Temperature and Freshwater as Methods for Controlling D. Vexillum Biofouling

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Contents

Abstract

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Libraries

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.5     v dplyr   1.0.7
## v tidyr   1.1.4     v stringr 1.4.0
## v readr   2.0.2     v forcats 0.5.1

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()

##
## Attaching package: 'patchwork'

## The following object is masked from 'package:cowplot':
##
##     align_plots
```

```
## here() starts at /Users/laurengill/Github/tunicate-heat-immersion

## This is DHARMa 0.4.4. For overview type '?DHARMa'. For recent changes, type news(package = 'DHARMa')

## Loading required package: MASS

##
## Attaching package: 'MASS'

## The following object is masked from 'package:patchwork':
##
##     area

## The following object is masked from 'package:dplyr':
##
##     select

## Loading required package: survival

## Loading required package: splines

## Loading required package: gamlss.data

##
## Attaching package: 'gamlss.data'

## The following object is masked from 'package:datasets':
##
##     sleep

## Loading required package: gamlss.dist

## Loading required package: nlme

##
## Attaching package: 'nlme'

## The following object is masked from 'package:dplyr':
##
##     collapse

## Loading required package: parallel

##  **********   GAMLSS Version 5.3-4  **********

## For more on GAMLSS look at https://www.gamlss.com/

## Type gamlssNews() to see new features/changes/bug fixes.
```

```
##
## Attaching package: 'gamlss'

## The following object is masked from 'package:DHARMa':
##
##     getQuantile

## ## FSA v0.9.1. See citation('FSA') if used in publication.
## ## Run fishR() for related website and fishR('IFAR') for related book.

## Loading required package: sn

## Loading required package: stats4

##
## Attaching package: 'sn'

## The following object is masked from 'package:stats':
##
##     sd

##
## Attaching package: 'ordinal'

## The following objects are masked from 'package:nlme':
##
##     ranef, VarCorr

## The following object is masked from 'package:dplyr':
##
##     slice
```

Analyses and Graphs

Reading in Data

```
tunidata <- read.csv("tunicate_master.csv")
tunidata$temperature_c <- as.factor(tunidata$temperature_c)
tunidata$water_type <- as.factor(tunidata$water_type)
tunidata$exposure_time_s <- as.factor(tunidata$exposure_time_s)
```

Change in mean RGB values

First Check for normality, p-value = 0.3433 normal distribution!

```
shapiro.test(tunidata$X48hr_rgb)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  tunidata$X48hr_rgb
## W = 0.98249, p-value = 0.3433
```

Using a linear model for normal distribution - allows you to use random effects and nested effects

```
tunirgb <- tunidata %>%
  mutate(exposure_time_s = as.factor(exposure_time_s)) %>%
  mutate(temperature_c = as.factor(temperature_c)) %>%
  mutate(change_rgb = X48hr_rgb-initial_rgb)

modrgb <- lm(X48hr_rgb ~ exposure_time_s + water_type + temperature_c + exposure_time_s*water_type*tempe
```

```
## Warning: In lm.fit(x, y, offset = offset, singular.ok = singular.ok, ...) :
##  extra argument 'family' will be disregarded
```

```
summary(modrgb)
```

Since model is not influenced by random effects, take this out of the model (stepAIC does not work with random effects) and then reduce model

```
modrgb <- lm(X48hr_rgb ~ exposure_time_s + water_type + temperature_c + exposure_time_s*water_type*tempe
stepmodrgb <- stepAIC(modrgb, direction = "backward", trace = F)
formula(stepmodrgb)
```

```
## X48hr_rgb ~ water_type + temperature_c
```

stepAIC has outputted its final, reduced model. Final p values for model output

```
newmodrgb <- lm(X48hr_rgb ~ water_type + temperature_c, data = tunirgb)
summary(newmodrgb)
```

```
##
## Call:
## lm(formula = X48hr_rgb ~ water_type + temperature_c, data = tunirgb)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -29.518  -9.107   0.730   8.664  36.323
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         157.279      3.518  44.710  < 2e-16 ***
## water_typeseawater   -6.260      3.146  -1.990  0.05026 .
## temperature_c50       2.627      4.450   0.590  0.55664
## temperature_c70      13.720      4.450   3.083  0.00286 **
```

```
## temperature_c90      10.645      4.450   2.392  0.01925 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 14.07 on 75 degrees of freedom
## Multiple R-squared:  0.1823, Adjusted R-squared:  0.1387
## F-statistic:  4.18 on 4 and 75 DF,  p-value: 0.004138
```

Graph

```
deltachangergb <- ggplot(tunirgb, aes(x = temperature_c, y = change_rgb, fill = water_type))+
  labs(x = "Temperature (°C)", y = "Change in mean RGB", fill = "Water type")+
#labs(title = "Temperature (°C)")+
  geom_jitter(position = position_dodge(width=0.75))+
  geom_boxplot(alpha = 0.6)+
  theme_bw()

#ggsave("change-in-mean-rgb.jpg")
```

Mold Cover

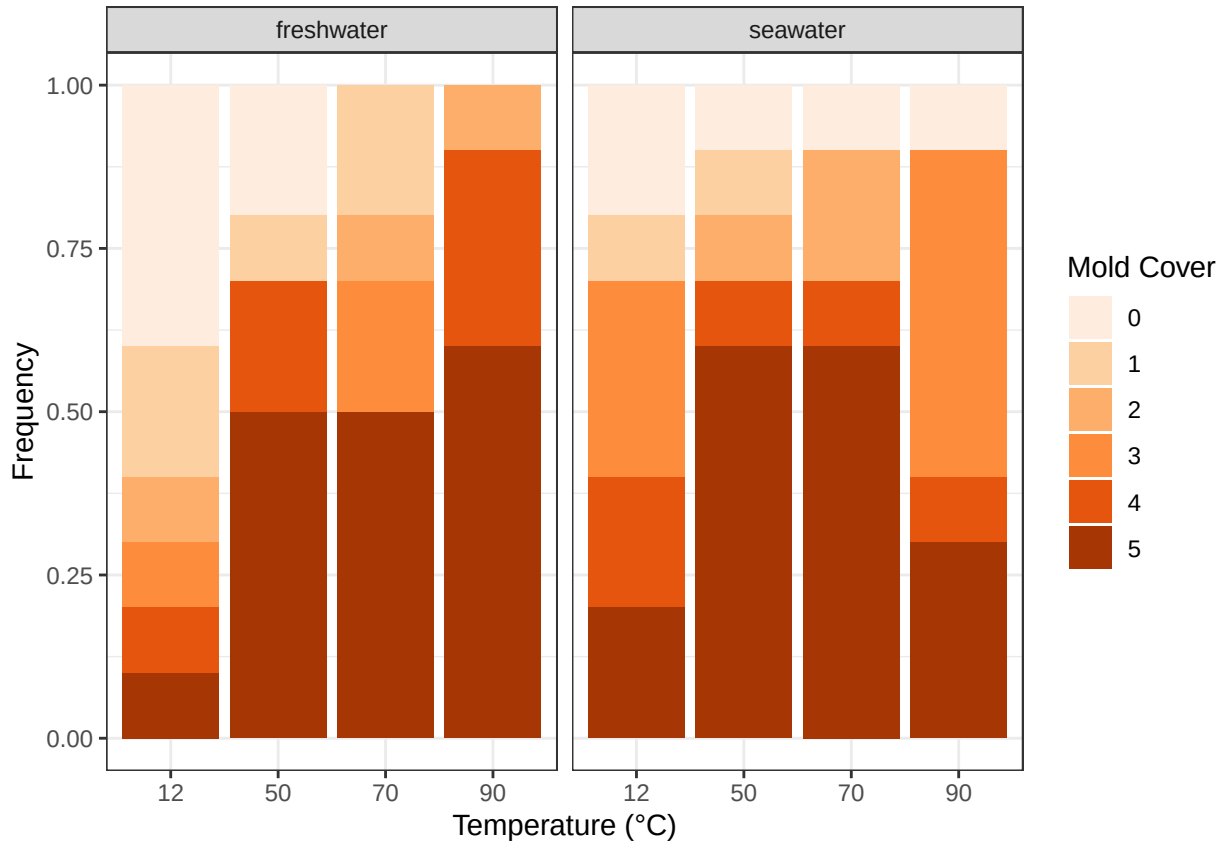
Displaying the data as a proportion of overall score with given mold cover scores

```
tuni_stacked = tunidata %>%
  group_by(mold_cover, temperature_c, water_type, exposure_time_s) %>%
  summarise(frequency = n())%>%
  mutate(temperature_c=  as.factor(temperature_c))
```

