Ulva lactuca and Hypnea musciformis rETRmax Mixed Model

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Run5&6 rETRmax Analysis, Script Chunks, and Plots

This is the analysis of the final run PLUS the additional run 6 of the Ulva and Hypnea salinity and nutrient experiments conducted on the lanai in St. John 616. These experiments incorporated three temperature levels and with the additional data in run 6 includes very low Nitrogen values at seawater salinity levels. Model was run on parameter rETRmax

Packages loaded:

```
library(lme4)
library(lmerTest)
library(effects)
library(car)
library(MuMIn)
library (dplyr)
library(emmeans)
library(DHARMa)
library(performance)
library(patchwork)
```

Load and prepare the dataset

Open the photosynthesis dataset that was created in R using the package "Phytotools" Several parameters are available but are not independent of one another thus for this analysis, we chose to use rETRmax to represent the results for photosynthetic efficiency

```
run5_6_photosyn_data <- read.csv("data/run5_run6_all_photosyn.csv")
```

Write function that returns the run number based on the range of dates input

```
get_run_number <- function(d) {
  # returns the run number based on the input date
  run = NA
  if ("2021-09-20" <= d && d <= "2021-09-29") {
    run <- 1
  } else if ("2021-09-30" <= d && d <= "2021-10-09") {
```

```
    run <- 2
  } else if ("2021-10-11" <= d && d <= "2021-10-29") {
    run <- 3
  } else if ("2021-11-01" <= d && d <= "2021-11-12") {
    run <- 4
  } else if ("2022-02-11" <= d && d <= "2022-03-01") {
    run <- 0
  } else {
    print("The date is %s not in a recognized range", d)
  }
  run
}
```

Make new column adding run to the dataset

```
run5_6_photosyn_data$run <- sapply(run5_6_photosyn_data$Date, get_run_number)
run5_6_photosyn_data$run <- as.factor(run5_6_photosyn_data$run)
```

Change levels to factors

```
#assigns temperature as a factor
run5_6_photosyn_data$Temperature <- as.factor(run5_6_photosyn_data$Temperature)

#assigns treatment as characters from integers then to factors
run5_6_photosyn_data$Treatment <- as.factor(as.character(run5_6_photosyn_data$Treatment))
```

Make a subset of the data to isolate the species and use only Day 9 (final day) results

```
hypnea <- subset(run5_6_photosyn_data, Species == "hm" & RLC.Day == 9)
ulva <- subset(run5_6_photosyn_data, Species == "ul" & RLC.Day == 9)
```

