

Host activity before and during infection influences resulting parasite intensities

David R. Clark

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```
# Clear the working environment
rm(list = ls())
# Visualization
library(ggplot2)
library(visreg)
library(gridExtra)
source("http://highstat.com/Books/BGS/GAMM/RCodeP2/HighstatLibV6.R")
# (generalized) Linear mixed modeling
library(lme4)
library(glmmTMB)
library(lmodel2)
# Statistical analysis reporting and model validation
library(performance)
library(car)
library(lmtest)
library(DHARMa)
# Data wrangling
library(dplyr)
library(plyr)
library(tidyverse)
library(tidylog)
library(splancs)
```

Visualize some patterns in the raw data

```
# Load in dataset from above. This bit of code is meant to save time so people
# dont have to rerun the entire data parsing and calculating step above. Code
# will be saved for reproducibility.
IndBehav7 <- read_csv("IndividualBehaviors_20240501_WA.csv")

## Rows: 347 Columns: 27
## -- Column specification -----
## Delimiter: ","
## chr  (3): TrialTime, Sex, Treatment
## dbl  (23): fishID, BehavGroup, AvgVel, BehavVig, VarvelBef, PreWeight, PreLen...
## lgl  (1): ContrPeriod
##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.
```

```

IndBehav7 <- IndBehav7 %>%
  rename(Wormaf = Wormbf, AUC = AUC2)

## rename: renamed 2 variables (AUC, Wormaf)

# Subsetting down to Infected individuals
IndBehav7I <- IndBehav7 %>%
  filter(Infection == 1 & wormJump >= 0)

## filter: removed 172 rows (50%), 175 rows remaining

# getting the mean and SD of worms jumped
summary(IndBehav7I$wormJump)

##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      0.000   2.000   2.000   1.909   2.000   3.000

sqrt(var(IndBehav7I$wormJump))

## [1] 0.4189282

# Setting some of the factors back to factors
IndBehav7$fishID <- as.factor(IndBehav7$fishID)
IndBehav7$TrialTime <- as.factor(IndBehav7$TrialTime)
IndBehav7$Sex <- as.factor(IndBehav7$Sex)
IndBehav7$Infection <- as.factor(IndBehav7$Infection)
IndBehav7$ContInf <- as.factor(IndBehav7$ContInf)
IndBehav7$Died <- as.factor(IndBehav7$Died)
IndBehav7$Treatment <- as.factor(IndBehav7$Treatment)

# Calculating tissue tolerance for each individual.
IndBehav7 <- IndBehav7 %>%
  mutate(ChSMI = LateSMI - PreSMI) %>%
  mutate(TisTol = ChSMI/Totworm)

## mutate: new variable 'ChSMI' (double) with 85 unique values and 0% NA
## mutate: new variable 'TisTol' (double) with 47 unique values and 46% NA

# Subsetting down to female and males only to scale their SMI Males
IndBehavM <- IndBehav7 %>%
  filter(Sex == "M") %>%
  mutate(ScPSMI = scale(PreSMI), ScLSMI = scale(LateSMI))

## filter: removed 180 rows (52%), 167 rows remaining
## mutate: new variable 'ScPSMI' (double) with 41 unique values and 0% NA
##          new variable 'ScLSMI' (double) with 41 unique values and 0% NA

```

```

# Females
IndBehavF <- IndBehav7 %>%
  filter(Sex == "F") %>%
  mutate(ScPSMI = scale(PreSMI), ScLSMI = scale(LateSMI))

## filter: removed 167 rows (48%), 180 rows remaining
## mutate: new variable 'ScPSMI' (double) with 45 unique values and 0% NA
##          new variable 'ScLSMI' (double) with 45 unique values and 0% NA

# Combine the two separate dataframes together
IndBehav8 <- rbind(IndBehavF, IndBehavM)

# Scaling some variables to make them biologically comparable and better for
# model fitting
IndBehav8 <- IndBehav8 %>%
  mutate(ScVarvelBef = c(scale(VarvelBef)), ScNRatebf = c(scale(NRatebf)), ScBehavTol = c(scale(BehavTol)),
         ScTotworm = c(scale(Totworm)), ScAUC = c(scale(AUC)), ScBehavVig = c(scale(BehavVig)),
         ScChSMI = c(scale(ChSMI)), ScTisTol = c(scale(TisTol)))

## mutate: new variable 'ScVarvelBef' (double) with 86 unique values and 2% NA
##          new variable 'ScNRatebf' (double) with 85 unique values and 37% NA
##          new variable 'ScBehavTol' (double) with 49 unique values and 1% NA
##          new variable 'ScTotworm' (double) with 47 unique values and 46% NA
##          new variable 'ScAUC' (double) with 49 unique values and 43% NA
##          new variable 'ScBehavVig' (double) with 87 unique values and 1% NA
##          new variable 'ScChSMI' (double) with 85 unique values and 0% NA
##          new variable 'ScTisTol' (double) with 47 unique values and 46% NA

# Filtering down to only infected and only individuals who have VIE injections
# for sample size questions
IndBehavind <- IndBehav8 %>%
  distinct(fishID, .keep_all = TRUE) %>%
  filter(Treatment == "VIE") %>%
  filter(Infection == "1")

## distinct: removed 260 rows (75%), 87 rows remaining
## filter: removed 52 rows (60%), 35 rows remaining
## filter: removed 18 rows (51%), 17 rows remaining

```

What hypotheses we want to test with these data and what data we can use to test them?

- Does host behavioral vigor trade-off with their ability to resist parasite infection?
- Does higher host activity during infection lead to higher parasite infection?

Does infection or sex variation impact the average activity of individuals?

Individuals had 3 behavioral trials per time period of infection (i.e. 3 behavioral trials before infection) and therefore using preliminary analysis we showed that there is no difference due to time of day of these recordings so we averaged and quantified the variance of the velocities for that day to get an average activity per trial time.

This analysis uses the average activity for each individual at each trial point.

Description, development, and fitting of linear model for the analysis

We will use a linear mixed model to analyze how average activity differs by infection status and sexual variation. FishID is included as a random term to allow for non-independence of individuals due to multiple measurements per individual across time.

- Deterministic
- $AvgVel_{det} = a + b_1 \text{TrialTime} + b_2 \text{Infection} * b_3 \text{Sex} + b_4 \text{ScPSMI} + b_5 \text{ScRPLength} + b_6 \text{Treatment} + a_i$
- Stochastic
 - $AvgVel \sim N(AvgVel_{det}, \sigma^2)$
 - $a_i \sim N(0, \sigma_{fishID}^2)$
- Fixed
 - TrialTime
 - Infection status
 - Sex
 - An interaction between Sex and Infection status
 - Scaled Pre-infection SMI
 - Scaled residuals from length and sex
 - VIE Treatment
- Random
 - fishID

```
# Fit a linear model for checking what explanatory factors are important for  
# Average activity Note this is a linear mixed model because we have multiple  
# measures per fish and therefore, need to account for non-independence between  
# measures.  
AvgVelLM <- lmer(AvgVel ~ TrialTime + Infection * Sex + ScPSMI + ScRPLength + Treatment +  
  (1 | fishID), IndBehav8)
```

```
# Summary to see the relationship of the variables.  
summary(AvgVelLM)
```

```
## Linear mixed model fit by REML ['lmerMod']  
## Formula: AvgVel ~ TrialTime + Infection * Sex + ScPSMI + ScRPLength +  
##      Treatment + (1 | fishID)  
##      Data: IndBehav8  
##  
## REML criterion at convergence: 561.2  
##  
## Scaled residuals:  
##      Min      1Q  Median      3Q      Max  
## -2.3311 -0.5476 -0.1322  0.5362  2.7978  
##  
## Random effects:  
## Groups   Name              Variance Std.Dev.
```

```
## fishID (Intercept) 0.3020 0.5496
## Residual 0.3373 0.5808
## Number of obs: 250, groups: fishID, 86
##
## Fixed effects:
## Estimate Std. Error t value
## (Intercept) 2.01656 0.16934 11.908
## TrialTimeLate 0.13613 0.08857 1.537
## TrialTimeLater -0.20354 0.09157 -2.223
## Infection1 -0.09947 0.19610 -0.507
## SexM 0.43701 0.20882 2.093
## ScPSMI -0.11313 0.12255 -0.923
## ScRPLength -0.04706 0.11945 -0.394
## TreatmentVIE 0.07066 0.14596 0.484
## Infection1:SexM -0.25897 0.28652 -0.904
##
## Correlation of Fixed Effects:
## (Intr) TrilTmLt TrlTmLtr Infct1 SexM ScPSMI ScRPLn TrtVIE
## TrialTimeLt -0.262
## TrialTimLtr -0.265 0.484
## Infection1 -0.675 0.000 0.005
## SexM -0.611 0.000 0.005 0.528
## ScPSMI 0.063 0.000 -0.004 -0.009 0.046
## ScRPLength -0.074 0.000 0.005 0.085 0.040 -0.803
## TreatmntVIE -0.419 0.000 0.001 0.088 0.028 -0.156 0.053
## Infctn1:SxM 0.444 0.000 0.013 -0.692 -0.742 -0.084 -0.037 -0.007
```

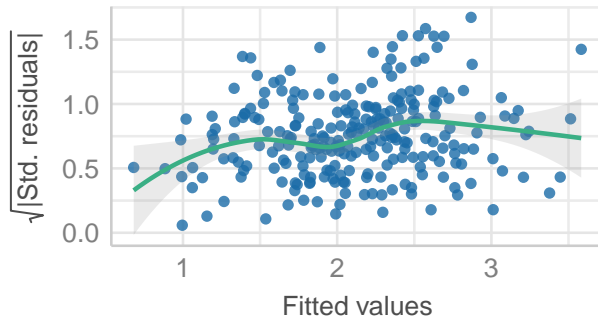
Validate that the model fits well and there are no problems

```
# Using the check_model function from the perforamnce package to check the
# model validation
```

```
check_model(AvgVellM, check = c("qq", "normality", "homogeneity"))
```

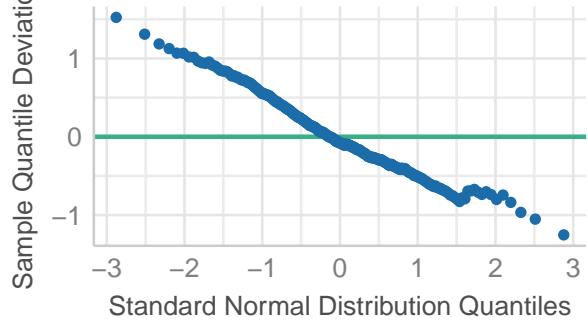
Homogeneity of Variance

Reference line should be flat and horizontal



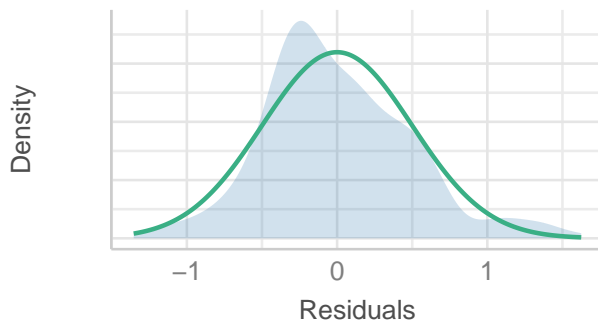
Normality of Residuals

Dots should fall along the line



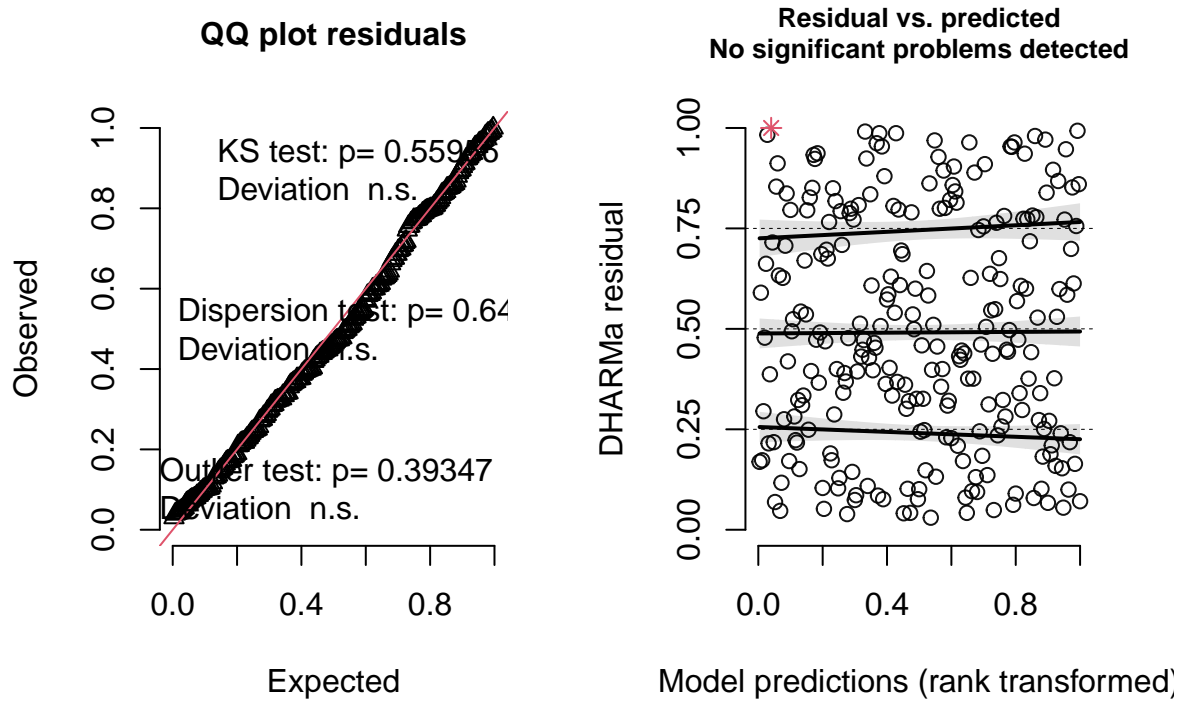
Normality of Residuals

Distribution should be close to the normal curve



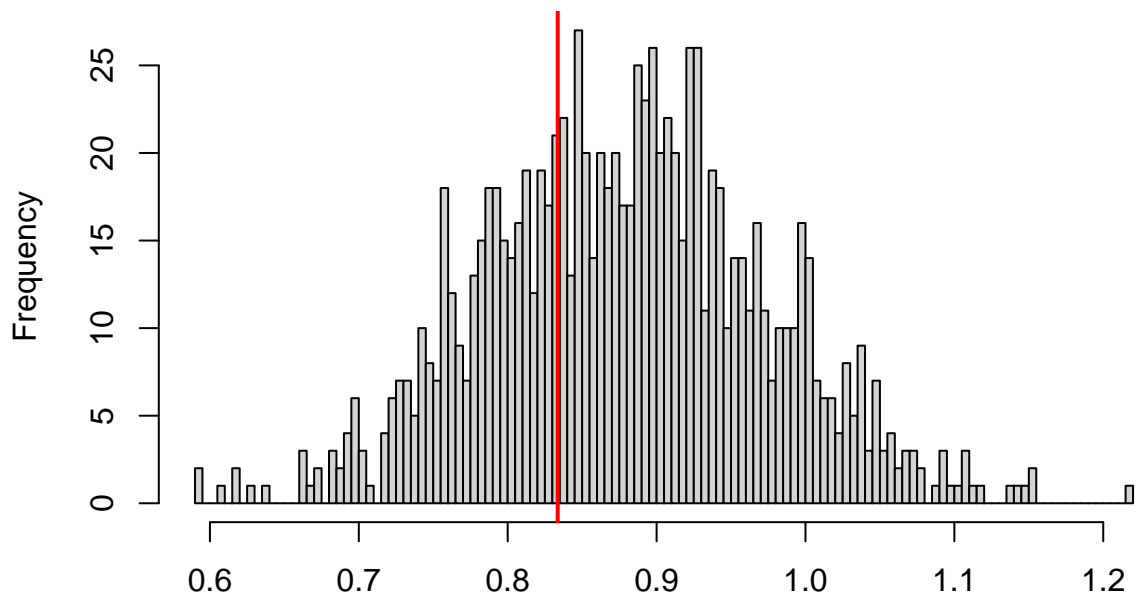
```
# Using the Dharma package to check quantile residuals First simulating the  
# quantile residuals  
sim_residuals_AvgVelLM <- simulateResiduals(AvgVelLM, 1000)  
# Plotting the quantile residuals to test how quantile residuals look  
plot(sim_residuals_AvgVelLM)
```

DHARMa residual



