

Ulva and Hypnea Photosynthesis and GROWTH Run5 - Sept-Nov 2021

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run5.6 GROWTH RATE Analysis, Script Chunks, and Plots

This is the analysis of the final run of the Ulva and Hypnea salinity and nutrient experiments conducted on the lanai in St. John 616. These experiments incorporated three temperature levels. Data gaps for both species filled by end of April 2022. This output reflects all data totally five treatments for each species.

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 growth/weight dataset

```
run5.6_growth <- read.csv("/Users/Angela/src/work/limu/algal_growth_photosynthesis/data_input/run5-6_gr
```

Make a new column for weight change (difference final from initial)

```
run5.6_growth$growth_rate_percent <-
  (run5.6_growth$final.weight - run5.6_growth$Initial.weight) / run5.6_growth$Initial.weight * 100
```

Also make a new column for daily growth rate from 8 day study (steady growth rate assumed rather than exponential), which may or may not be used

```
run5.6_growth$steady_growth_daily <- run5.6_growth$growth_rate_percent / 8
```

Make a new column that keeps only the numerical values for temperature (removes C)

```
run5.6_growth$temp_clean <- as.factor(substr(run5.6_growth$temperature, 1, 2))
```

Change levels to factors

```
run5.6_growth$temperature <- as.factor(run5.6_growth$temp_clean)
run5.6_growth$run <- as.factor(run5.6_growth$run)
run5.6_growth$treatment <- as.factor(as.character(run5.6_growth$treatment))
```

Create subset of the data to isolate the species

```
hypnea <- subset(run5.6_growth, Species == "Hm")
ulva <- subset(run5.6_growth, Species == "Ul")
```

Run the model

ULVA

run model without interaction since interaction caused collinearity issues

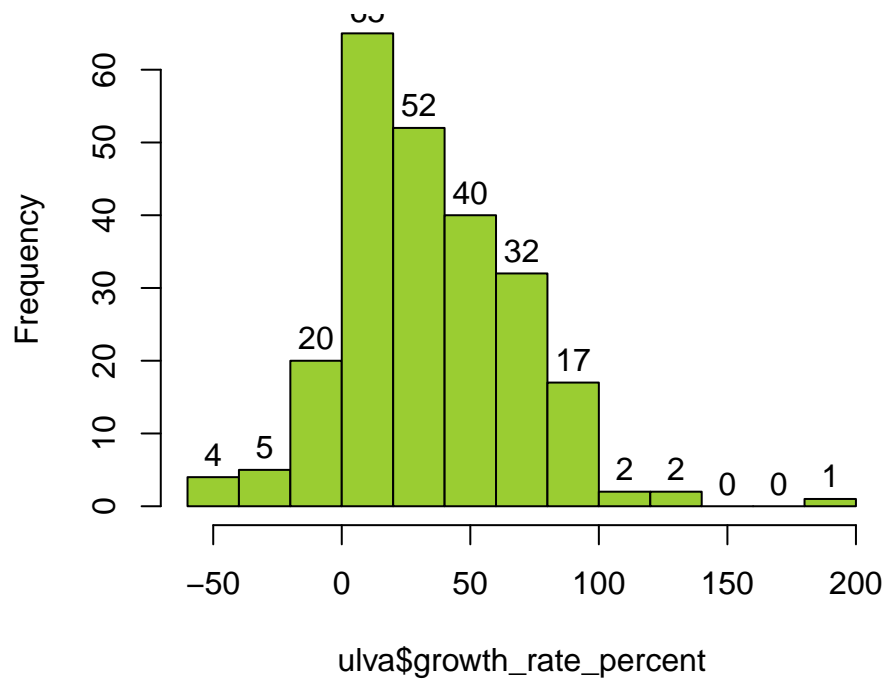
```
run5.6_growth_model_noint <- lmer(formula = growth_rate_percent ~ treatment +
                                temperature + (1 | run) + (1 | plant.ID) +
                                (1 | RLC.order), data = ulva)
```

```
## boundary (singular) fit: see help('isSingular')
```