```
## `summarise()` has grouped output by 'mold_cover', 'temperature_c', 'water_type'. You can override usi
```

Stacked bar graphs showing mold cover

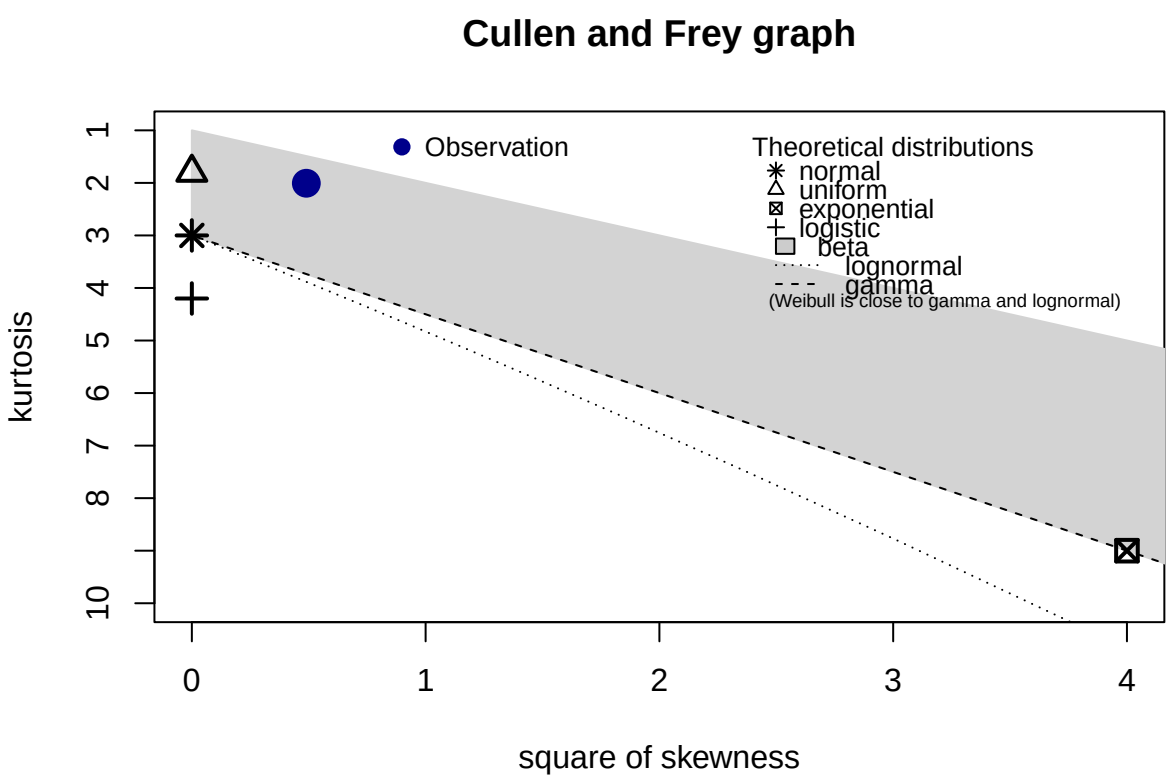
```
ggplot(tuni_stacked, aes(y = frequency, x = temperature_c, fill = as.factor(mold_cover))) +
  geom_bar(stat = "identity", position = "fill") +
  facet_grid(.~water_type) +
  labs(x ="Temperature (°C)", y = "Frequency")+
  scale_fill_brewer(palette = "Oranges", direction=1) +
  labs(fill = "Mold Cover") +
  theme_bw()
```




```
#ggsave("mold-cover.jpg")
```

Examining mold cover data to find the best distribution

```
descdist(tunidata$mold_cover)
```




```
## summary statistics
## ------
## min:  0   max:  5
## median:  4
## mean:  3.325
## estimated sd:  1.854007
## estimated skewness:  -0.6997944
## estimated kurtosis:  2.006652
```

```
fit <- fitDist(mold_cover, data = tunidata, type = "realAll", try.gamlss = T)
```

```
##   |                                                                      |
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0
##   |                                                                      |==========
##   Lapack routine dgesv: system is exactly singular: U[4,4] = 0
##   |                                                                      |==========
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0
##   |                                                                      |==========
```

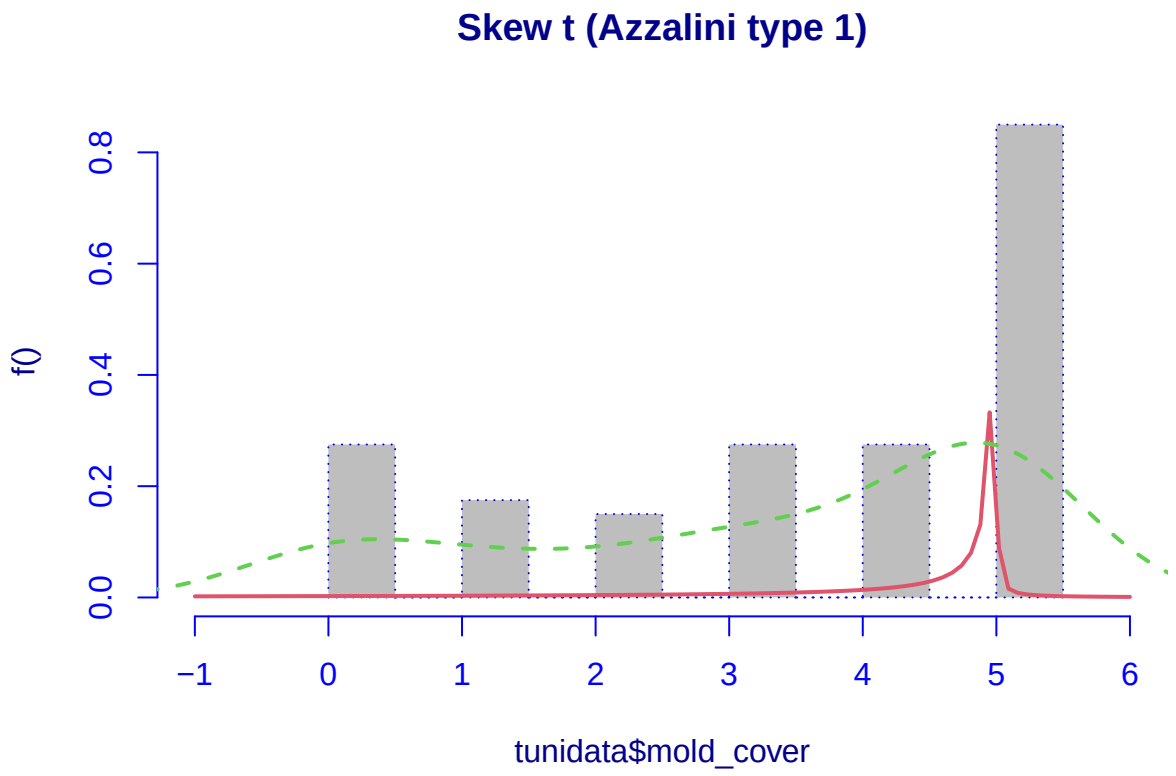
```
fit$fits
```