```
# Testing for dispersion
testDispersion(sim_residuals_AvgVelLM)
```

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated



```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94836, p-value = 0.642
## alternative hypothesis: two.sided

# All model validation looks good.
```

Testing the significance of factors in our model using a Kenward-Rodgers F test

```
# F test to test for significance of slope of variables
Anova(AvgVelLM, test = "F", type = 3)
```

```
## Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
##
## Response: AvgVel
##
```

	F	Df	Df.res	Pr(>F)
(Intercept)	141.8069	1	94.033	< 2.2e-16 ***
TrialTime	6.9196	2	163.528	0.001304 **
Infection	0.2573	1	77.719	0.613431
Sex	4.3793	1	77.704	0.039642 *
ScPSMI	0.8522	1	78.960	0.358746
ScRPLength	0.1552	1	78.107	0.694694
Treatment	0.2343	1	79.162	0.629676
Infection:Sex	0.8169	1	78.805	0.368842

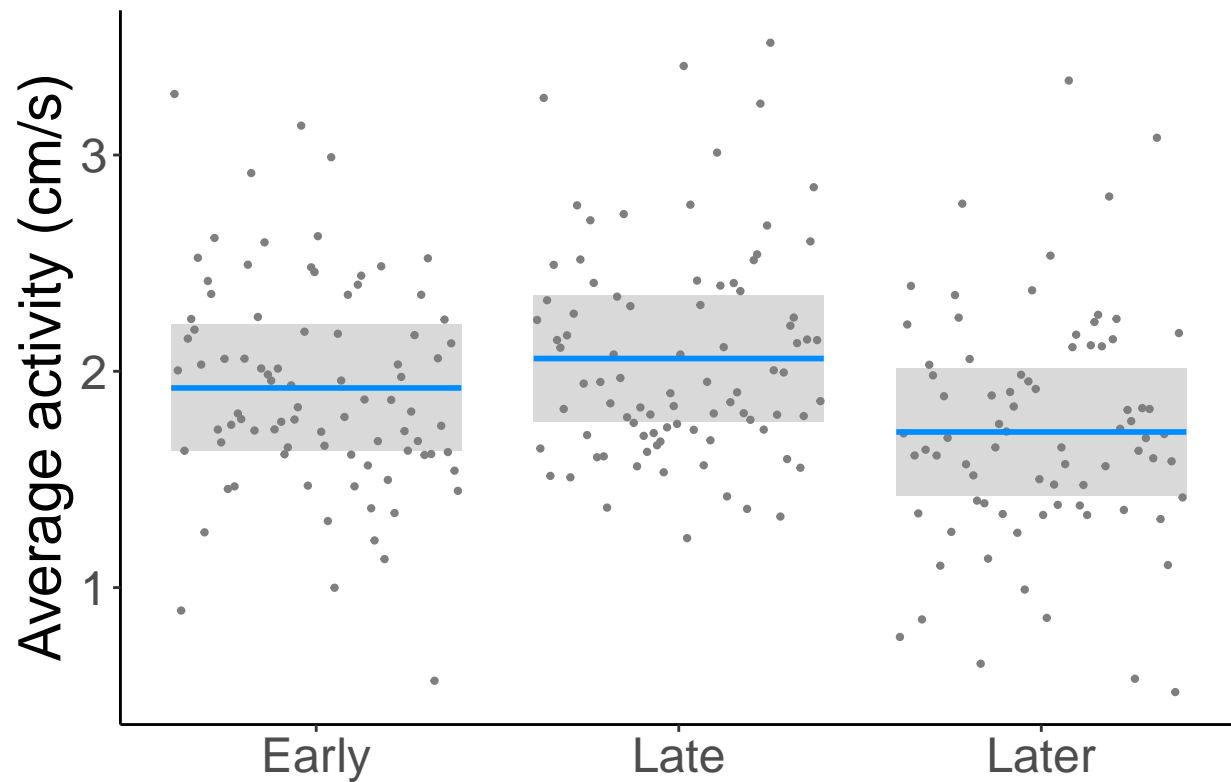
```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Visualize the important explanatory factors for average activity

```
# TrialTimeGraph
AvgVelbyTT = visreg(AvgVelLM, scale = "response", "TrialTime", partial = T, gg = TRUE) +
  theme_classic() + theme(legend.position = "none") + ylab("Average activity (cm/s)") +
  xlab(" ") + theme(text = element_text(size = 22))
```

```
## Conditions used in construction of plot
## Infection: 1
## Sex: F
## ScPSMI: -0.07158284
## ScRPLength: 0.04746859
## Treatment: UNTOUCHED
## fishID: 1
```

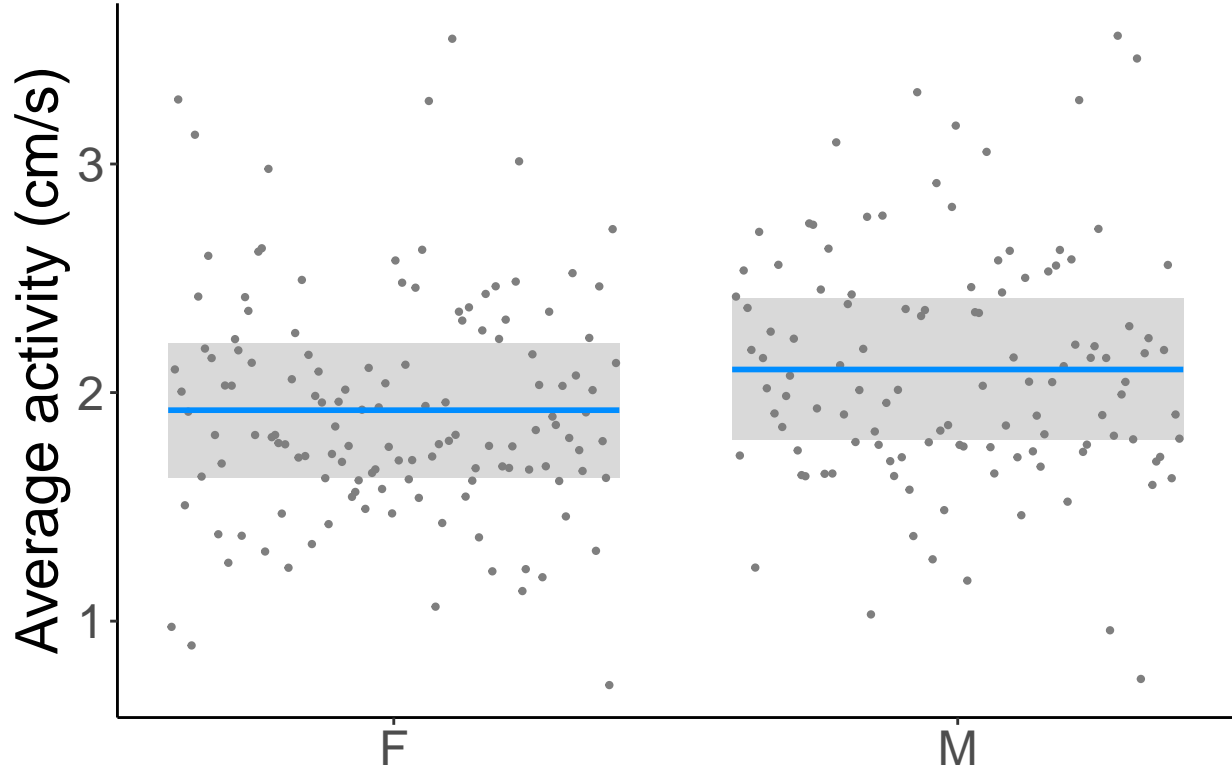
```
# Print the graph
print(AvgVelbyTT)
```

```
# TrialTimeGraph
AvgVelbySex = visreg(AvgVelLM, scale = "response", "Sex", partial = T, gg = TRUE) +
  theme_classic() + theme(legend.position = "none") + ylab("Average activity (cm/s)") +
  xlab(" ") + theme(text = element_text(size = 22))
```

```
## Conditions used in construction of plot
## TrialTime: Early
## Infection: 1
## ScPSMI: -0.07158284
## ScRPLength: 0.04746859
## Treatment: UNTOUCHED
## fishID: 1
```

```
# Print the graph
print(AvgVelbySex)
```



->

Description, development, and fitting of linear model for the analysis

We will use a linear mixed model to analyze how Change in activity differs by infection status and sexual variation. FishID is included as a random term to allow for non-independence of individuals due to multiple measurements per individual across time.

- Deterministic
- $ChVel_{det} = a + b_1Sex + b_2ScResidPLSMI + b_3ScVarvelBef + b_4ScRPLength + b_5ScBehavVig + b_6Treatment + b_8Infection + b_7Infection:Treatment + a_i$
- Stochastic
 - $ChVel \sim N(ChVel_{det}, \sigma^2)$
 - $a_i \sim N(0, \sigma_{fishID}^2)$
- Fixed
 - Sex
 - Scaled Residuals from body condition and length
 - Scaled variance in velocity before infection
 - Scaled residuals from length and sex
 - Infection
 - Interaction between VIE treatment and infection status
 - VIE treatment of the fish

- Random
 - fishID

```
IndBehavCh <- IndBehav8 %>%
  filter(TrialTime != "Before") %>%
  mutate(TrialTime = recode(TrialTime, Early = "1", Late = "2", Later = "3"))
```

```
## filter: no rows removed
```

```
## mutate: changed 261 values (100%) of 'TrialTime' (0 new NA)
```

```
IndBehavCh$TrialTime <- as.factor(IndBehavCh$TrialTime)
IndBehavCh$Infection <- as.factor(IndBehavCh$Infection)
```

```
IndBehavChI <- IndBehavCh %>%
  filter(Infection == "1")
```

```
## filter: removed 117 rows (45%), 144 rows remaining
```

```
IndBehavChU <- IndBehavCh %>%
  filter(Infection == "0")
```

```
## filter: removed 144 rows (55%), 117 rows remaining
```

```
# Fit a linear model for checking what explanatory factors are important for
# Variance in activity Note this is a linear mixed model because we have
# multiple measures per fish and therefore, need to account for
# non-independence between measures.
ChVelLM <- lmer(ChBehav ~ Sex + ScChSMI + ScPSMI + Treatment + ScBehavVig + Infection +
  TrialTime + Infection:ScBehavVig + ScBehavVig:Infection + Infection:TrialTime +
  Infection:Sex + TrialTime:Sex + TrialTime:Sex:Infection + TrialTime:Infection:ScBehavVig +
  (1 | fishID), IndBehavCh)

# Summary to see the relationship of the variables.
summary(ChVelLM)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## ChBehav ~ Sex + ScChSMI + ScPSMI + Treatment + ScBehavVig + Infection +
##   TrialTime + Infection:ScBehavVig + ScBehavVig:Infection +
##   Infection:TrialTime + Infection:Sex + TrialTime:Sex + TrialTime:Sex:Infection +
##   TrialTime:Infection:ScBehavVig + (1 | fishID)
## Data: IndBehavCh
##
## REML criterion at convergence: 370.8
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.38935 -0.53312 -0.02744  0.53279  2.58157
```

```
##
## Random effects:
##   Groups   Name      Variance Std.Dev.
## fishID    (Intercept) 0.1491   0.3861
## Residual                0.1319   0.3631
## Number of obs: 250, groups: fishID, 86
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                        0.50869    0.13002   3.912
## SexM                              0.09268    0.17459   0.531
## ScChSMI                           -0.07553    0.04960  -1.523
## ScPSMI                            -0.06499    0.05225  -1.244
## TreatmentVIE                      0.01237    0.10248   0.121
## ScBehavVig                       -0.19721    0.09414  -2.095
## Infection1                       -0.25889    0.16164  -1.602
## TrialTime2                        0.05679    0.11558   0.491
## TrialTime3                       -0.30160    0.11560  -2.609
## ScBehavVig:Infection1             -0.19062    0.12019  -1.586
## Infection1:TrialTime2             -0.03576    0.15463  -0.231
## Infection1:TrialTime3             0.28581    0.15562   1.837
## SexM:Infection1                   0.13788    0.23569   0.585
## SexM:TrialTime2                   0.21458    0.16821   1.276
## SexM:TrialTime3                   0.21344    0.17069   1.250
## SexM:Infection1:TrialTime2        -0.11109    0.22563  -0.492
## SexM:Infection1:TrialTime3        -0.50627    0.23463  -2.158
## ScBehavVig:Infection0:TrialTime2  0.01548    0.09098   0.170
## ScBehavVig:Infection1:TrialTime2  0.03507    0.07113   0.493
## ScBehavVig:Infection0:TrialTime3 -0.14388    0.09219  -1.561
## ScBehavVig:Infection1:TrialTime3 -0.03645    0.07477  -0.488
```

```
##
## Correlation matrix not shown by default, as p = 21 > 12.
## Use print(x, correlation=TRUE) or
##      vcov(x)          if you need it
```

```
# ChVelLMI<-lmer(log10(ChBehav)~Sex+ScResidPLSMI+ScChSMI+TrialTime+ScRPLength+ScBehavVig*Sex+Treatment+
# IndBehavChI) summary(ChVelLMI) Anova(ChVelLMI, type='3', test='F')
```

```
# ChVelLMU<-lmer(ChBehav~Sex+ScResidPLSMI+ScSMI+ScRPLength+Sex*ScBehavVig+Treatment+(1|BehavGroup),
# IndBehavChU) summary(ChVelLMU) Anova(ChVelLMU, type='3', test='F')
```

```
# ChVelLMI<-lmer(ChBehav~ScBehavVig+Sex+ScChSMI+ScRPLength+Treatment+(1|fishID),
# IndBehavI3) summary(ChVelLMI) Anova(ChVelLMI, test='F', type=3)
# ChVelLMU<-lmer(ChBehav~ScBehavVig+Sex+ScChSMI+ScRPLength+Treatment+(1|fishID),
# IndBehavU3) summary(ChVelLMU) Anova(ChVelLMU, test='F', type=3)
```

```
# IndBehavI3<- IndBehav8 %>% filter(Infection == '1') IndBehavU3<- IndBehav8
# %>% filter(Infection == '0')
```

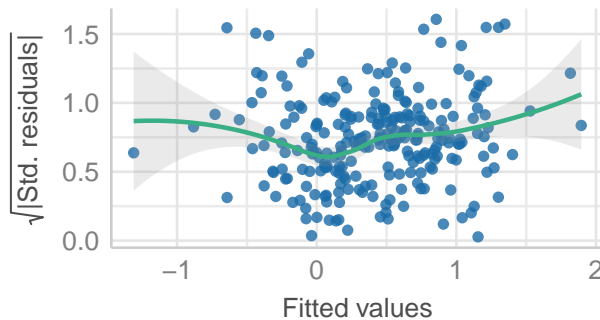
Validate that the model fits well and there are no problems

```
# Using the check_model function from the performamnce package to check the  
# model validation
```

```
check_model(ChVeLLM, check = c("qq", "normality", "homogeneity"))
```