ULVA – 240 observations 15 different treatment/temperature combos n = 16

Run model without interaction between the dependent variables
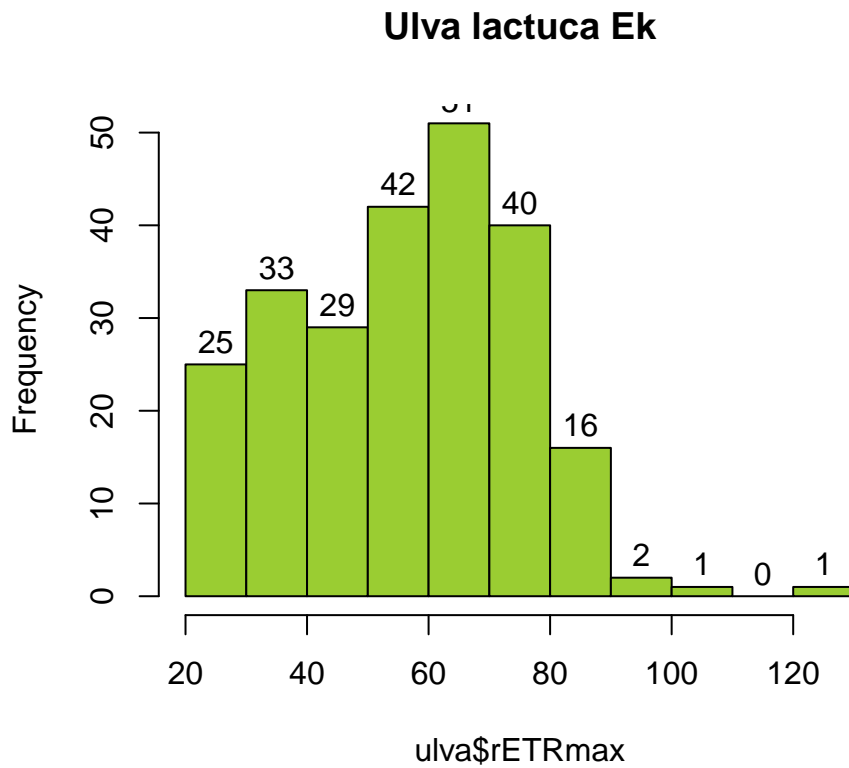
```
run5_6_photosyn_model_noint <- lmer(formula = rETRmax ~ Treatment + Temperature + (1 | run), data = ulva
```
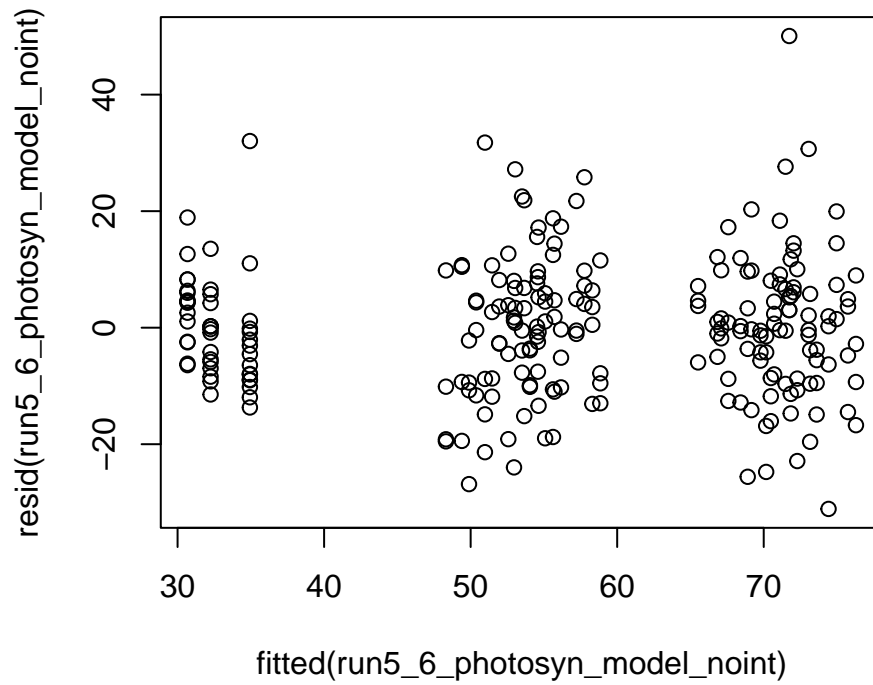
Make a histogram and Normal and residual plots of the data for ulva

```
hist(ulva$rETRmax, main = paste("Ulva lactuca Ek"), col = "olivedrab3", labels = TRUE)
```
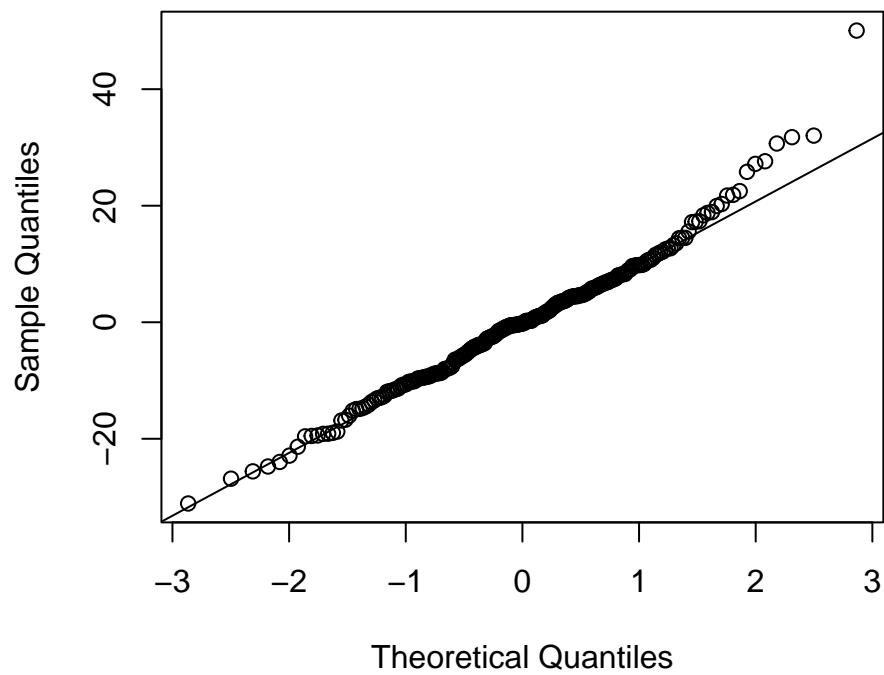
Ulva lactuca Ek


```
plot(resid(run5_6_photosyn_model_noint) ~ fitted(run5_6_photosyn_model_noint))
```

```
qqnorm(resid(run5_6_photosyn_model_noint))
qqline(resid(run5_6_photosyn_model_noint))
```
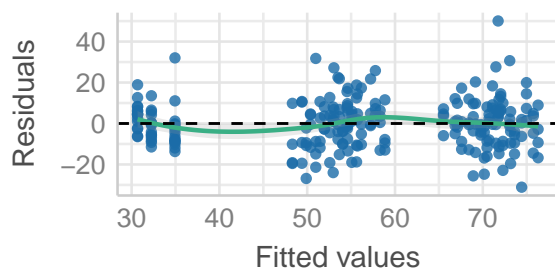
Normal Q−Q Plot

