# The effects of ocean acidification and increased nutrients on invasive amphipod herbivory of Zostera marina (eelgrass) in San Francisco Bay

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**Aim:**

Examine the impact of ocean acidification and increased nutrients on Ampithoe valida consumption of eelgrass plant tissue.

**Hypothesis:**

a. A. valida will exhibit increased rates of consumption on eelgrass grown in higher nutrient conditions due to increased plant palatability of eelgrass and algae via reduced C:N ratios.
b. A. valida exposed to low pH conditions will exhibit higher rates of consumption due to unknown physiological changes that alter the amphipods.

**Null Hypothesis:**

A. valida will exhibit no change in rates of consumption on eelgrass grown in high nutrient and low pH bay conditions than eelgrass grown in ambient bay conditions.

## Statistical Approach: (Rough Draft)

A Generalized Linear Mixed Model was used to include both fixed and random effects in the experiment. A Shapiro-wilks normality test showed that the data was not normal (p-value= 1.086e-08.) The "Kenward Roger's F test" method was used to calculate the degrees of freedom in the statistical analysis. I performed an ANOVA test with Kenward Roger's method and there were no significant interactions between treatments. There was one interaction that was had a slightly significant effect on A.valida consumption and that was between eelgrass nutrient treatment (ambient or reduced) and A.valida pH treatment (ambient or reduced), p-value= 0.06. (We see this interaction in..reference plots. )

## Code in R

```r
library(tidyverse)
```

```
## Warning: package 'ggplot2' was built under R version 4.3.1
```

```
## Warning: package 'tidyr' was built under R version 4.3.1

## Warning: package 'readr' was built under R version 4.3.1

## Warning: package 'dplyr' was built under R version 4.3.1

## Warning: package 'stringr' was built under R version 4.3.1

## Warning: package 'lubridate' was built under R version 4.3.1
```

```r
library(openintro)
library(nlme)
```

```
## Warning: package 'nlme' was built under R version 4.3.1
```

```r
library(lme4)
```

```
## Warning: package 'lme4' was built under R version 4.3.1

## Warning: package 'Matrix' was built under R version 4.3.1
```

```r
library(emmeans)
```

```
## Warning: package 'emmeans' was built under R version 4.3.1
```

```r
library(ggpubr)
library(lmerTest)
```

## Data Preparation & Plots in R

```r
#set working directory
setwd("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub/NSF Pilot Project/tl

#import data
choicei <- read.csv("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub/NSF P:

choicedat <- read.csv("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub/NSF

choicedat2 <- read.csv("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub/NSI

choicedat2man <- read.csv("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub,

avmassdat <- read.csv("~/Library/Mobile Documents/com~apple~CloudDocs/SFSU/Thesis/My Thesis/Git-Hub/NSF

#data preparation
choiceid <- choicei %>%
  pivot_longer(A:D,
```

```r
                  names_to = "treatment",
                  values_to = "section_ID") %>%
    relocate(c(treatment, section_ID), .after = AV_control)

#av mass
avmassdata <- avmassdat %>%
  mutate(av_drymass_g = Boat_AV_dry_mass_g - Boat_mass_g) %>%
  dplyr::select(-Boat_AV_dry_mass_g, -Boat_mass_g) %>%
  rename(unit_ID = Container_ID)

#dataframes that join the data to IDs

choicedata <- left_join(choiceid, choicedat) %>%
  left_join(choicedat2man) %>%
  left_join(avmassdata, by = "unit_ID") %>%
  mutate(AVeel_treat = paste(tankpH_treat, "_", treatment),
         pH_treat = ifelse(treatment %in% c("A", "B"), "ambient",
                           ifelse(treatment %in% c("C", "D"), "reduced", NA)),
         nut_treat = ifelse(treatment %in% c("A", "C"), "ambient",
                           ifelse(treatment %in% c("B", "D"), "increased", NA)),
         plant_ID = str_extract(section_ID, '[0-9]+.[0-9]+'),
         section_age = str_remove(section_ID, "^([^.]+.){2}"),
         area_change_adj = area_change/av_drymass_g) %>%
  relocate(c(AVeel_treat, pH_treat, nut_treat), .after = treatment) %>%
  relocate(c(AV_no, mass_mg, area_change, area_change_adj, plant_ID, section_age), .before = Notes)

## Joining with `by = join_by(treatment, section_ID, Notes)`
## Joining with `by = join_by(section_ID)`
```