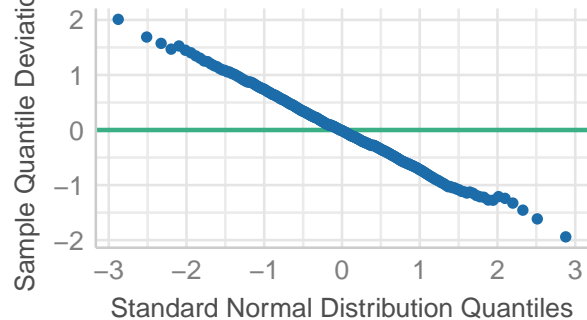
Homogeneity of Variance

Reference line should be flat and horizontal



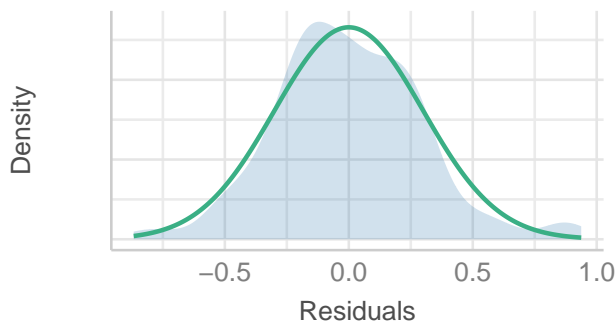
Normality of Residuals

Dots should fall along the line



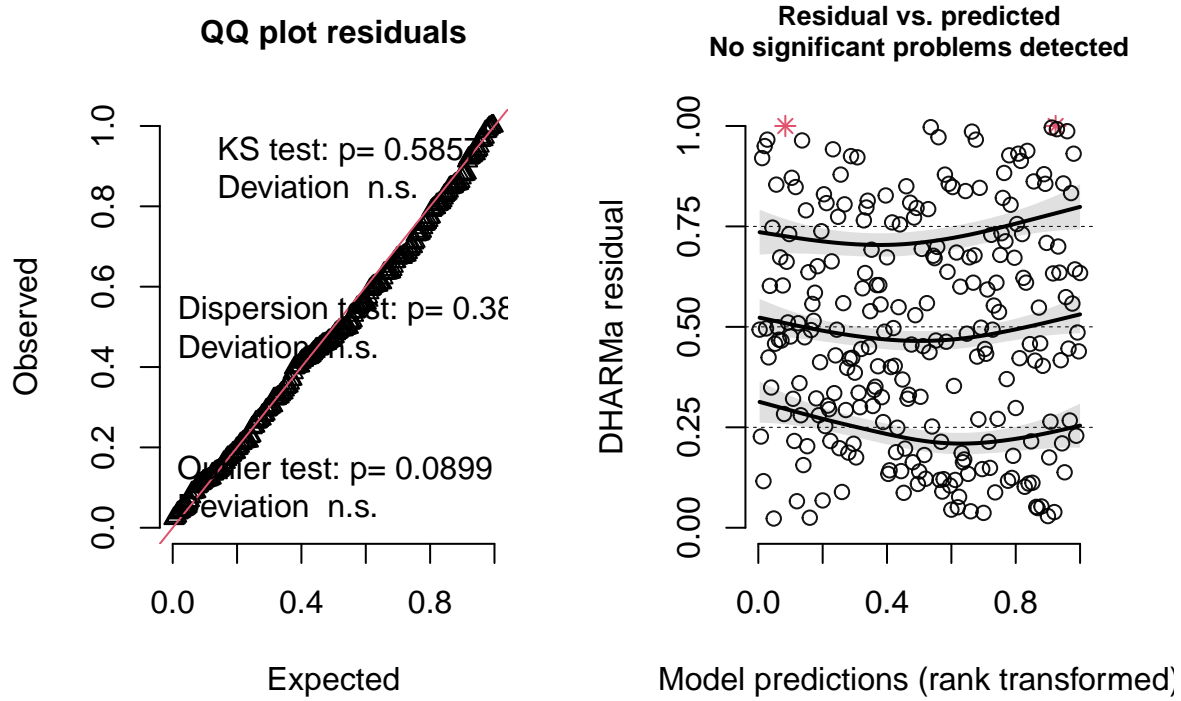
Normality of Residuals

Distribution should be close to the normal curve



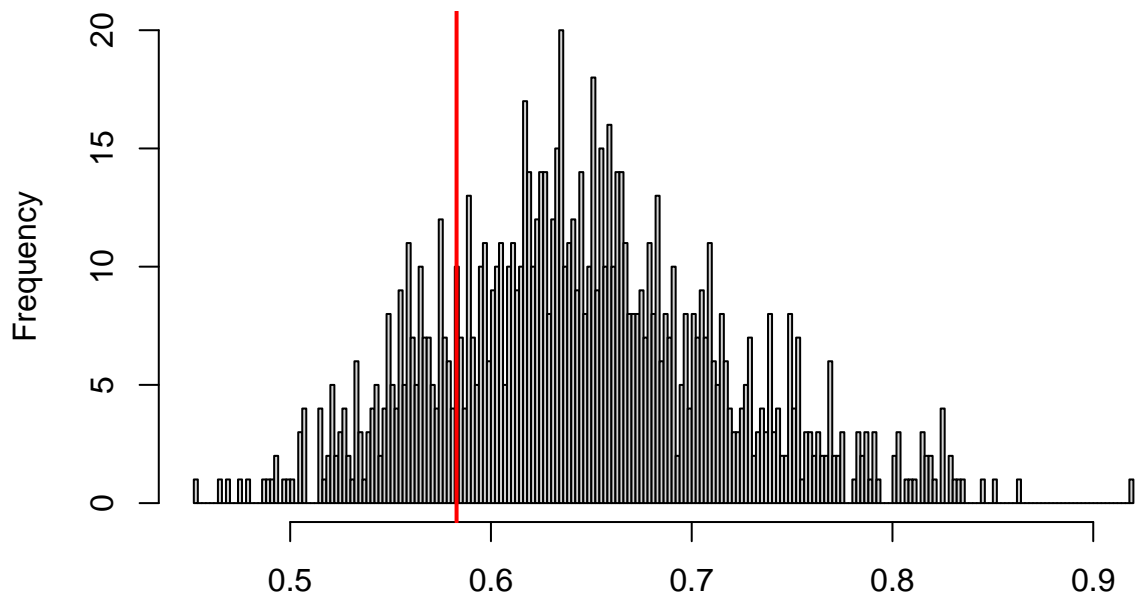
```
# Using the Dharma package to check quantile residuals First simulating the  
# quantile residuals  
sim_residuals_ChVeLLM <- simulateResiduals(ChVeLLM, 1000)  
# Plotting the quantile residuals to test how quantile residuals look  
plot(sim_residuals_ChVeLLM)
```

DHARMA residual



```
# Testing for dispersion
testDispersion(sim_residuais_ChVeLLM)
```

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated



```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.90301, p-value = 0.384
## alternative hypothesis: two.sided
```

*# There are some problems with this model validation. It doesnt look model
breaking but definitely should look at other model error structures to
resolve the issues.*

Testing the significance of factors in our model using a Kenward-Rodgers F test

```
# F test to test for significance of slope of variables
Anova(ChVelLM, test = "F", type = 3)
```

```
## Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
##
## Response: ChBehav
##
##          F Df  Df.res    Pr(>F)
## (Intercept) 15.3059 1 131.751 0.0001458 ***
## Sex          0.2818 1 144.564 0.5963388
## ScChSMI      2.3185 1  78.235 0.1318779
## ScPSMI       1.5471 1  77.705 0.2172983
## Treatment    0.0146 1  77.142 0.9042483
## ScBehavVig   4.3881 1 145.124 0.0379280 *
## Infection    2.5654 1 143.280 0.1114269
## TrialTime     5.5526 2 152.077 0.0047044 **
## ScBehavVig:Infection 2.5152 1 144.093 0.1149481
## Infection:TrialTime 2.5549 2 152.327 0.0810283 .
## Sex:Infection 0.3422 1 143.456 0.5594580
## Sex:TrialTime 1.0689 2 152.654 0.3459287
## Sex:Infection:TrialTime 2.5193 2 153.591 0.0838363 .
## ScBehavVig:Infection:TrialTime 1.1301 4 153.201 0.3444655
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
cpinf = c("gray25", "purple")
labellers <- labeller(Sex = c(F = "Females", M = "Males"))

# TrialTimeGraph
ChVelbySexUnfVR = visreg(ChVelLM, scale = "response", "TrialTime", by = "Sex", cond = list(Infection = 1,
partial = T, plot = FALSE))

ChVelbySexUnfVRfit <- ChVelbySexUnfVR$fit
ChVelbySexUnfVRres <- ChVelbySexUnfVR$res

ChVelbySexInfVR = visreg(ChVelLM, scale = "response", "TrialTime", "Sex", cond = list(Infection = "1"),
partial = T, plot = FALSE)

ChVelbySexInfVRfit <- ChVelbySexInfVR$fit
```

```

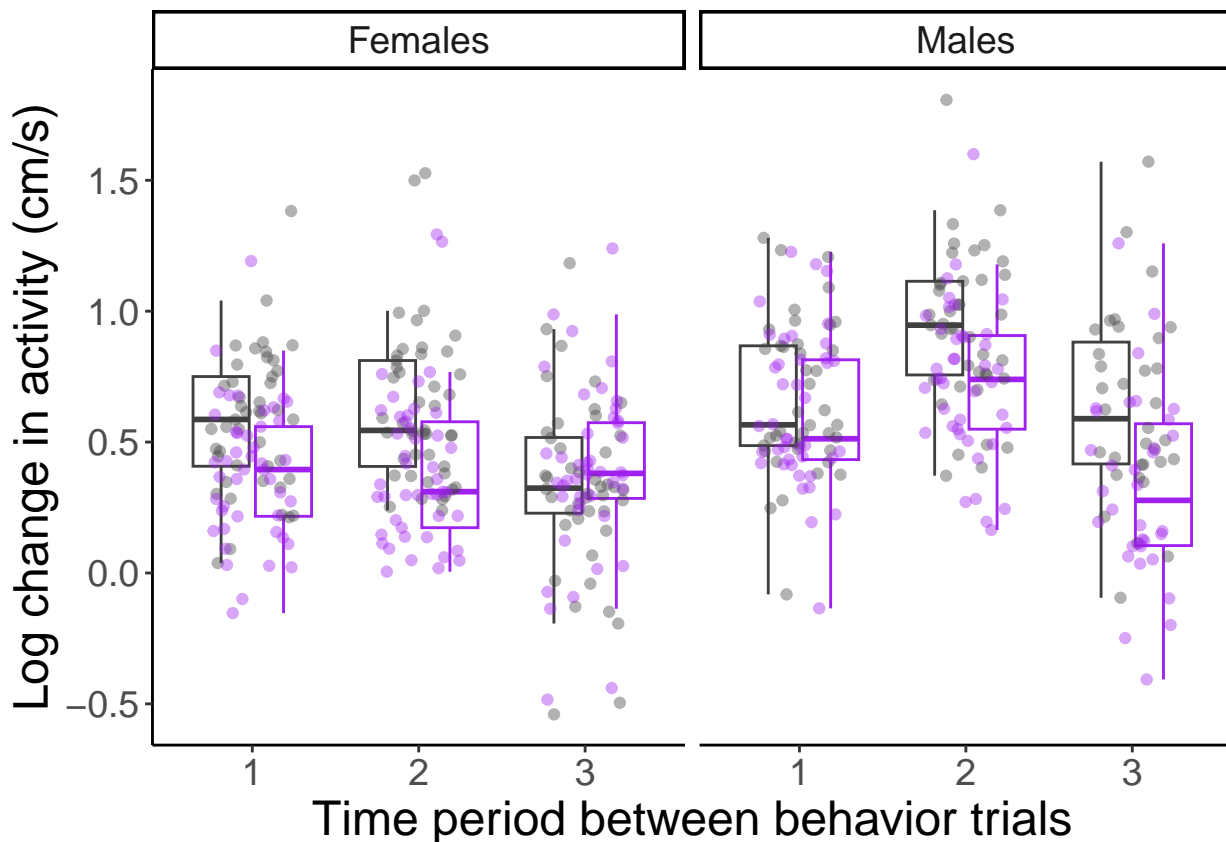
ChVelbySexInfVRres <- ChVelbySexInfVR$res

ChVelbySexVRres <- rbind(ChVelbySexUnfVRres, ChVelbySexInfVRres)

ChActSx <- ggplot(ChVelbySexVRres, aes(x = TrialTime, y = visregRes, color = Infection),
  group = Infection) + geom_boxplot(outliers = FALSE) + geom_jitter(aes(x = TrialTime,
  y = visregRes, color = Infection, group = Infection), width = 0.25, alpha = 0.4) +
  theme_classic() + theme(legend.position = "none") + ylab("Log change in activity (cm/s)") +
  xlab("Time period between behavior trials") + theme(text = element_text(size = 18)) +
  scale_color_manual(values = cpinf) + scale_fill_manual(values = cpinf) + facet_wrap(~Sex,
  labeller = labellers)

print(ChActSx)

```



```

# Sex graph by trialtime and infection for infected individuals
# ChVelbySexInfVR=visreg(ChVelLM, scale='response', 'TrialTime','Sex',
# cond=list(Infection='1'), partial=T, plot=FALSE)
# ChVelbySexInfVRfit<-ChVelbySexInfVR$fit
# ChVelbySexInfVRres<-ChVelbySexInfVR$res ChActSxI<-ggplot(ChVelbySexInfVRres,
# aes(x=Sex, y=visregRes, group=Sex, color=Sex))+
# geom_boxplot(data=ChVelbySexInfVRres, aes(x=Sex, y=visregRes, group=Sex),
# method='lm')+ geom_jitter(data=ChVelbySexInfVRres, aes(x=Sex,y=visregRes))+
# theme_classic()+ #theme(legend.position='none')+ ylab('Change in Activity
# (cm/s)')+ xlab('Sex')+ theme(text=element_text(size=18))+
# scale_color_manual(values=cpsex)+
# scale_fill_manual(values=cpsex)+facet_wrap(~TrialTime)+ggtitle('Infected')+

```



```
# ylim(-1.7,0.7) print(ChActSxI) grid.arrange(ChActSxI, ChActSxU, nrow=1)
```

->

What factors are important for host behavioral vigor and do is there any sexual variation in host behavioral vigor?

Description, development, and fitting of linear model for the analysis

We will use a linear model to analyze how behavioral vigor differs by sexual variation and other important host traits. Given each host only has one behavioral vigor measure, we do not need the fishID random effect used in previous models/

- Deterministic
 - $BehavVig_{det} = a + b_1Sex + b_2ScResidPLSMI + b_3ScVarVelBef + b_4ScRPLength + b_5Treatment + b_6Sex:ScResidPLSMI + b_7Sex:ScRPLength + a_i$
- Stochastic
 - $BehavVig \sim N(BehavVig_{det}, \sigma^2)$
 - $a_i \sim N(0, \sigma_{BehavGroup}^2)$
- Fixed
 - Sex
 - Scaled residuals from Pre-infection SMI and length
 - Scaled variance in velocity before infection
 - Scaled residuals from length and sex
 - VIE treatment
 - An interaction between sex and Pre-infection SMI
 - An interaction between sex and Pre-infection Length
- Random
 - Behavior group of recording

```
# Fit a linear model for checking what explanatory factors are important for
# Variance in Velocity Note this is a linear mixed model because we have
# multiple measures per fish and therefore, need to account for
# non-independence between measures.
BehavVigLM <- glmmTMB(BehavVig ~ Sex * ScResidPLSMI + ScVarvelBef + ScRPLength +
  Treatment + Sex:ScRPLength + Infection + (1 | BehavGroup), family = Gamma("log"),
  IndBehav8)

# Summary to see the relationship of the variables.
summary(BehavVigLM)
```

```
## Family: Gamma ( log )
## Formula:
## BehavVig ~ Sex * ScResidPLSMI + ScVarvelBef + ScRPLength + Treatment +
## Sex:ScRPLength + Infection + (1 | BehavGroup)
## Data: IndBehav8
```

```
##
##      AIC      BIC   logLik deviance df.resid
##    459.2    498.2   -218.6   437.2     244
##
## Random effects:
##
## Conditional model:
##   Groups      Name      Variance Std.Dev.
## BehavGroup (Intercept) 0.006146 0.0784
## Number of obs: 255, groups: BehavGroup, 7
##
## Dispersion estimate for Gamma family (sigma^2): 0.167
##
## Conditional model:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    0.40490    0.06149   6.585 4.54e-11 ***
## SexM           -0.07774    0.05364  -1.449  0.14725
## ScResidPLSMI    0.08681    0.04855   1.788  0.07377 .
## ScVarvelBef     0.27656    0.03084   8.967 < 2e-16 ***
## ScRPLength     -0.07254    0.03157  -2.298  0.02157 *
## TreatmentVIE    -0.03154    0.05857  -0.538  0.59025
## Infection1      0.05146    0.05356   0.961  0.33662
## SexM:ScResidPLSMI -0.17873    0.05836  -3.062  0.00220 **
## SexM:ScRPLength  -0.20957    0.06983  -3.001  0.00269 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Post-hoc analysis where we split by sex

```
IndBehav8M <- IndBehav8 %>%
  filter(Sex == "M")
```

```
## filter: removed 135 rows (52%), 126 rows remaining
```

```
IndBehav8F <- IndBehav8 %>%
  filter(Sex == "F")
```

```
## filter: removed 126 rows (48%), 135 rows remaining
```

```
# Fit a linear model for checking what explanatory factors are important for
# Variance in Velocity Note this is a linear mixed model because we have
# multiple measures per fish and therefore, need to account for
# non-independence between measures. Females
BehavVigLMF <- glmmTMB(BehavVig ~ ScResidPLSMI + ScVarvelBef + Treatment + ScRPLength +
  (1 | BehavGroup), family = Gamma("log"), IndBehav8F)
# Males
BehavVigLMM <- glmmTMB(BehavVig ~ ScResidPLSMI + ScVarvelBef + ScRPLength + Treatment +
  (1 | BehavGroup), family = Gamma("log"), IndBehav8M)

