## Guppy Network Infection Experiment

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## 1 Introduction

#### 1.1 Overall summary

This is an analysis to examine how host traits, particularly host sociality and sex, impacts the spread of disease within experimental epidemics of Trinidadian guppies and Monogenean parasites.

### 1.2 Quick experimental summary

We released 6 female guppies and 3 male guppies into experimental behavioral enclosures together and recorded their baseline sociality (Here defined as Contact Rate) for 2 days. We then introduced an infected female index fish on day 3 to see how quickly parasites spread within the group of fish and how likely specific individuals to pick up parasite infection. We repeated recording behavior for 7 days post infection for a total of 9 days of behavioral recordings, however the analysis within this document is limited to days 1-3.

#### 1.3 Questions

#### 1.3.1 Individual level

- Are females more likely to be infected than male hosts?
- Does host worm contact rate explain individual level probability of infection?
- Do infected index fish receive less contact rate from uninfected conspecifics relative to uninfected index fish?

-Do infected index fish with higher infection intensity receive less contact rate from uninfected conspecifics?

#### 1.4 Data description, structure and type

This analysis uses a single master dataframe titled:DyadindividualContacts\_20240726.csv that is subset into different portions based on what questions/analysis we are addressing. Variables within the data frame as follows:

**Ind**: The individual ID of each fish used in the trial.

ContactRate: The daily average contact rate for each individual (contacts/dyad/min)

**Day3ContactRatewoI**: The daily average contact rate for each non-index individual with other non-index individuals. Index fish is the fish introduced on Day 3 with infection (or not for uninfected conrols) (contacts/dyad/min)

**IndexContact**: The daily average contact rate with the index individual. Repeated for each individual since they only had one day with the index individual. (contacts/dyad/min)

**Day:** The day in which the behavior was taken. Days 1 and 2 are days without the infected index individual and day three is with the infected index individual. 3 levels. One level for each day.

**Popavg:** The average contact rate for each group (Contacts/dyads/min/fish)

**Pop**: The group ID for each replicate. One level per group.

**Treatment**: Whether the group received an infected or uninfected index fish at the beginning of day 3. 2 levels.

**TempTreatment**: The treatment (infected or uninfected) of each group for each day. For every group it will be Unf on days 1 and 2 due to not having an index, but Inf for Infected treatments for days 3. 2 levels. **Sex**: The sex of each individual. 2 levels, F for female, M for male.

**Inf.**: This is renamed to Infection in some subset data sets. Whether or not an individual was infected at the end of day 3. 2 levels, 1 - infected, 0-uninfected

worms: The number of worms on each individual at the end of day 3.

**prev**: Prevalence (proportion of infected individuals) for the group at the end of day 3.

**IndexWorm**: Number of worms on the index fish at the end of day 3. This is used as a metric for the amount of worms an individual will have been exposed to during intitial epidemic spread.

Index: Whether the individual was an index fish or not. 2 levels, 0-not index, 1-index

**ChConWithIn**: Change in contact with including the contacts with the index between days 2 and 3.

ChConWOIn: Change in contact with with only non-index individuals between days 2 and 3.

**ChConPop:** Change in the group average contact rate between days 2 and 3

 $\mathbf{PreSVL}$ : The snout to vent length of the guppy prior to introduction into behavioral arena. Measured in (Millimeters)

**PreWeight**: The weight of the individual prior to introduction into the behavior box. Measured in (grams) **PostSVL**: The snout to vent length of the guppy after wrapping up entire experiment. Measured in (Millimeters)

PostWeight: The Weight of the guppy after wrapping up entire experiment. Measured in (grams)

```
# Load in libraries for analysis and data wrangling Visualization
library(ggplot2)
library(visreg)
source("http://highstat.com/Books/BGS/GAMM/RCodeP2/HighstatLibV6.R")
# (generalized) Linear mixed modeling
library(lme4)
library(glmmTMB)
# Statistcal analysis reporting and model validation
library(performance)
library(car)
library(lmtest)
library(DHARMa)
# Data wrangling
library(dplyr)
library(plyr)
library(tidyverse)
library(emmeans)
# Importing the master data sheet check intro for description of
# factors Choose X datasheet (specify when finished)
IndContacts <- read.csv("DyadindividualContacts_20240726.csv")</pre>
# This is creating a variable to calcualte the contact rate with the
# infected individual for non-infected fish and combining them back
# together. Subsetting to inidivudals who are not the index
# Addtitionally this code subsets down to only focusing on dyads with
# the index fish as the recipient
IndContactsNI <- IndContacts %>%
    filter(Index != "1" & day == "3" & str_detect(recipID2, "_10")) %>%
    dplyr::rename(IndexContactR = ContactInitR) %>%
    select(c(fishID2, recipID2, IndexContactR))
# Subseting to individuals who are the index fish
IndContactsI <- IndContacts %>%
    filter(Index == "1") %>%
    mutate(IndexContactR = NA) %>%
    select(c(fishID2, recipID2, IndexContactR))
# Rbinding our two variables together
IndContactsNI <- rbind(IndContactsNI, IndContactsI)</pre>
# Merging our dataframe back together by fishID to add in the
# indexcontactrate
IndContacts2 <- merge(IndContacts, IndContactsNI, by = c("fishID2"), .keep_all = TRUE) %>%
    # select(-c(recipID2.y, day.y, population.y))%>%
dplyr::rename(recipID2 = recipID2.x) %>%
    distinct(fishID2, recipID2, population, day, .keep_all = TRUE)
# Sum contact rate across all videos in a day for each dyad We want
# to combine all contacts across a day. We added 0.001 to each of
# these to help with further model fit without impacting the
# distribution of the data
```