## Plots

```r
# **Plots for Eelgrass Area**

#bulk area loss for AV *not control, not adjusted*
boxplota1 <- ggplot(data = choicedata[choicedata$AV_control == "AV",],
                    aes(x = treatment,
                        y = area_change,
                        color = tankpH_treat)) +
  introdataviz::geom_split_violin(alpha = .5, trim = FALSE) +
  geom_hline(yintercept = 0,
             color = "black",
             size = 0.8) +
  geom_boxplot(position = position_dodge(0.8),
               size = 0.5) +
  geom_point(position = position_dodge(0.8),
             size = 1)  +
  # geom_text(aes(label = area_change),
  #           position = position_dodge(0.8),
  #           vjust = -0.5,
  #           size = 3) + # Adjust position and size of text
  scale_color_manual(name = expression(paste(italic("A. valida") ~ "pH")),
```

```
                breaks = c("ambient", "reduced"),
                values = c("grey70", "cyan4")) +
scale_x_discrete(breaks = c("A", "B", "C", "D"),
                labels = c("ambient pH\nambient nut.", "ambient pH\nincreased nut.",
                           "reduced pH\nambient nut.", "reduced pH\nincreased nut.")) +
labs(x = "Eelgrass treatment",
     y = expression(paste("Change in Segment Area ("*cm^2*")")),
     title = "A. Valida Choice Bulk Area Loss by Treatments") +
theme_bw(base_size = 20) +
theme(axis.text = element_text(color = "black", size = 10))+
 theme(plot.title = element_text(size = 14))
```
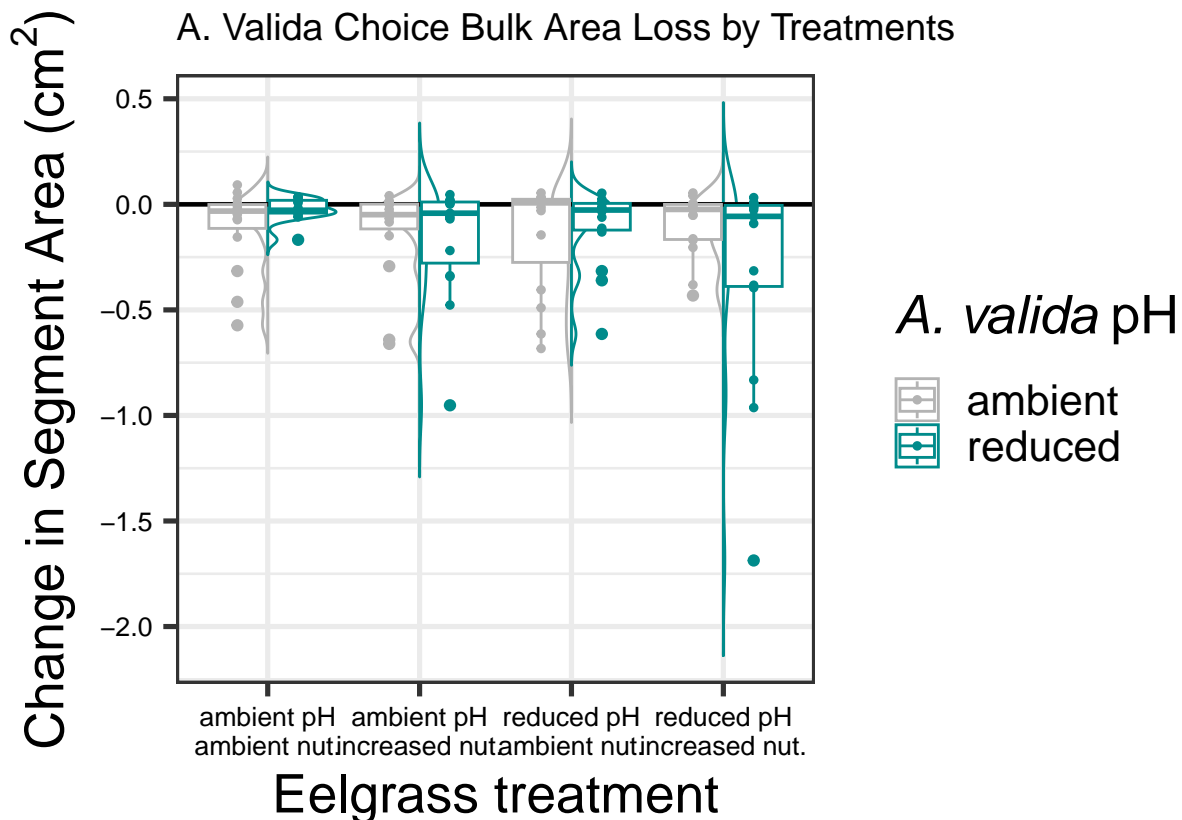
```
## Warning: Using 'size' aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use 'linewidth' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```

boxplota1