# Summary to see the relationship of the variables for females
summary(BehavVigLMF)
```

```
## Family: Gamma ( log )
## Formula:
## BehavVig ~ ScResidPLSMI + ScVarvelBef + Treatment + ScRPLength +
## (1 | BehavGroup)
## Data: IndBehav8F
##
##      AIC      BIC    logLik deviance df.resid
##    205.3    225.5    -95.6    191.3      125
##
## Random effects:
##
## Conditional model:
## Groups      Name      Variance Std.Dev.
## BehavGroup (Intercept) 0.06519  0.2553
## Number of obs: 132, groups: BehavGroup, 7
##
## Dispersion estimate for Gamma family (sigma^2): 0.112
##
## Conditional model:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)   0.51769    0.10826   4.782 1.73e-06 ***
## ScResidPLSMI  0.13492    0.04603   2.931 0.00338 **
## ScVarvelBef   0.22637    0.04584   4.938 7.87e-07 ***
## TreatmentVIE -0.15760    0.07191  -2.192 0.02841 *
## ScRPLength   -0.07310    0.02742  -2.666 0.00768 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

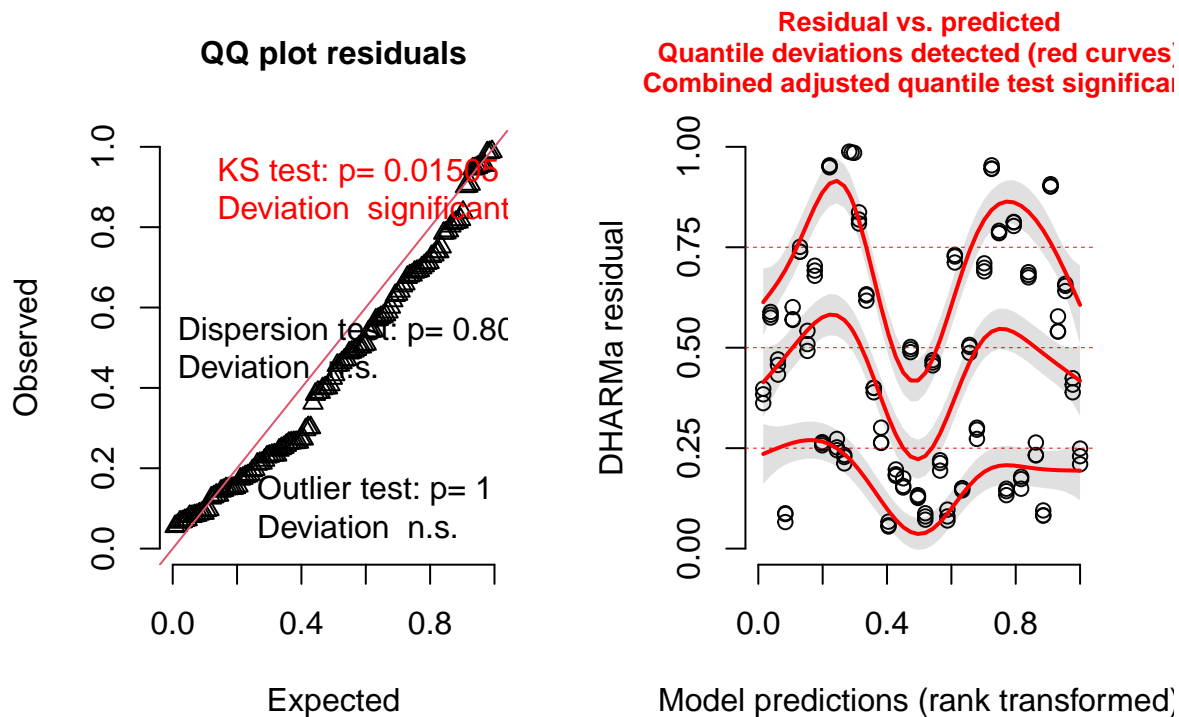
```
# Summary to see the relationship of the variables for males.
summary(BehavVigLMM)
```

```
## Family: Gamma ( log )
## Formula:
## BehavVig ~ ScResidPLSMI + ScVarvelBef + ScRPLength + Treatment +
## (1 | BehavGroup)
## Data: IndBehav8M
##
##      AIC      BIC    logLik deviance df.resid
##    232.4    252.1   -109.2    218.4      116
##
## Random effects:
##
## Conditional model:
## Groups      Name      Variance Std.Dev.
## BehavGroup (Intercept) 0.03055  0.1748
## Number of obs: 123, groups: BehavGroup, 7
##
## Dispersion estimate for Gamma family (sigma^2): 0.178
##
## Conditional model:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)   0.27679    0.08762   3.159 0.00158 **
## ScResidPLSMI -0.07309    0.03834  -1.907 0.05657 .
## ScVarvelBef   0.27408    0.03869   7.084 1.40e-12 ***
```

```
## ScRPLength    -0.29818    0.07138   -4.178 2.95e-05 ***
## TreatmentVIE   0.16286    0.10435    1.561 0.11860
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

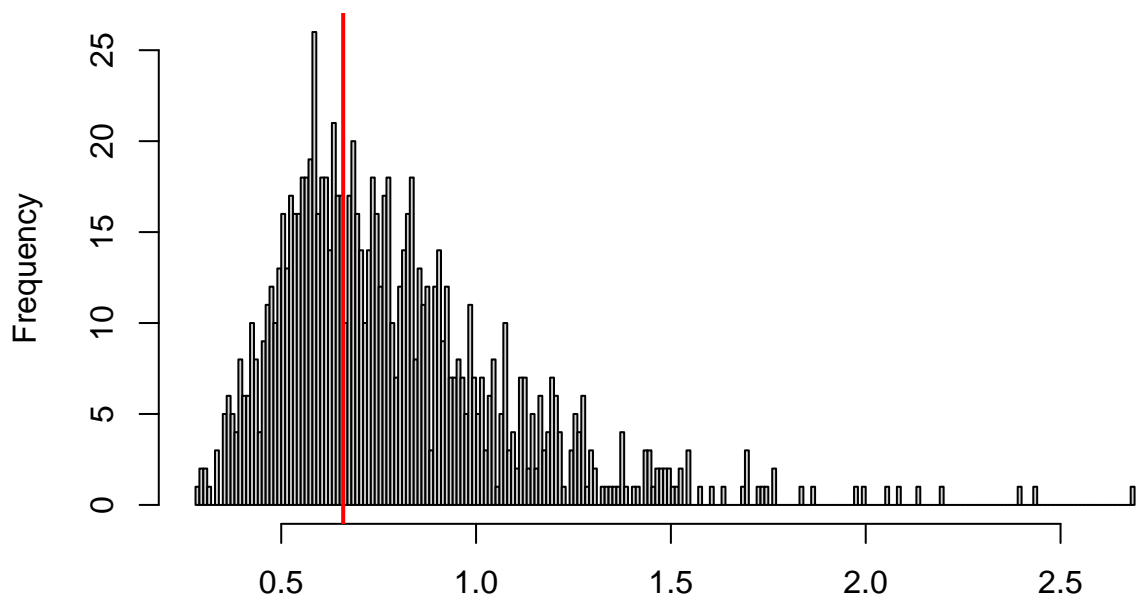
```
# Using the Dharma package to check quantile residuals for female vigor model
# First simulating the quantile residuals
sim_residuals_BehavVigLMF <- simulateResiduals(BehavVigLMF, 1000)
# Plotting the quantile residuals to test how quantile residuals look
plot(sim_residuals_BehavVigLMF)
```

DHARMA residual



```
# Testing for dispersion
testDispersion(sim_residuals_BehavVigLMF)
```

**DHARMa nonparametric dispersion test via sd of
residuals fitted vs. simulated**

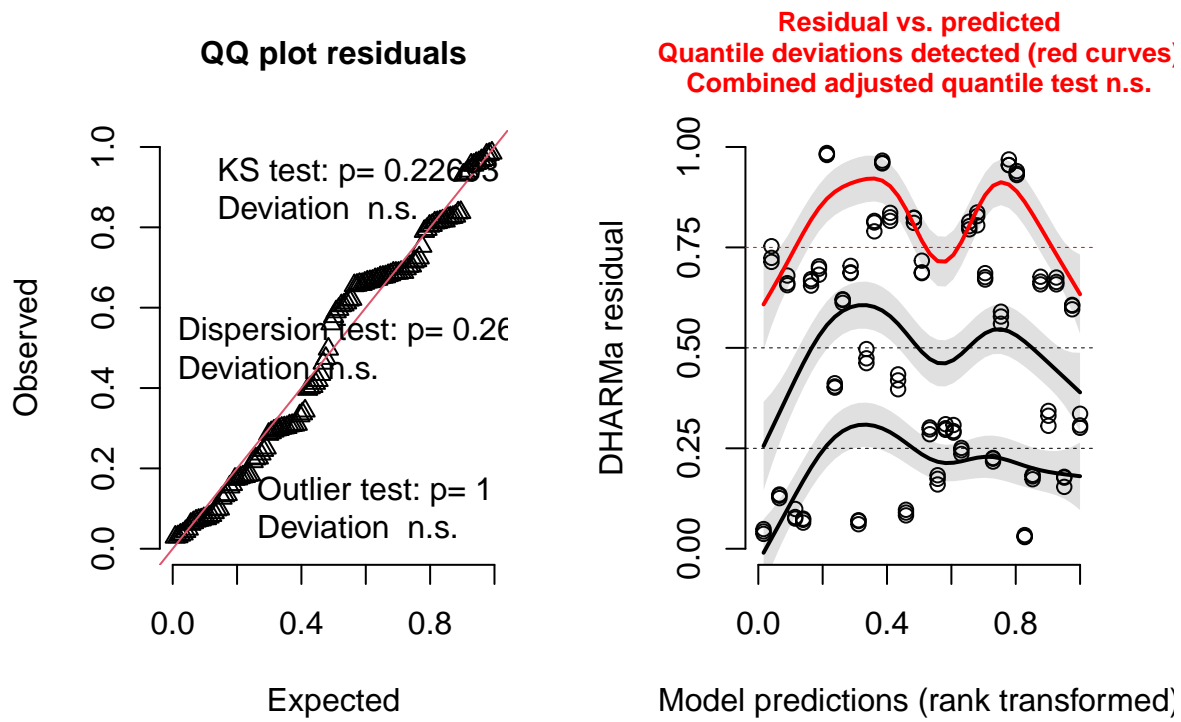


Simulated values, red line = fitted model. p-value (two.sided) = 0.806

```
##
## DHARMa nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data:  simulationOutput
## dispersion = 0.83647, p-value = 0.806
## alternative hypothesis: two.sided

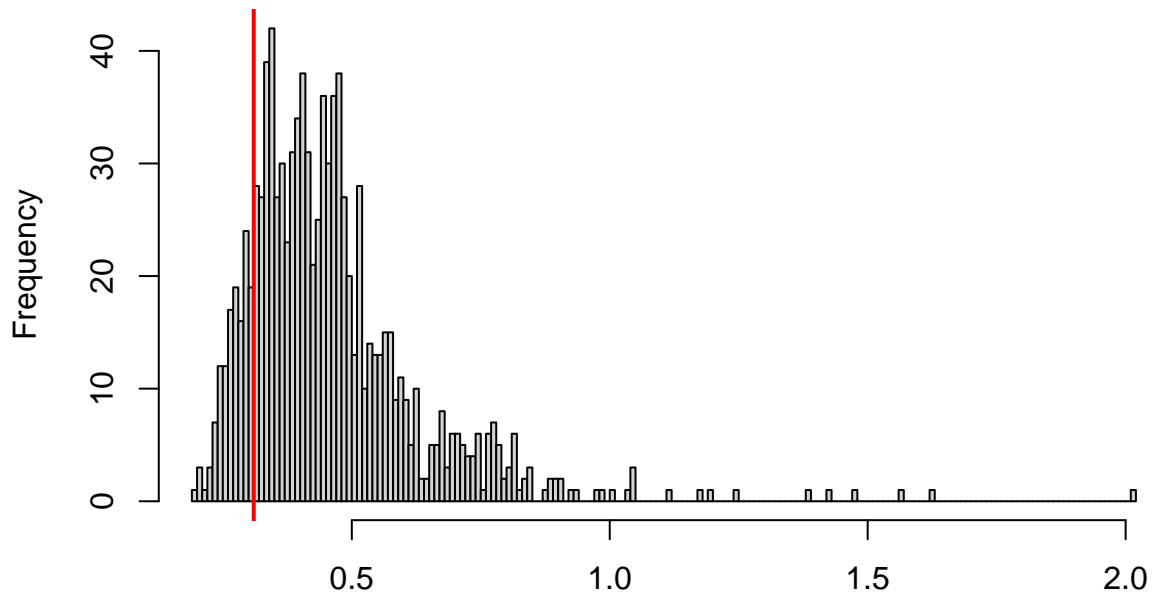
# Using the Dharma package to check quantile residuals for female vigor model
# First simulating the quantile residuals
sim_residuals_BehavVigLMM <- simulateResiduals(BehavVigLMM, 1000)
# Plotting the quantile residuals to test how quantile residuals look
plot(sim_residuals_BehavVigLMM)
```

DHARMa residual



```
# Testing for dispersion
testDispersion(sim_residuals_BehavVigLMM)
```

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated



Simulated values, red line = fitted model. p -value (two.sided) = 0.268

```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.67304, p-value = 0.268
## alternative hypothesis: two.sided
```

Testing the significance of factors in our model

```
Anova(BehavVigLMF, type = 2, test = "Chisq")
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: BehavVig
##           Chisq Df Pr(>Chisq)
## ScResidPLSMI  8.5926  1  0.003375 **
## ScVarvelBef  24.3883  1  7.875e-07 ***
## Treatment     4.8032  1  0.028406 *
## ScRPLength    7.1076  1  0.007676 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova(BehavVigLMM, type = 2, test = "Chisq")
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: BehavVig
##           Chisq Df Pr(>Chisq)
## ScResidPLSMI  3.6353  1  0.05657 .
## ScVarvelBef  50.1810  1  1.402e-12 ***
## ScRPLength   17.4528  1  2.945e-05 ***
## Treatment     2.4357  1  0.11860
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

What factors are important for host behavioral tolerance and do is there any sexual variation in host behavioral tolerance?