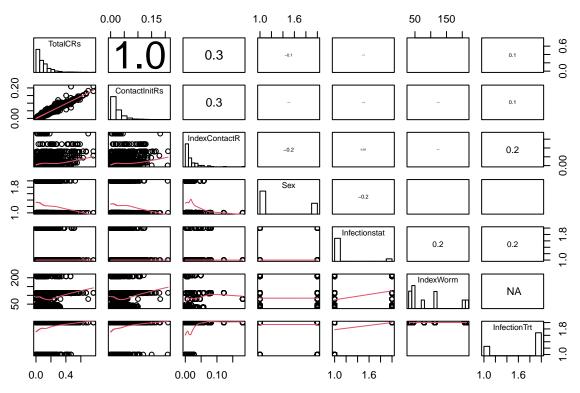
```
IndContactsSum <- IndContacts2 %>%
    select(-c(X, fishID, frame_num, recipID)) %>%
    mutate(ContactInitR = ContactInitR + 1e-04, TotalCR = TotalCR + 1e-04,
        RecipCR = RecipCR + 1e-04) %>%
    group_by(fishID2, recipID2, day) %>%
    dplyr::summarise(ContactInitRs = sum(ContactInitR), TotalCRs = sum(TotalCR),
        RecipCRs = sum(RecipCR), conttactinit = sum(contactinit), RecipfledCR = sum(RecipfledCR))
# Remove unneeded columns
IndContactssub <- IndContacts2 %>%
    select(-c(X, fishID, frame_num, recipID, ContactInitR, TotalCR, RecipCR,
        RecipfledCR)) %>%
    group_by(fishID2, day)
# Merge sheets together so that new data frame has all variables
IndContacts3 <- merge(IndContactsSum, IndContactssub, by = c("fishID2",</pre>
    "recipID2", "day"), .keep_all = TRUE)
# Going down to one row per indiviual
IndContacts3 <- IndContacts3 %>%
    group_by(fishID2, recipID2, population, day) %>%
    distinct(fishID2, recipID2, population, day, .keep_all = TRUE) %>%
    filter(fishID2 != "RCinf1 NA")
# Calculating important metrics that may apply across all subsetting
# of data
# Wormcontacts = Number of contacts a fish has had with infectious
# agents. So the multiplicative of contact rate with the infected
# index with the number of worms on the infected index
IndContacts3 <- IndContacts3 %>%
   mutate(WormContact = IndexContactR * IndexWorm)
# Assigning a fish label to the fish so that if we want to subset
# down to one metric for fish because not all metrics change across
# days. This currently doesn't work due to index only have day 3
# measure Fish<-c('1','0','0')
# Adding this factor to overall data frame
# PopulationContacts$Fish<-as.factor(Fish)
# Overall summary just checking that everything looks okay.
summary(IndContacts3)
```

```
##
                                                     ContactInitRs
     fishID2
                      recipID2
                                           day
                     Length:3213
## Length:3213
                                      Min. :1.000
                                                     Min. :0.000100
## Class :character Class :character
                                      1st Qu.:1.000
                                                     1st Qu.:0.005731
## Mode :character Mode :character
                                      Median :2.000
                                                     Median: 0.015294
##
                                      Mean :2.098
                                                     Mean
                                                            :0.023234
                                                     3rd Qu.:0.031961
                                      3rd Qu.:3.000
##
##
                                      Max. :3.000
                                                     Max. :0.210114
##
##
      TotalCRs
                       RecipCRs
                                      conttactinit
                                                     RecipfledCR
                                    Min. : 0.0 Min. :0.00000
## Min. :0.00010
                   Min. :0.00010
```

```
1st Qu.:0.02560
                     1st Qu.:0.00585
                                       1st Qu.: 195.0
                                                        1st Qu.:0.00450
  Median :0.06421
                     Median :0.01518
                                       Median : 516.0
                                                       Median: 0.01226
                                                        Mean
  Mean
         :0.09574
                     Mean :0.02325
                                       Mean : 766.5
                                                               :0.01860
   3rd Qu.:0.13188
                     3rd Qu.:0.03188
                                       3rd Qu.:1060.0
                                                        3rd Qu.:0.02548
##
   Max.
         :0.76783
                     Max. :0.21011
                                       Max.
                                             :7558.0
                                                        Max.
                                                               :0.19029
##
##
                      TotalContacts
    population
                                       contactinit
                                                         recipinit
## Length:3213
                      Min. :
                                  0
                                      Min. : 0.0
                                                       Min. : 0.0
   Class : character
                      1st Qu.: 897
                                      1st Qu.: 195.0
                                                       1st Qu.: 202.0
##
   Mode :character
                      Median: 2192
                                      Median : 516.0
                                                       Median : 515.0
##
                      Mean
                            : 3178
                                      Mean
                                            : 766.5
                                                       Mean : 767.1
##
                      3rd Qu.: 4405
                                      3rd Qu.:1060.0
                                                       3rd Qu.:1061.0
##
                      Max.
                             :27629
                                      Max.
                                             :7558.0
                                                       Max.
                                                              :7558.0
##
##
                        Sex
     recipfled
                                         recipsex
                                                              worms
##
         : 0.0
                    Length: 3213
                                       Length: 3213
                                                          Min. : 0.00
   1st Qu.: 154.0
                                       Class : character
##
                    Class : character
                                                          1st Qu.: 0.00
   Median : 416.0
                    Mode :character
                                       Mode :character
                                                          Median: 0.00
   Mean
         : 615.7
                                                          Mean : 10.16
##
##
   3rd Qu.: 852.0
                                                          3rd Qu.: 1.00
##
   Max. :5987.0
                                                          Max.
                                                                 :214.00
##
                                                          NA's
                                                                 :2313
##
                                                         InfectionTrt
     Recipworm
                        Index
                                      Infectionstat
   Min. : 0.00
                    Min. :0.00000
                                      Min.
                                             :0.00000
                                                               :0.0000
##
                                                        Min.
##
   1st Qu.: 0.00
                    1st Qu.:0.00000
                                      1st Qu.:0.00000
                                                        1st Qu.:0.0000
  Median: 0.00
                    Median :0.00000
                                      Median :0.00000
                                                        Median :1.0000
## Mean
         : 10.16
                    Mean
                          :0.03922
                                      Mean
                                             :0.09804
                                                        Mean
                                                               :0.7087
   3rd Qu.: 1.00
                    3rd Qu.:0.00000
                                      3rd Qu.:0.00000
                                                        3rd Qu.:1.0000
##
  Max.
         :214.00
                           :1.00000
                                      Max.
                                             :1.00000
                                                               :1.0000
                    Max.
                                                        Max.
  NA's
##
          :2313
##
     IndexWorm
                     recipID2.y
                                       IndexContactR
                                                          WormContact
##
  Min.
          : 32.00
                    Length: 3213
                                       Min.
                                              :0.00000
                                                         Min.
                                                               : 0.0000
##
  1st Qu.: 43.00
                    Class : character
                                       1st Qu.:0.00336
                                                         1st Qu.: 0.2600
## Median : 79.00
                                       Median :0.00948
                                                         Median: 0.8368
                    Mode :character
## Mean
         : 94.05
                                       Mean
                                              :0.01789
                                                         Mean
                                                                : 2.1099
## 3rd Qu.:114.00
                                       3rd Qu.:0.02156
                                                         3rd Qu.: 2.4295
## Max. :214.00
                                       Max.
                                             :0.18141
                                                         Max. :14.3317
## NA's
           :936
                                       NA's
                                              :126
                                                         NA's
                                                                :1026
# Set data struuture for some factors
# Setting Day as a factor instead of a numeric
IndContacts3$day <- as.factor(IndContacts3$day)</pre>
# Setting whether a fish was an index (intitial infection) or not as
# factor
IndContacts3$Index <- as.factor(IndContacts3$Index)</pre>
# Setting whether an fish was infected or not as factor
IndContacts3$Infectionstat <- as.factor(IndContacts3$Infectionstat)</pre>
# Setting whether fish were male or female as a factor
IndContacts3$Sex <- as.factor(IndContacts3$Sex)</pre>
IndContacts3$recipsex <- as.factor(IndContacts3$recipsex)</pre>
# Setting the treatment of fish group (infected index or not) as
# factor
IndContacts3$InfectionTrt <- as.factor(IndContacts3$InfectionTrt)</pre>
```

```
# Setting population the fish were from as a factor
IndContacts3$population <- as.factor(IndContacts3$population)
# Setting fish ID as a factor
IndContacts3$fishID2 <- as.factor(IndContacts3$fishID2)
# Setting recipient ID as a factor
IndContacts3$recipID2 <- as.factor(IndContacts3$recipID2)</pre>
```

#### 1.5 Initial data exploration



```
## Family: beta ( logit )
## Formula: TotalCRs ~ day + (1 | population)
## Data: IndContacts3
##
## AIC BIC logLik deviance df.resid
## -10194.6 -10164.3 5102.3 -10204.6 3208
```

```
##
## Random effects:
##
## Conditional model:
## Groups
              Name
                          Variance Std.Dev.
## population (Intercept) 0.1611 0.4014
## Number of obs: 3213, groups: population, 14
## Dispersion parameter for beta family ():
##
## Conditional model:
              Estimate Std. Error z value Pr(>|z|)
##
## (Intercept) -1.71784
                          0.10950 -15.69 <2e-16 ***
              -0.85657
                          0.03289 -26.04
                                            <2e-16 ***
## day2
## day3
              -1.02701
                          0.03251 -31.59 <2e-16 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
# Testing for significance
drop1(daylm, test = "Chisq")
## Single term deletions
##
## Model:
## TotalCRs ~ day + (1 | population)
         Df AIC
                        LRT Pr(>Chi)
           -10194.6
## <none>
          2 -9273.4 925.29 < 2.2e-16 ***
## day
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
# It seems we have some high contact rate on day 1, we have to
# eliminate it due to acclimation
# Ploting behavior by individual across groups
daylmVR <- visreg(daylm, "day", scale = "response", partial = TRUE, plot = FALSE)
# Generate the fitted relationship
daylmVRfit <- daylmVR$fit</pre>
# Generate the residuals
daylmVRres <- daylmVR$res</pre>
# Plot this relationship
ggplot(daylmVRres, aes(x = day, y = visregRes)) + geom_boxplot(outliers = FALSE) +
   geom_jitter(alpha = 0.2) + theme_classic() + theme(legend.position = "none",
   text = element_text(size = 22)) + ylab("Total contact rate") + xlab("Day")
```

```
## Adding missing grouping variables: 'recipID2', 'population'
## Adding missing grouping variables: 'population'
```

```
# %>% filter(population == 'ADinf1' | population == 'ADunf1' |
# population == 'GZinf1' | population == 'GZunf1' | population ==
# 'SBinf1' | population == 'SBunf1')
```