```
## trying a violin plot out for fun!
# ggplot(data = choicedata[choicedata$AV_control == "AV",],
#                  aes(x = treatment,
#                      y = area_change,
```

```r
#                           color = tankpH_treat)) +
#   introdataviz::geom_split_violin(alpha = .5, trim = FALSE)+
#   geom_boxplot(width=0.1) + theme_minimal() +
#   scale_x_discrete(breaks = c("A", "B", "C", "D"),
#                    labels = c("ambient pH\nambient nut.", "ambient pH\nincreased nut.",
#                               "reduced pH\nambient nut.", "reduced pH\nincreased nut.")) +
#   labs(x = "Eelgrass treatment",
#       y = expression(paste("Change in Segment Area ("*cm^2*")")),
#       title = "A. Valida Choice Bulk Area Loss by Treatments")+
#   scale_color_manual(name = expression(paste(italic("A. valida") ~ "pH")),
#       breaks = c("ambient", "reduced")
```

```r
#Plot for area loss adjusted by total mass of AV
boxplota2 <- ggplot(data = choicedata[choicedata$AV_control == "AV",],
                    aes(x = treatment,
                        y = area_change_adj,
                        color = tankpH_treat)) +
  geom_hline(yintercept = 0,
             color = "black",
             size = 0.8) +
  geom_boxplot(position = position_dodge(0.9),
               size = 0.8) +
  geom_point(position = position_dodge(0.9),
             size = 2) +
  scale_color_manual(name = expression(paste(italic("A. valida") ~ "pH")),
                     breaks = c("ambient", "reduced"),
                     values = c("grey70", "cyan4")) +
  scale_x_discrete(breaks = c("A", "B", "C", "D"),
                   labels = c("ambient pH\nambient nut.", "ambient pH\nincreased nut.",
                              "reduced pH\nambient nut.", "reduced pH\nincreased nut.")) +
  labs(x = "Eelgrass treatment",
      y = expression(paste("Change in segment area ("*cm^2*"/ g AV)")),
      title = "A. Valida Choice Adjusted Area Loss by Treatments") +
  theme_bw(base_size = 20) +
  theme(axis.text = element_text(color = "black", size=10))+
  theme(plot.title = element_text(size = 14))
boxplota2
```