Description, development, and fitting of linear model for the analysis

We will use a linear model to analyze how behavioral tolerance differs by sexual variation and other important host traits. Given each host only has one behavioral tolerance measure, we do not need the fishID random effect used in previous models/

- Deterministic
- $BehavTol_{det} = a + b_1Sex + b_2ScRPLength + b_3ScVarVelBef + b_4ScResidPLSMI + b_5ScBehavVig + b_5ScTisTol + b_6Sex:ScRPLength + a_i$
- Stochastic

- $\text{BehavTol} \sim N(\text{BehavTol}_{\text{det}}, \sigma^2)$
- $a_i \sim N(0, \sigma_{\text{BehavGroup}}^2)$

- Fixed

- Sex
- Scaled residual from length and sex
- Scaled Pre-infection SMI
- Scaled variance in velocity before infection
- Scaled behavioral vigor
- Scaled Tissue Tolerance
- Sex by length residuals interaction

We have some outliers that make interpreting the results for behavioral tolerance a pain so were removing them from the analysis

Fit a linear model for behavioral tolerance Note this is a linear mixed model because we have multiple measures per fish and therefore, need to account for non-independence between measures.

```
BehavTolLM <- lmer(BehavTol ~ Sex + ScResidPLSMI + ScChSMI + ScVarvelBef + ScRPLength +
  ScBehavVig + Treatment + (1 | BehavGroup), IndBehavI)
```

Summary to see the relationship of the variables.

```
summary(BehavTolLM)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: BehavTol ~ Sex + ScResidPLSMI + ScChSMI + ScVarvelBef + ScRPLength +
##       ScBehavVig + Treatment + (1 | BehavGroup)
## Data: IndBehavI
##
## REML criterion at convergence: -356.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.6868 -0.3811 -0.1960  0.1658  3.0866
##
## Random effects:
## Groups      Name                Variance Std.Dev.
## BehavGroup (Intercept) 1.043e-07 0.000323
## Residual              1.793e-06 0.001339
## Number of obs: 45, groups: BehavGroup, 7
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)  3.457e-04  3.723e-04  0.929
## SexM         -1.200e-04  4.228e-04 -0.284
## ScResidPLSMI  2.729e-05  2.480e-04  0.110
## ScChSMI       1.720e-04  2.349e-04  0.732
## ScVarvelBef  -1.742e-04  3.108e-04 -0.560
## ScRPLength   -5.887e-05  2.381e-04 -0.247
## ScBehavVig    1.916e-04  2.517e-04  0.761
## TreatmentVIE -4.660e-05  4.746e-04 -0.098
```



```
##
## Correlation of Fixed Effects:
##          (Intr) SexM   SRPLSM ScCSMI ScVrvB ScRPLn ScBhvV
## SexM      -0.590
## ScResdPLSMI 0.121 -0.132
## ScChSMI     0.103 -0.015  0.235
## ScVarvelBef 0.331 -0.145  0.318  0.103
## ScRPLength  0.083 -0.121 -0.150 -0.216 -0.228
## ScBehavVig  -0.097  0.079 -0.048 -0.068 -0.547  0.351
## TreatmntVIE -0.544  0.110 -0.163 -0.034 -0.375 -0.066 -0.014
```

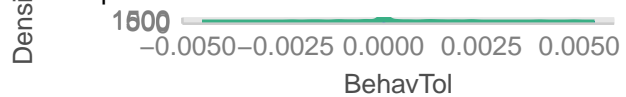
Validate that the model fits well and there are no problems

```
# Using the check_model function from the performamnce package to check the
# model validation
```

```
check_model(BehavToLLM)
```

Posterior Predictive Check

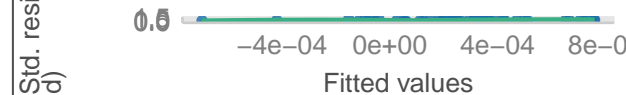
Model-predicted lines should resemble observed data



— Observed data — Model-predicted

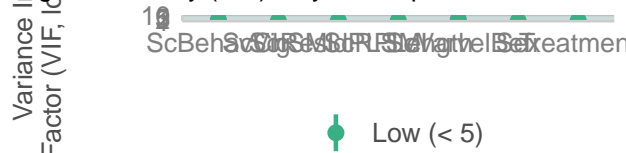
Homogeneity of Variance

Reference line should be flat and horizontal



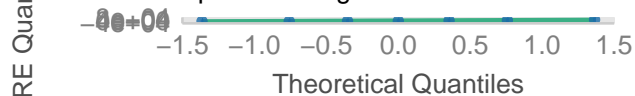
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



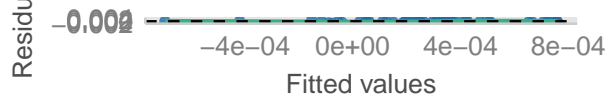
Normality of Random Effects (BehavGroup)

Dots should be plotted along the line



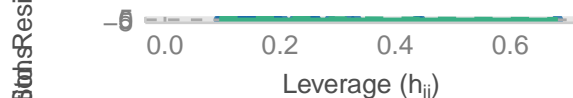
Linearity

Reference line should be flat and horizontal



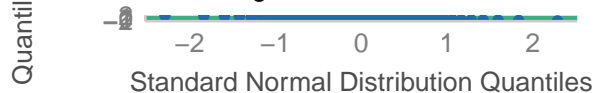
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



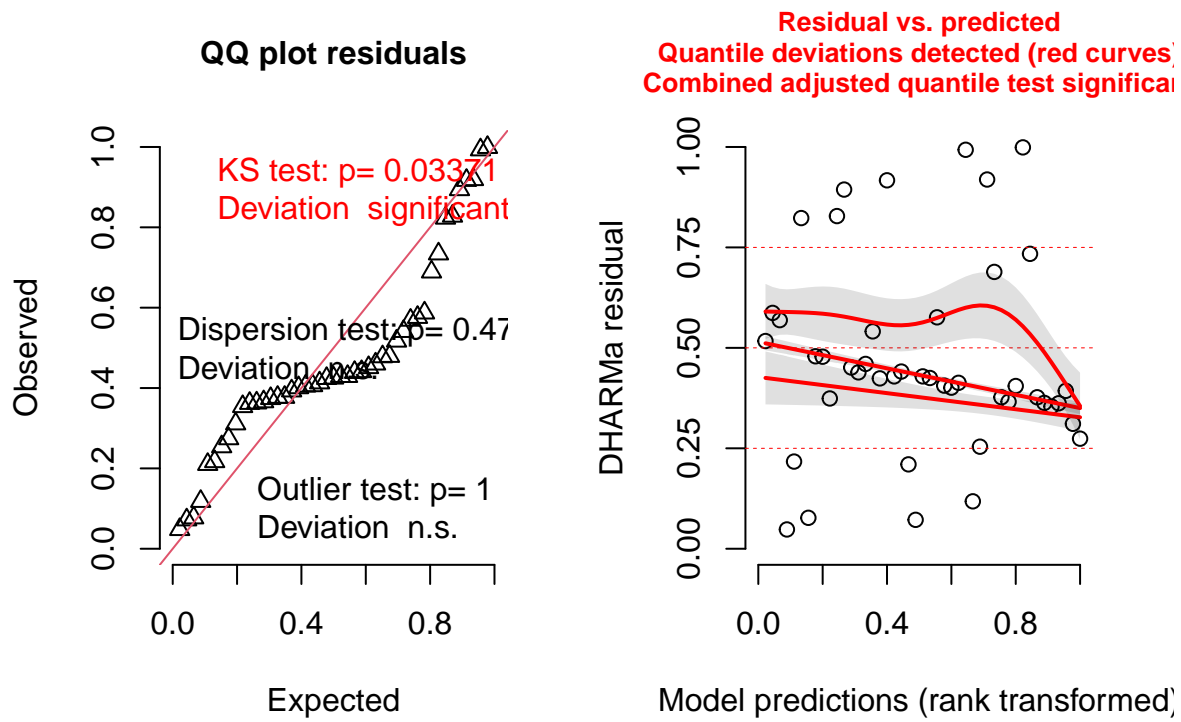
```
# Using the Dharma package to check quantile residuals First simulating the
# quantile residuals
```

```
sim_residuals_BehavToLLM <- simulateResiduals(BehavToLLM, 1000)
```

```
# Plotting the quantile residuals to test how quantile residuals look
```

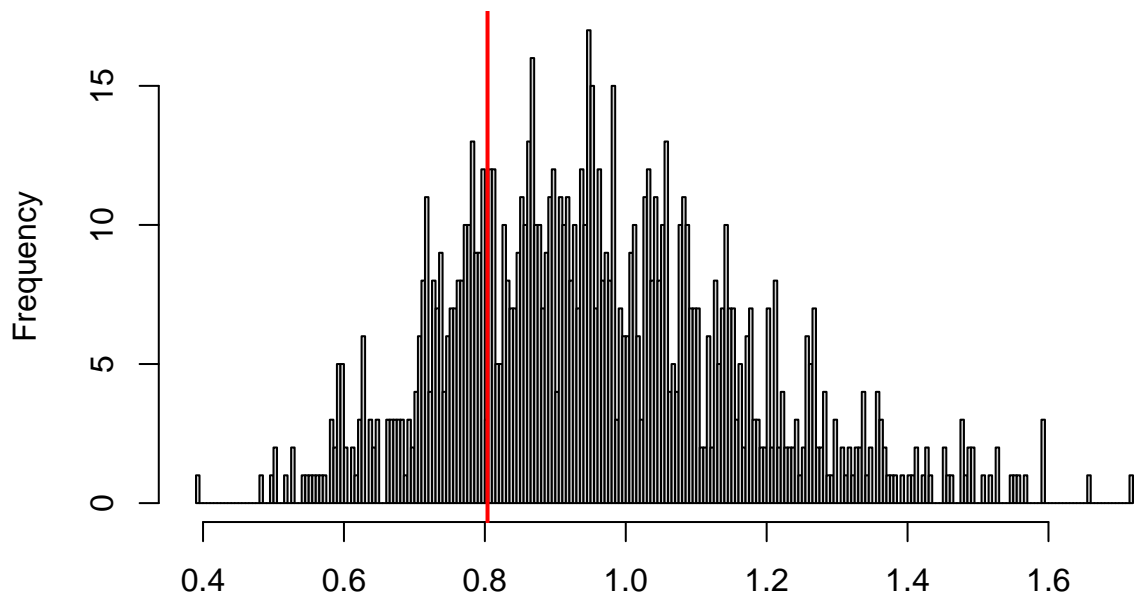
```
plot(sim_residuals_BehavToLLM)
```

DHARMa residual



```
# Testing for dispersion
testDispersion(sim_residuals_BehavToLLM)
```

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated



```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.83643, p-value = 0.472
## alternative hypothesis: two.sided
```

Testing the significance of factors in our model

```
Anova(BehavTolLM, Type = 3, test = "F")
```

```
## Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
##
## Response: BehavTol
##              F Df Df.res Pr(>F)
## Sex          0.0711  1 36.820 0.7912
## ScResidPLSMI 0.0104  1 35.645 0.9193
## ScChSMI      0.4784  1 36.987 0.4935
## ScVarvelBef  0.2812  1 36.951 0.5991
## ScRPLength   0.0567  1 36.439 0.8131
## ScBehavVig   0.5320  1 36.568 0.4704
## Treatment    0.0092  1 34.611 0.9242
```

Visualize the important explanatory factors for behavioral tolerance

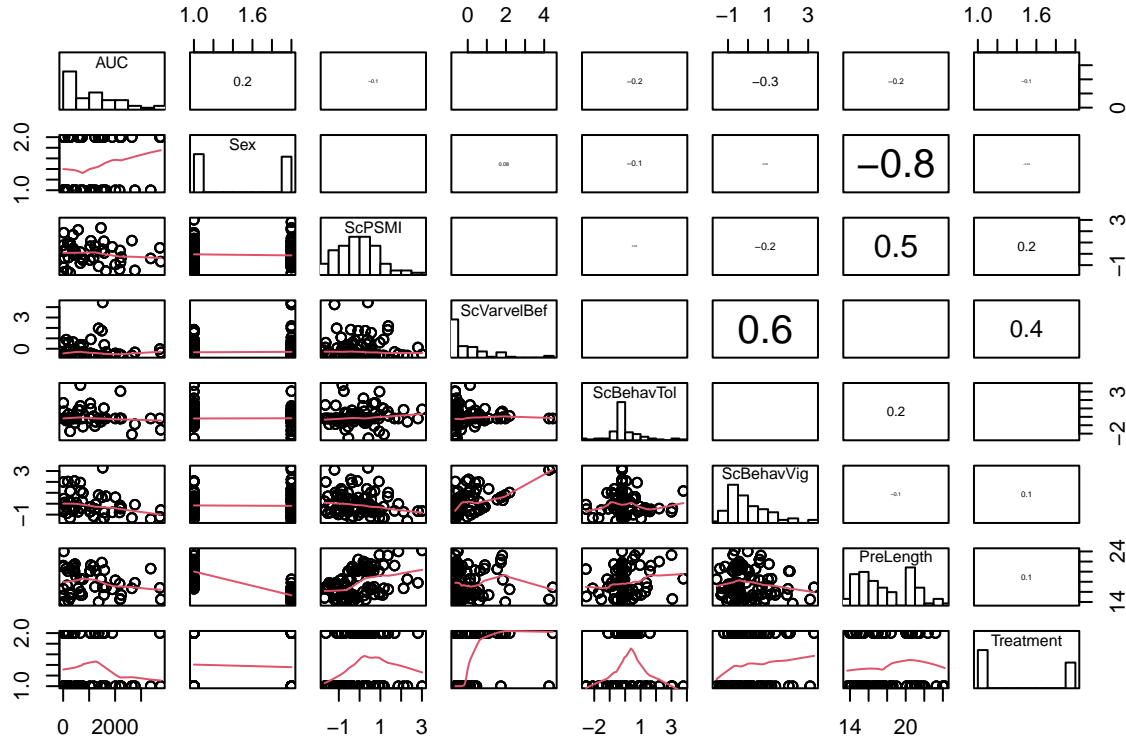
Post-hoc analysis for behavioral tolerance by sex

->

What factors are important for host infection intensity over the course of infection and do is there any sexual variation in host infection intensity?

Visually inspection of the explanatory variables that will be used in the analyses

```
pairs(~AUC + Sex + ScPSMI + ScVarvelBef + ScBehavTol + ScBehavVig + PreLength + Treatment,
      lower.panel = panel.smooth, diag.panel = panel.hist, upper.panel = panel.cor,
      data = IndBehav8)
```



Description, development, and fitting of linear model for the analysis

We will use a linear model to analyze how infection intensity differs by sexual variation and other important host traits. Given each host only has one infection intensity measure, we do not need the fishID random effect used in previous models.

- Deterministic
 - $AUC_{det} = a + b_1 \text{Sex} + b_2 \text{ScBehavVig} + b_3 \text{ScVarVelBef} + b_4 \text{ScResidPLSMI} + b_5 \text{ScRPLength} + b_6 \text{Sex:ScBehavVig} + b_7 \text{Sex:ScResidPLSMI}$
- Stochastic
 - $AUC \sim N(AUC_{det}, \sigma^2)$
- Fixed
 - Sex
 - Scaled behavioral vigor
 - Scaled Pre-infection SMI
 - Scaled variance in velocity before infection
 - Scaled Residuals of Length and Sex
 - VIE treatment
 - Interaction between Sex and behavioral vigor
 - Interaction between sex and body condition

```
# Scaling our pre-infection scaled mass index
IndBehavI$ScPSMI <- as.numeric(IndBehavI$ScPSMI)
# Fit a linear model for infection integral Fitting a glm because we only have
# one measure of behavioral vigor and infection integral
```

```
AUCLM <- glm(AUC ~ Sex + ScBehavVig + ScVarvelBef + ScRPLength + Treatment + wormJump +
  Sex:ScRPLength, family = Gamma(link = "log"), IndBehavI)

# Summary to see the relationship of the variables.
summary(AUCLM)
```

```
##
## Call:
## glm(formula = AUC ~ Sex + ScBehavVig + ScVarvelBef + ScRPLength +
##     Treatment + wormJump + Sex:ScRPLength, family = Gamma(link = "log"),
##     data = IndBehavI)
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)    6.19754    0.61592  10.062 9.95e-12 ***
## SexM           0.53484    0.26311   2.033  0.04995 *
## ScBehavVig     -0.55887    0.15749  -3.549  0.00116 **
## ScVarvelBef     0.45869    0.18094   2.535  0.01601 *
## ScRPLength     -0.55197    0.16684  -3.308  0.00222 **
## TreatmentVIE   -0.05194    0.29171  -0.178  0.85975
## wormJump        0.20270    0.30905   0.656  0.51631
## SexM:ScRPLength 0.51726    0.29635   1.745  0.08994 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Gamma family taken to be 0.689384)
##
## Null deviance: 69.030  on 41  degrees of freedom
## Residual deviance: 55.847  on 34  degrees of freedom
## (6 observations deleted due to missingness)
## AIC: 675.03
##
## Number of Fisher Scoring iterations: 9
```

Validate that the model fits well and there are no problems

```
# Using the check_model function from the performamnce package to check the
# model validation
check_model(AUCLM)
```