Check the performance of the model

```
performance::check_model(run5_6_photosyn_model_noint)
```
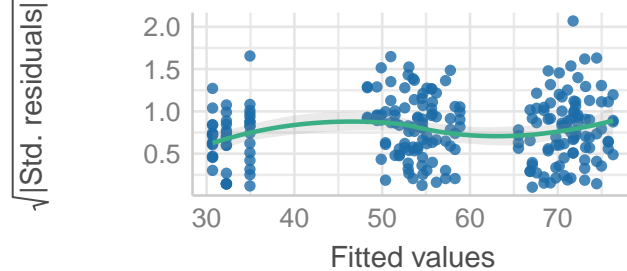
Linearity
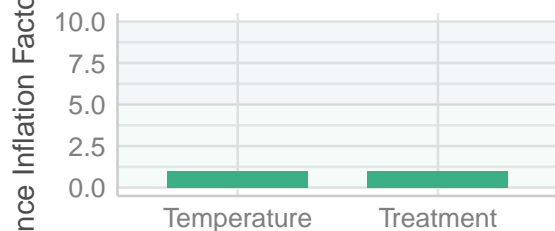Reference line should be flat and horizontal

Homogeneity of Variance
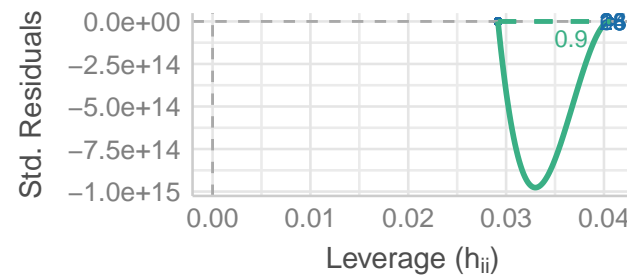Reference line should be flat and horizontal

Collinearity
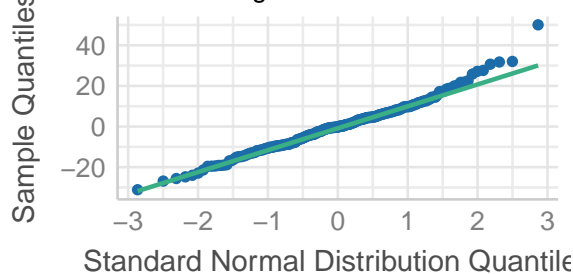Higher bars (>5) indicate potential collinearity is

low (< 5)   moderate (< 10)   high (≥

Influential Observations
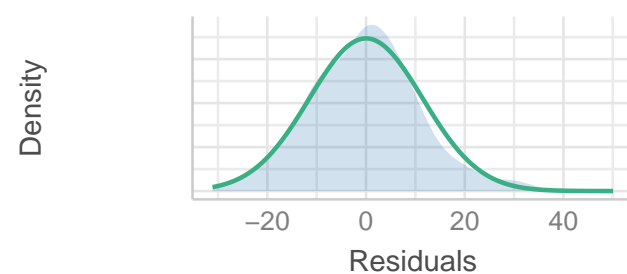Points should be inside the contour lines

