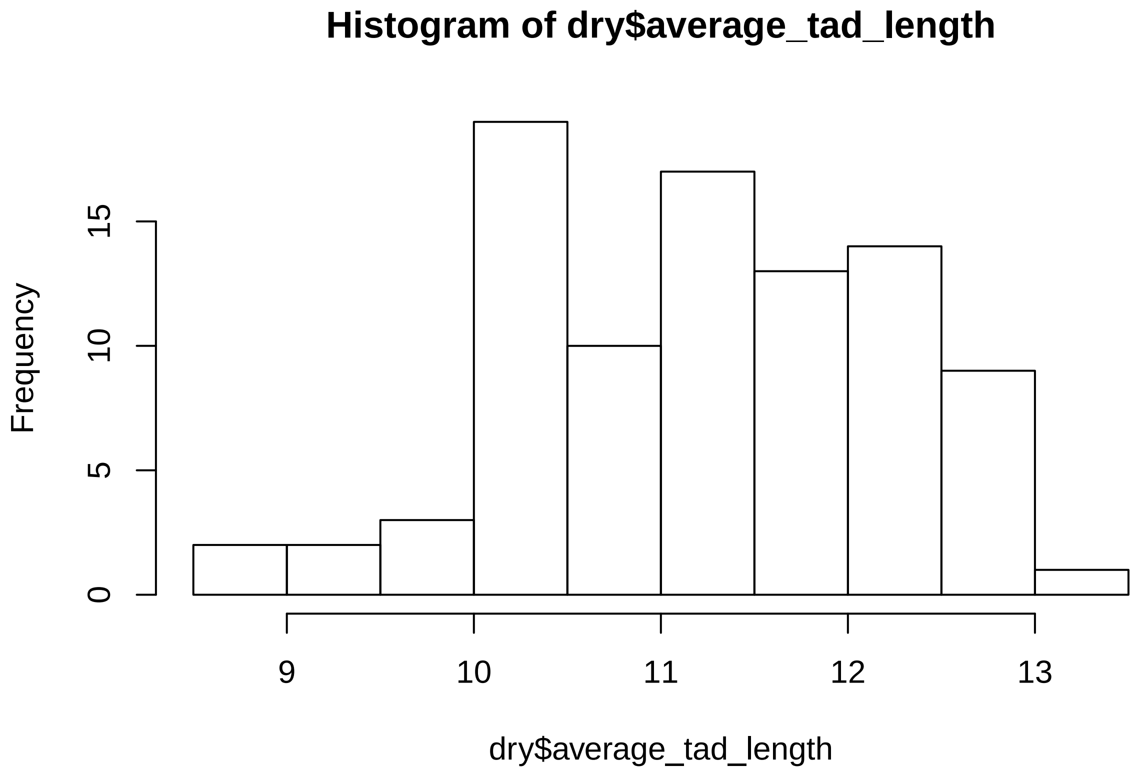
```
  theme_classic(base_size = 9) +
  theme(axis.text = element_text(color="black", size = 9), axis.ticks = element_line(color = "black"))
  theme(legend.title.align=0.5, legend.justification = c(0,.5), legend.background = element_rect(color
  scale_x_continuous(limits=c(4,8.5),breaks= pretty_breaks(n=5)) +
  scale_y_continuous(limits=c(8.5,14), breaks= pretty_breaks(n=6)) +
  scale_color_manual(legend_title, values =  c("darkblue", "dimgray")) +
  scale_fill_manual(guide = FALSE, values =  c("darkblue", "dimgray"))
tadpole_length
```



STATS

LMM

```
library(lme4)
library(car)
library(lmerTest)

hist(dry$average_tad_length)
```



```
#all models
hatchling_size_full = lmer(average_tad_length~age_t*treatment +  (1|clutch), data = dry)
hatchling_size_nointeraction = lmer(average_tad_length~age_t+treatment +  (1|clutch),  data = dry)
hatchling_size_notreatment = lmer(average_tad_length~age_t +  (1|clutch),  data = dry)
hatchling_size_noage = lmer(average_tad_length~treatment +  (1|clutch), data = dry)

hatchling_size_full2 = lmer(average_tad_length~age_t*treatment +  (1|clutch) + (1|block), data = dry)

#block effect?
average_tad_length_2_block = lmer(average_tad_length~block + (1|clutch), data = dry)
Anova(average_tad_length_2_block)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: average_tad_length
##        Chisq Df Pr(>Chisq)
## block 0.1177  1     0.7315
```

```
#Response: average_tad_length
#       Chisq Df Pr(>Chisq)
#block 0.1177  1     0.7315

#AIC comparison
library(AICcmodavg)
print(aictab(list(hatchling_size_full, hatchling_size_full2, hatchling_size_nointeraction, hatchling_si
```

```
##
## Model selection based on AICc:
##
##                               K   AICc Delta_AICc AICcWt Cum.Wt
## hatchling_size_nointeraction 5 171.82       0.00   0.77   0.77
## hatchling_size_full          6 174.74       2.92   0.18   0.94
## hatchling_size_full2         7 177.09       5.27   0.05   1.00
## hatchling_size_noage         4 184.80      12.98   0.00   1.00
## hatchling_size_notreatment   4 221.08      49.26   0.00   1.00
```

```
# AIC lower in full model without time blocks as random effect

summary(hatchling_size_full)
```