Model-predicted lines should resemble observed d

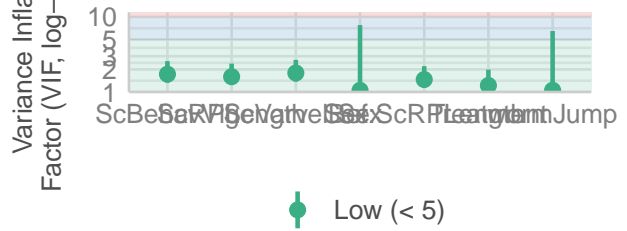


Reference line should be flat and horizontal

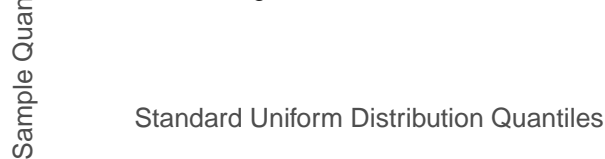


High collinearity (VIF) may inflate parameter uncertainty

Points should be inside the contour lines

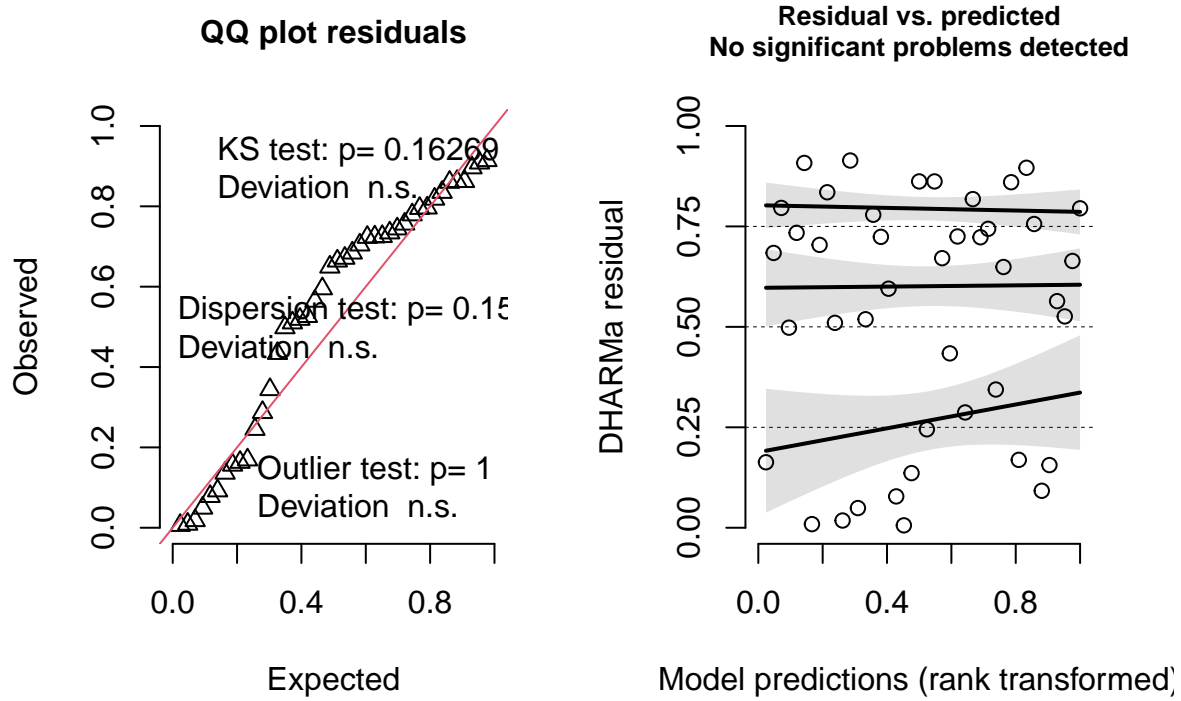


Dots should fall along the line



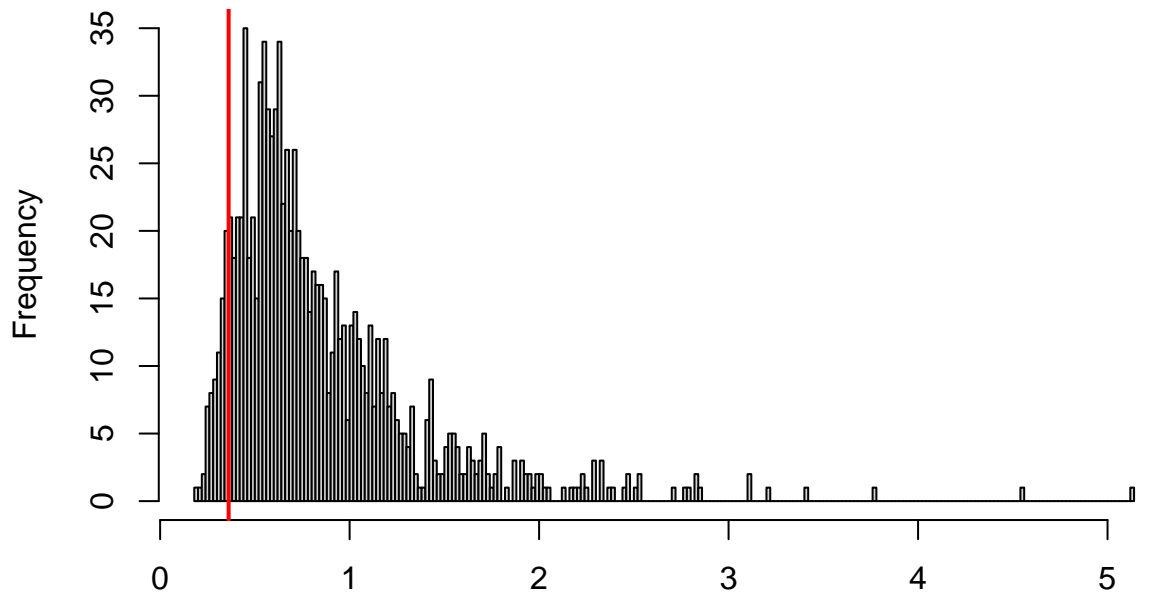
30

DHARMA residual



```
# Testing for dispersion
testDispersion(sim_residuais_AUCLM)
```

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated



```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.42402, p-value = 0.154
## alternative hypothesis: two.sided
```

Testing the significance of factors in our model

```
Anova(AUCLM, type = 3)
```

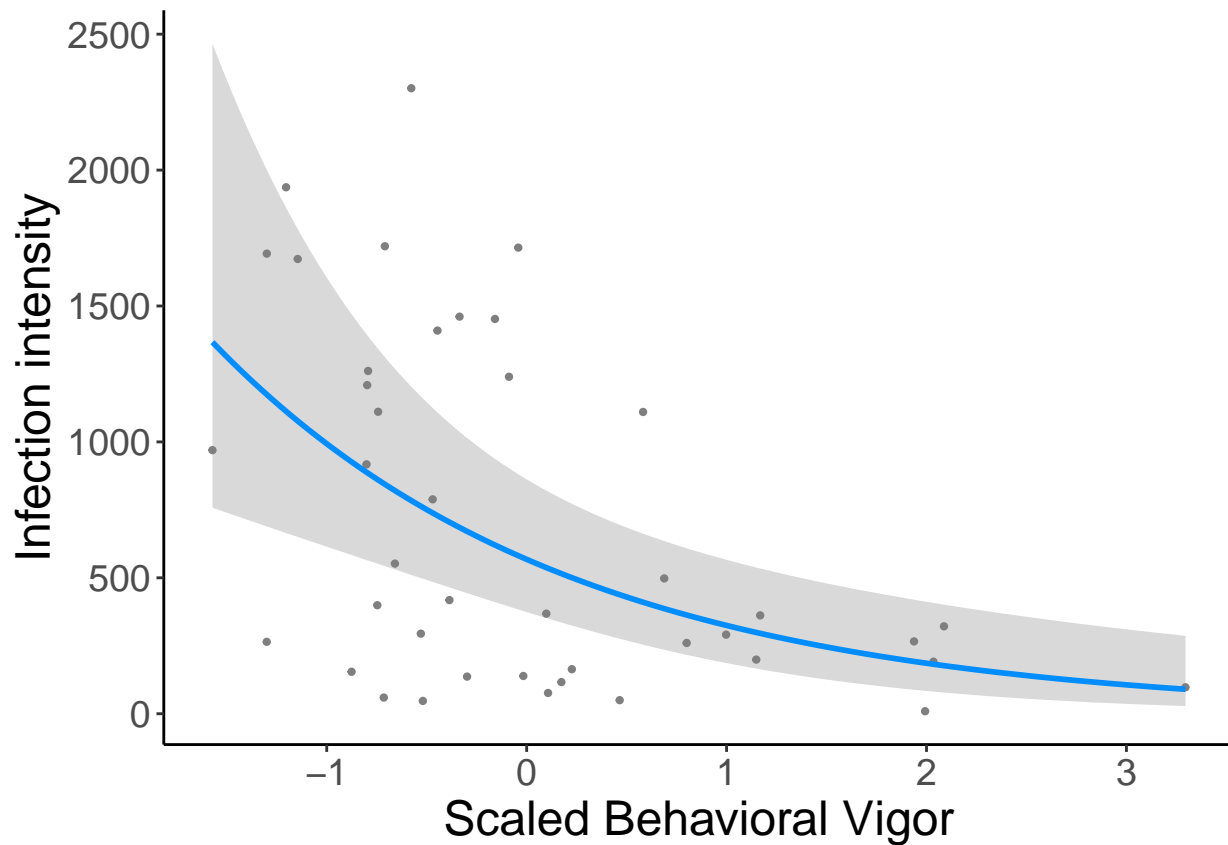
```
## Analysis of Deviance Table (Type III tests)
##
## Response: AUC
##              LR Chisq Df Pr(>Chisq)
## Sex              3.9769  1  0.046127 *
## ScBehavVig       10.4695  1  0.001214 **
## ScVarvelBef       7.2344  1  0.007152 **
## ScRPLength        6.8738  1  0.008747 **
## Treatment         0.0325  1  0.856908
## wormJump          0.5585  1  0.454867
## Sex:ScRPLength    2.5644  1  0.109294
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Visualize the important explanatory factors for infection intensity

```
# Behavioral Vigor graph
InfIntbyVig = visreg(AUCLM, scale = "response", "ScBehavVig", partial = T, gg = TRUE) +
  theme_classic() + theme(legend.position = "none") + ylab("Infection intensity") +
  xlab("Scaled Behavioral Vigor") + theme(text = element_text(size = 18))
```

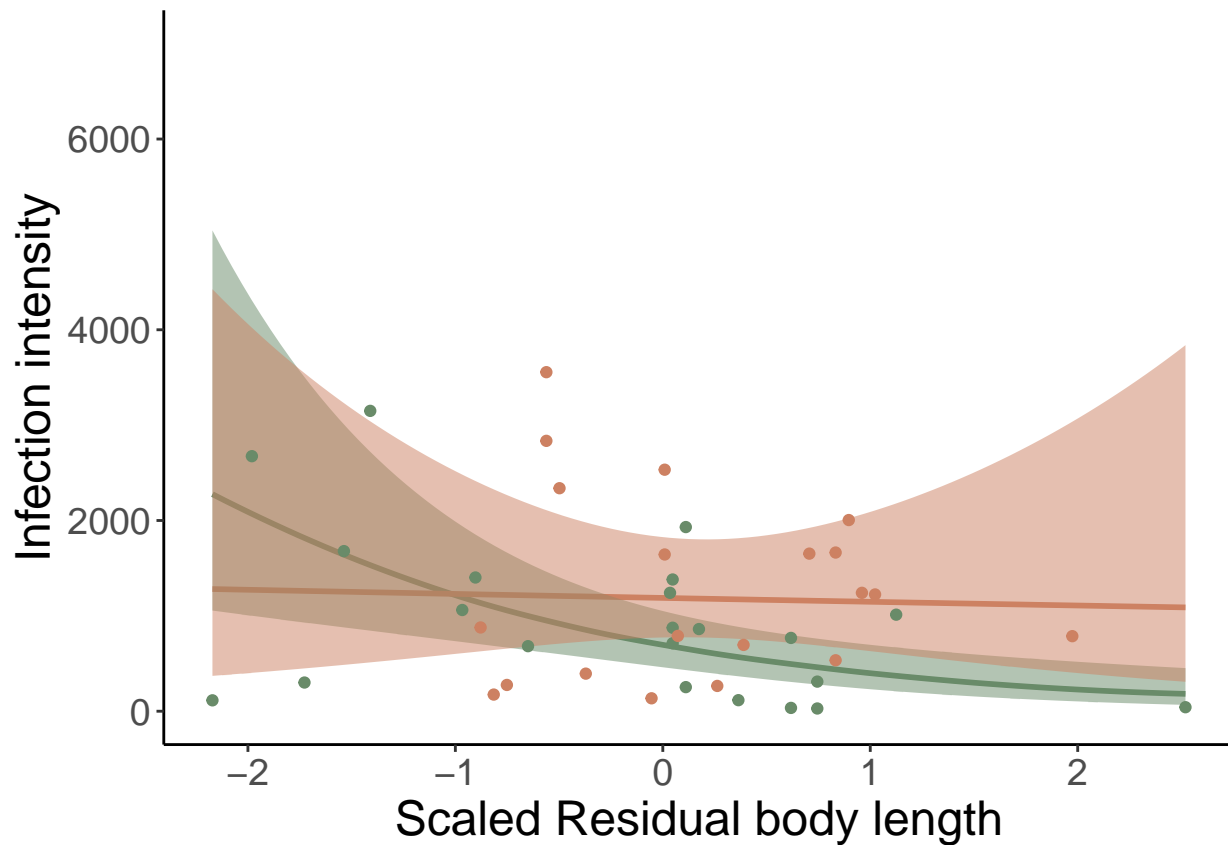
```
## Conditions used in construction of plot
## Sex: F
## ScVarvelBef: -0.5119451
## ScRPLength: 0.04746859
## Treatment: UNTOUCHED
## wormJump: 2
```

```
# Print the graph
print(InfIntbyVig)
```

```
# Behavioral Vigor graph Extracting fit and residuals from model
InfIntbyLen = visreg(AUCLM, scale = "response", "ScRPLength", "Sex", partial = T,
  plot = FALSE, overlay = T)
# Extracting fit
InfIntbyLenfit <- InfIntbyLen$fit
# Extracting residuals
InfIntbyLenres <- InfIntbyLen$res
cpsex = c("darkseagreen4", "lightsalmon3")
InfIntbyLengraph <- ggplot() + geom_smooth(data = InfIntbyLenfit, aes(x = ScRPLength,
  y = visregFit, group = Sex, color = Sex)) + geom_ribbon(data = InfIntbyLenfit,
  aes(x = ScRPLength, y = visregFit, ymin = visregLwr, ymax = visregUpr, fill = Sex),
  alpha = 0.5) + geom_point(data = InfIntbyLenres, aes(x = ScRPLength, y = visregRes,
  group = Sex, color = Sex)) + theme_classic() + theme(legend.position = "none") +
  ylab("Infection intensity") + xlab("Scaled Residual body length") + theme(text = element_text(size = 12)) +
  scale_color_manual(values = cpsex) + scale_fill_manual(values = cpsex) + ylim(0,
  7000)
# Print the graph
print(InfIntbyLengraph)
```

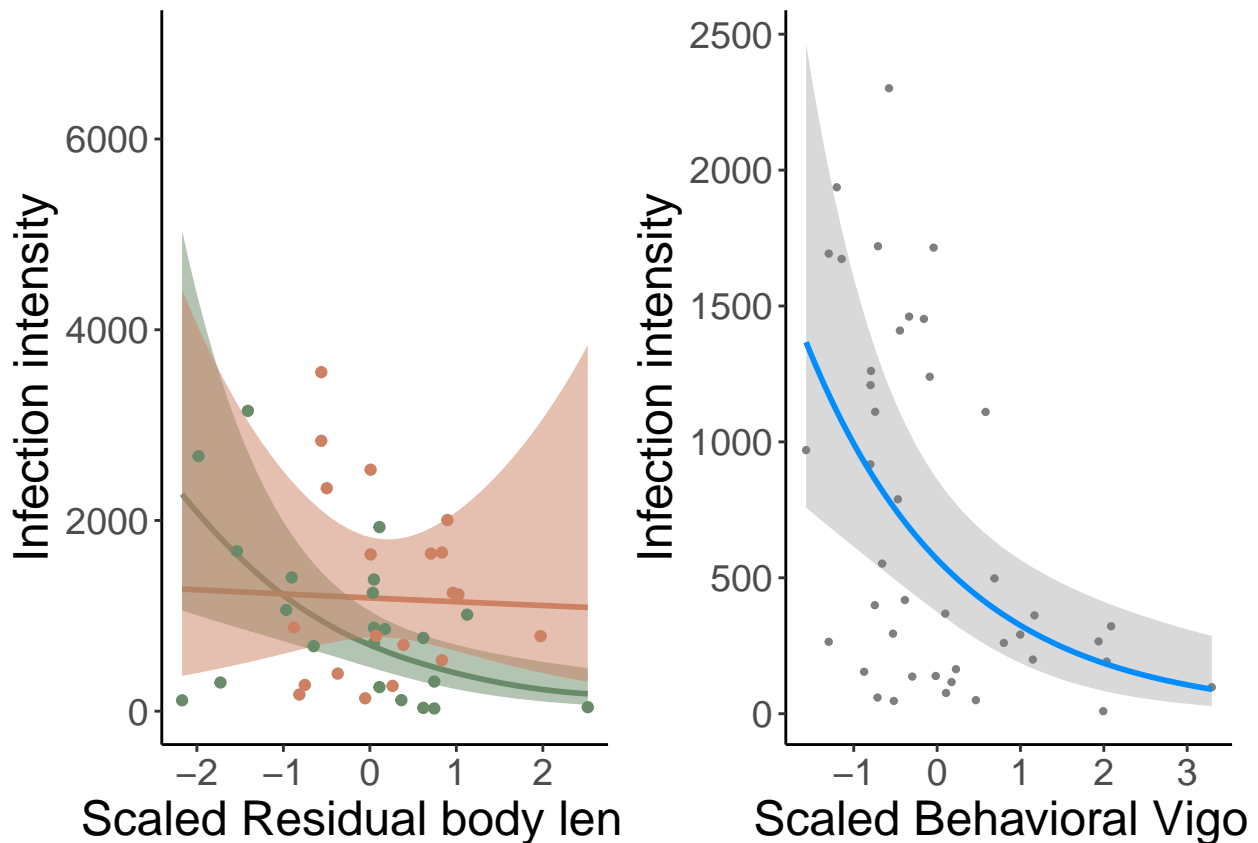
```
## 'geom_smooth()' using method = 'loess' and formula = 'y ~ x'
```