Normality of Residuals
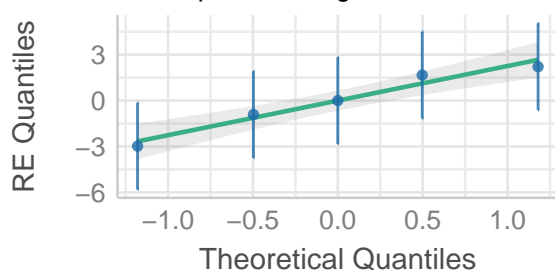Dots should fall along the line

Normality of Residuals
Distribution should be close to the normal curve

Normality of Random Effects (run)
Dots should be plotted along the line

```
r.squaredGLMM(run5_6_photosyn_model_noint)
```

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

```
##             R2m        R2c
## [1,] 0.588114 0.6105791
```

```
summary(run5_6_photosyn_model_noint)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: rETRmax ~ Treatment + Temperature + (1 | run)
##    Data: ulva
##
## REML criterion at convergence: 1838.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.6598 -0.6944 -0.0180  0.5500  4.2806
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  run      (Intercept)   7.889   2.809
##  Residual             136.754  11.694
## Number of obs: 240, groups:  run, 5
##
## Fixed effects:
##                Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)      34.936      3.446   3.670  10.137 0.000816 ***
## Treatment2-35%   20.611      3.945   4.030   5.225 0.006275 **
## Treatment3-28%   21.696      3.945   4.030   5.500 0.005213 **
## Treatment4-18%   37.818      3.945   4.030   9.587 0.000638 ***
## Treatment5-11%   39.142      3.945   4.030   9.923 0.000558 ***
## Temperature27    -4.258      1.849 230.000  -2.303 0.022189 *
## Temperature30    -2.682      1.849 230.000  -1.451 0.148243
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Warning in abbreviate(rn, minlength = 11): abbreviate used with non-ASCII chars
```

```
##
## Correlation of Fixed Effects:
```

```
## Warning in abbreviate(rn, minlength = 6): abbreviate used with non-ASCII chars
```

```
##             (Intr) T2-35% T3-28% T4-18% T5-11% Tmpr27
## Trtmnt2-35% -0.790
## Trtmnt3-28% -0.790  0.817
## Trtmnt4-18% -0.790  0.817  0.817
## Trtmnt5-11% -0.790  0.817  0.817  0.817
## Temperatr27 -0.268  0.000  0.000  0.000  0.000
## Temperatr30 -0.268  0.000  0.000  0.000  0.000  0.500
```