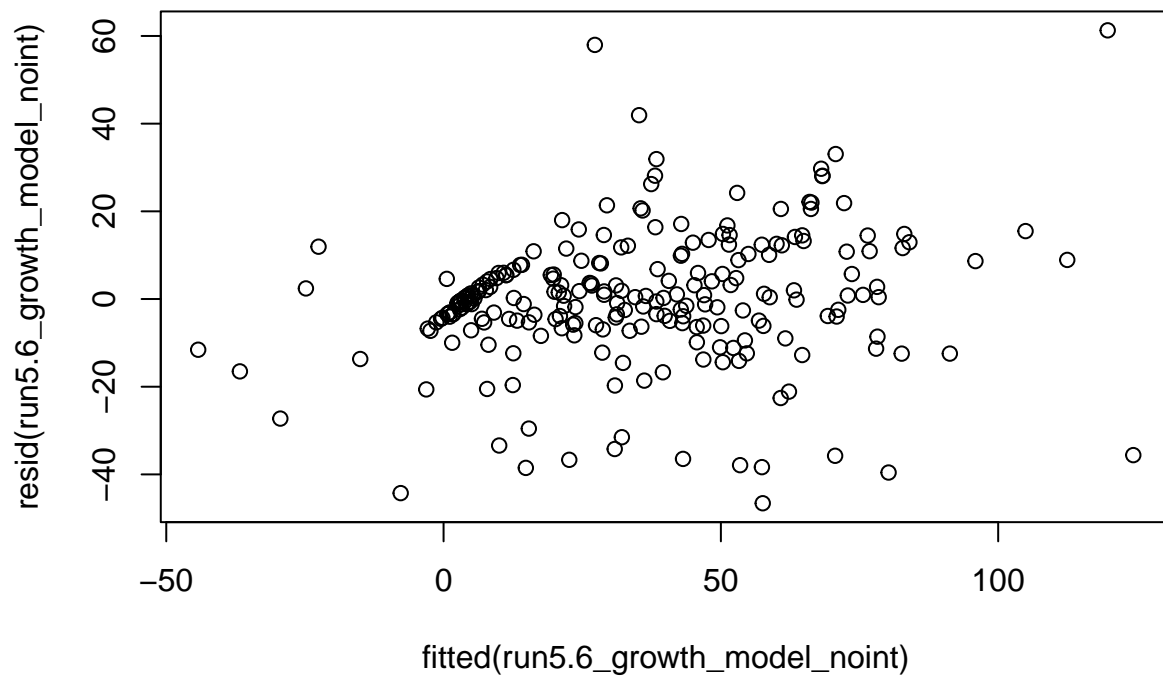
```
#make a histogram of the data for Ulva
```

```
hist(ulva$growth_rate_percent, main = paste("Ulva lactuca Growth Rate (%)"),
     col = "olivedrab3", labels = TRUE)
```

Ulva lactuca Growth Rate (%)

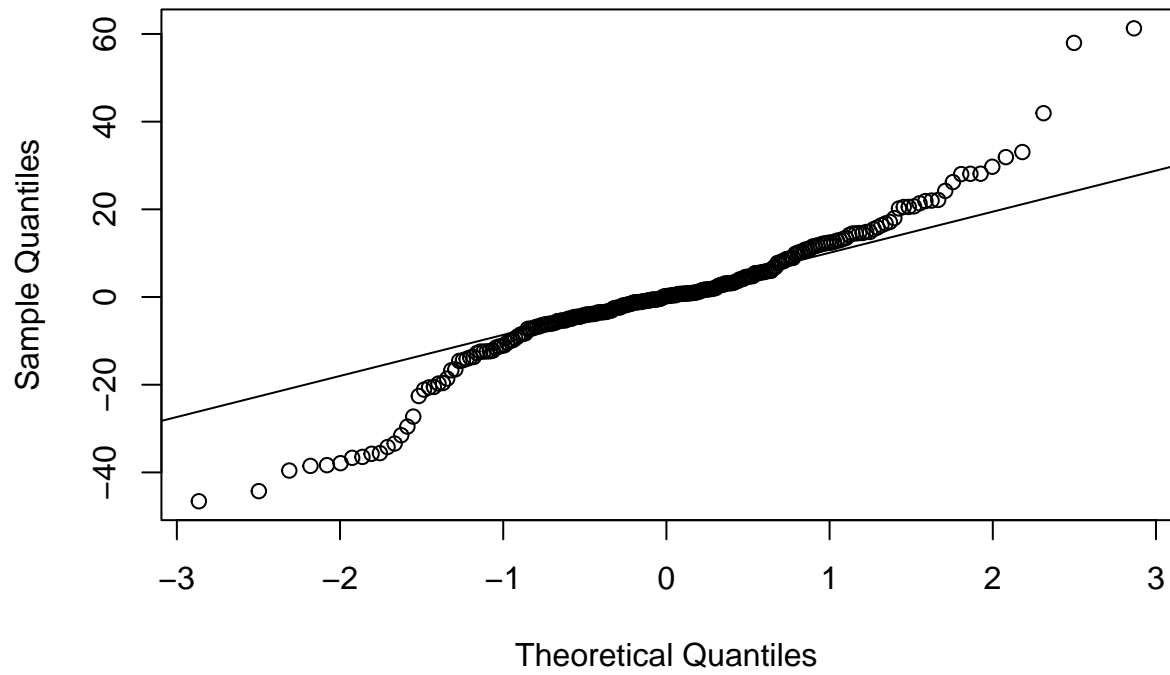


```
plot(resid(run5.6_growth_model_noint) ~ fitted(run5.6_growth_model_noint))
```



```
qqnorm(resid(run5.6_growth_model_noint))  
qqline(resid(run5.6_growth_model_noint))
```