## 2 Individual Level Analysis

### 2.1 Are hosts with higher worm contact rate more likely to become infected?

For this first bit we need to select the data we need to answer this specific question

#### 2.1.1 Fitting the contact rate versus probability of infection model

We're fitting a binomial generalized linear mixed model with whether an individual was infected or not at the end of day 3 as our response variable and worm contact rate as predictor variables. We have population as a random effect to control for non-independence of worm contact rate between individuals within the same group.

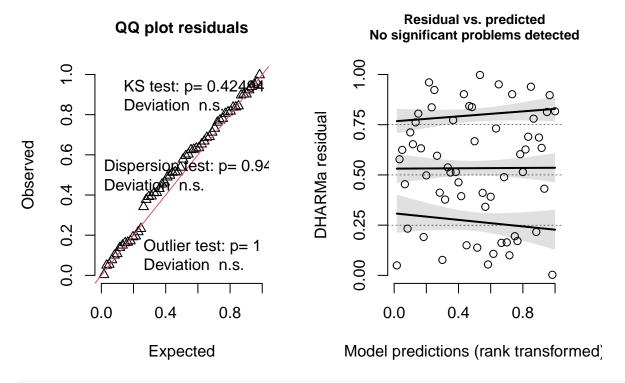
In addition to this, we fit a second GLMM examining the same relationship but with host contact rate instead of the product term of worm contact rate to see if it better fits our data.

```
## Family: binomial ( logit )
## Formula:
## Infectionstat ~ WormContact + I(WormContact^2) + (1 | population)
## Data: IndContactsPI1
```

```
##
##
       ATC
                       logLik deviance df.resid
                BIC
##
       72.9
                81.3
                       -32.4
                                  64.9
##
## Random effects:
##
## Conditional model:
## Groups
              Name
                           Variance Std.Dev.
   population (Intercept) 0.4255
                                    0.6523
## Number of obs: 60, groups: population, 10
## Conditional model:
                    Estimate Std. Error z value Pr(>|z|)
## (Intercept)
                                0.57614 -3.051 0.00228 **
                    -1.75787
## WormContact
                     1.03939
                                0.37157
                                         2.797 0.00515 **
## I(WormContact^2) -0.07959
                                0.03400 -2.341 0.01925 *
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
summary(ProbinfLMFSC)
## Family: binomial (logit)
## Formula:
## Infectionstat ~ ContactInitRs + I(ContactInitRs^2) + (1 | population)
## Data: IndContactsPI1
##
##
       AIC
                 BIC
                       logLik deviance df.resid
##
       78.3
                        -35.1
                                  70.3
                86.7
                                             56
##
## Random effects:
##
## Conditional model:
## Groups
              Name
                           Variance Std.Dev.
## population (Intercept) 1.702
## Number of obs: 60, groups: population, 10
##
## Conditional model:
##
                       Estimate Std. Error z value Pr(>|z|)
## (Intercept)
                                    0.8086 -1.850
                       -1.4958
                                                     0.0643 .
## ContactInitRs
                        69.5154
                                   44.7783
                                           1.552
                                                     0.1206
## I(ContactInitRs^2) -615.9132
                                 453.3311 -1.359
                                                     0.1743
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
2.1.2 Next we see will validate the model to make sure everything is looking good.
```

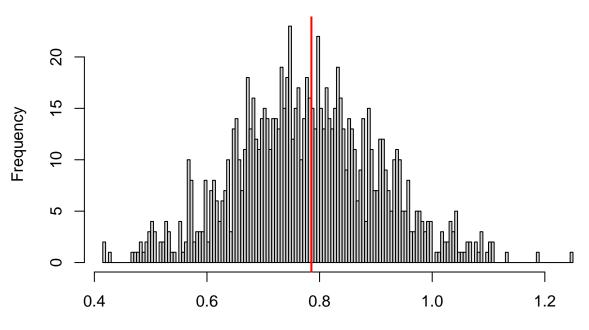
```
# Using DHARMa package to look at quantile residuals to validate the
# worm contact rate model fit
sim_residuals_ProbinfLM <- simulateResiduals(ProbinfLMF, 1000)
# Quantile residuals Ploting the quantile residuals
plot(sim_residuals_ProbinfLM)</pre>
```

### DHARMa residual



# Testing dispersion
testDispersion(sim\_residuals\_ProbinfLM)

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

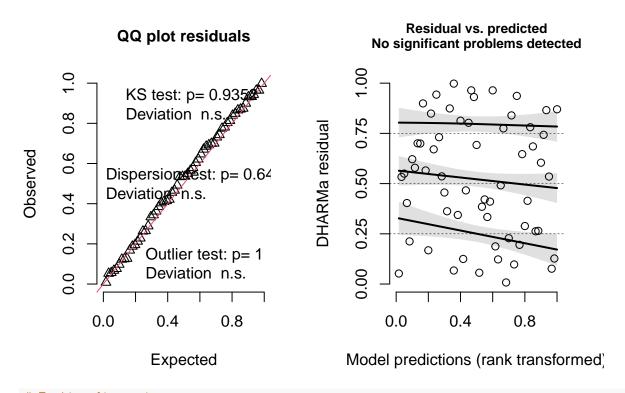


Simulated values, red line = fitted model. p-value (two.sided) = 0.942

```
##
## DHARMa nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 1.0087, p-value = 0.942
## alternative hypothesis: two.sided

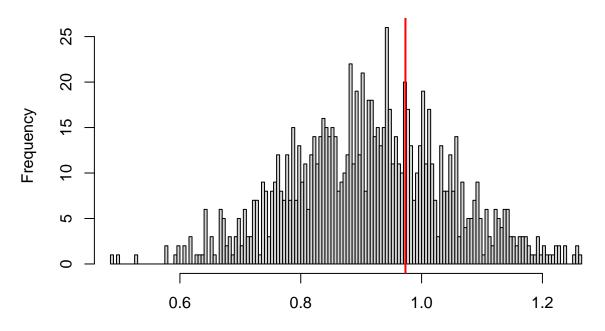
# Using DHARMa package to look at quantile residuals to validate the
# contact rate model fit
sim_residuals_ProbinfLMSC <- simulateResiduals(ProbinfLMFSC, 1000)
# Quantile residuals Ploting the quantile residuals
plot(sim_residuals_ProbinfLMSC)</pre>
```