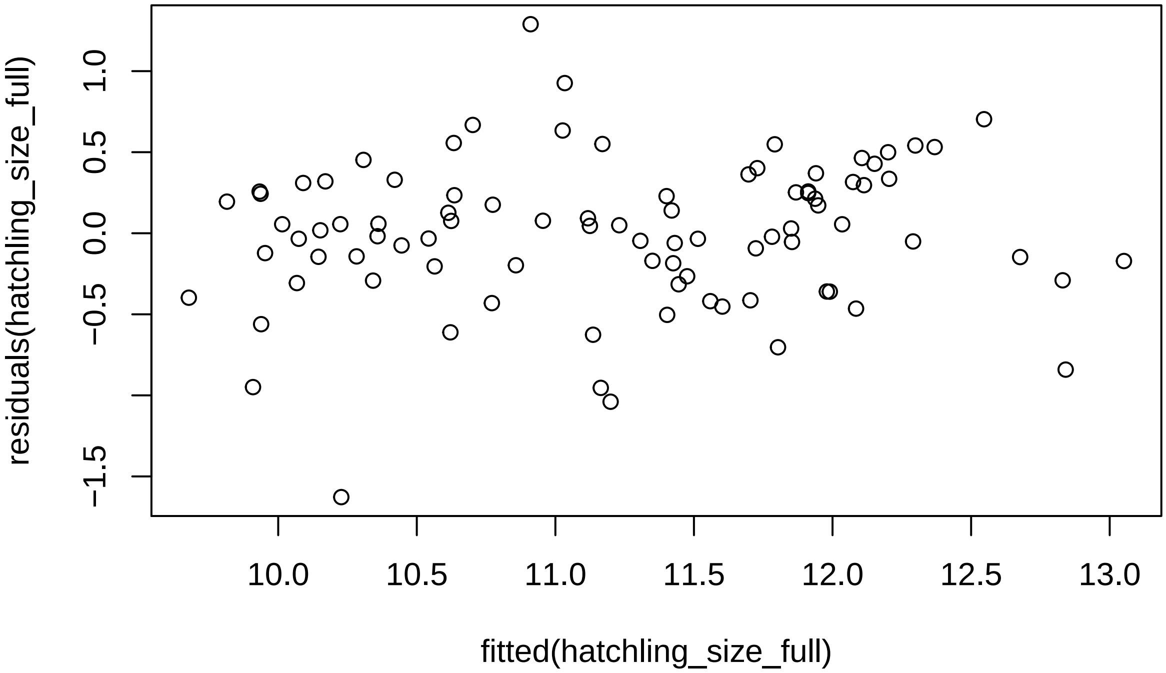
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: average_tad_length ~ age_t * treatment + (1 | clutch)
##    Data: dry
##
## REML criterion at convergence: 161.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.2509 -0.4999  0.0748  0.5729  2.5754
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clutch   (Intercept) 0.1388   0.3726
##  Residual             0.2507   0.5007
## Number of obs: 90, groups:  clutch, 23
##
## Fixed effects:
##                                  Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)                        9.5057     0.6473 82.7432  14.686  < 2e-16 ***
## age_t                              0.3782     0.1050 81.3939   3.603  0.00054 ***
## treatmentMedium humidity          -2.1105     1.1738 70.4731  -1.798  0.07645 .
## age_t:treatmentMedium humidity     0.1718     0.2077 69.9832   0.827  0.41085
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) age_t  trtmMh
## age_t       -0.986
## trtmntMdmhm -0.498  0.497
## ag_t:trtmMh  0.450 -0.456 -0.994
```