\#run ANOVA and pairwise comparisons (sig differences for both random variables)

```
anova(run5_6_photosyn_model_noint, type = c("III"), ddf = "Satterthwaite")
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF   DenDF F value     Pr(>F)
## Treatment   23537.8  5884.4     4   5.948  43.029 0.0001577 ***
## Temperature   741.5   370.7     2 230.000   2.711 0.0685972 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ulva_photosyn_model_aov <- aov(rETRmax ~ Treatment + Temperature, data = ulva)
TukeyHSD(ulva_photosyn_model_aov, "Treatment", ordered = FALSE)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = rETRmax ~ Treatment + Temperature, data = ulva)
##
## $Treatment
##                   diff       lwr       upr      p adj
## 2-35%-1-35%N 20.610833 13.932211 27.289455 0.0000000
## 3-28%-1-35%N 21.696250 15.017628 28.374872 0.0000000
## 4-18%-1-35%N 37.818125 31.139503 44.496747 0.0000000
## 5-11%-1-35%N 39.141667 32.463045 45.820289 0.0000000
## 3-28%-2-35%   1.085417 -5.593205  7.764039 0.9917098
## 4-18%-2-35%  17.207292 10.528670 23.885914 0.0000000
## 5-11%-2-35%  18.530833 11.852211 25.209455 0.0000000
## 4-18%-3-28%  16.121875  9.443253 22.800497 0.0000000
## 5-11%-3-28%  17.445417 10.766795 24.124039 0.0000000
## 5-11%-4-18%   1.323542 -5.355080  8.002164 0.9824820
```

```
TukeyHSD(ulva_photosyn_model_aov, "Temperature", ordered = FALSE)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = rETRmax ~ Treatment + Temperature, data = ulva)
##
## $Temperature
##            diff       lwr       upr      p adj
## 27-20 -4.25775 -8.696249 0.1807495 0.0631868
## 30-20 -2.68225 -7.120749 1.7562495 0.3294726
## 30-27  1.57550 -2.862999 6.0139995 0.6802496
```
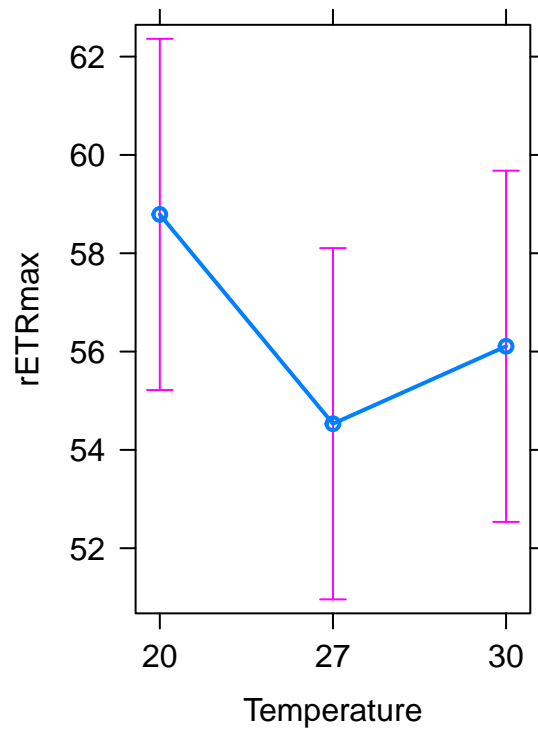
Plot the random effects for Ulva

```
plot(allEffects(run5_6_photosyn_model_noint))
```

Treatment effect plot
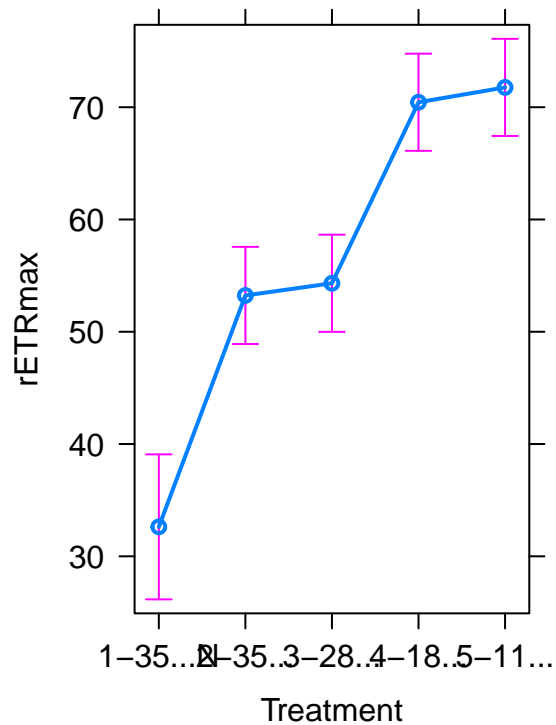
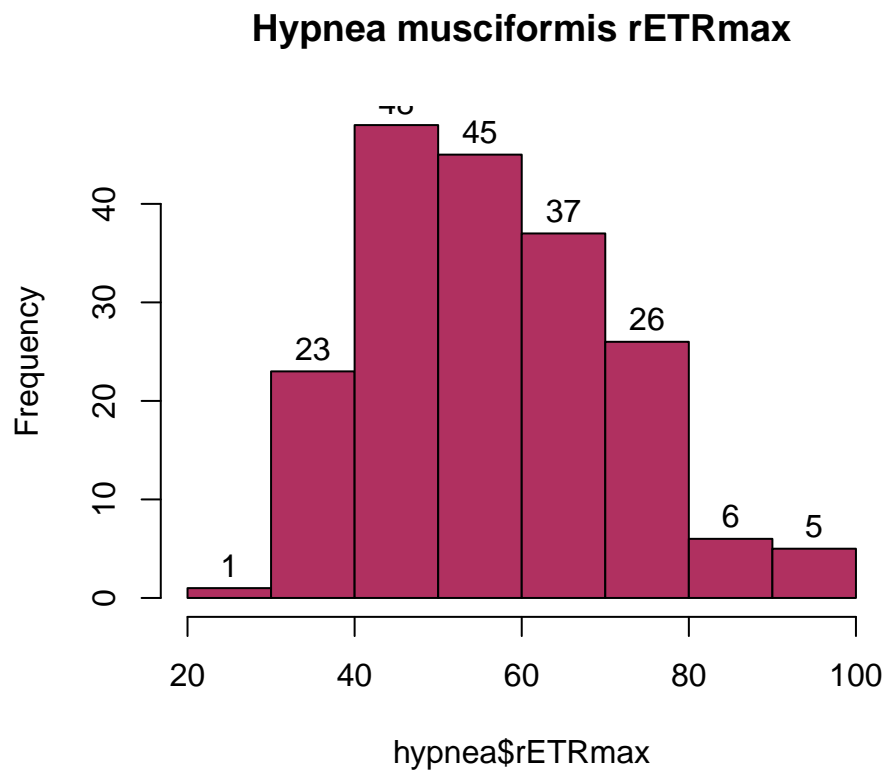## Temperature effect plot