#### DHARMa residual



# Testing dispersion
testDispersion(sim\_residuals\_ProbinfLMSC)

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



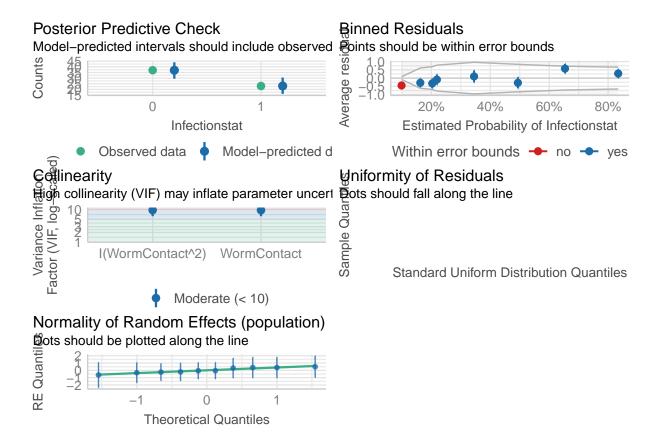
Simulated values, red line = fitted model. p-value (two.sided) = 0.648

```
##
## DHARMa nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 1.065, p-value = 0.648
## alternative hypothesis: two.sided

# Below we are exmaining the quantile residuals among the fixed
# variables to see if there are any funky patterns. Excluded from
# main run for space but remove # to see plots
# plotResiduals(sim_residuals_InfCRlm, IndividualInfDFD2$Sex)
# plotResiduals(sim_residuals_InfCRlm, IndividualInfDFD2$ContactRate)

check_model(ProbinfLMF)
```

## 'check\_outliers()' does not yet support models of class 'glmmTMB'.

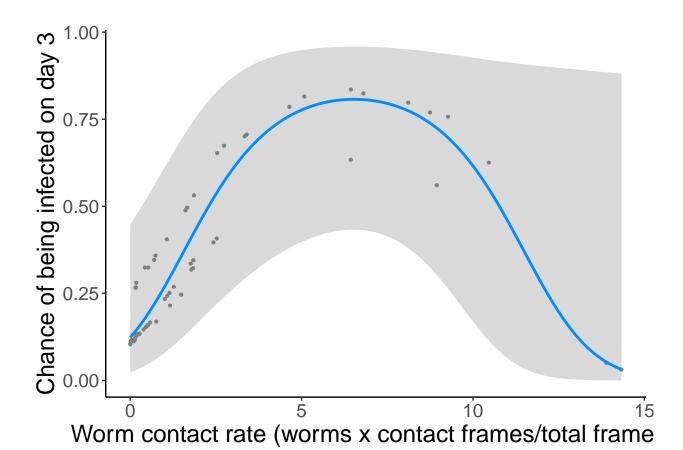


#### 2.1.3 Likelihood ratio test to test for significance in our model

```
# Drop 1 with chisq test to do a likelihood ratio test for our model
drop1(ProbinfLMF, test = "Chisq")
## Single term deletions
##
## Infectionstat ~ WormContact + I(WormContact^2) + (1 | population)
                          AIC
                                LRT Pr(>Chi)
##
                    Df
                       72.889
## <none>
                     1 80.081 9.1926 0.002430 **
## WormContact
                     1 79.620 8.7310 0.003128 **
## I(WormContact^2)
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

#### 2.1.4 Visualization of the relationships between worm contact rate and probability of infection

```
# Visreg of contact rate effect
InfWCRvr <- visreg(ProbinfLMF, "WormContact", scale = "response", partial = T,
    overlay = TRUE, gg = TRUE) + xlab("Worm contact rate (worms x contact frames/total frames) ") +
    ylab("Chance of being infected on day 3") + theme_classic() + theme(text = element_text(size = 18))
print(InfWCRvr)</pre>
```



2.2 Do hosts contact uninfected index as much as infected index individuals?

```
# Filtering down to only Getting index contact rate
IndContactsIndexrecip <- IndContacts4 %>%
    filter(Index == "1")
```

2.2.1 Fitting a model to test for total contact rate for infected versus uninfected index individuals

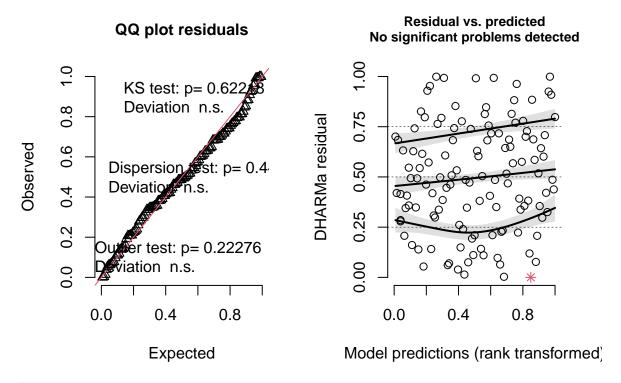
```
## Family: beta (logit)
```

```
## Formula:
## RecipCRs ~ InfectionTrt + recipsex + ScContactInitRs + I(ScContactInitRs^2) +
       (1 | fishID2)
## Data: IndContactsIndexrecip
##
                BIC logLik deviance df.resid
## -1025.0 -1005.2
                       519.5 -1039.0
##
## Random effects:
##
## Conditional model:
## Groups Name
                       Variance Std.Dev.
## fishID2 (Intercept) 0.01133 0.1064
## Number of obs: 126, groups: fishID2, 14
## Dispersion parameter for beta family (): 478
##
## Conditional model:
##
                       Estimate Std. Error z value Pr(>|z|)
                                  0.10340 -46.71 < 2e-16 ***
## (Intercept)
                       -4.82983
## InfectionTrt1
                       -0.06979
                                   0.11463
                                           -0.61 0.542613
## recipsexM
                        0.34583
                                   0.08221
                                             4.21 2.59e-05 ***
## ScContactInitRs
                                             20.56 < 2e-16 ***
                        1.30765
                                   0.06359
## I(ScContactInitRs^2) 0.14887
                                  0.03936
                                           3.78 0.000155 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```

### 2.2.2 Validating the recipient initiated contact with index model

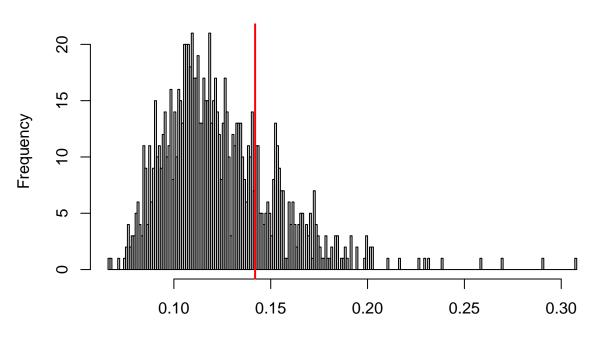
```
sim_residuals_IndexTClm <- simulateResiduals(IndexTClm, 1000) #Quantile residuals
# Ploting the quantile residuals
plot(sim_residuals_IndexTClm)</pre>
```

### DHARMa residual



# Testing dispersion
testDispersion(sim\_residuals\_IndexTClm)

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



Simulated values, red line = fitted model. p-value (two.sided) = 0.44

```
##
##
             DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##
             simulated
##
## data: simulationOutput
## dispersion = 1.1523, p-value = 0.44
## alternative hypothesis: two.sided
# using the checkmodel function to validate model
check_model(IndexTClm)
## 'check_outliers()' does not yet support models of class 'glmmTMB'.
  Posterior Predictive Check
                                                                                                                                                                        Homogeneity of Variance
  Model-predicted lines should resemble observed data Reference line should be flat and horizontal
  Density
                                                                                                                                                                          residu
                                                                                0.05
                                                                                                                                                                                                                  0.03
                                   0.00
                                                                                                                             0.10
                                                                                                                                                                                   0.00
                                                                                                                                                                                                                                                 0.06
                                                                                                                                                                                                                                                                                0.09
                                                                                   RecipCRs
                                                                                                                                                                                                                                Fitted values
                                           Observed data — Model-predicted data
   Collinearity
                                                                                                                                                                        Iniformity of Residuals
 Collinearity (VIF) may inflate parameter uncertain to the line of 
                                                                                                                                                                                        Standard Uniform Distribution Quantiles
                                                                                            Low (< 5)
   Normality of Random Effects (fishID2)
  Sots should be plotted along the line
  RE
                                                                                                 0
                                                                  Theoretical Quantiles
```

```
# sim_residuals_IndexInitTClm <-simulateResiduals(IndexInitTClm,
# 1000) #Quantile residuals #Ploting the quantile residuals
# plot(sim_residuals_IndexInitTClm) #Testing dispersion
# testDispersion(sim_residuals_IndexInitTClm)</pre>
```