```
Anova(hatchling_size_full)
```

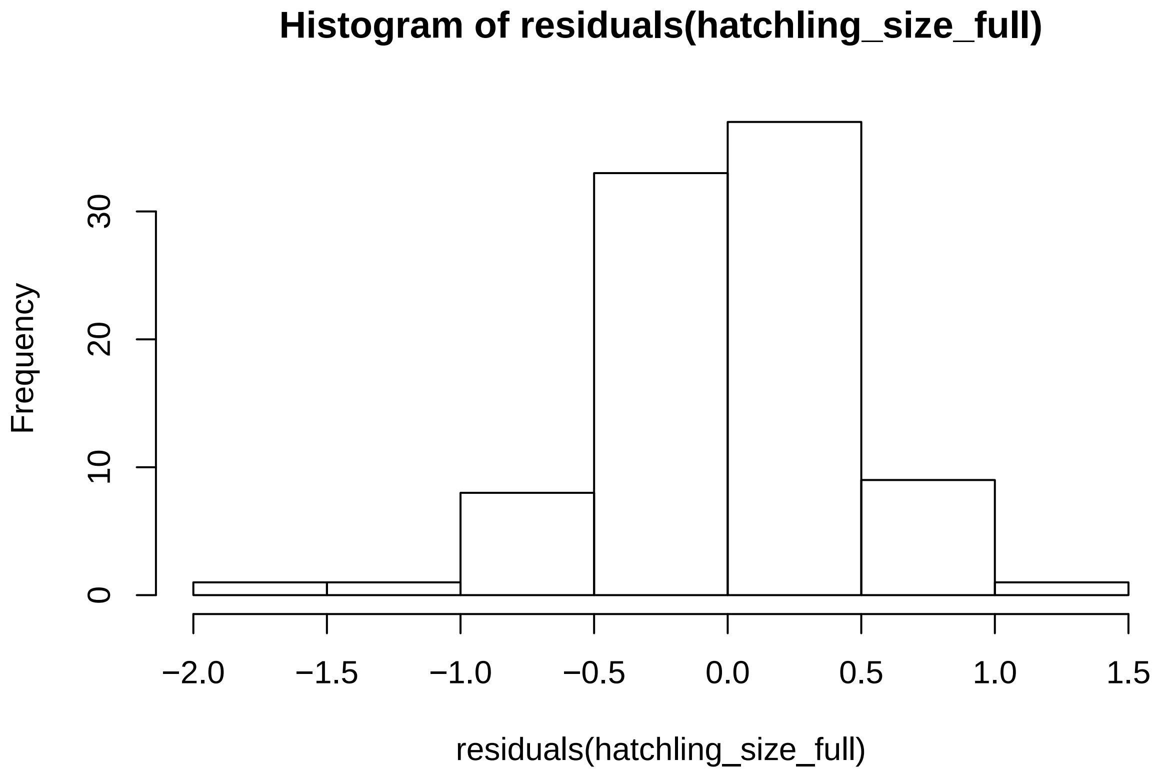
```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: average_tad_length
##                    Chisq Df Pr(>Chisq)
## age_t            20.0098  1  7.704e-06 ***
```

```
## treatment        76.1679  1  < 2.2e-16 ***
## age_t:treatment   0.6845  1      0.408
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