```
## Warning: Removed 4 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```
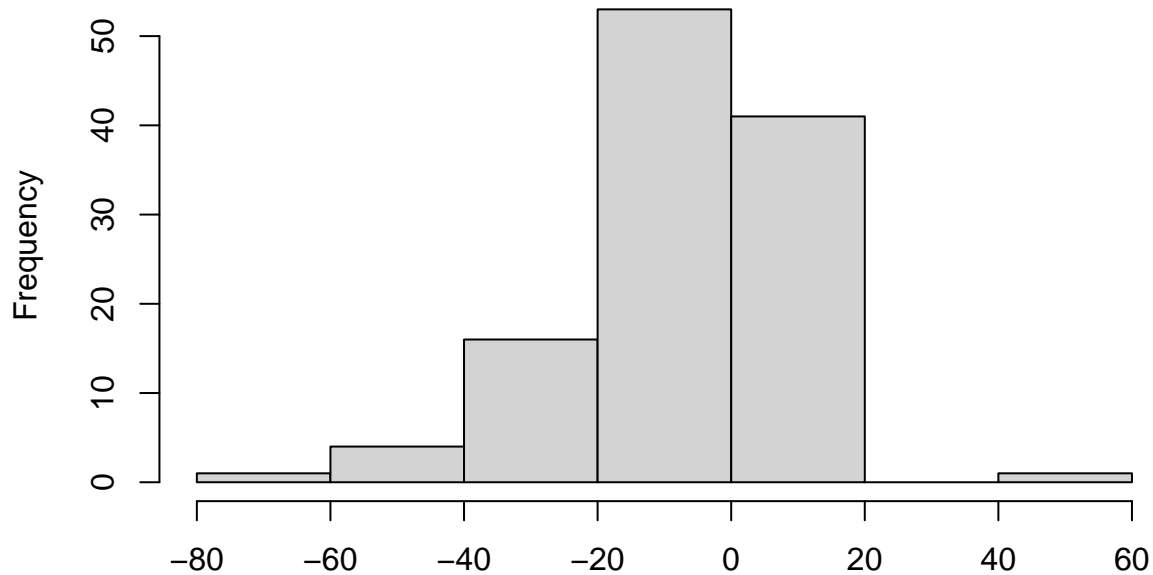
```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## ('geom_point()').
```

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A. Valida Choice Adjusted Area Loss by Treatments
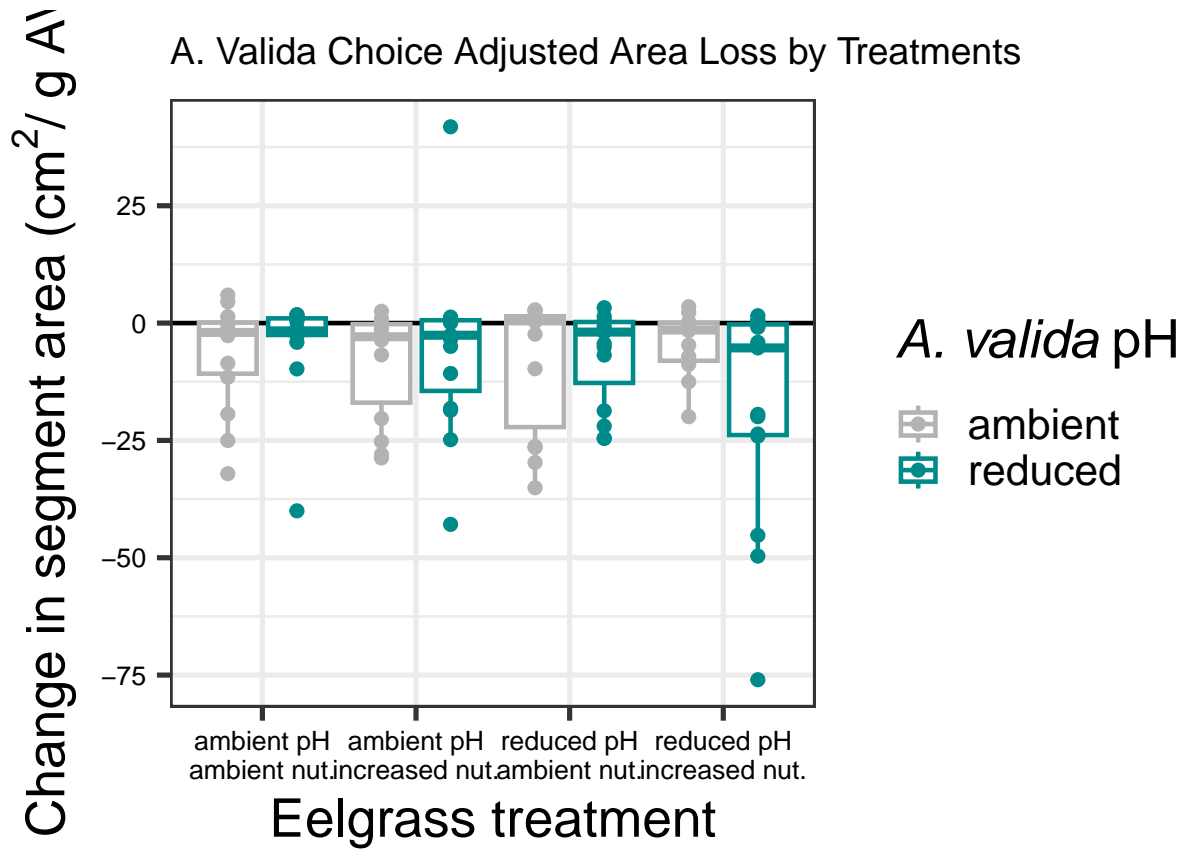
## Analysis

```
#bulk area loss analysis

hist((choicedata$area_change_adj[choicedata$AV_control == "AV"]))
```