Normal Q-Q Plot

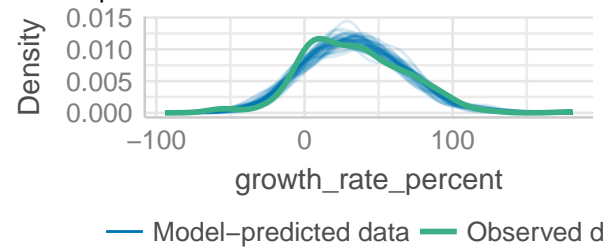


Check the performance of the model for Ulva

```
performance::check_model(run5.6_growth_model_noint)
```

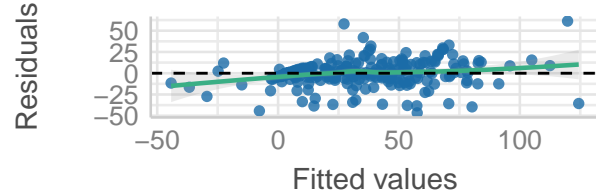
Posterior Predictive Check

Model-predicted lines should resemble observed data



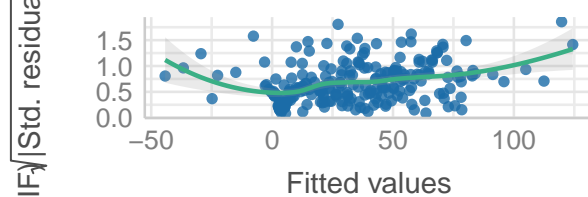
Linearity

Reference line should be flat and horizontal



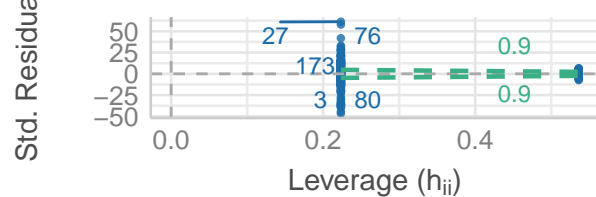
Homogeneity of Variance

Reference line should be flat and horizontal



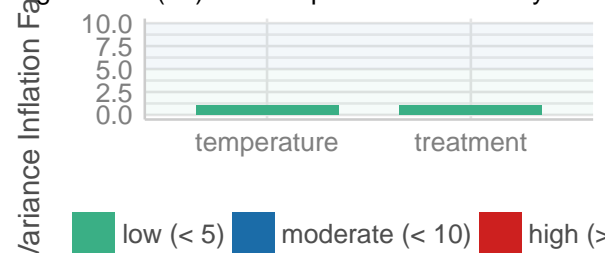
Influential Observations

Points should be inside the contour lines



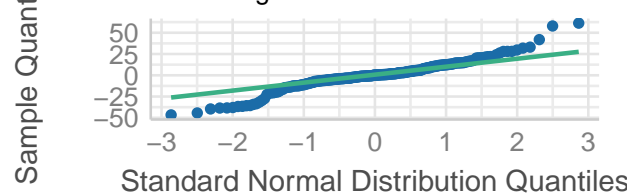
Collinearity

Higher bars (>5) indicate potential collinearity issues



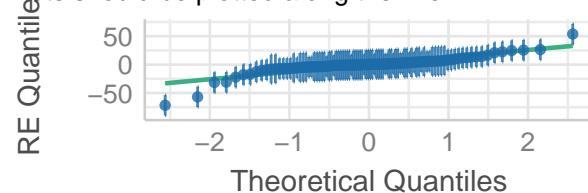
Normality of Residuals

Dots should fall along the line



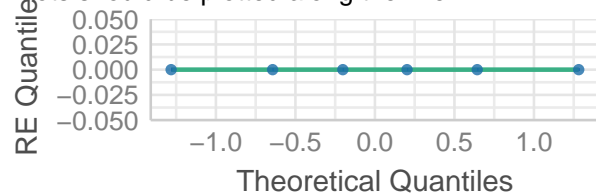
Normality of Random Effects (plant.ID)