```
##           ST1       SHASHo2        SHASHo           JSUo           JSU
## -7.438242e+02 -7.212029e+02 -5.941774e+02 -5.670796e+02  3.353055e+00
##           ST5           ST3           SN2           SST           ST2
```

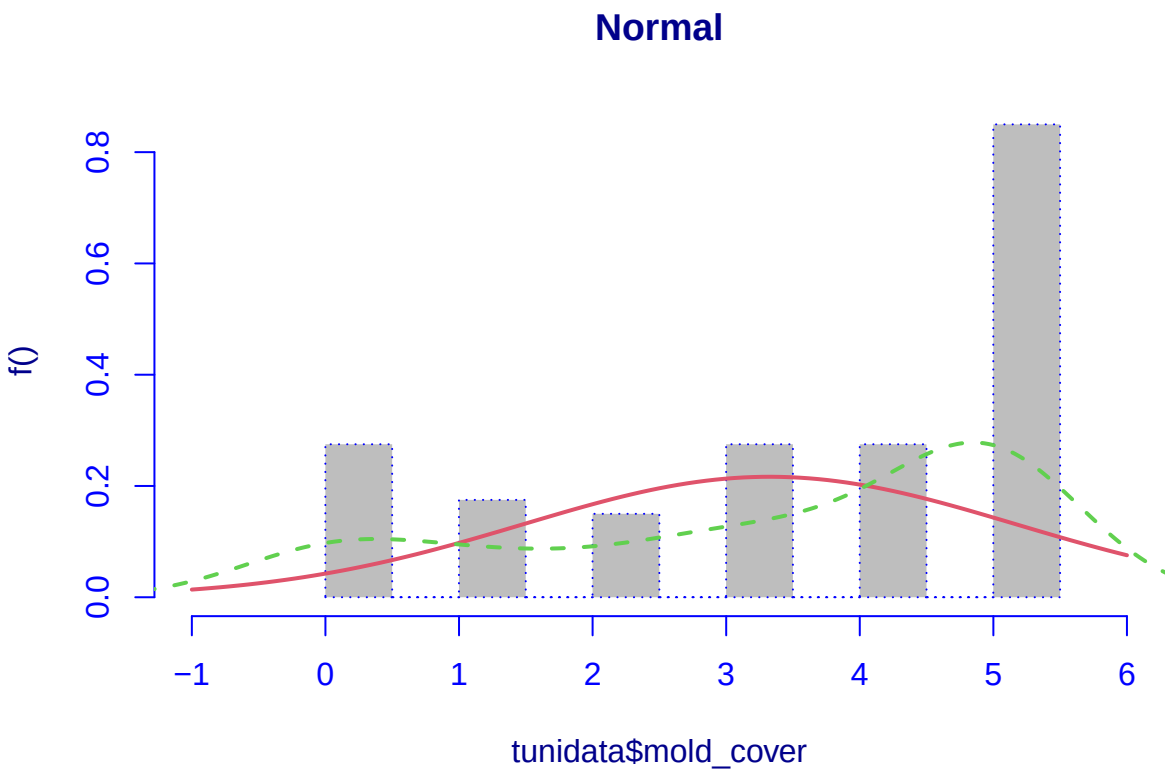
```
##  1.033464e+01  2.521045e+02  2.681226e+02  2.698031e+02  2.700922e+02
##            PE           SEP4           NET           PE2          EGB2
##  2.712465e+02  2.713103e+02  2.730814e+02  2.835127e+02  2.895608e+02
##            GU            NO           TF2            TF           SN1
##  3.112873e+02  3.287997e+02  3.307997e+02  3.307997e+02  3.307997e+02
##        exGAUS           ST4           LO            RG          EXP
##  3.308045e+02  3.327997e+02  3.343529e+02  3.492131e+02  3.542351e+02
##       PARETO2      PARETO2o         SHASH          SEP1           GT
##  3.562351e+02  3.562352e+02  4.069489e+02  1.185033e+05  6.144562e+08
```

Fitdist determined ST1 to be the best distribution, comparing it against a normal distribution to make sure AIC value is lower. AIC value of ST1 = -743.8 while AIC value of normal = 328

```
mST1 <- histDist(tunidata$mold_cover, "ST1", density = T, main = "Skew t (Azzalini type 1)")
```




```
mNO <- histDist(tunidata$mold_cover, "NO", density = T, main = "Normal")
```




```
GAIC(mST1, mNO)
```

```
##      df       AIC
## mST1  4 -743.8242
## mNO   2  328.7997
```

Now creating a full model, reduced model is the same as full model. No factors are significant

```
tunirgb$colony_id <- as.factor(tunirgb$colony_id)
tunirgb$mold_cover <- as.factor(tunirgb$mold_cover)

ordinalmodfull <- clmm2(mold_cover ~ water_type + temperature_c + exposure_time_s +
                      exposure_time_s*water_type*temperature_c,
                      random = colony_id, data = tunirgb, Hess = TRUE)
summary(ordinalmodfull)
```

```
## Cumulative Link Mixed Model fitted with the Laplace approximation
##
## Call:
## clmm2(location = mold_cover ~ water_type + temperature_c + exposure_time_s +
##     exposure_time_s * water_type * temperature_c, random = colony_id,
##     data = tunirgb, Hess = TRUE)
##
## Random effects:
```

```
##                  Var   Std.Dev
## colony_id 0.08633119 0.2938217
##
## Location coefficients:
##                                                          Estimate Std. Error
## water_typeseawater                                        1.4322   1.0358
## temperature_c50                                           1.5210   1.0377
## temperature_c70                                           0.7898   0.5905
## temperature_c90                                           2.4114   1.1833
## exposure_time_s120                                       -1.3967   1.1751
## water_typeseawater:exposure_time_s120                    -0.2186   1.5864
## temperature_c50:exposure_time_s120                        1.0243   1.7631
## temperature_c70:exposure_time_s120                        2.4574   1.4489
## temperature_c90:exposure_time_s120                        1.0751   1.7042
## water_typeseawater:temperature_c50                       -1.4843   1.5749
## water_typeseawater:temperature_c70                       -1.9368   1.0619
## water_typeseawater:temperature_c90                       -2.5154   1.6410
## water_typeseawater:temperature_c50:exposure_time_s120     1.0916   2.4101
## water_typeseawater:temperature_c70:exposure_time_s120    23.2430      NaN
## water_typeseawater:temperature_c90:exposure_time_s120    -0.1900   2.3047
##                                                          z value Pr(>|z|)
## water_typeseawater                                        1.3827 0.166767
## temperature_c50                                           1.4657 0.142722
## temperature_c70                                           1.3376 0.181027
## temperature_c90                                           2.0378 0.041566
## exposure_time_s120                                       -1.1886 0.234606
## water_typeseawater:exposure_time_s120                    -0.1378 0.890380
## temperature_c50:exposure_time_s120                        0.5810 0.561255
## temperature_c70:exposure_time_s120                        1.6960 0.089881
## temperature_c90:exposure_time_s120                        0.6308 0.528142
## water_typeseawater:temperature_c50                       -0.9424 0.345968
## water_typeseawater:temperature_c70                       -1.8239 0.068175
## water_typeseawater:temperature_c90                       -1.5328 0.125328
## water_typeseawater:temperature_c50:exposure_time_s120     0.4529 0.650610
## water_typeseawater:temperature_c70:exposure_time_s120        NaN NA
## water_typeseawater:temperature_c90:exposure_time_s120    -0.0825 0.934289
##
## No scale coefficients
##
## Threshold coefficients:
##     Estimate Std. Error z value
## 0|1 -1.0939   0.7416    -1.4752
## 1|2 -0.3827   0.7253    -0.5277
## 2|3  0.0931   0.7269     0.1281
## 3|4  0.8816   0.7201     1.2242
## 4|5  1.6307   0.7279     2.2404
##
## log-likelihood: -112.6399
## AIC: 267.2799
## Condition number of Hessian: 3.058746e+12
```

```
#temperature_c90 p=0.041566
```

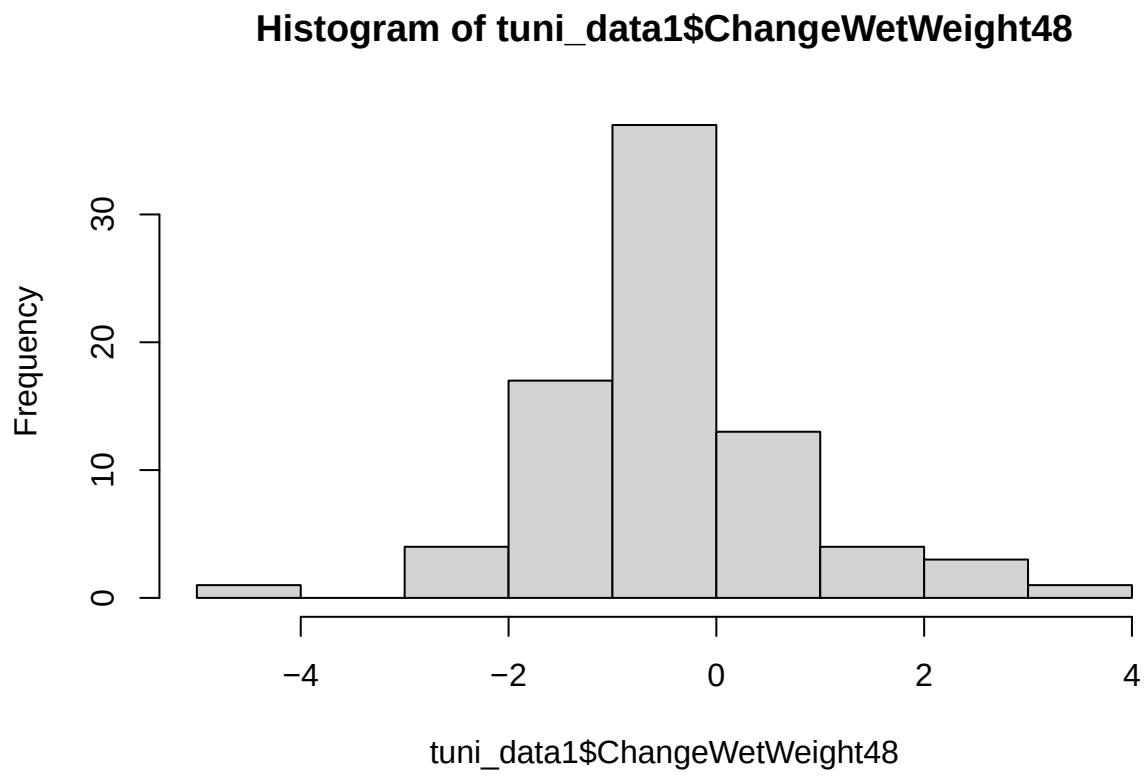
Change in weight after 48 hours

Data Visualization