## Histogram of (choicedata$area_change_adj[choicedata$AV_control == "



(choicedata$area_change_adj[choicedata$AV_control == "AV"])

```
glmm2 <- lmer((area_change) ~ (pH_treat * nut_treat * tankpH_treat) +
                (1 | tank_ID:unit_ID) + (1 | plant_ID),
              data = choicedata[choicedata$AV_control == "AV",])
glmm2
```
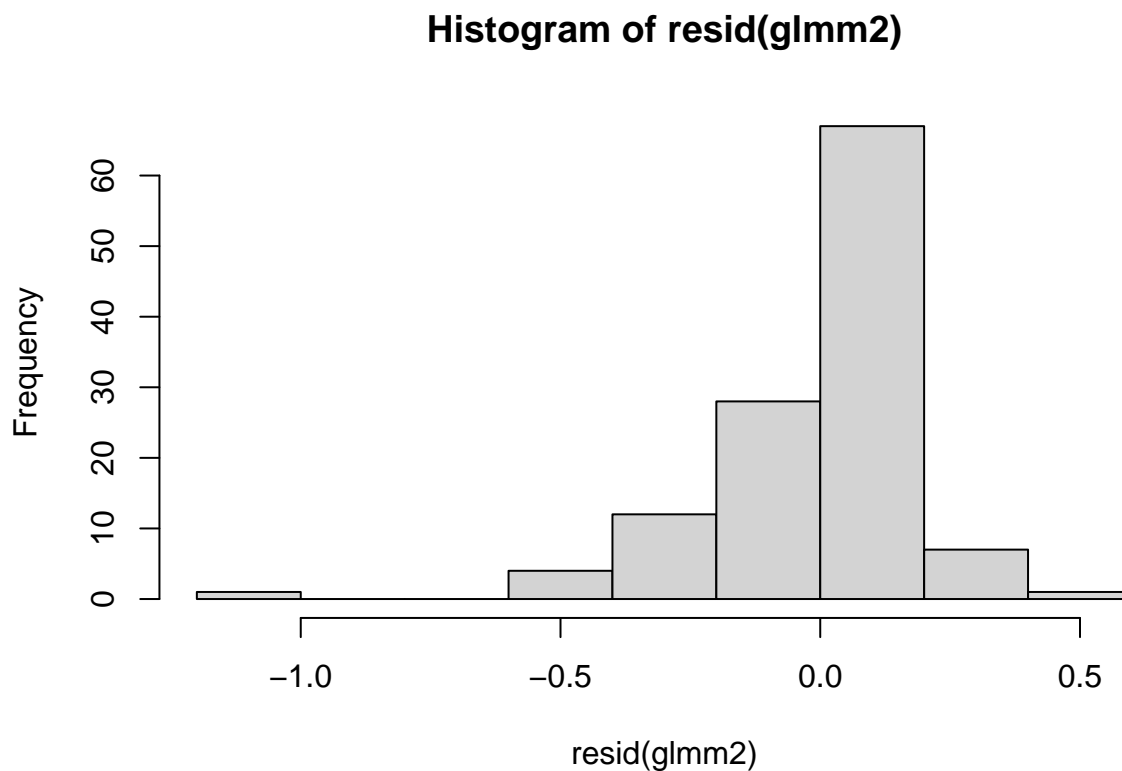
```
## Linear mixed model fit by REML ['lmerModLmerTest']
## Formula: (area_change) ~ (pH_treat * nut_treat * tankpH_treat) + (1 |
##     tank_ID:unit_ID) + (1 | plant_ID)
##    Data: choicedata[choicedata$AV_control == "AV", ]
## REML criterion at convergence: 30.4803
## Random effects:
##  Groups          Name        Std.Dev.
##  tank_ID:unit_ID (Intercept) 0.07206
##  plant_ID        (Intercept) 0.10636
##  Residual                    0.22697
## Number of obs: 120, groups:  tank_ID:unit_ID, 30; plant_ID, 24
## Fixed Effects:
##                                 (Intercept)
##                                    -0.10425
##                              pH_treatreduced
##                                    -0.03782
##                             nut_treatincreased
##                                    -0.04193
##                            tankpH_treatreduced
##                                     0.07962
```

```
##                       pH_treatreduced:nut_treatincreased
##                                                  0.08195
##                       pH_treatreduced:tankpH_treatreduced
##                                                 -0.04103
##              nut_treatincreased:tankpH_treatreduced
##                                                 -0.08669
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced
##                                                 -0.15250
```

```r
#use the shapiro test
shapiro.test(resid(glmm2)) #ns = good
```
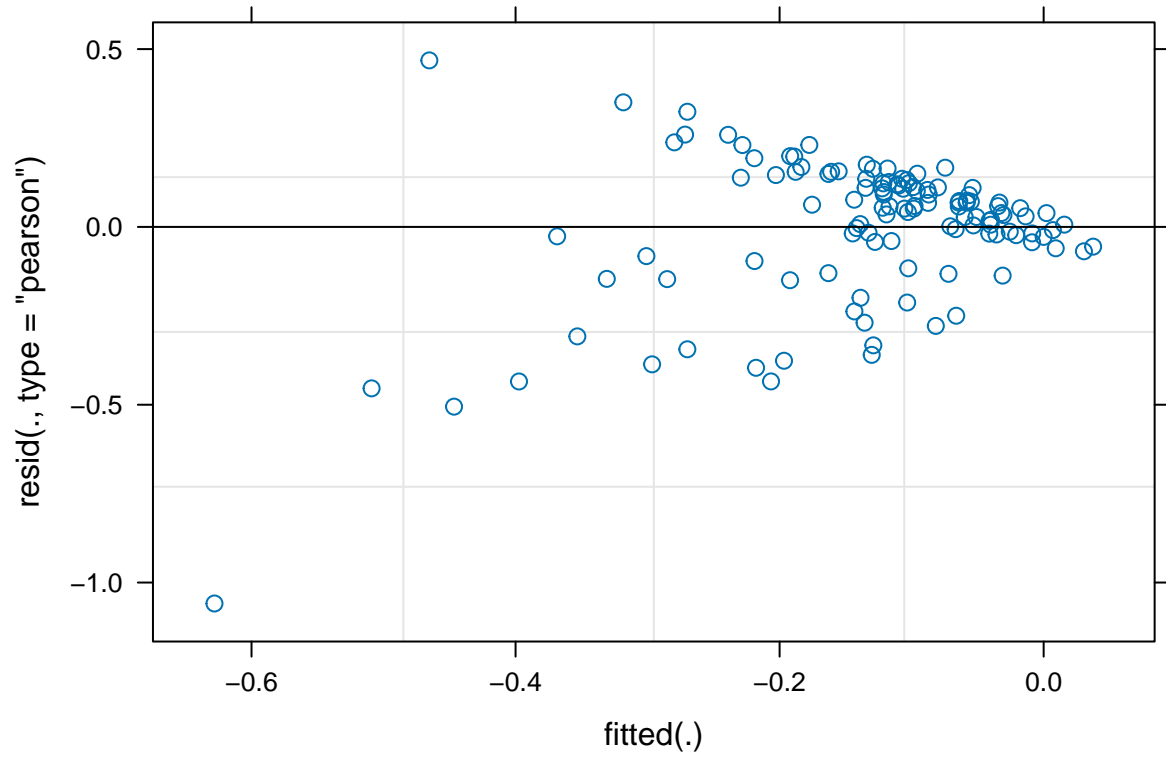
```
##
##  Shapiro-Wilk normality test
##
## data:  resid(glmm2)
## W = 0.87359, p-value = 1.086e-08
```

```r
#plot the residuals
hist(resid(glmm2))
```
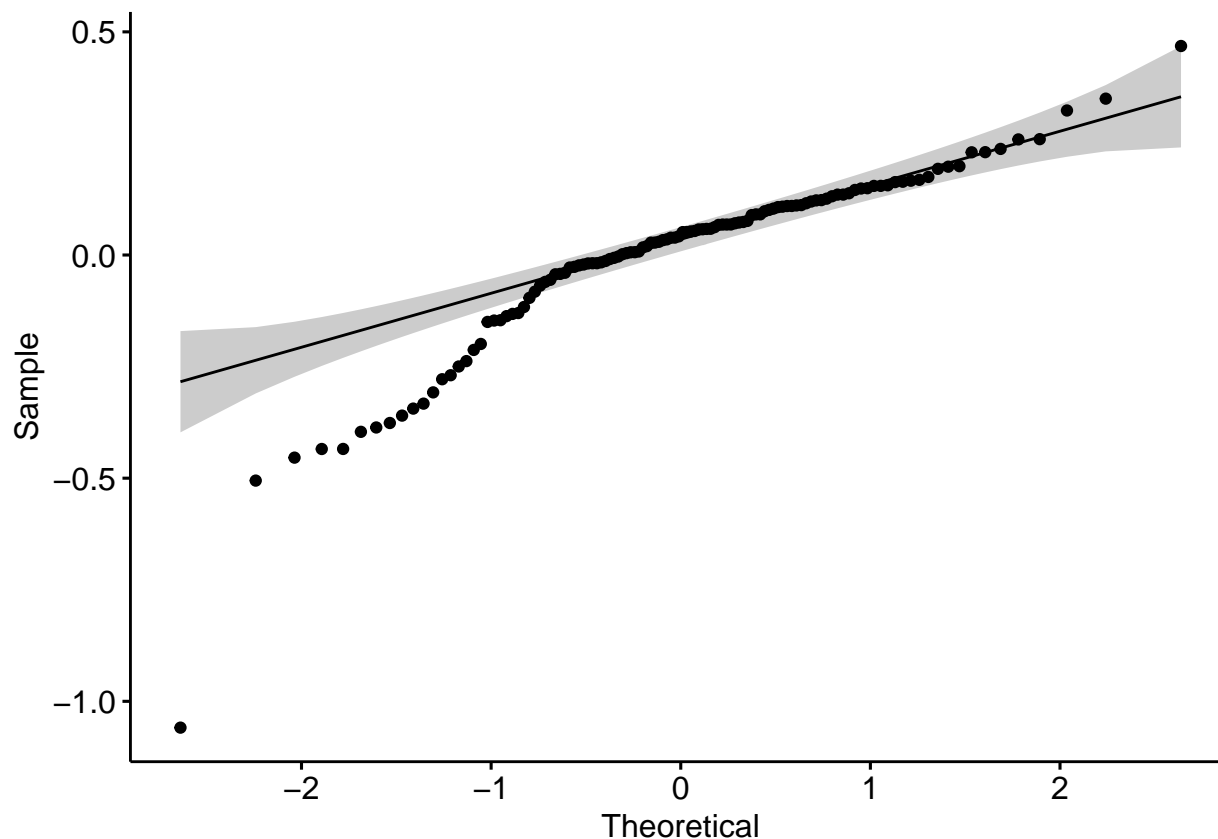
## Histogram of resid(glmm2)



```r
plot(glmm2)
```

```
ggqqplot(resid(glmm2))
```

```
#use the Kenward-Roger test to find degrees of freedom

summary(glmm2, ddf = "Kenward-Roger")
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: (area_change) ~ (pH_treat * nut_treat * tankpH_treat) + (1 |
##     tank_ID:unit_ID) + (1 | plant_ID)
##     Data: choicedata[choicedata$AV_control == "AV", ]
##
## REML criterion at convergence: 30.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.6654 -0.2039  0.2052  0.5152  2.0632
##
## Random effects:
##  Groups          Name        Variance Std.Dev.
##  tank_ID:unit_ID (Intercept) 0.005192 0.07206
##  plant_ID        (Intercept) 0.011312 0.10636
##  Residual                    0.051518 0.22697
## Number of obs: 120, groups:  tank_ID:unit_ID, 30; plant_ID, 24
##
## Fixed effects:
##                                          Estimate Std. Error
## (Intercept)                              -0.10425    0.07694
```

```