Dots should be plotted along the line



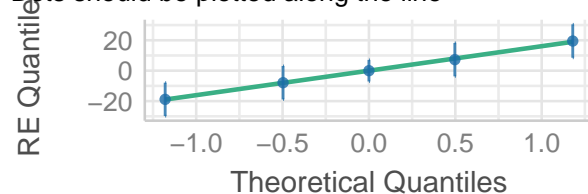
Normality of Random Effects (RLC.order)

Dots should be plotted along the line



Normality of Random Effects (run)

Dots should be plotted along the line



These outputs show the model is acceptable

##Run ANOVA and Tukey's comparison

ANOVA shows that there is no significant difference between temperatures but the salinity/nutrient treatments are very close to significant. Tukey's is run on treatments to see pairwise comparisons

```
anova(run5.6_growth_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  11448.5  2862.13     4    4.823   9.0470 0.01793 *
## temperature    35.5    17.76     2  106.302   0.0562 0.94542
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ulva_growth_model_aov <- aov(growth_rate_percent ~ treatment + temperature, data = ulva)
TukeyHSD(ulva_growth_model_aov, "treatment", ordered = FALSE)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = growth_rate_percent ~ treatment + temperature, data = ulva)
##
## $treatment
##      diff      lwr      upr    p adj
## 1-0 26.122972  8.891291 43.35465 0.0004144
## 2-0 33.643252 16.411571 50.87493 0.0000019
## 3-0 40.933674 23.701993 58.16536 0.0000000
## 4-0 45.547350 28.315668 62.77903 0.0000000
## 2-1  7.520280 -9.711401 24.75196 0.7514921
## 3-1 14.810702 -2.420979 32.04238 0.1293111
## 4-1 19.424377  2.192696 36.65606 0.0183743
## 3-2  7.290422 -9.941259 24.52210 0.7723133
## 4-2 11.904098 -5.327584 29.13578 0.3206085
## 4-3  4.613675 -12.618006 21.84536 0.9478415
```

```
r.squaredGLMM(run5.6_growth_model_noint)
```

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

```
##           R2m      R2c
## [1,] 0.2104576 0.7429601
```

```
summary(run5.6_growth_model_noint)
```

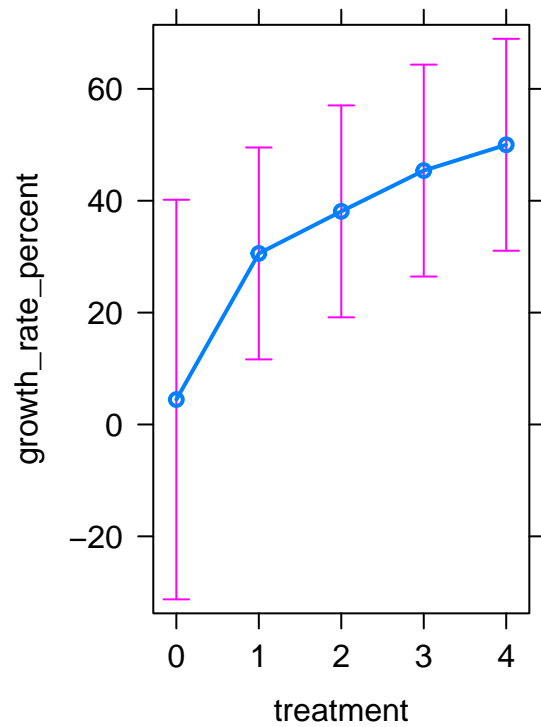
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: growth_rate_percent ~ treatment + temperature + (1 | run) + (1 |
## plant.ID) + (1 | RLC.order)
## Data: ulva
##
## REML criterion at convergence: 2146.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.6188 -0.3140  0.0118  0.3964  3.4453
```