```
# Sex by SMI graph Extracting the fit and residuals
InfIntbySex = visreg(AUCLM, scale = "response", "Sex", partial = T, plot = FALSE)
# Extract fit
InfIntbySexFit <- InfIntbySex$fit
# Extract residuals
InfIntbySexRes <- InfIntbySex$res
# Add colorscheme by sex
cpsex = c("darkseagreen4", "lightsalmon3")
# Plot the graph
InfSexgraph <- ggplot(data = InfIntbySexRes, aes(x = Sex, y = visregRes)) + geom_boxplot(aes(fill = Sex),
  geom_jitter(alpha = 0.5) + theme_classic() + theme(legend.position = "none") +
  ylab("Infection intensity") + xlab(" ") + theme(text = element_text(size = 18)) +
  scale_fill_manual(values = cpsex) + scale_x_discrete(breaks = c("F", "M"), labels = c("Females",
    "Males")))
# Loading in gridExtra for multiple graphs in one image
library(gridExtra)

#
InfIntgraphs <- grid.arrange(InfIntbyLengraph, InfIntbyVig, nrow = 1)

## 'geom_smooth()' using method = 'loess' and formula = 'y ~ x'
```



Fitting posthoc model to test for pattern within sex

```
# Subsetting down to females only
IndBehavIF <- IndBehavI %>%
  filter(Sex == "F")
```

```
## filter: removed 23 rows (48%), 25 rows remaining
```

```
# Subsetting down to males only
IndBehavIM <- IndBehavI %>%
  filter(Sex == "M")
```

```
## filter: removed 25 rows (52%), 23 rows remaining
```

```
# Fit a linear model for infection intensity by sex Female model
AUCLMF <- glm(AUC ~ ScBehavVig + ScVarvelBef + ScResidPLSMI + ScRPLength + Treatment,
  family = Gamma(link = "log"), IndBehavIF)
# Male model
AUCLMM <- glm(AUC ~ ScBehavVig + ScVarvelBef + ScResidPLSMI + ScRPLength + Treatment,
  family = Gamma(link = "log"), IndBehavIM)
```

```
# Summary to see the relationship of the variables. Females
summary(AUCLMF)
```

```
##
## Call:
## glm(formula = AUC ~ ScBehavVig + ScVarvelBef + ScResidPLSMI +
##       ScrPLength + Treatment, family = Gamma(link = "log"), data = IndBehavIF)
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    6.3853     0.2365  27.004 5.12e-16 ***
## ScBehavVig     -0.2783     0.1928  -1.444 0.166036
## ScVarvelBef     0.3585     0.3033   1.182 0.252570
## ScResidPLSMI   -0.4320     0.2557  -1.689 0.108417
## ScrPLength     -0.6701     0.1582  -4.237 0.000496 ***
## TreatmentVIE    0.3790     0.3909   0.970 0.345034
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Gamma family taken to be 0.6339407)
##
## Null deviance: 44.276 on 23 degrees of freedom
## Residual deviance: 35.674 on 18 degrees of freedom
## (1 observation deleted due to missingness)
## AIC: 376.28
##
## Number of Fisher Scoring iterations: 22
```

```
# Males
summary(AUCLMM)
```

```
##
## Call:
## glm(formula = AUC ~ ScBehavVig + ScVarvelBef + ScResidPLSMI +
##       ScrPLength + Treatment, family = Gamma(link = "log"), data = IndBehavIM)
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    7.21005     0.25048  28.785 3.28e-15 ***
## ScBehavVig     -0.69585     0.26406  -2.635  0.0180 *
## ScVarvelBef     0.51982     0.25857   2.010  0.0616 .
## ScResidPLSMI   -0.27102     0.19305  -1.404  0.1795
## ScrPLength     0.03954     0.27736   0.143  0.8884
## TreatmentVIE   -0.30223     0.44366  -0.681  0.5055
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Gamma family taken to be 0.701784)
##
## Null deviance: 23.688 on 21 degrees of freedom
## Residual deviance: 18.147 on 16 degrees of freedom
## (1 observation deleted due to missingness)
## AIC: 368.34
##
## Number of Fisher Scoring iterations: 9
```

```
# Testing for significance for females
Anova(AUCLMF, type = 3, test = "LR")
```

```
## Analysis of Deviance Table (Type III tests)
##
## Response: AUC
##          LR Chisq Df Pr(>Chisq)
## ScBehavVig    1.5676 1  0.210552
## ScVarvelBef    1.5190 1  0.217771
## ScResidPLSMI    3.1025 1  0.078170 .
## ScRPLength     8.3693 1  0.003816 **
## Treatment      0.7356 1  0.391066
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Testing for significance for males
Anova(AUCLMM, type = 3, test = "LR")
```

```
## Analysis of Deviance Table (Type III tests)
##
## Response: AUC
##          LR Chisq Df Pr(>Chisq)
## ScBehavVig    6.3953 1  0.01144 *
## ScVarvelBef    3.9527 1  0.04680 *
## ScResidPLSMI    1.3761 1  0.24077
## ScRPLength     0.0158 1  0.90005
## Treatment      0.3521 1  0.55291
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Examining change in activity for only infected individuals

```
# Setting some variables as factors to confirm they are this way for the
# analysis
```

```
IndBehavChI$Sex <- as.factor(IndBehavChI$Sex)
IndBehavChI$Infection <- as.factor(IndBehavChI$Infection)
IndBehavChI$Treatment <- as.factor(IndBehavChI$Treatment)
```

```
# Subsetting our dataframe to relevant data and making some
```

```
IndBehavChI <- IndBehavChI %>%
  mutate(ScWormaf = scale(Wormaf)) %>%
  mutate(ScChBehav = scale(ChBehav))
```

```
## mutate: new variable 'ScWormaf' (double) with 43 unique values and 53% NA
```

```
## mutate: new variable 'ScChBehav' (double) with 135 unique values and 7% NA
```

```
pairs(~ChBehav + Sex + Infection + TrialTime + PreSMI + AUC + Treatment + ResidPLength +
  NRatebf + Wormaf, lower.panel = panel.smooth, diag.panel = panel.hist, upper.panel = panel.cor,
  data = IndBehavChI)
```

```

## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter

## Warning in cor(x, y, use = "pairwise.complete.obs"): the standard deviation is
## zero
## Warning in cor(x, y, use = "pairwise.complete.obs"): the standard deviation is
## zero

## Warning in par(usr): argument 1 does not name a graphical parameter

## Warning in cor(x, y, use = "pairwise.complete.obs"): the standard deviation is
## zero
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## zero

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## zero
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## zero

## Warning in par(usr): argument 1 does not name a graphical parameter
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## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter

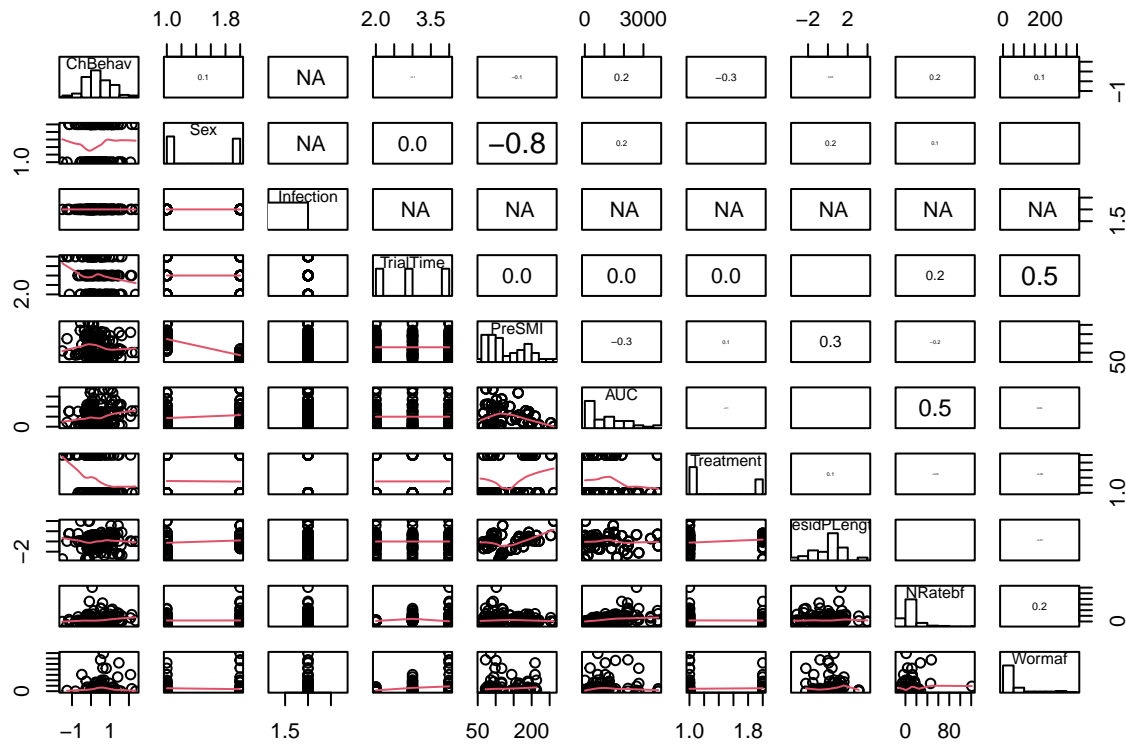
## Warning in cor(x, y, use = "pairwise.complete.obs"): the standard deviation is
## zero
## Warning in cor(x, y, use = "pairwise.complete.obs"): the standard deviation is
## zero

## Warning in par(usr): argument 1 does not name a graphical parameter
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## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter

```



```
## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter
## Warning in par(usr): argument 1 does not name a graphical parameter
```



```
cpsex = c("darkseagreen4", "lightsalmon3")
```

We will use a linear mixed model to analyze how Change in activity differs by sexual variation and how infected an individual fish was. FishID is included as a random term to allow for non-independence of individuals due to multiple measurements per individual across time.

- Deterministic
- $\text{Log}_{10}(\text{InfInt}_{det} + 1) = a + b_1 \text{TrialTime} + b_2 \text{ChBehav} + b_3 \text{Sex} + b_4 \text{ScPSMI} + b_5 \text{Treatment} + b_6 \text{wormJump} + b_7 \text{ScChSMI} + b_8 \text{BehavVig} + a_i$
- Stochastic
 - $\text{ChVel} \sim N(\text{Log}_{10}(\text{InfInt}_{det} + 1), \sigma^2)$
 - $a_i \sim N(0, \sigma_{fishID}^2)$
- Fixed
 - TrialTime
 - Change in behavior between time points
 - Sex
 - Scaled pre-infection scaled mass index
 - VIE treatment of the fish
 - number of worms that initially started the infection
 - Scaled change in scaled mass index
 - Behavior vigor of each fish

- Random
 - fishID

```

# Fit a linear model for checking what explanatory factors are important for
# Variance in activity Note this is a linear mixed model because we have
# multiple measures per fish and therefore, need to account for
# non-independence between measures.
InfIntLMInf <- lmer(log10(Wormaf + 1) ~ Sex + ScPSMI + ScChSMI + TrialTime + ScBehavVig +
  Treatment + ChBehav + wormJump + (1 | fishID), IndBehavChI)

# Summary to see the relationship of the variables.
summary(InfIntLMInf)

## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(Wormaf + 1) ~ Sex + ScPSMI + ScChSMI + TrialTime + ScBehavVig +
##      Treatment + ChBehav + wormJump + (1 | fishID)
##      Data: IndBehavChI
##
## REML criterion at convergence: 129.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.03509 -0.69817 -0.04025  0.72865  1.60854
##
## Random effects:
##   Groups      Name                Variance Std.Dev.
##  fishID      (Intercept)  0.005027  0.0709
##   Residual                0.406932  0.6379
## Number of obs: 61, groups:  fishID, 21
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   0.55319    0.41074   1.347
## SexM          -0.11606    0.18735  -0.619
## ScPSMI        -0.06753    0.14011  -0.482
## ScChSMI       -0.07540    0.13309  -0.567
## TrialTime2     0.77036    0.19797   3.891
## TrialTime3     1.35524    0.20857   6.498
## ScBehavVig    0.07273    0.13822   0.526
## TreatmentVIE  0.20701    0.22032   0.940
## ChBehav       0.45893    0.19621   2.339
## wormJump     -0.18333    0.17373  -1.055
##
## Correlation of Fixed Effects:
##              (Intr) SexM   ScPSMI ScCSMI TrlTm2 TrlTm3 ScBhvV TrtVIE ChBehv
## SexM          0.015
## ScPSMI        0.228 -0.281
## ScChSMI       0.193 -0.100  0.559
## TrialTime2    -0.197  0.018 -0.008 -0.020
## TrialTime3    -0.334 -0.011  0.007  0.016  0.444
## ScBehavVig   0.149 -0.018  0.523  0.334 -0.049  0.095
## TreatmntVIE -0.377  0.004 -0.440 -0.412  0.007 -0.023 -0.541

```

```
## ChBehav      -0.388 -0.168  0.072  0.190 -0.105  0.240  0.468 -0.068
## wormJump     -0.860 -0.256 -0.073 -0.051 -0.029  0.074 -0.118  0.175  0.277
```

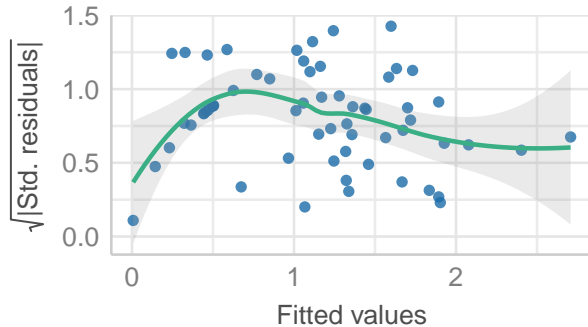
Validate that the model fits well and there are no problems

```
# Using the check_model function from the performamnce package to check the
# model validation
```

```
check_model(InfIntLMInf, check = c("qq", "normality", "homogeneity"))
```