2.2.3 Using a likelihood ratio test to test for significance in the recipient initiated contact with index model

```
drop1(IndexTClm, test = "Chisq")
```

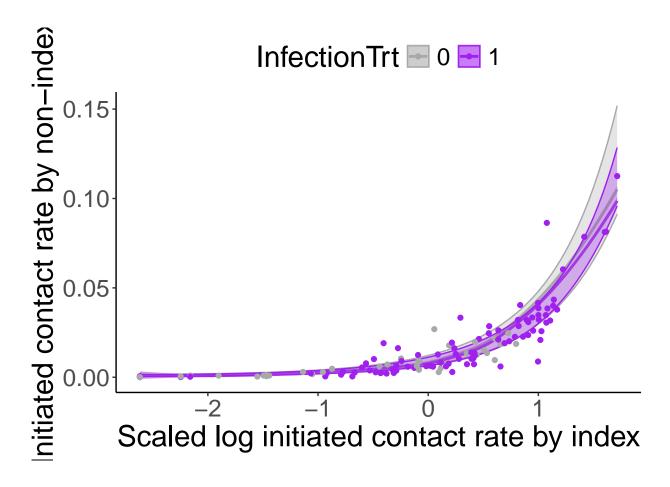
## Single term deletions

```
##
## Model:
## RecipCRs ~ InfectionTrt + recipsex + ScContactInitRs + I(ScContactInitRs^2) +
       (1 | fishID2)
##
                       Df
                               AIC
                                       LRT Pr(>Chi)
## <none>
                          -1025.04
## InfectionTrt
                        1 -1026.68
                                   0.359 0.5491749
                        1 -1011.25 15.794 7.063e-05 ***
## recipsex
                        1 -844.04 183.003 < 2.2e-16 ***
## ScContactInitRs
## I(ScContactInitRs^2) 1 -1013.99 13.054 0.0003027 ***
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

#### 2.2.4 Visualizing the relationship between recipient sex and total contacts with index

```
# Generating fitted relationship and residuals for contact rate and
# infection treatment
RCind <- visreg(IndexTClm, "ScContactInitRs", "InfectionTrt", scale = "response",
   partial = T, plot = FALSE)
# Grabbing the fitted relationship
RCindfit <- RCind$fit
# Grabbing the partial residuals
RCinfres <- RCind$res
# Setting the color scheme for infected and uninfected groups
cpinf = c("darkgray", "purple")
# Plotting the relationship for received contact rate and infection
# treatment
ggplot(RCindfit, aes(x = ScContactInitRs, y = visregFit, group = InfectionTrt,
    colour = InfectionTrt, fill = InfectionTrt)) + geom_smooth(data = RCindfit,
   method = "loess", aes(x = ScContactInitRs, y = visregFit)) + geom_ribbon(aes(ymin = visregLwr,
   ymax = visregUpr), alpha = 0.3) + geom_point(data = IndContactsIndexrecip,
    aes(x = ScContactInitRs, y = RecipCRs)) + theme_classic() + xlab("Scaled log initiated contact rate
   ylab("Initiated contact rate by non-index") + theme(text = element_text(size = 22)) +
   theme(legend.position = "top") + scale_color_manual(values = cpinf) +
    scale_fill_manual(values = cpinf)
```

## 'geom\_smooth()' using formula = 'y ~ x'



### 2.3 Do index fish with higher infection intensity have less recipient contacts?

Subsetting the data frame down for this analysis

```
# subsetting down
IndContactsIndexinfrecip <- IndContactsIndexrecip %>%
    filter(InfectionTrt == "1")

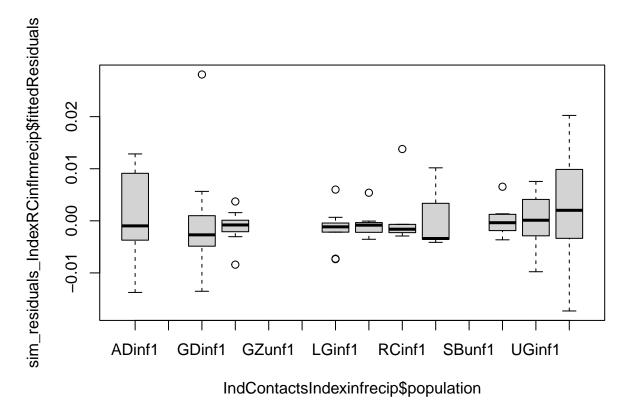
# Sclaing some of our variables for the analysis Index infection
# intensity
IndContactsIndexinfrecip$ScIndexWorm <- scale(IndContactsIndexinfrecip$IndexWorm)</pre>
```

### 2.3.1 Fitting our GLM for the receipient contacts by infection intensity

```
# Fitting the glmmtmb for index received contacts by infection
# intensity
IndexRCinflmrecip <- glmmTMB(RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) +
    recipsex + ScContactInitRs + I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs +
    ScIndexWorm:recipsex + I(ScIndexWorm^2):recipsex, family = beta_family(link = "logit"),
    data = IndContactsIndexinfrecip)
# summary of model
summary(IndexRCinflmrecip)</pre>
```

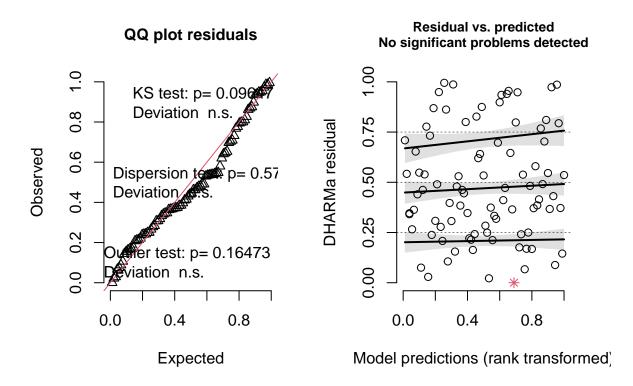
```
## Family: beta (logit)
## Formula:
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + recipsex + ScContactInitRs +
      I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs + ScIndexWorm:recipsex +
##
      I(ScIndexWorm^2):recipsex
## Data: IndContactsIndexinfrecip
##
                      logLik deviance df.resid
       AIC
                BIC
##
    -714.2
             -689.2
                       367.1
                             -734.2
##
##
## Dispersion parameter for beta family (): 506
## Conditional model:
##
                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)
                              -4.78131
                                         0.10348 -46.20 < 2e-16 ***
## ScIndexWorm
                             -0.12330
                                         0.10519
                                                  -1.17 0.24112
                                                  -2.45 0.01419 *
## I(ScIndexWorm^2)
                             -0.12916
                                         0.05267
## recipsexM
                              0.50868
                                         0.11469
                                                   4.44 9.2e-06 ***
## ScContactInitRs
                                                  18.34 < 2e-16 ***
                              1.37431
                                         0.07492
                              0.10111 0.04347
## I(ScContactInitRs^2)
                                                    2.33 0.02004 *
## ScIndexWorm:ScContactInitRs 0.19357
                                         0.09342
                                                  2.07 0.03825 *
                              0.36632
                                                    2.72 0.00646 **
## ScIndexWorm:recipsexM
                                         0.13452
## I(ScIndexWorm^2):recipsexM -0.18727
                                        0.09899
                                                  -1.89 0.05852 .
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```