```
#Making new columns for changes in weight
tuni_data1 <- tunidata %>% mutate(ChangeWetWeight = final_weight_g-post_acclimation_weight_g)
tuni_data1 <- tuni_data1 %>% mutate(ChangeWetWeight48 = `X48hr_weight`-post_acclimation_weight_g)

p2 <- ggplot(tuni_data1, aes(x = exposure_time_s, y = ChangeWetWeight48, fill = water_type, ))+
  labs(x = "Immersion time (s)", y = "Change in wet weight (g)", fill = "Water type")+
  geom_boxplot(alpha = 0.6)+
  theme_bw()

p3 <- p2 + facet_grid(cols = vars(temperature_c))
```

Testing for Normality - p-value = 0.01824 so not a normal distribution

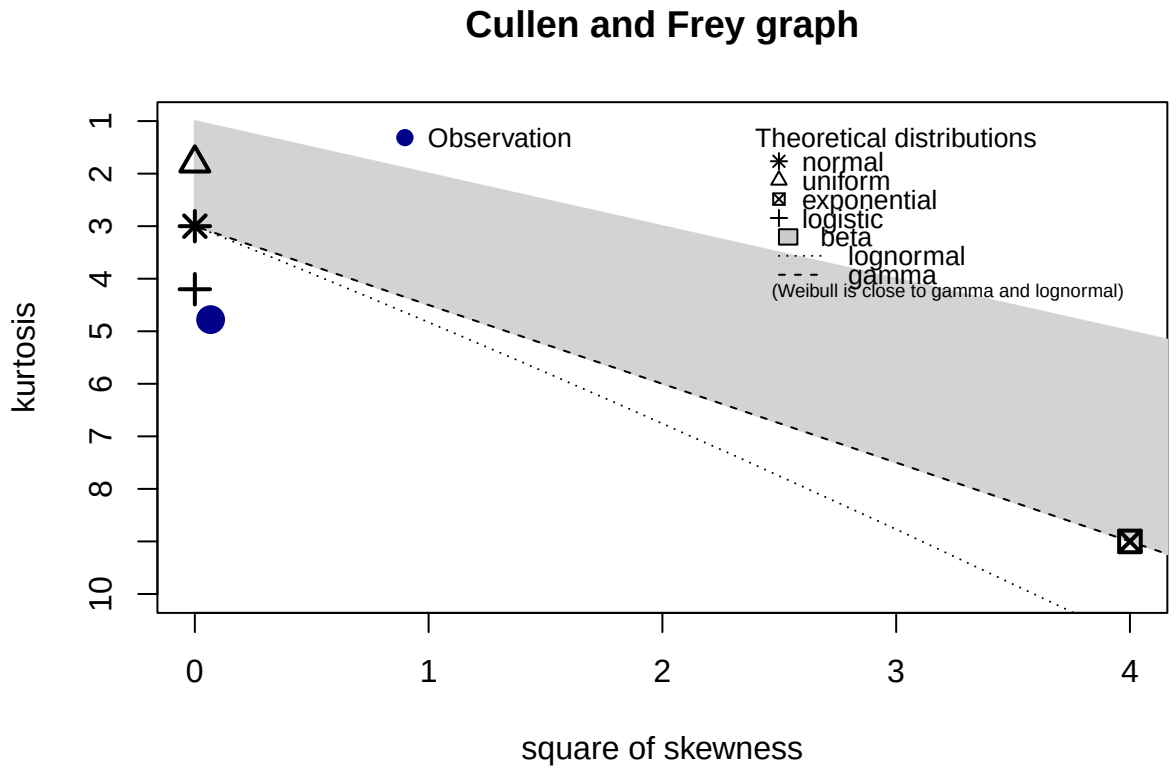
```
hist(tuni_data1$ChangeWetWeight48)
```




```
shapiro.test(tuni_data1$ChangeWetWeight48)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  tuni_data1$ChangeWetWeight48
## W = 0.96216, p-value = 0.01824
```

```
#p-value = 0.01824, not normal
descdist(tuni_data1$ChangeWetWeight48)
```




```
## summary statistics
## ------
## min:  -4.27   max:  3.09
## median:  -0.355
## mean:  -0.419375
## estimated sd:  1.189221
## estimated skewness:  0.2597728
## estimated kurtosis:  4.778191
```

```
#might be logistic
```

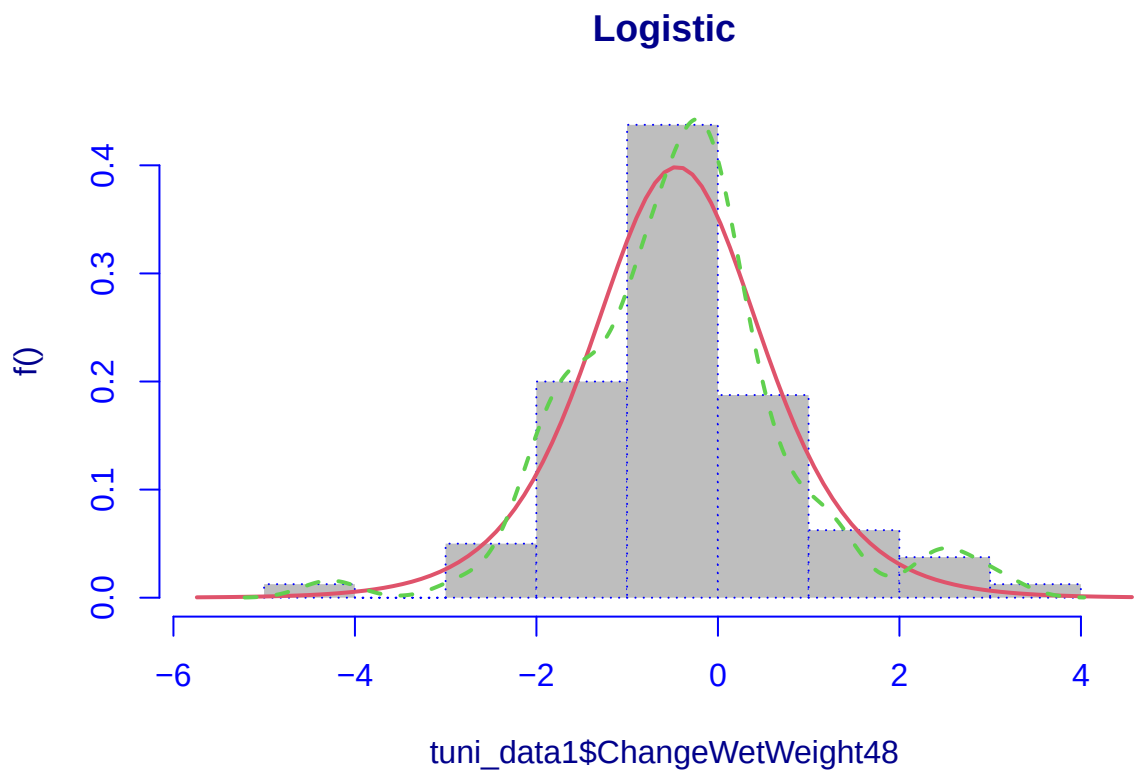
Distribution Fitting - used FitDist function - followed a logistic distribution

```
fitDist(ChangeWetWeight48, data=tuni_data1, type="realAll", try.gamlss = T)
```

```
##   |                                                                      |
```

```
##
## Family:  c("LO", "Logistic")
## Fitting method: "nlminb"
##
## Call:  gamlssML(formula = y, family = DIST[i])
##
## Mu Coefficients:
## [1]  -0.4483
## Sigma Coefficients:
## [1]  -0.4662
##
##  Degrees of Freedom for the fit: 2 Residual Deg. of Freedom   78
## Global Deviance:     247.968
##             AIC:     251.968
##             SBC:     256.732
```

```
#family=LO, logistic

mLOG_weight <- histDist(tuni_data1$ChangeWetWeight48, "LO", density = T, main = "Logistic")
```




```
GAIC(mLOG_weight)
```

```
## [1] 251.9677
```

GAMLSS Model

```
mod_weight <- gamlss(ChangeWetWeight48 ~ water_type + temperature_c + exposure_time_s + water_type*tempe
```

```
## GAMLSS-RS iteration 1: Global Deviance = 213.2525
## GAMLSS-RS iteration 2: Global Deviance = 212.7865
## GAMLSS-RS iteration 3: Global Deviance = 212.7863
```