```
##
## Random effects:
##   Groups   Name      Variance Std.Dev.
## plant.ID  (Intercept) 340.4    18.45
## RLC.order (Intercept)  0.0     0.00
## run       (Intercept) 315.0    17.75
## Residual                316.4    17.79
## Number of obs: 240, groups: plant.ID, 96; RLC.order, 6; run, 5
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)    5.429    18.421    2.821  0.295    0.789
## treatment1    26.123    20.521    2.780  1.273    0.299
## treatment2    33.643    20.521    2.780  1.639    0.207
## treatment3    40.934    20.521    2.780  1.995    0.147
## treatment4    45.547    20.521    2.780  2.220    0.120
## temperature27 -1.019     5.657  106.302 -0.180    0.857
## temperature30 -1.894     5.657  106.302 -0.335    0.738
##
## Correlation of Fixed Effects:
##              (Intr) trtmn1 trtmn2 trtmn3 trtmn4 tmpr27
## treatment1  -0.869
## treatment2  -0.869  0.984
## treatment3  -0.869  0.984  0.984
## treatment4  -0.869  0.984  0.984  0.984
## temperatr27 -0.154  0.000  0.000  0.000  0.000
## temperatr30 -0.154  0.000  0.000  0.000  0.000  0.500
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

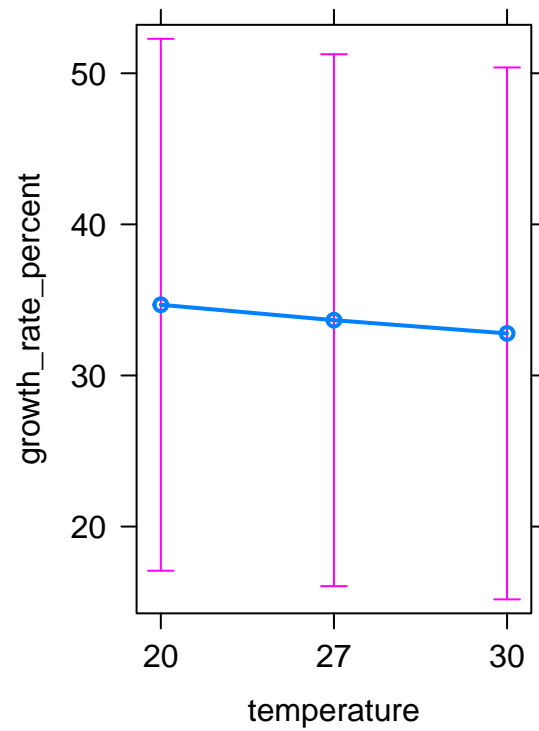
Effects Plots

```
plot(allEffects(run5.6_growth_model_noint))
```

treatment effect plot



temperature effect plot



HYPNEA

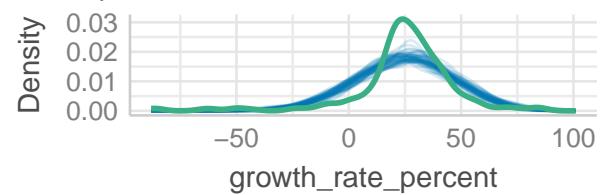
```
run5.6_growth_model_noint <- lmer(formula = growth_rate_percent ~ treatment +
  temperature + (1 | run) + (1 | plant.ID) +
  (1 | RLC.order), data = hypnea)
```

Check the performance of the model for Hypnea

```
performance::check_model(run5.6_growth_model_noint)
```


Posterior Predictive Check

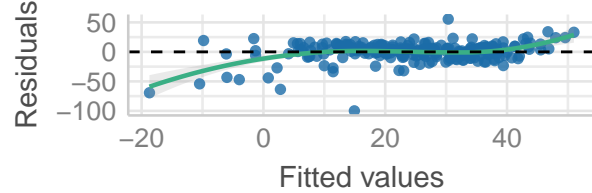
Model-predicted lines should resemble observed data