### 2.3.2 Validating the recipient contact rate by worm load model



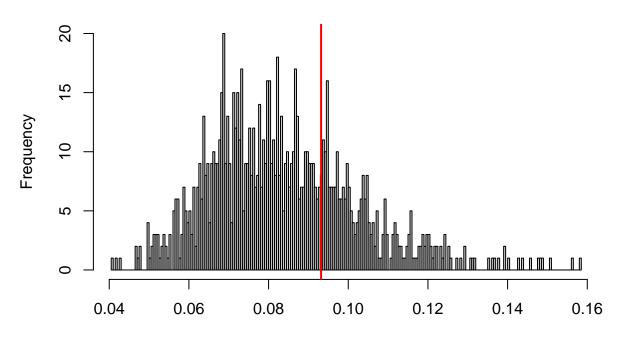
# Ploting the quantile residuals
plot(sim\_residuals\_IndexRCinflmrecip)

## DHARMa residual



```
# Testing dispersion
testDispersion(sim_residuals_IndexRCinflmrecip)
```

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



Simulated values, red line = fitted model. p-value (two.sided) = 0.574

```
##
## DHARMa nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 1.1119, p-value = 0.574
## alternative hypothesis: two.sided

# sim_residuals_IndexRCinflminit
# <-simulateResiduals(IndexRCinflminit, 1000) #Quantile residuals
# #Ploting the quantile residuals
# plot(sim_residuals_IndexRCinflminit) #Testing dispersion
# testDispersion(sim_residuals_IndexRCinflminit)</pre>
```

## 2.3.3 Using a likelihood ratio test to test for significance in recipient contact by index worm load model

```
drop1(IndexRCinflmrecip, test = "Chisq")

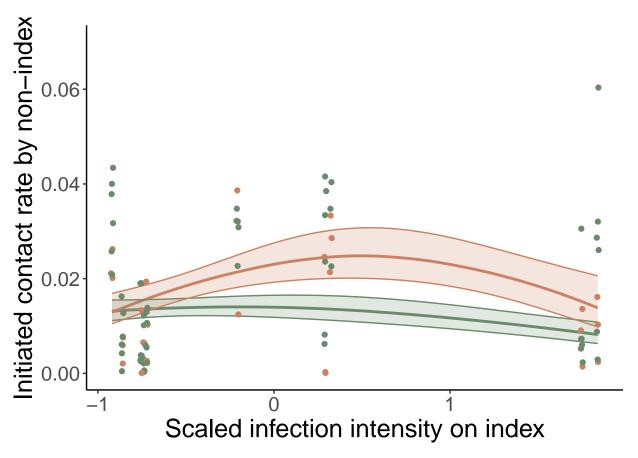
## Single term deletions
##
## Model:
```

```
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + recipsex + ScContactInitRs +
      I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs + ScIndexWorm:recipsex +
##
##
      I(ScIndexWorm^2):recipsex
                                            LRT Pr(>Chi)
##
                                     AIC
## <none>
                                 -714.22
## I(ScContactInitRs^2)
                              1 -711.54 4.6840 0.030446 *
## ScIndexWorm:ScContactInitRs 1 -711.70 4.5242 0.033419 *
## ScIndexWorm:recipsex
                               1 -708.94 7.2791 0.006976 **
## I(ScIndexWorm^2):recipsex 1 -712.71 3.5147 0.060827 .
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

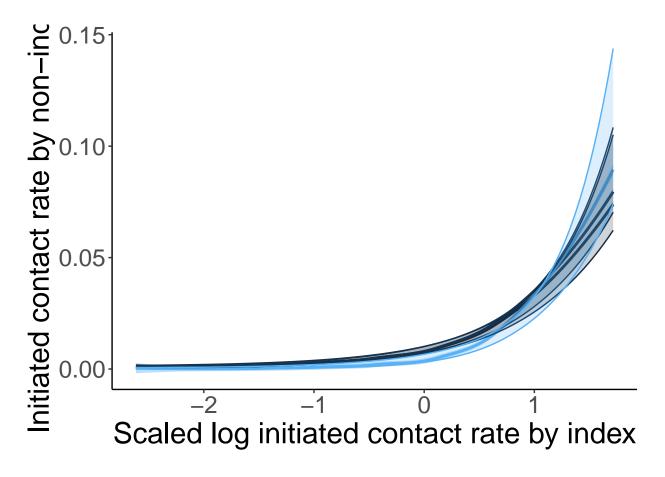
## 2.3.4 Visualizing the relationship between recipient sex and recipient contact and worm load model

```
# Extracting fit and residuals from our model
IndexRCinflmVR <- visreg(IndexRCinflmrecip, "ScIndexWorm", "recipsex",</pre>
    scale = "response", partial = T, plot = FALSE)
# Extracting fit
IndexRCinflmVRfit <- IndexRCinflmVR$fit</pre>
# Extracting residuals
IndexRCinflmVRres <- IndexRCinflmVR$res</pre>
# Setting sex colorscheme
cpsex = c("darkseagreen4", "lightsalmon3")
# Plotting our relationship
ggplot(IndexRCinflmVRfit, aes(x = ScIndexWorm, y = visregFit, group = recipsex,
   fill = recipsex, color = recipsex)) + geom_smooth(method = "loess") +
   geom_ribbon(aes(ymin = visregLwr, ymax = visregUpr), alpha = 0.2) +
   geom_jitter(data = IndContactsIndexinfrecip, aes(x = ScIndexWorm, y = RecipCRs)) +
   theme_classic() + xlab("Scaled infection intensity on index") + ylab("Initiated contact rate by non
   theme(text = element_text(size = 19)) + theme(legend.position = "none") +
    scale_fill_manual(values = cpsex) + scale_color_manual(values = cpsex) +
   vlim(0, 0.07)
## 'geom_smooth()' using formula = 'y ~ x'
## Warning: Removed 5 rows containing missing values or values outside the scale range
```

## ('geom\_point()').



## 'geom\_smooth()' using method = 'loess' and formula = 'y ~ x'



#### 2.3.5 Post hoc analysis for the popularity of index based on sex and index worms

```
# Subsetting down to females
IndContactsIndexinfrecipF <- IndContactsIndexinfrecip %>%
    filter(recipsex == "F")
# Subsetting down to males
IndContactsIndexinfrecipM <- IndContactsIndexinfrecip %>%
    filter(recipsex == "M")
# Fitting a GLMM for females only for post hoc analysis
IndexRCinflmrecipF <- glmmTMB(RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) +</pre>
   ScContactInitRs + I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs,
    family = beta_family(), data = IndContactsIndexinfrecipF)
# Summary for the female model model
summary(IndexRCinflmrecipF)
   Family: beta (logit)
## Formula:
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + ScContactInitRs +
       I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs
## Data: IndContactsIndexinfrecipF
##
##
        AIC
                       logLik deviance df.resid
                 BIC
     -453.4
                                -467.4
              -438.8
                        233.7
##
```