```
summary(mod_weight)
```

```
## ******************************************************************
## Family:  c("LO", "Logistic")
##
## Call:  gamlss(formula = ChangeWetWeight48 ~ water_type + temperature_c +
##     exposure_time_s + water_type * temperature_c *
##     exposure_time_s, family = LO, data = tuni_data1)
##
## Fitting method: RS()
##
## ------------------------------------------------------------------
## Mu link function:  identity
## Mu Coefficients:
##                                                      Estimate Std. Error
## (Intercept)                                         -0.419560   0.342951
## water_typeseawater                                  -0.085236   0.550895
## temperature_c50                                     -0.658363   0.551229
## temperature_c70                                     -0.009943   0.597957
## temperature_c90                                      0.239046   0.508074
## exposure_time_s120                                  -0.592382   0.611791
## water_typeseawater:temperature_c50                   1.770892   0.824653
## water_typeseawater:temperature_c70                  -0.002100   0.843915
## water_typeseawater:temperature_c90                  -0.658816   0.766084
## water_typeseawater:exposure_time_s120                0.954808   0.828360
## temperature_c50:exposure_time_s120                   1.056345   0.843771
## temperature_c70:exposure_time_s120                   0.714326   0.872074
## temperature_c90:exposure_time_s120                   0.438147   0.805839
## water_typeseawater:temperature_c50:exposure_time_s120 -0.055741   1.200594
## water_typeseawater:temperature_c70:exposure_time_s120 -2.154567   1.194720
## water_typeseawater:temperature_c90:exposure_time_s120 -0.704037   1.109788
##                                                     t value Pr(>|t|)
## (Intercept)                                          -1.223   0.2257
## water_typeseawater                                   -0.155   0.8775
## temperature_c50                                      -1.194   0.2368
## temperature_c70                                      -0.017   0.9868
## temperature_c90                                       0.470   0.6396
## exposure_time_s120                                   -0.968   0.3366
## water_typeseawater:temperature_c50                    2.147   0.0356 *
## water_typeseawater:temperature_c70                   -0.002   0.9980
## water_typeseawater:temperature_c90                   -0.860   0.3931
## water_typeseawater:exposure_time_s120                 1.153   0.2534
## temperature_c50:exposure_time_s120                    1.252   0.2152
## temperature_c70:exposure_time_s120                    0.819   0.4158
## temperature_c90:exposure_time_s120                    0.544   0.5886
## water_typeseawater:temperature_c50:exposure_time_s120  -0.046   0.9631
```

```
## water_typeseawater:temperature_c70:exposure_time_s120  -1.803   0.0761 .
## water_typeseawater:temperature_c90:exposure_time_s120  -0.634   0.5281
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ------------------------------------------------------------------
## Sigma link function:  log
## Sigma Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.66316    0.09298  -7.133 1.17e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ------------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  17
##       Residual Deg. of Freedom:  63
##                       at cycle:  3
##
## Global Deviance:     212.7863
##             AIC:     246.7863
##             SBC:     287.2807
## ******************************************************************
```

Model Selection - final formula for change

```
stepmodweight48 <- stepGAIC(mod_weight, direction = "backward", trace = F)
```

```
## Start:  AIC= 246.79
##  ChangeWetWeight48 ~ water_type + temperature_c + exposure_time_s +
##     water_type * temperature_c * exposure_time_s
```

```
summary(stepmodweight48)
```

```
## ******************************************************************
## Family:  c("LO", "Logistic")
##
## Call:  gamlss(formula = ChangeWetWeight48 ~ water_type + temperature_c +
##     water_type:temperature_c, family = LO, data = tuni_data1,
##     trace = FALSE)
##
## Fitting method: RS()
##
## ------------------------------------------------------------------
## Mu link function:  identity
## Mu Coefficients:
##                                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)                      -0.6259     0.2951  -2.121  0.03741 *
## water_typeseawater                0.3364     0.4134   0.814  0.41849
## temperature_c50                  -0.1969     0.4225  -0.466  0.64260
## temperature_c70                   0.2711     0.4315   0.628  0.53192
## temperature_c90                   0.3656     0.4030   0.907  0.36745
```

```
## water_typeseawater:temperature_c50   1.7036     0.6363   2.677  0.00922 **
## water_typeseawater:temperature_c70  -1.0045     0.6109  -1.644  0.10453
## water_typeseawater:temperature_c90  -0.9499     0.5662  -1.678  0.09778 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ----------------------------------------------------------------------
## Sigma link function:  log
## Sigma Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.60404    0.09281  -6.509 9.23e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ----------------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  9
##       Residual Deg. of Freedom:  71
##                       at cycle:  3
##
## Global Deviance:     221.8059
##             AIC:     239.8059
##             SBC:     261.2441
## **********************************************************************
```

```
#water_typeseawater:temperature_c50 p-value = 0.00922 significant

formula(stepmodweight48)
```

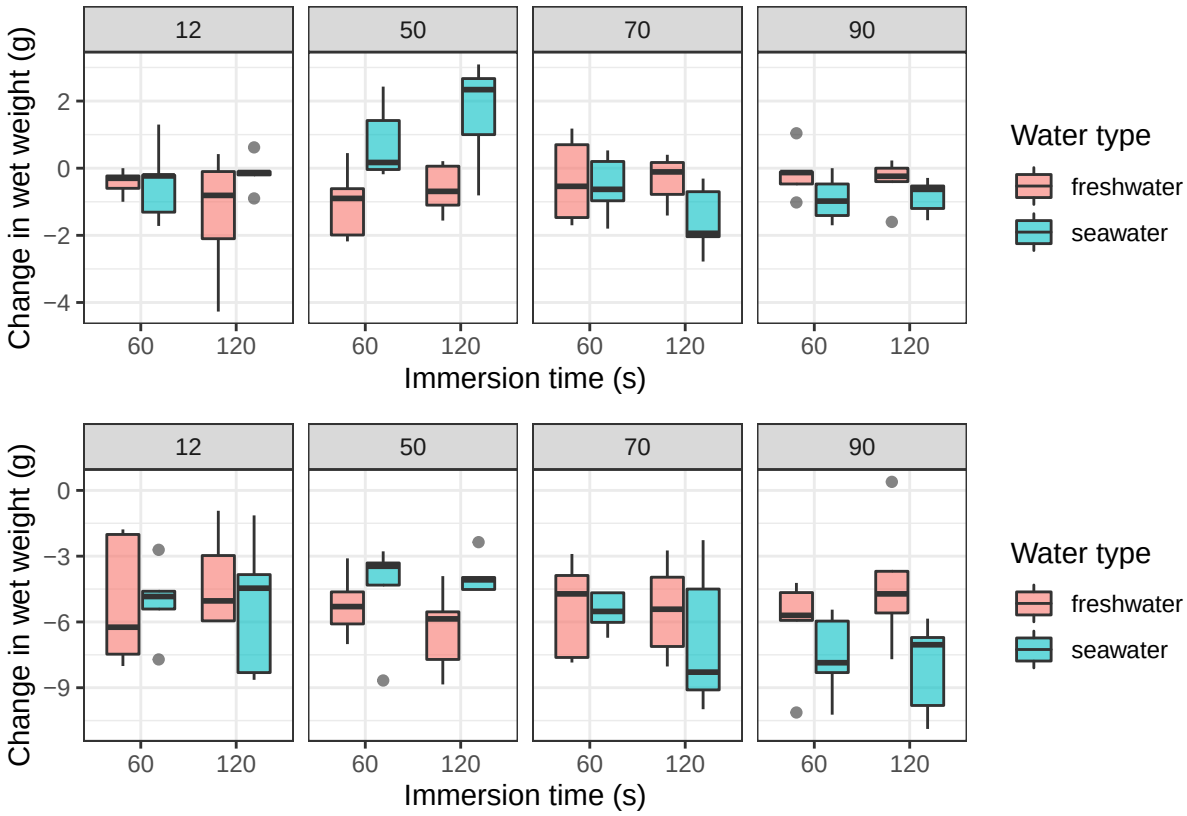
```
## ChangeWetWeight48 ~ water_type + temperature_c + water_type:temperature_c
```