HYPNEA – 191 observations 12 different treatment/temperature combos (missing low N) n = 16

Run model without interaction between the dependent variables
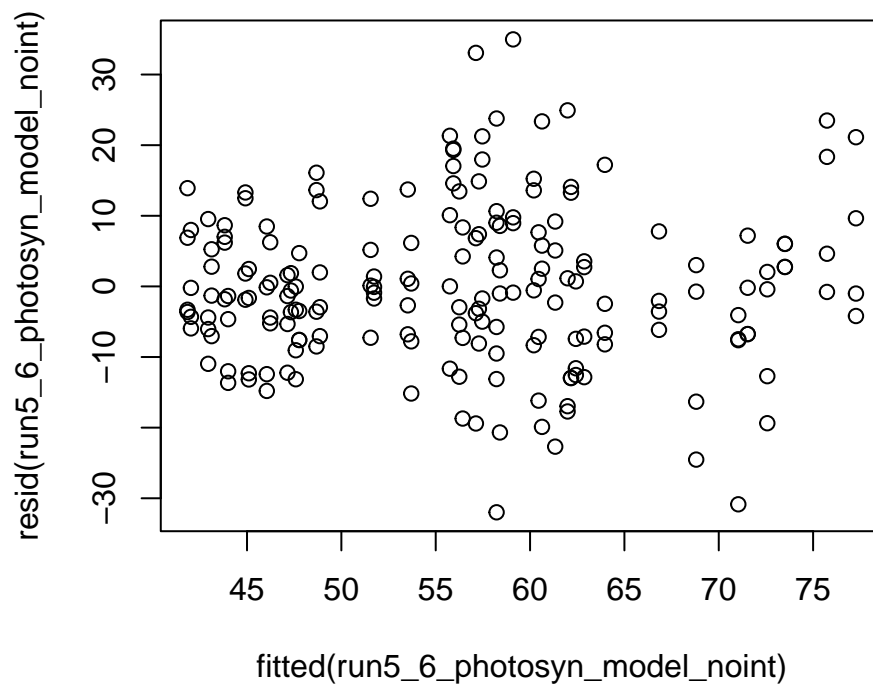
```
run5_6_photosyn_model_noint <- lmer(formula = rETRmax ~ Treatment + Temperature + (1 | run),
                                    data = hypnea)
```