```
##
##
## Dispersion parameter for beta family (): 476
## Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
##
## (Intercept)
                                          0.11525 -41.31
                              -4.76123
                                                            <2e-16 ***
                                                   -1.27
                                                            0.2034
## ScIndexWorm
                              -0.16894
                                          0.13282
## I(ScIndexWorm^2)
                              -0.13398
                                          0.05521
                                                    -2.43
                                                            0.0152 *
## ScContactInitRs
                               1.34848
                                          0.13756
                                                     9.80
                                                            <2e-16 ***
## I(ScContactInitRs^2)
                               0.10363
                                          0.08248
                                                     1.26
                                                            0.2089
## ScIndexWorm:ScContactInitRs 0.25914
                                                     2.03
                                                            0.0421 *
                                          0.12749
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
# Likelihood ratio test for significance in the female model
drop1(IndexRCinflmrecipF, test = "Chisq")
## Single term deletions
##
## Model:
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + ScContactInitRs +
       I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs
##
                              Df
                                     AIC
                                            LRT Pr(>Chi)
                                  -453.41
## <none>
                               1 -449.79 5.6269 0.01769 *
## I(ScIndexWorm^2)
## I(ScContactInitRs^2)
                               1 -453.99 1.4190 0.23356
## ScIndexWorm:ScContactInitRs 1 -450.91 4.5031 0.03383 *
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
# Fitting a GLMM for male post hoc analysis
IndexRCinflmrecipM <- glmmTMB(RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) +</pre>
    ScContactInitRs + I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs,
    , family = beta_family(), data = IndContactsIndexinfrecipM)
# Summary for the male model
summary(IndexRCinflmrecipM)
## Family: beta (logit)
## Formula:
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + ScContactInitRs +
       I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs
## Data: IndContactsIndexinfrecipM
##
##
                      logLik deviance df.resid
       AIC
                BIC
##
     -256.9 -247.1
                       135.5 -270.9
                                            23
##
## Dispersion parameter for beta family (): 668
## Conditional model:
                               Estimate Std. Error z value Pr(>|z|)
                              -4.390565 0.139093 -31.566 < 2e-16 ***
## (Intercept)
```

```
## ScIndexWorm
                             0.280794
                                       0.105600 2.659 0.007837 **
## I(ScIndexWorm^2)
                            ## ScContactInitRs
                             1.494855    0.124637    11.994    < 2e-16 ***
                             0.150132 0.079416
## I(ScContactInitRs^2)
                                                 1.890 0.058698 .
## ScIndexWorm:ScContactInitRs -0.009143 0.156285 -0.059 0.953349
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
# Likelihood ratio test for significance in the male
drop1(IndexRCinflmrecipM, test = "Chisq")
## Single term deletions
## Model:
## RecipCRs ~ ScIndexWorm + I(ScIndexWorm^2) + ScContactInitRs +
      I(ScContactInitRs^2) + ScIndexWorm:ScContactInitRs
##
                            Df
                                   AIC
                                         LRT Pr(>Chi)
## <none>
                               -256.95
## I(ScIndexWorm^2)
                             1 -249.41 9.5334 0.002018 **
## I(ScContactInitRs^2)
                             1 -255.60 3.3430 0.067491 .
## ScIndexWorm:ScContactInitRs 1 -258.94 0.0034 0.953382
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

# 2.4 Do groups with infected versus uninfected index fish change their contact rate different across days 2 and 3 and does this vary by sex?

Subsetting down to the data we need for this analysis

```
IndContacts4ch <- IndContacts4 %>%
         distinct(fishID2, recipID2, .keep_all = TRUE)
# Subsetting down to females
IndContacts4chF <- IndContacts4ch %>%
         filter(Sex == "F")
# Subsetting down to males
IndContacts4chM <- IndContacts4ch %>%
         filter(Sex == "M")
```

We are fitting a linear mixed model with change in contact rate between dyas 2 and 3 for all uninfected fish in the experiment.

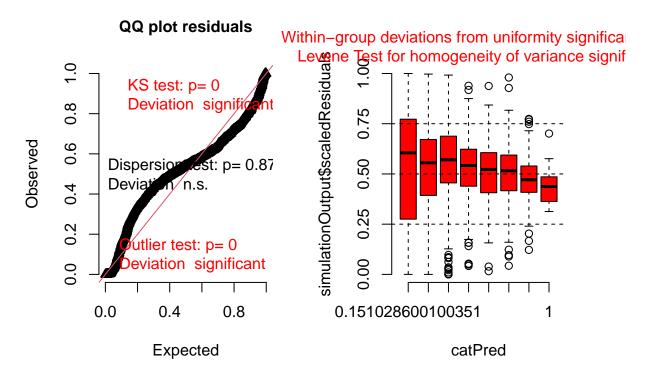
#### 2.4.1 Fitting our linear model for the change in behavior across days 2 and 3

```
# fitting glmmtmb for total contacts over sex and infection treatment
Contactsmodel <- lmer(ChContactinit ~ InfectionTrt + Sex + recipsex + (1 |
    population) + (1 | fishID2), data = IndContacts4ch)

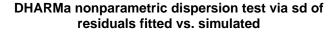
# Summary for our model
summary(Contactsmodel)</pre>
```

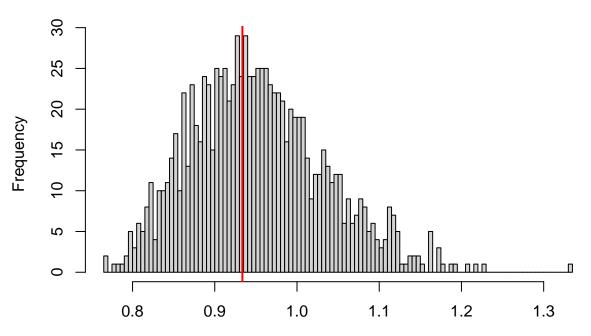
```
## Linear mixed model fit by REML ['lmerMod']
## Formula: ChContactinit ~ InfectionTrt + Sex + recipsex + (1 | population) +
      (1 | fishID2)
##
##
     Data: IndContacts4ch
## REML criterion at convergence: -5040.4
## Scaled residuals:
      Min
            1Q Median
                              3Q
                                      Max
## -4.8411 -0.3423 0.0428 0.4171 6.0639
## Random effects:
## Groups
                          Variance Std.Dev.
              Name
              (Intercept) 5.565e-05 0.007460
## fishID2
## population (Intercept) 9.093e-05 0.009536
## Residual
                          3.314e-04 0.018204
## Number of obs: 1008, groups: fishID2, 126; population, 14
##
## Fixed effects:
                 Estimate Std. Error t value
## (Intercept) -0.002680 0.005101 -0.525
## InfectionTrt1 -0.006581
                            0.005967 -1.103
## SexM
                 0.003802
                            0.001868
                                       2.035
## recipsexM
                 0.005012 0.001226
                                      4.088
##
## Correlation of Fixed Effects:
              (Intr) InfcT1 SexM
## InfectnTrt1 -0.835
## SexM
              -0.129 0.000
## recipsexM -0.090 0.000 0.082
sim_residuals_Contactsmodel <- simulateResiduals(Contactsmodel, 1000) #Quantile residuals
# Ploting the quantile residuals
plot(sim_residuals_Contactsmodel)
```