```
#ChangeWetWeight48 ~ water_type + temperature_c + water_type:temperature_c
```

Change in Weight over 3 Weeks (compare to post-acclimation)

Data Visualization

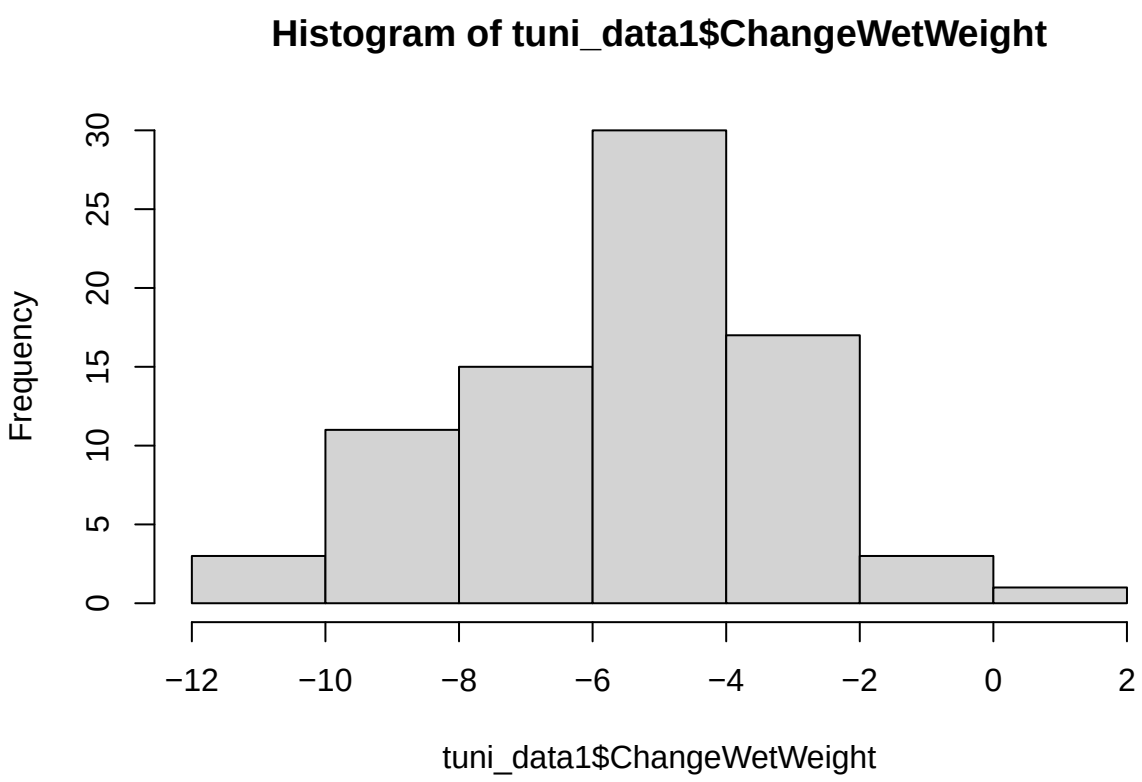
```
p <- ggplot(tuni_data1, aes(x = exposure_time_s, y = ChangeWetWeight, fill = water_type))+
  labs(x = "Immersion time (s)", y = "Change in wet weight (g)", fill = "Water type")+
#labs(title = "Temperature (°C)")+
  geom_boxplot(alpha = 0.6)+
  theme_bw()

p4 <- p + facet_grid(cols = vars(temperature_c))

p3 + p4 + plot_layout(ncol=1)
```




```
#ggsave("change-in-wet-weight.jpg")
```

Testing for Normality

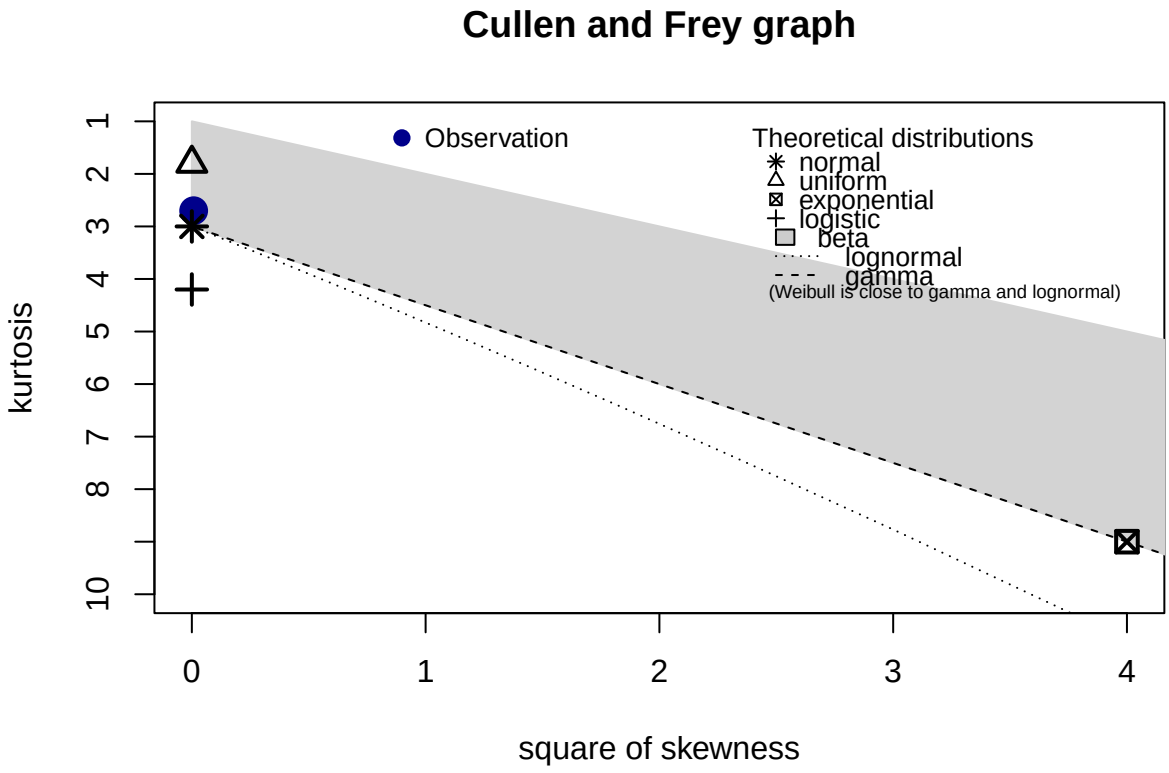
```
hist(tuni_data1$ChangeWetWeight)
```




```
shapiro.test(tuni_data1$ChangeWetWeight)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  tuni_data1$ChangeWetWeight
## W = 0.98952, p-value = 0.7646
```

```
#p-value = 0.7646 thus follows a normal distribution!
descdist(tuni_data1$ChangeWetWeight)
```




```
## summary statistics
## ------
## min:  -10.88   max:  0.39
## median:  -5.43
## mean:  -5.55025
## estimated sd:  2.36077
## estimated skewness:  -0.08817917
## estimated kurtosis:  2.700809
```

Distribution Fitting

```
fitDist(ChangeWetWeight, data = tuni_data1, type = "realline", try.gamlss = T)
```

```
##   |                                                                      |
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0
##   |                                                                      |=================
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0
##   |                                                                      |==================
##   Lapack routine dgesv: system is exactly singular: U[4,4] = 0
##   |                                                                      |==================
##   Lapack routine dgesv: system is exactly singular: U[4,4] = 0
##   |                                                                      |==================
##   Lapack routine dgesv: system is exactly singular: U[4,4] = 0
##   |                                                                      |==================
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0
```

```
##   |                                                                      |==================
##   Lapack routine dgesv: system is exactly singular: U[3,3] = 0

##
## Family:  c("NO", "Normal")
## Fitting method: "nlminb"
##
## Call:  gamlssML(formula = y, family = NO)
##
## Mu Coefficients:
## [1]  -5.55
## Sigma Coefficients:
## [1]  0.8527
##
##  Degrees of Freedom for the fit: 2 Residual Deg. of Freedom   78
## Global Deviance:     363.462
##             AIC:     367.462
##             SBC:     372.226
```

```
#this also gives normal distribution
```

Linear Model

```
mod_changeweight <- lm(ChangeWetWeight ~ exposure_time_s + water_type + temperature_c + exposure_time_s
summary(mod_changeweight)
```

```
##
## Call:
## lm(formula = ChangeWetWeight ~ exposure_time_s + water_type +
##     temperature_c + exposure_time_s * water_type * temperature_c,
##     data = tuni_data1, family = gaussian)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -4.162 -1.688  0.111  1.474  4.652
##
## Coefficients:
##                                                    Estimate Std. Error
## (Intercept)                                          -5.102      1.023
## exposure_time_s120                                    0.930      1.447
## water_typeseawater                                    0.048      1.447
## temperature_c50                                      -0.124      1.447
## temperature_c70                                      -0.292      1.447
## temperature_c90                                      -1.026      1.447
## exposure_time_s120:water_typeseawater                -1.154      2.047
## exposure_time_s120:temperature_c50                   -2.078      2.047
## exposure_time_s120:temperature_c70                   -0.990      2.047
## exposure_time_s120:temperature_c90                    0.936      2.047
## water_typeseawater:temperature_c50                    0.670      2.047
## water_typeseawater:temperature_c70                   -0.168      2.047
## water_typeseawater:temperature_c90                   -1.480      2.047
## exposure_time_s120:water_typeseawater:temperature_c50  2.918      2.895
## exposure_time_s120:water_typeseawater:temperature_c70 -0.100      2.895
```