Make a histogram and Normal and residual plots of the data for hypnea

```
hist(hypnea$rETRmax, main = paste("Hypnea musciformis rETRmax"), col = "maroon", labels = TRUE)
```
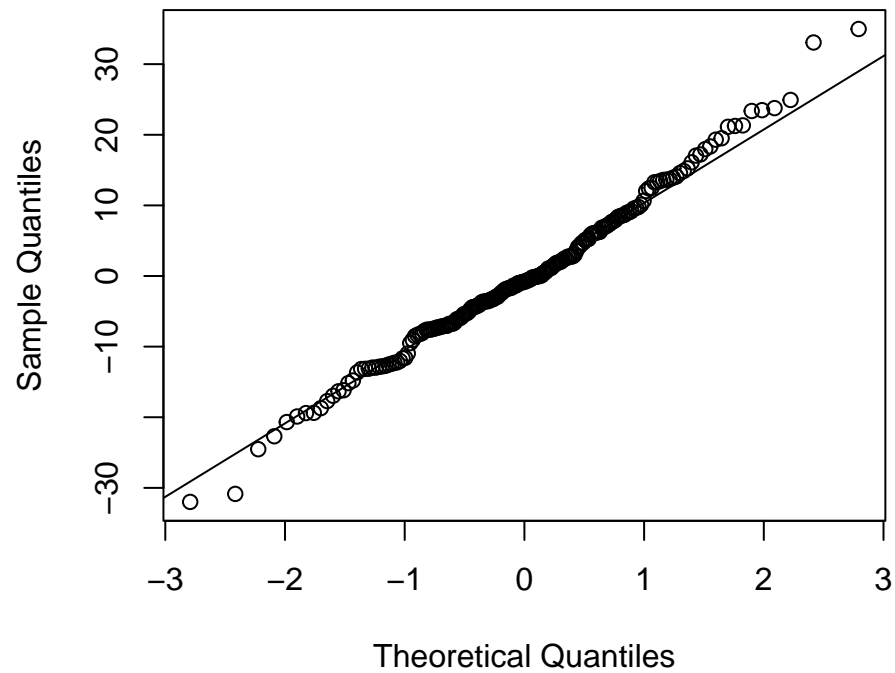
Hypnea musciformis rETRmax


```
plot(resid(run5_6_photosyn_model_noint) ~ fitted(run5_6_photosyn_model_noint))
```

```
qqnorm(resid(run5_6_photosyn_model_noint))
qqline(resid(run5_6_photosyn_model_noint))
```
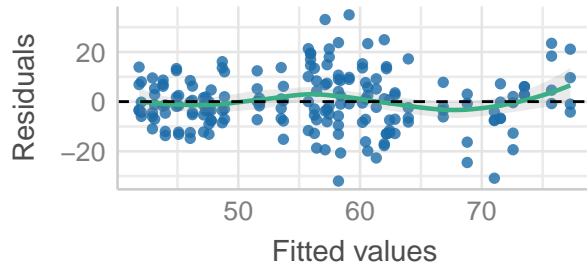
Normal Q–Q Plot

Check the performance of the model

```
performance::check_model(run5_6_photosyn_model_noint)
```
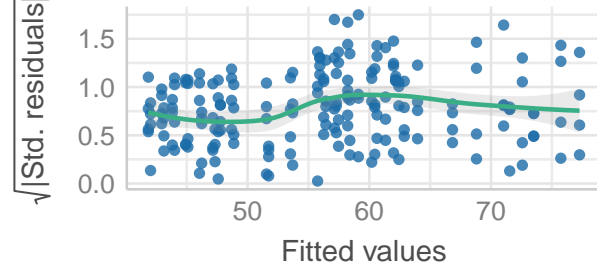
Linearity
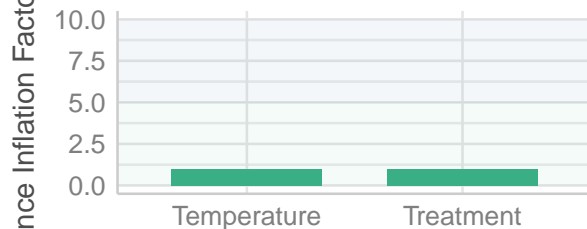Reference line should be flat and horizontal

Homogeneity of Variance
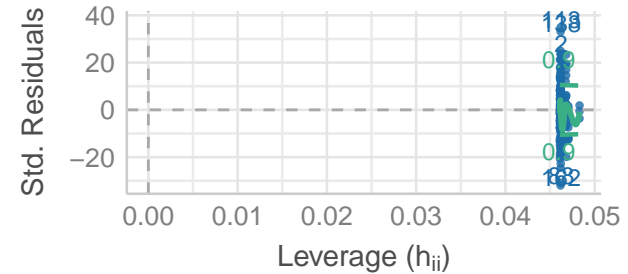Reference line should be flat and horizontal