— Model-predicted data — Observed data

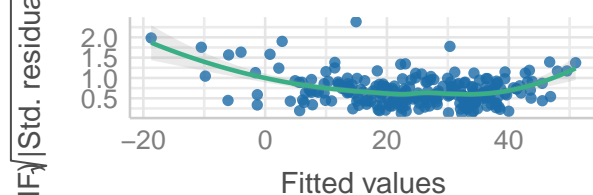
Linearity

Reference line should be flat and horizontal



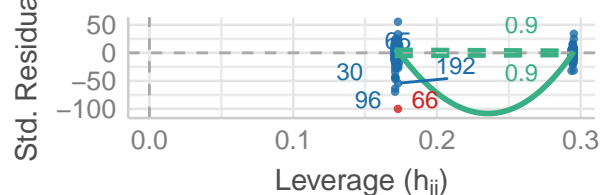
Homogeneity of Variance

Reference line should be flat and horizontal



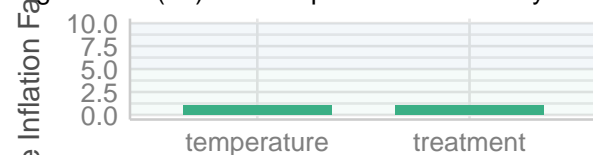
Influential Observations

Points should be inside the contour lines



Collinearity

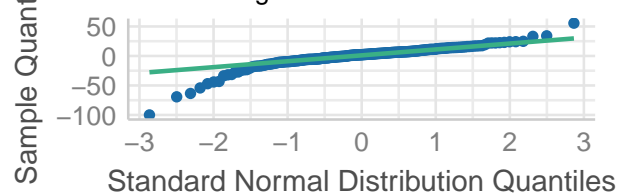
Higher bars (>5) indicate potential collinearity issue



low (< 5) moderate (< 10) high (> 10)

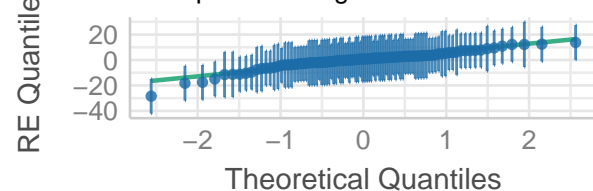
Normality of Residuals

Dots should fall along the line



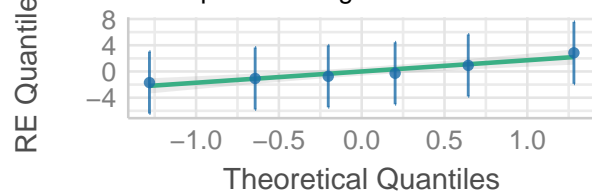
Normality of Random Effects (plant.ID)

Dots should be plotted along the line



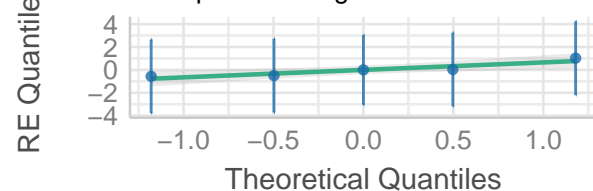
Normality of Random Effects (RLC.order)

Dots should be plotted along the line



Normality of Random Effects (run)