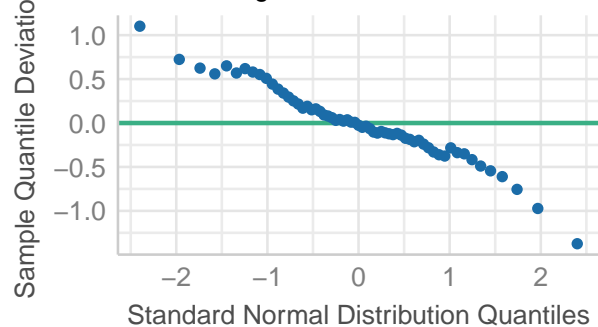
Homogeneity of Variance

Reference line should be flat and horizontal



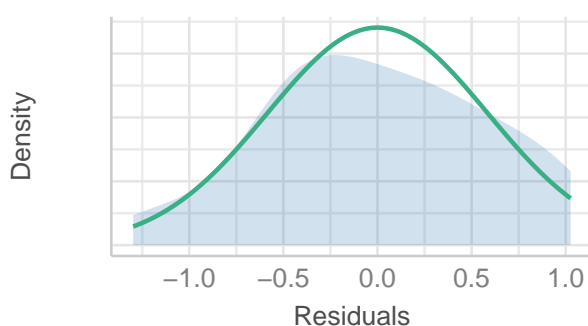
Normality of Residuals

Dots should fall along the line



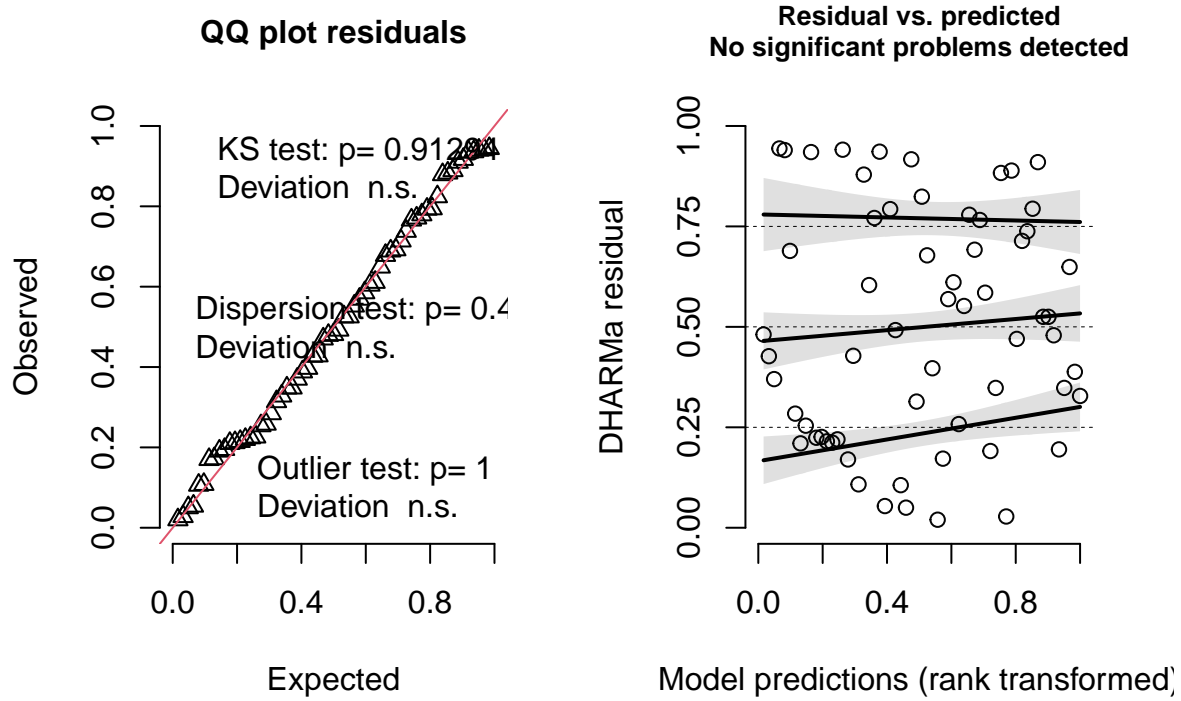
Normality of Residuals

Distribution should be close to the normal curve



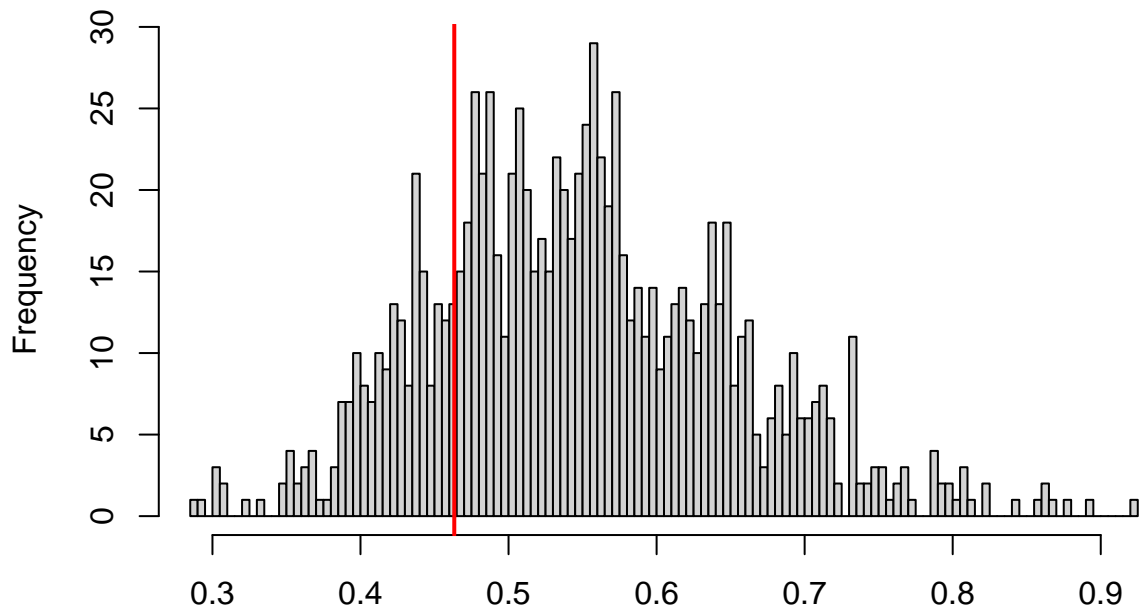
```
# Using the Dharma package to check quantile residuals First simulating the
# quantile residuals
sim_residuals_InfIntLMInf <- simulateResiduals(InfIntLMInf, 1000)
# Plotting the quantile residuals to test how quantile residuals look
plot(sim_residuals_InfIntLMInf)
```

DHARMa residual



```
# Testing for dispersion
testDispersion(sim_residuals_InfIntLMInf)
```

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated



```
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.84406, p-value = 0.4
## alternative hypothesis: two.sided
```

```
# There are some problems with this model validation. It doesnt look model
# breaking but definitely should look at other model error structures to
# resolve the issues.
```

Testing the significance of factors in our model using a Kenward-Rodgers F test for infected only infection intensity model

```
# F test to test for signifcance of slope of variables
Anova(InfIntLMInf, test = "F", type = 3)
```

```
## Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
##
## Response: log10(Wormaf + 1)
##
```

	F	Df	Df.res	Pr(>F)
(Intercept)	1.7844	1	17.834	0.19841
Sex	0.3819	1	13.514	0.54686
ScPSMI	0.2320	1	12.774	0.63816
ScChSMI	0.3180	1	15.068	0.58110
TrialTime	21.5646	2	40.186	4.341e-07 ***
ScBehavVig	0.2706	1	16.492	0.60982
Treatment	0.8818	1	12.696	0.36524
ChBehav	4.9482	1	42.669	0.03146 *
wormJump	1.1043	1	13.713	0.31148

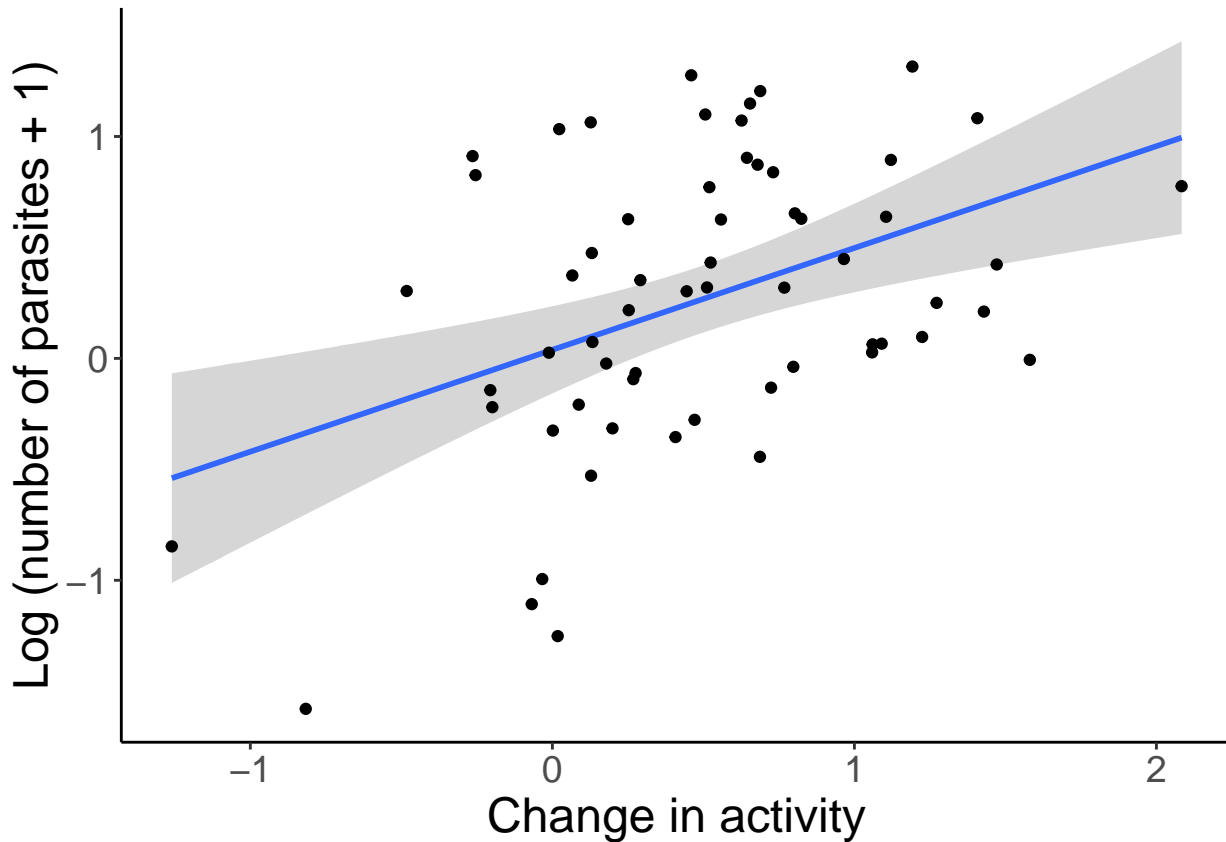
```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Visualize the important explanatory factors for infection intensity for infected individuals

```
# Change in behavior by Extracting fit and residual
ChVelbyRate = visreg(InfIntLMInf, scale = "response", "ChBehav", partial = T, overlay = TRUE,
  plot = FALSE)
# Extracting fit
ChVelbyRatefit <- ChVelbyRate$fit
# Extracting residual
ChVelbyRateres <- ChVelbyRate$res
# Constructing the graph
ChVelbyRategraph <- ggplot(ChVelbyRateres, aes(x = ChBehav, y = visregRes)) + geom_smooth(method = "lm"
  geom_point() + theme_classic() + theme(legend.position = "none") + ylab("Log (number of parasites +
  xlab("Change in activity") + theme(text = element_text(size = 18))

# Print the graph
print(ChVelbyRategraph)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
# Subset to only infected females
IndBehavChIF <- IndBehavChI %>%
  filter(Sex == "F" & Infection == "1") %>%
  mutate(ScWormaf = scale(Wormaf))
```

```
## filter: removed 69 rows (48%), 75 rows remaining
```

```
## mutate: changed 32 values (43%) of 'ScWormaf' (0 new NA)
```

```
# Subset to only infected males
IndBehavChIM <- IndBehavChI %>%
  filter(Sex == "M" & Infection == "1") %>%
  mutate(ScWormaf = scale(Wormaf))
```

```
## filter: removed 75 rows (52%), 69 rows remaining
```

```
## mutate: changed 36 values (52%) of 'ScWormaf' (0 new NA)
```

```
# Fit linear mixed models to post hoc check for significance for male and
# female slopes
ChVeLLMInfF <- lmer(ChBehav ~ ScPSMI + ScChSMI + TrialTime + ScBehavVig + Treatment +
  ScNRatebf + (1 | fishID), IndBehavChIF)
```

```

# Fit linear mixed models to post hoc check for significance for male and
# female slopes
ChVeLLMInfM <- lmer(ChBehav ~ ScPSMI + ScChSMI + TrialTime + ScBehavVig + Treatment +
  ScNRatebf + (1 | fishID), IndBehavChIM)

# Summary to see the relationship of the variables for female model.
summary(ChVeLLMInfF)

```

```

## Linear mixed model fit by REML ['lmerMod']
## Formula: ChBehav ~ ScPSMI + ScChSMI + TrialTime + ScBehavVig + Treatment +
##       ScNRatebf + (1 | fishID)
## Data: IndBehavChIF
##
## REML criterion at convergence: 115.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.40025 -0.59721  0.01767  0.39831  2.49141
##
## Random effects:
## Groups Name Variance Std.Dev.
## fishID (Intercept) 0.1516  0.3893
## Residual          0.1465  0.3827
## Number of obs: 74, groups: fishID, 25
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   0.35892   0.13500   2.659
## ScPSMI         0.03171   0.19273   0.165
## ScChSMI       -0.12455   0.20790  -0.599
## TrialTime2     -0.12062   0.12073  -0.999
## TrialTime3     -0.08185   0.11226  -0.729
## ScBehavVig    -0.29924   0.09676  -3.093
## TreatmentVIE -0.14657   0.20917  -0.701
## ScNRatebf      0.20238   0.07599   2.663
##
## Correlation of Fixed Effects:
##              (Intr) ScPSMI ScChSMI TrlTm2 TrlTm3 ScBhvV TrtVIE
## ScPSMI         0.239
## ScChSMI       -0.110 -0.849
## TrialTime2     -0.419 -0.040  0.004
## TrialTime3     -0.423 -0.024  0.008  0.525
## ScBehavVig     0.035 -0.134  0.264 -0.057 -0.030
## TreatmntVIE   -0.553 -0.435  0.344 -0.012  0.010  0.015
## ScNRatebf      0.134  0.090 -0.008 -0.443 -0.210  0.128  0.026

```

```

# Summary to see the relationship of the variables for male model.
summary(ChVeLLMInfM)

```

```

## Linear mixed model fit by REML ['lmerMod']
## Formula: ChBehav ~ ScPSMI + ScChSMI + TrialTime + ScBehavVig + Treatment +
##       ScNRatebf + (1 | fishID)

```

```
## Data: IndBehavChIM
##
## REML criterion at convergence: 87.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.77079 -0.56125 -0.08803  0.54617  2.27980
##
## Random effects:
##   Groups   Name      Variance Std.Dev.
## fishID    (Intercept) 0.1907   0.4366
## Residual                0.1050   0.3240
## Number of obs: 60, groups: fishID, 22
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   0.55518    0.14960   3.711
## ScPSMI        -0.13224    0.18568  -0.712
## ScChSMI        -0.10664    0.17887  -0.596
## TrialTime2      0.13984    0.10301   1.358
## TrialTime3     -0.28284    0.12219  -2.315
## ScBehavVig    -0.42170    0.10922  -3.861
## TreatmentVIE  -0.19015    0.24076  -0.790
## ScNRatebf     -0.02104    0.03640  -0.578
##
## Correlation of Fixed Effects:
##              (Intr) ScPSMI ScChSMI TrlTm2 TrlTm3 ScBhvV TrtVIE
## ScPSMI        -0.175
## ScChSMI        -0.120  0.769
## TrialTime2     -0.335 -0.007 -0.010
## TrialTime3     -0.302 -0.027 -0.029  0.518
## ScBehavVig     0.243  0.240 -0.007 -0.040 -0.094
## TreatmentVIE  -0.603  0.134  0.221  0.003  0.013 -0.400
## ScNRatebf      0.078  0.021  0.032 -0.317 -0.439  0.127 -0.010
```

```
# Anova to test for significance for females
Anova(ChVelLMInfF, test = "F", type = 2)
```

```
## Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
##
## Response: ChBehav
##              F Df Df.res    Pr(>F)
## ScPSMI        0.0271  1 19.801 0.870991
## ScChSMI        0.3589  1 19.547 0.555988
## TrialTime      0.5239  2 47.777 0.595551
## ScBehavVig    9.5583  1 20.050 0.005741 **
## Treatment     0.4909  1 19.840 0.491657
## ScNRatebf     6.8075  1 61.242 0.011393 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Anova to test for significance for males
Anova(ChVelLMInfM, test = "F", type = 2)
```

```
## Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
##
## Response: ChBehav
##           F Df Df.res  Pr(>F)
## ScPSMI      0.5070  1 16.615 0.486299
## ScChSMI      0.3553  1 16.990 0.558975
## TrialTime    7.1061  2 36.341 0.002484 **
## ScBehavVig 14.8948  1 17.070 0.001250 **
## Treatment    0.6236  1 16.776 0.440748
## ScNRatebf    0.3246  1 41.558 0.571916
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```