### DHARMa residual



# Testing dispersion
testDispersion(sim\_residuals\_Contactsmodel)





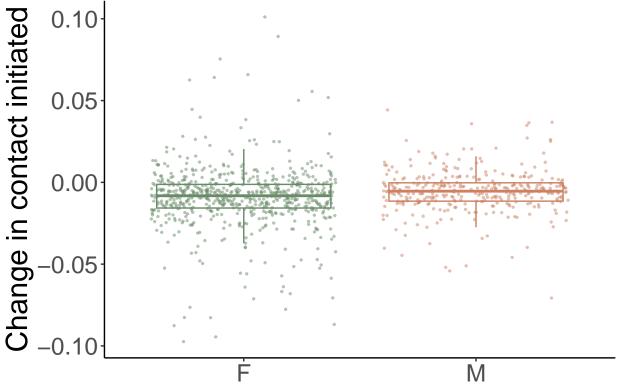
Simulated values, red line = fitted model. p-value (two.sided) = 0.878

```
##
##
    DHARMa nonparametric dispersion test via sd of residuals fitted vs.
    simulated
##
##
## data: simulationOutput
## dispersion = 0.98044, p-value = 0.878
## alternative hypothesis: two.sided
check_model(Contactsmodel)
Posterior Predictive Check
                                                 Linearity
Model-predicted lines should resemble observed da Reference line should be flat and horizontal
              -0.10
                     -0.05
                             0.00
                                     0.05
                                             0.10
                                                             -0.03 -0.02 -0.01 0.00
                       ChContactinit
                                                                      Fitted values

    Observed data — Model-predicted

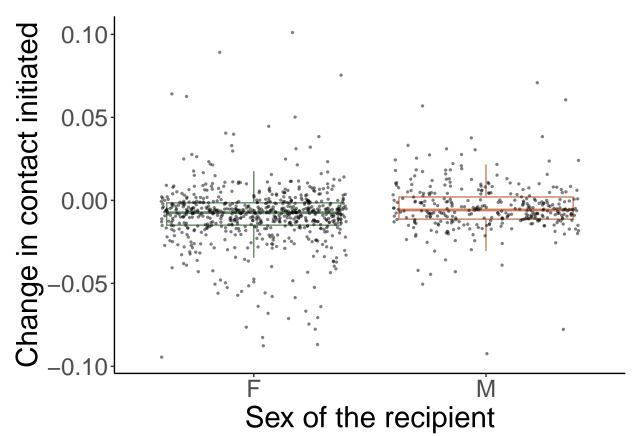
Homogeneity of Variance
                                                 Ѩfluential Observations
Reference line should be flat and horizontal
                                                 Points should be inside the contour lines
                                                 a Stoths Res
        8.5
                                                     _9.8
              -0.03 -0.02 -0.01 0.00
                                                          0.00
                                                                  0.02
                                                                                  0.06
                                                                                          0.08
                        Fitted values
                                                                      Leverage (h<sub>ii</sub>)
 arity
                                                 Normality of Residuals
⊞igh collinearity (VIF) may inflate parameter uncerta Dots should fall along the line
Variance II
Factor (VIF, I6
                                                 Quanti
            InfectionTrtecipsex
                                         NA
                                                          Standard Normal Distribution Quantiles
                                                 Φ
                       Low (< 5)
                                     NA
Mormality of Random Effects (fishID2)
                                                 Mormality of Random Effects (population)
 the line
                                                 bots should be plotted along the line
Quar
                                                 Quai
      -0.09
                                                    -0.02 -
                -2
                      _1
                                                                  _1
                                                                           ()
RE
                                                 RE
                    Theoretical Quantiles
                                                                  Theoretical Quantiles
Anova(Contactsmodel, type = "2", test = "F")
## Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df)
## Response: ChContactinit
                       F Df Df.res
                                        Pr(>F)
## InfectionTrt 1.2165 1 12.00
                                        0.2917
## Sex
                  4.1414
                          1 112.51
                                        0.0442 *
## recipsex
                 16.7139 1 881.00 4.743e-05 ***
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
# Anova(ContactsmodelF, type='2', test='F') Anova(ContactsmodelM,
# type='2', test='F')
```

```
# Setting a labeller to change axis names in the plot
labellerI <- labeller(InfectionTrt = c(`1` = "Infected Treatment", `0` = "Uninfected Treatment"))</pre>
# Setting colorscheme for infection and sex categorical variables
cpinf = c("darkgray", "purple")
cpsex = c("darkseagreen4", "lightsalmon3")
# Exporting the fit and residual for sex in our model
ContactsmodelVR <- visreg(Contactsmodel, "Sex", scale = "response", partial = T,</pre>
    plot = FALSE)
# Extracting fit
ContactsmodelVRfit <- ContactsmodelVR$fit</pre>
# Extracting residuals
ContactsmodelVRres <- ContactsmodelVR$res</pre>
# Plotting out the relationship
ggplot(ContactsmodelVRres, aes(x = Sex, y = visregRes, color = Sex)) +
    geom_boxplot(aes(color = Sex), outliers = FALSE) + geom_jitter(alpha = 0.5,
    size = 0.5) + theme_classic() + theme(text = element_text(size = 22)) +
    ylab("Change in contact initiated") + xlab("Sex of the initiator") +
    scale_color_manual(values = cpsex) + theme(legend.position = "none")
```



## Sex of the initiator

```
# Plotting the residuals
ggplot(ContactsmodelrecipVRres, aes(x = recipsex, y = visregRes)) + geom_boxplot(aes(color = recipsex),
   outliers = FALSE) + geom_jitter(alpha = 0.5, size = 0.5) + theme_classic() +
   theme(text = element_text(size = 22)) + ylab("Change in contact initiated") +
   xlab("Sex of the recipient") + scale_color_manual(values = cpsex) +
   theme(legend.position = "none")
```



2.4.2 Post-hoc analyses for exploring sex differences in the change in contact rate between days 2 and 3

```
# Fitting our post-hoc analysis for female change in contact model
ContactsmodelF <- lmer(ChContactinit ~ (1 | population) + (1 | fishID2),
    data = IndContacts4chF)

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

# Summary for the female model
summary(ContactsmodelF)

## Linear mixed model fit by REML ['lmerMod']
## Formula: ChContactinit ~ (1 | population) + (1 | fishID2)
## Data: IndContacts4chF

##
## REML criterion at convergence: -3278.5</pre>
```

```
##
## Scaled residuals:
##
      Min
                1Q Median
## -4.5939 -0.3498 0.0270 0.4104 5.5912
##
## Random effects:
## Groups
               Name
                           Variance Std.Dev.
               (Intercept) 0.0000000 0.00000
## fishID2
   population (Intercept) 0.0001584 0.01259
## Residual
                           0.0004134 0.02033
## Number of obs: 672, groups: fishID2, 84; population, 14
##
## Fixed effects:
##
                Estimate Std. Error t value
## (Intercept) -0.005501
                           0.003454 -1.593
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
# One sample t-test to test if the change in contact rate is
# different than zero
t.test(IndContacts4chF$ChContactinit)
##
##
   One Sample t-test
## data: IndContacts4chF$ChContactinit
## t = -6.0216, df = 671, p-value = 2.845e-09
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
## -0.007294344 -0.003707031
## sample estimates:
     mean of x
##
## -0.005500688
# FItting our post-hoc analysis for male change in contact model
ContactsmodelM <- lmer(ChContactinit ~ (1 | population) + (1 | fishID2),</pre>
   data = IndContacts4chM)
## boundary (singular) fit: see help('isSingular')
# Summary for the male model
summary(ContactsmodelM)
## Linear mixed model fit by REML ['lmerMod']
## Formula: ChContactinit ~ (1 | population) + (1 | fishID2)
     Data: IndContacts4chM
##
## REML criterion at convergence: -1859.1
## Scaled residuals:
##
      Min
                1Q Median
                                3Q
                                       Max
## -4.4324 -0.3528 -0.0027 0.3729 4.0355
```

```
##
## Random effects:
                          Variance Std.Dev.
## Groups
## fishID2 (Intercept) 0.0001385 0.01177
## population (Intercept) 0.0000000 0.00000
## Residual
                          0.0001754 0.01325
## Number of obs: 336, groups: fishID2, 42; population, 14
##
## Fixed effects:
##
               Estimate Std. Error t value
## (Intercept) -0.002325 0.001954 -1.19
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
\# One sample t-test to test if the change in contact rate is
# different than zero
t.test(IndContacts4chM$ChContactinit)
##
##
  One Sample t-test
##
## data: IndContacts4chM$ChContactinit
## t = -2.4168, df = 335, p-value = 0.01619
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
```

## -0.0042177269 -0.0004326831

## sample estimates:
## mean of x
## -0.002325205