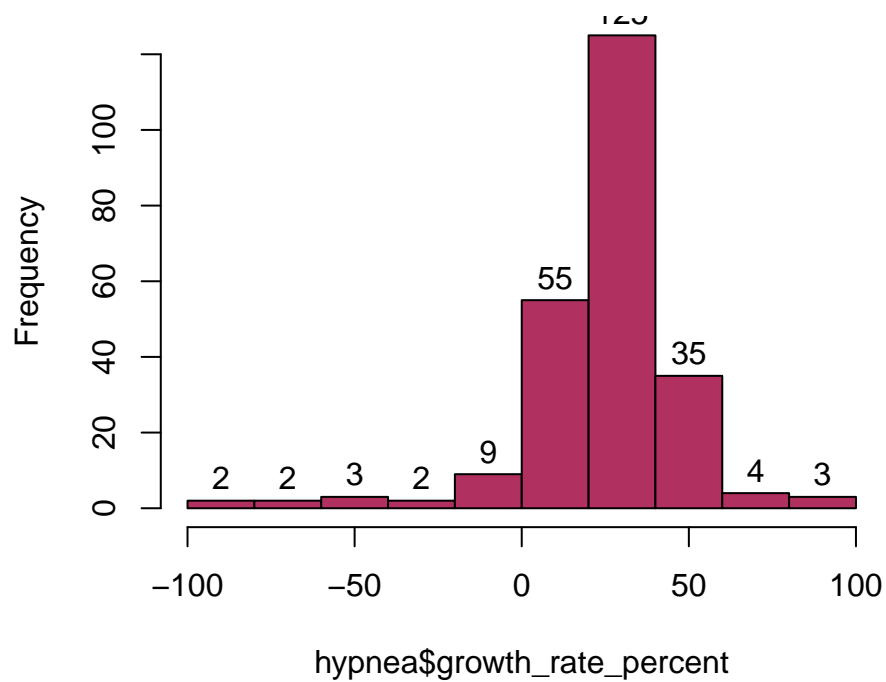
Dots should be plotted along the line



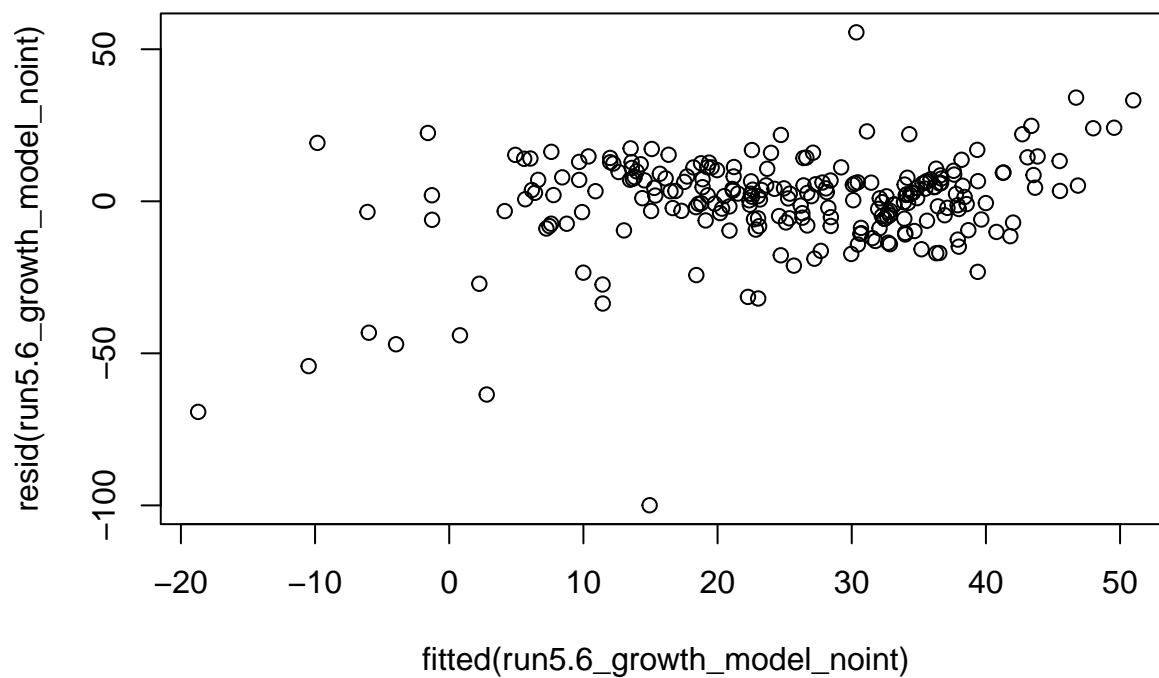
These outputs show the model is acceptable for the data

```
hist(hypnea$growth_rate_percent, main = paste("Hypnea musciformis Growth Rate (%)"),
     col = "maroon", labels = TRUE)
```

Hypnea musciformis Growth Rate (%)

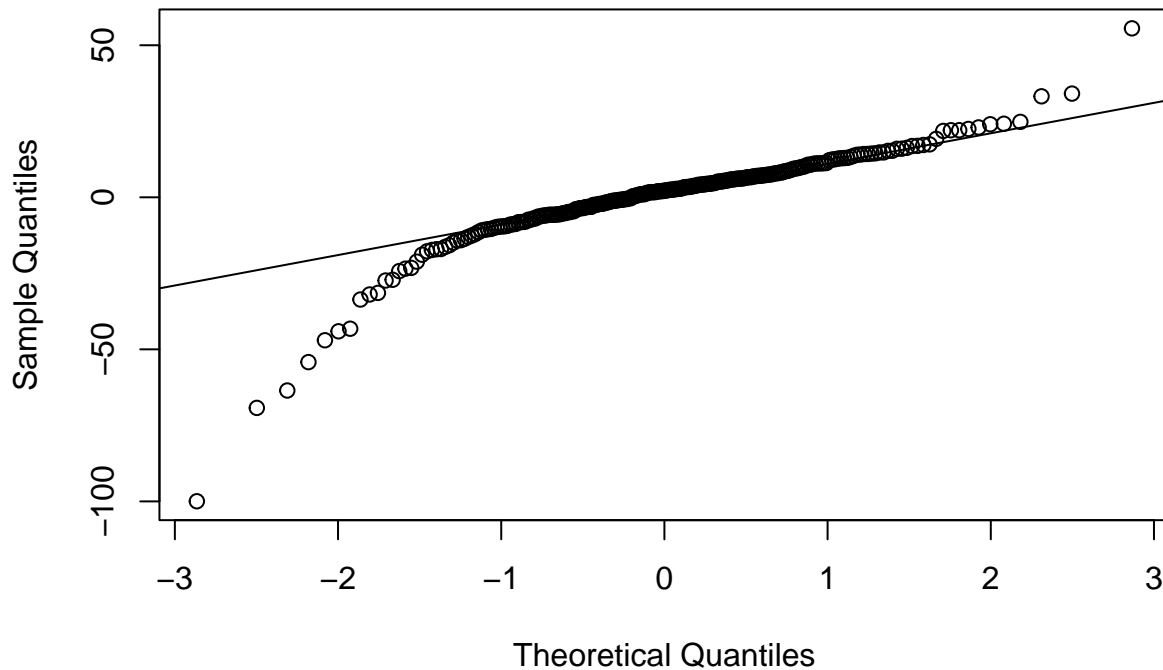


```
plot(resid(run5.6_growth_model_noint) ~ fitted(run5.6_growth_model_noint))
```



```
qqnorm(resid(run5.6_growth_model_noint))  
qqline(resid(run5.6_growth_model_noint))
```