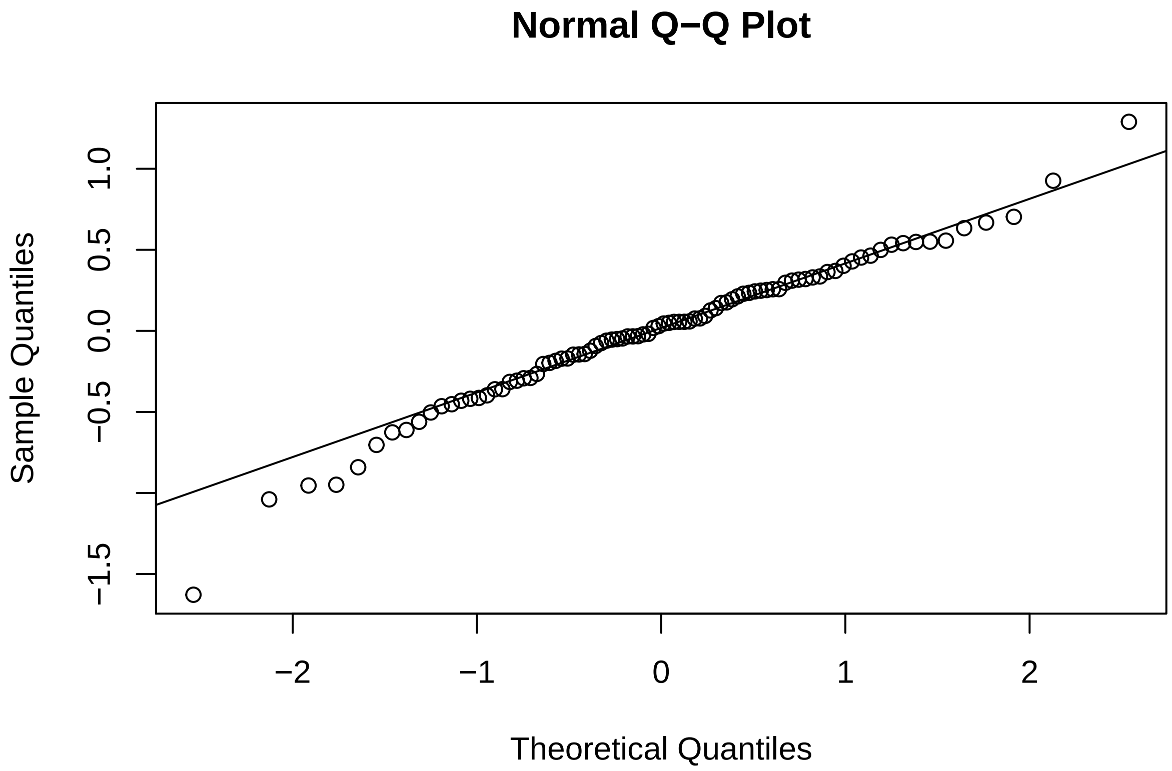
```
plot(fitted(hatchling_size_full), residuals(hatchling_size_full))
```



```
hist(residuals(hatchling_size_full))
```



```
qqnorm(residuals(hatchling_size_full))
qqline(residuals(hatchling_size_full))
```



```r
shapiro.test(residuals(hatchling_size_full))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(hatchling_size_full)
## W = 0.97532, p-value = 0.08426
```

Shapiro-Wilk normality test

data: residuals(hatchling_size_full) W = 0.97532, p-value = 0.08426

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: average_tad_length Chisq Df Pr(>Chisq)
age_t 20.0098 1 7.704e-06 ***treatment 76.1679 1 < 2.2e-16*** age_t:treatment 0.6845 1 0.408

Likelihood ratio tests

```r
anova(hatchling_size_full, hatchling_size_nointeraction, test = "Chisq")
```

```
## Data: dry
## Models:
## hatchling_size_nointeraction: average_tad_length ~ age_t + treatment + (1 | clutch)
## hatchling_size_full: average_tad_length ~ age_t * treatment + (1 | clutch)
##                               npar    AIC    BIC  logLik deviance  Chisq Df
```

```
## hatchling_size_nointeraction    5 162.84 175.34 -76.419   152.84
## hatchling_size_full             6 164.14 179.14 -76.071   152.14 0.6965  1
##                              Pr(>Chisq)
## hatchling_size_nointeraction
## hatchling_size_full               0.404
```

```
anova(hatchling_size_full, hatchling_size_notreatment, test = "Chisq")
```

```
## Data: dry
## Models:
## hatchling_size_notreatment: average_tad_length ~ age_t + (1 | clutch)
## hatchling_size_full: average_tad_length ~ age_t * treatment + (1 | clutch)
##                            npar    AIC    BIC   logLik deviance  Chisq Df
## hatchling_size_notreatment    4 215.42 225.42 -103.712   207.42
## hatchling_size_full           6 164.14 179.14  -76.071   152.14 55.283  2
##                            Pr(>Chisq)
## hatchling_size_notreatment
## hatchling_size_full         9.895e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(hatchling_size_full, hatchling_size_noage, test = "Chisq")
```

```
## Data: dry
## Models:
## hatchling_size_noage: average_tad_length ~ treatment + (1 | clutch)
## hatchling_size_full: average_tad_length ~ age_t * treatment + (1 | clutch)
##                      npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## hatchling_size_noage    4 179.48 189.48 -85.739   171.48
## hatchling_size_full     6 164.14 179.14 -76.071   152.14 19.337  2  6.323e-05
##
## hatchling_size_noage
## hatchling_size_full  ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EFFECT OF INTERACTION:

- **X = 0.6965; df = 1**
- **p-value = 0.404 NS**

EFFECT OF TREATMENT:

- **X = 55.283; df = 2**
- **p-value = 9.895e-13**

EFFECT OF AGE:

- **X = 19.337; df = 2**
- **p-value = 6.323e-05**

Combined Figure 6

```
library(gridExtra)
library(cowplot)

# 1 column is 3.43 inches wide

#  @ 3.43 x 3.75 portrait

figure6 = plot_grid(hatching_timing, tadpole_length, labels = "AUTO", label_x = -.01, label_y = 1, scal
figure6
```