## pH_treatreduced                                              -0.03782     0.10533
## nut_treatincreased                                           -0.04193     0.10558
## tankpH_treatreduced                                           0.07962     0.09077
## pH_treatreduced:nut_treatincreased                            0.08195     0.14953
## pH_treatreduced:tankpH_treatreduced                          -0.04103     0.12347
## nut_treatincreased:tankpH_treatreduced                       -0.08669     0.12200
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced       -0.15250     0.17342
##                                                                   df t value
## (Intercept)                                                 40.29616   -1.355
## pH_treatreduced                                             35.05382   -0.359
## nut_treatincreased                                          35.04796   -0.397
## tankpH_treatreduced                                        102.34402    0.877
## pH_treatreduced:nut_treatincreased                          34.75253    0.548
## pH_treatreduced:tankpH_treatreduced                         79.71632   -0.332
## nut_treatincreased:tankpH_treatreduced                      76.68212   -0.711
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced      78.58825   -0.879
##                                                             Pr(>|t|)
## (Intercept)                                                    0.183
## pH_treatreduced                                                0.722
## nut_treatincreased                                             0.694
## tankpH_treatreduced                                            0.382
## pH_treatreduced:nut_treatincreased                             0.587
## pH_treatreduced:tankpH_treatreduced                            0.741