Collinearity
Higher bars (>5) indicate potential collinearity issue

Influential Observations
Points should be inside the contour lines
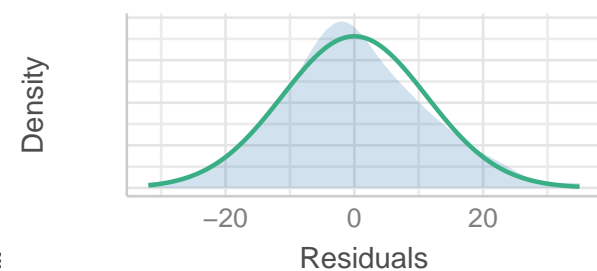
low (< 5) moderate (< 10) high (>=

Normality of Residuals
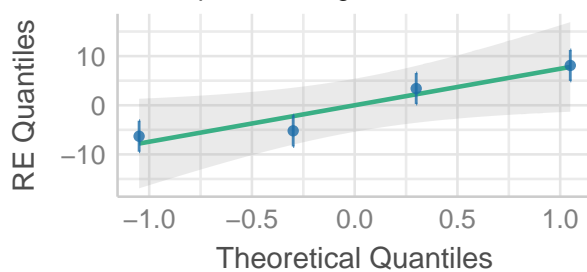Dots should fall along the line

Normality of Residuals
Distribution should be close to the normal curve

Normality of Random Effects (run)
Dots should be plotted along the line

```
r.squaredGLMM(run5_6_photosyn_model_noint)
```

```
##              R2m       R2c
## [1,] 0.2375505 0.4500714
```

```
summary(run5_6_photosyn_model_noint)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: rETRmax ~ Treatment + Temperature + (1 | run)
##    Data: hypnea
##
## REML criterion at convergence: 1458.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.79523 -0.61841 -0.06864  0.60795  3.05660
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  run      (Intercept)  50.61    7.114
##  Residual             130.95   11.443
## Number of obs: 191, groups:  run, 4
##
## Fixed effects:
##                Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)      65.403      4.092   4.726  15.982 2.70e-05 ***
## Treatment3-28%   -1.972      2.336 182.000  -0.844    0.400
## Treatment4-18%    3.767      2.336 182.000   1.613    0.109
## Treatment5-11%    2.230      2.349 182.004   0.950    0.344
## Temperature27   -15.283      2.023 182.000  -7.555 1.97e-12 ***
## Temperature30   -15.102      2.031 182.003  -7.435 3.96e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


## Warning in abbreviate(rn, minlength = 11): abbreviate used with non-ASCII chars


##
## Correlation of Fixed Effects:


## Warning in abbreviate(rn, minlength = 6): abbreviate used with non-ASCII chars


##            (Intr) T3-28% T4-18% T5-11% Tmpr27
## Trtmnt3-28% -0.285
## Trtmnt4-18% -0.285  0.500
## Trtmnt5-11% -0.285  0.497  0.497
## Temperatr27 -0.247  0.000  0.000  0.000
## Temperatr30 -0.247  0.000  0.000  0.009  0.498
```

#run ANOVA and pairwise comparisons (only significant diff for Temperature)

```
anova(run5_6_photosyn_model_noint, type = c("III"), ddf = "Satterthwaite")
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Treatment  910.4   303.5     3   182  2.3175   0.07712 .
```

```
## Temperature 9823.9  4912.0     2    182 37.5106 2.287e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hypnea_photosyn_model_aov <- aov(rETRmax ~ Treatment + Temperature, data = hypnea)
TukeyHSD(hypnea_photosyn_model_aov, "Temperature", ordered = FALSE)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = rETRmax ~ Treatment + Temperature, data = hypnea)
##
## $Temperature
##               diff        lwr       upr     p adj
## 27-20 -15.2834375 -20.732092 -9.834783 0.0000000
## 30-20 -15.0172137 -20.487447 -9.546981 0.0000000
## 30-27   0.2662238  -5.204009  5.736457 0.9927371
```

Plot the random effects for Hypnea

```
plot(allEffects(run5_6_photosyn_model_noint))
```


Treatment effect plot

Temperature effect plot