Normal Q-Q Plot



```
anova(run5.6_growth_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  17326.0  4331.5     4   3.0249  13.9859 0.02726 *
## temperature    56.8    28.4     2  28.7741  0.0917 0.91269
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
hypnea_growth_model_aov <- aov(growth_rate_percent ~ treatment + temperature, data = hypnea)
TukeyHSD(hypnea_growth_model_aov, "treatment", ordered = FALSE)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = growth_rate_percent ~ treatment + temperature, data = hypnea)
##
## $treatment
##           diff           lwr           upr         p adj
## 2-1   -15.4100923 -27.0807398  -3.73944481 0.0031807
## 3-1   -11.5769131 -23.2475606   0.09373443 0.0530291
## 3.5-1  -0.7223387 -12.3929862  10.94830879 0.9998110
## 4-1   -24.2978542 -35.9685017 -12.62720672 0.0000003
## 3-2     3.8331792  -7.8374683  15.50382674 0.8955467
## 3.5-2  14.6877536   3.0171061  26.35840110 0.0057452
## 4-2    -8.8877619 -20.5584094   2.78288559 0.2262656
## 3.5-3  10.8545744  -0.8160731  22.52522186 0.0819446
## 4-3   -12.7209412 -24.3915887  -1.05029365 0.0249597
## 4-3.5 -23.5755155 -35.2461630 -11.90486801 0.0000008
```

```
r.squaredGLMM(run5.6_growth_model_noint)
```

```
##           R2m           R2c
## [1,] 0.1644597 0.4022791
```

```
summary(run5.6_growth_model_noint)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: growth_rate_percent ~ treatment + temperature + (1 | run) + (1 |
##   plant.ID) + (1 | RLC.order)
##   Data: hypnea
##
## REML criterion at convergence: 2081.8
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -5.6796 -0.3253  0.1225  0.4421  3.1569
##
## Random effects:
##   Groups      Name      Variance Std.Dev.
##   plant.ID   (Intercept) 111.159  10.543
##   RLC.order  (Intercept)   8.876   2.979
##   run        (Intercept)   3.190   1.786
##   Residual                    309.705  17.598
## Number of obs: 240, groups:  plant.ID, 96; RLC.order, 6; run, 5
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)   35.7815    4.1631  17.1258   8.595 1.28e-07 ***
## treatment2   -15.4101    3.5923 161.5491  -4.290 3.07e-05 ***
## treatment3   -11.5769    3.5923 161.5491  -3.223 0.00154 **
## treatment3.5  -0.7223    4.6393   2.4459  -0.156 0.88820
## treatment4   -24.2979    3.5923 161.5491  -6.764 2.34e-10 ***
## temperature27  0.1798    4.4743  19.3094   0.040 0.96836
## temperature30 -1.5010    4.2516  53.3627  -0.353 0.72546
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trtmn2 trtmn3 trt3.5 trtmn4 tmpr27
## treatment2  -0.431
## treatment3  -0.431  0.500
## treatmnt3.5 -0.495  0.387  0.387
## treatment4  -0.431  0.500  0.500  0.387
## temperatr27 -0.524  0.000  0.000  0.000  0.000
## temperatr30 -0.514  0.000  0.000  0.000  0.000  0.486
```

Effects Plots

```
plot(allEffects(run5.6_growth_model_noint))
```