```
## exposure_time_s120:water_typeseawater:temperature_c90   -1.210      2.895
##                                                       t value Pr(>|t|)
## (Intercept)                                            -4.985 4.99e-06 ***
## exposure_time_s120                                      0.643    0.523
## water_typeseawater                                      0.033    0.974
## temperature_c50                                        -0.086    0.932
## temperature_c70                                        -0.202    0.841
## temperature_c90                                        -0.709    0.481
## exposure_time_s120:water_typeseawater                  -0.564    0.575
## exposure_time_s120:temperature_c50                     -1.015    0.314
## exposure_time_s120:temperature_c70                     -0.484    0.630
## exposure_time_s120:temperature_c90                      0.457    0.649
## water_typeseawater:temperature_c50                      0.327    0.744
## water_typeseawater:temperature_c70                     -0.082    0.935
## water_typeseawater:temperature_c90                     -0.723    0.472
## exposure_time_s120:water_typeseawater:temperature_c50   1.008    0.317
## exposure_time_s120:water_typeseawater:temperature_c70  -0.035    0.973
## exposure_time_s120:water_typeseawater:temperature_c90  -0.418    0.677
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.288 on 64 degrees of freedom
## Multiple R-squared:  0.2388, Adjusted R-squared:  0.06042
## F-statistic: 1.339 on 15 and 64 DF,  p-value: 0.2065
```

Model Selection

```
#Model Selection
step.mod_changeweight <- stepAIC(mod_changeweight, direction = "backward", trace = F)
summary(step.mod_changeweight)
```

```
##
## Call:
## lm(formula = ChangeWetWeight ~ water_type + temperature_c + water_type:temperature_c,
##     data = tuni_data1, family = gaussian)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.9350 -1.6263  0.1905  1.5020  5.5850
##
## Coefficients:
##                                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)                       -4.6370     0.7025  -6.601 5.97e-09 ***
## water_typeseawater                -0.5290     0.9935  -0.532    0.596
## temperature_c50                   -1.1630     0.9935  -1.171    0.246
## temperature_c70                   -0.7870     0.9935  -0.792    0.431
## temperature_c90                   -0.5580     0.9935  -0.562    0.576
## water_typeseawater:temperature_c50   2.1290     1.4050   1.515    0.134
## water_typeseawater:temperature_c70  -0.2180     1.4050  -0.155    0.877
## water_typeseawater:temperature_c90  -2.0850     1.4050  -1.484    0.142
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Residual standard error: 2.221 on 72 degrees of freedom
## Multiple R-squared:  0.193,  Adjusted R-squared:  0.1145
## F-statistic:  2.46 on 7 and 72 DF,  p-value: 0.0255
```

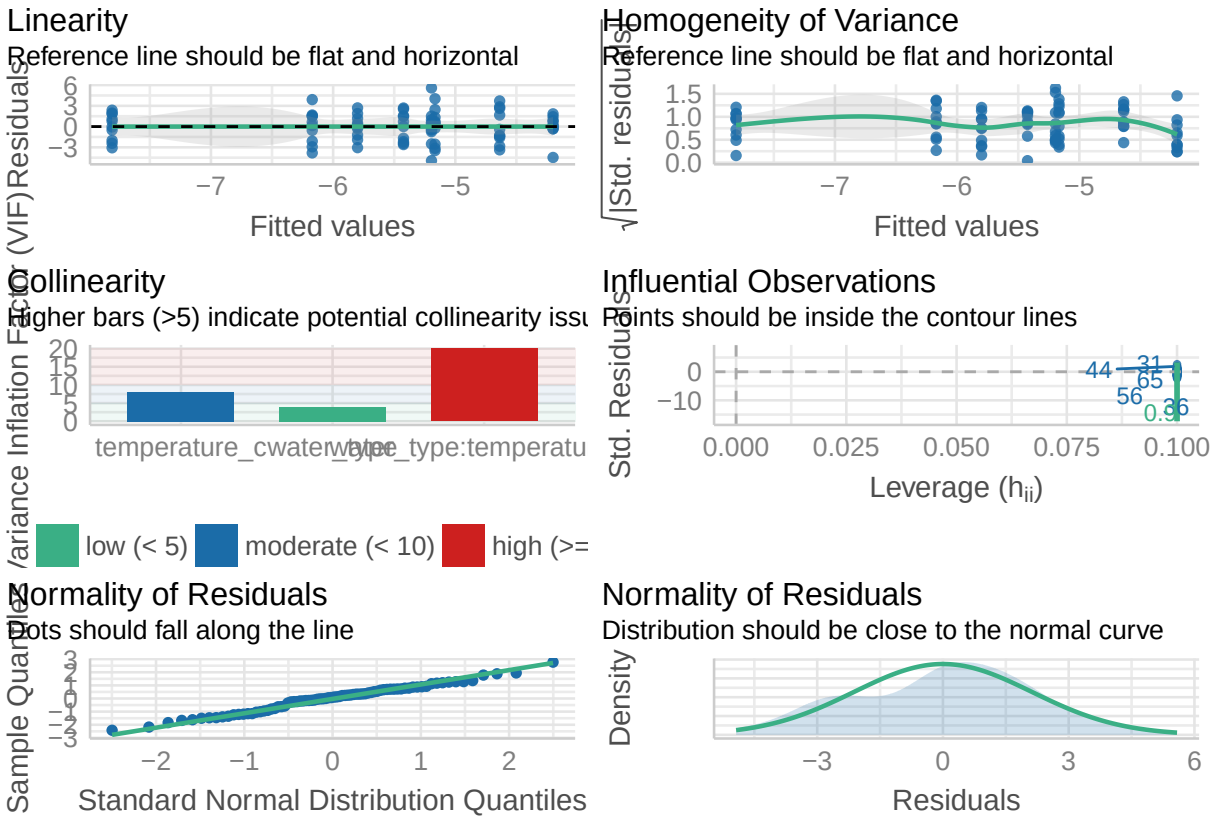
```
formula(step.mod_changeweight)
```

```
## ChangeWetWeight ~ water_type + temperature_c + water_type:temperature_c
```

```
#ChangeWetWeight ~ water_type + temperature_c + water_type:temperature_c
```

Check Model

```
check_model(step.mod_changeweight)
```




```
#check model when water_type:temperature_c was present and had major collinearity issues, thus removed
```

New Model

```
mod_changeweightv2 <- lm(ChangeWetWeight ~ water_type + temperature_c, family = gaussian, data = tuni_da
summary(mod_changeweightv2)
```

```
##
## Call:
```

```
## lm(formula = ChangeWetWeight ~ water_type + temperature_c, data = tuni_data1,
##     family = gaussian)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.1362 -1.6128  0.4318  1.5524  6.6058
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          -4.6152     0.5773  -7.995 1.22e-11 ***
## water_typeseawater   -0.5725     0.5163  -1.109   0.2711
## temperature_c50      -0.0985     0.7302  -0.135   0.8931
## temperature_c70      -0.8960     0.7302  -1.227   0.2237
## temperature_c90      -1.6005     0.7302  -2.192   0.0315 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.309 on 75 degrees of freedom
## Multiple R-squared:  0.09168,    Adjusted R-squared:  0.04324
## F-statistic: 1.893 on 4 and 75 DF,  p-value: 0.1205
```