## nut_treatincreased:tankpH_treatreduced                         0.480
## pH_treatreduced:nut_treatincreased:tankpH_treatreduced         0.382
##
## Correlation of Fixed Effects:
##            (Intr) pH_trt nt_trt tnkpH_ pH_t:_ pH_:H_ nt_:H_
## pH_tretrdcd -0.687
## nt_trtncrsd -0.686  0.501
## tnkpH_trtrd -0.595  0.398  0.397
## pH_trtrdc:_  0.485 -0.705 -0.706 -0.281
## pH_trtrd:H_  0.401 -0.584 -0.292 -0.674  0.412
## nt_trtnc:H_  0.405 -0.296 -0.587 -0.680  0.415  0.500
## pH_trt:_:H_ -0.285  0.416  0.413  0.479 -0.587 -0.711 -0.705
```

```r
#ANOVA for hypothesis testing using the Kenward-roger method
anova(glmm2, type = 3, ddf = "Kenward-Roger")
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##                                  Sum Sq  Mean Sq NumDF   DenDF F value  Pr(>F)
## pH_treat                       0.043616 0.043616     1  17.730  0.8466 0.36986
## nut_treat                      0.096228 0.096228     1  17.730  1.8679 0.18880
## tankpH_treat                   0.010017 0.010017     1  26.825  0.1944 0.66278
## pH_treat:nut_treat             0.000115 0.000115     1  17.728  0.0022 0.96286
## pH_treat:tankpH_treat          0.094284 0.094284     1  78.448  1.8301 0.18000
## nut_treat:tankpH_treat         0.182230 0.182230     1  77.766  3.5372 0.06375
## pH_treat:nut_treat:tankpH_treat 0.039843 0.039843     1  78.588  0.7734 0.38186
##
## pH_treat
## nut_treat
## tankpH_treat
## pH_treat:nut_treat
## pH_treat:tankpH_treat
```

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

## Notes

next, do analysis for adjusted then go through the plots and analysis and comment on each of them.