```
#significant p-value for temperature_c90 (p=value = 0.0315)
```

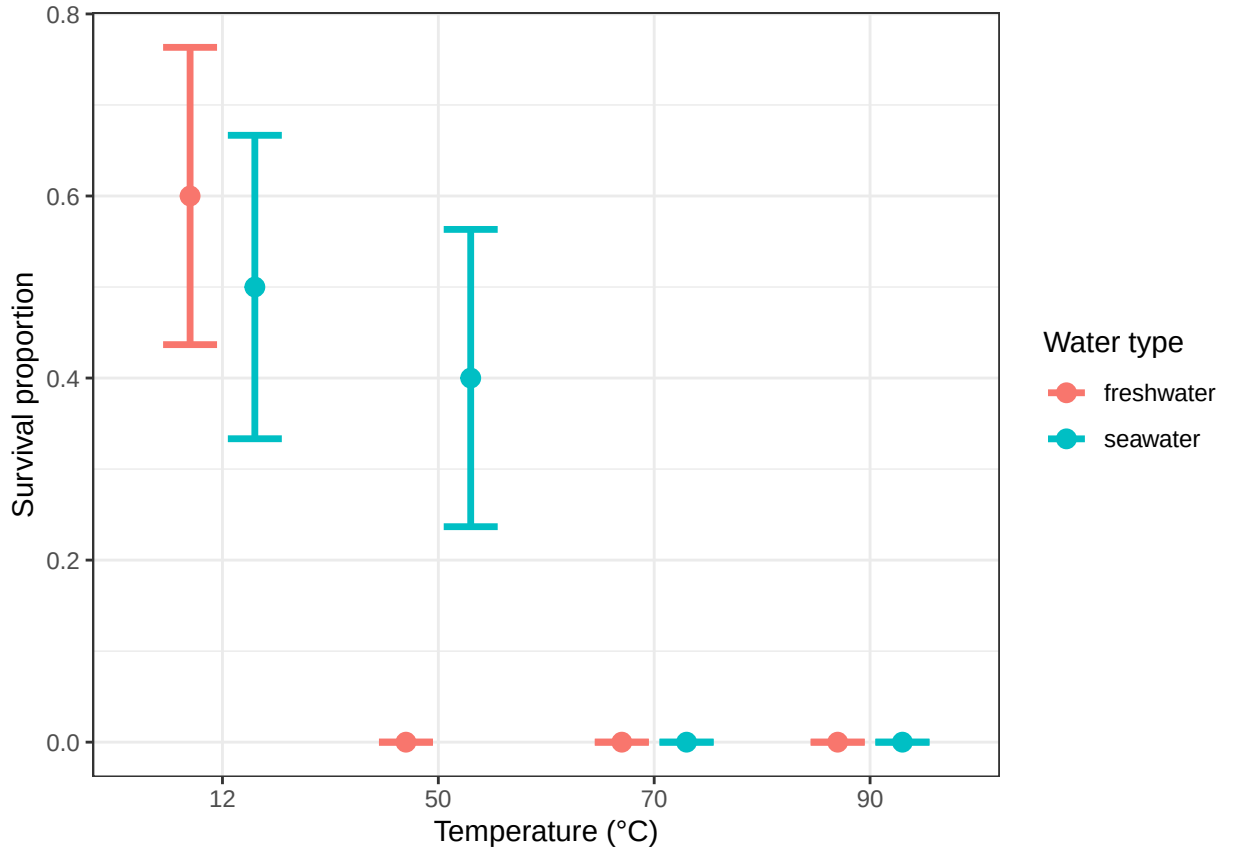
Survival

Visualizing Data

```
#making a column for the propotion of samples that survived per treatment
tunisurv<- tunidata%>%
  group_by(temperature_c, water_type)%>%
  summarize(proportion_survival=sum(survival)/10, sd=sd(survival), total= n(), SE = sd(survival)/sqrt(t
```

```
## `summarise()` has grouped output by 'temperature_c'. You can override using the `.groups` argument.
```

```
# divided by 10 because each combination of temperature and water type has 10 data points

#plotting
pd <- position_dodge(width = 0.6)
ggplot(tunisurv, aes(x=temperature_c, y=proportion_survival, colour=water_type))+
  geom_point(aes(colour=water_type), position = pd, size=3)+
  xlab("Temperature (°C)")+
  ylab("Survival proportion")+
  geom_errorbar(aes(ymin=proportion_survival-SE, ymax=proportion_survival+SE, width=0.5), size=1.2, posi
  theme_bw()+labs(colour="Water type")
```




```
#ggsave("survivalproportion.jpg")
```

Testing for Normality

```
shapiro.test(tunidata$survival)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  tunidata$survival
## W = 0.47548, p-value = 2.047e-15
```

```
#p-value = 2.047e-15
```

Checking Distribution

```
fitDist(survival, data = tunidata, type = "binom", try.gamlss = T)
```

```
##   |                                                                      |
##   system is computationally singular: reciprocal condition number = 5.31094e-21
##   |                                                                      |==================
```

```
##
```

```
## Family:  c("BI", "Binomial")
## Fitting method: "nlminb"
##
## Call:  gamlssML(formula = y, family = BI)
##
## Mu Coefficients:
## [1]  -1.466
##
##  Degrees of Freedom for the fit: 1 Residual Deg. of Freedom   79
## Global Deviance:     77.2124
##             AIC:     79.2124
##             SBC:     81.5944
```

```
#family = BI (binomial)
```

GAMLSS model

```
mod_survival <- gamlss(survival ~ water_type + temperature_c + exposure_time_s + water_type*temperature_
```

```
## GAMLSS-RS iteration 1: Global Deviance = 30.6187
## GAMLSS-RS iteration 2: Global Deviance = 30.6185
```

```
summary(mod_survival)
```

```
## ******************************************************************
## Family:  c("BI", "Binomial")
##
## Call:  gamlss(formula = survival ~ water_type + temperature_c +
##     exposure_time_s + water_type * temperature_c *
##     exposure_time_s + random(as.factor(colony_id)),
##     family = BI, data = tunidata)
##
## Fitting method: RS()
##
## ------------------------------------------------------------------
## Mu link function:  logit
## Mu Coefficients:
##                                                    Estimate Std. Error
## (Intercept)                                         -0.4979     0.9767
## water_typeseawater                                   0.9480     1.3928
## temperature_c50                                    -13.2564   378.1169
## temperature_c70                                    -13.2564   378.1889
## temperature_c90                                    -13.2564   378.1889
## exposure_time_s120                                   2.0913     1.5404
## water_typeseawater:temperature_c50                  11.2229   378.1200
## water_typeseawater:temperature_c70                  -0.9480   534.8400
## water_typeseawater:temperature_c90                  -0.9480   534.8400
## water_typeseawater:exposure_time_s120               -3.0393     2.0766
## temperature_c50:exposure_time_s120                  -2.0913   534.7300
## temperature_c70:exposure_time_s120                  -2.0913   534.8404
## temperature_c90:exposure_time_s120                  -2.0913   534.8404
## water_typeseawater:temperature_c50:exposure_time_s120   5.0728   534.7340
```

```
## water_typeseawater:temperature_c70:exposure_time_s120   3.0393   756.3722
## water_typeseawater:temperature_c90:exposure_time_s120   3.0393   756.3722
##                                                       t value Pr(>|t|)
## (Intercept)                                            -0.510    0.612
## water_typeseawater                                      0.681    0.499
## temperature_c50                                        -0.035    0.972
## temperature_c70                                        -0.035    0.972
## temperature_c90                                        -0.035    0.972
## exposure_time_s120                                      1.358    0.180
## water_typeseawater:temperature_c50                      0.030    0.976
## water_typeseawater:temperature_c70                     -0.002    0.999
## water_typeseawater:temperature_c90                     -0.002    0.999
## water_typeseawater:exposure_time_s120                  -1.464    0.149
## temperature_c50:exposure_time_s120                     -0.004    0.997
## temperature_c70:exposure_time_s120                     -0.004    0.997
## temperature_c90:exposure_time_s120                     -0.004    0.997
## water_typeseawater:temperature_c50:exposure_time_s120   0.009    0.992
## water_typeseawater:temperature_c70:exposure_time_s120   0.004    0.997
## water_typeseawater:temperature_c90:exposure_time_s120   0.004    0.997
##
## -----------------------------------------------------------------
## NOTE: Additive smoothing terms exist in the formulas:
##  i) Std. Error for smoothers are for the linear effect only.
## ii) Std. Error for the linear terms maybe are not accurate.
## -----------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  19.80211
##       Residual Deg. of Freedom:  60.19789
##                       at cycle:  2
##
## Global Deviance:     30.61848
##             AIC:     70.2227
##             SBC:     117.3918
## *****************************************************************
```

Model Selection

```
step.modsurvival <- stepGAIC(mod_survival, direction = "backward", trace = F)
```

```
## Start:  AIC= 70.22
##  survival ~ water_type + temperature_c + exposure_time_s + water_type *
##     temperature_c * exposure_time_s + random(as.factor(colony_id))
```

```
summary(step.modsurvival)
```

```
## *****************************************************************
## Family:  c("BI", "Binomial")
##
## Call:  gamlss(formula = survival ~ temperature_c, family = BI,
##     data = tunidata, trace = FALSE)
##
## Fitting method: RS()
```

```
##
## ------------------------------------------------------------------
## Mu link function:  logit
## Mu Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.2007     0.4495   0.446   0.6565
## temperature_c50  -1.5870     0.7173  -2.212   0.0299 *
## temperature_c70 -13.7667   197.3864  -0.070   0.9446
## temperature_c90 -13.7667   197.3864  -0.070   0.9446
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ------------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  4
##       Residual Deg. of Freedom:  76
##                       at cycle:  2
##
## Global Deviance:     47.54175
##             AIC:     55.54175
##             SBC:     65.06986
## ******************************************************************
```

```
formula(step.modsurvival)
```

```
## survival ~ temperature_c
```

```
#survival ~ temperature_c
```

Kruskal-Wallis Test

```
kruskal.test(survival ~ temperature_c, data = tunidata)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  survival by temperature_c
## Kruskal-Wallis chi-squared = 26.171, df = 3, p-value = 8.781e-06
```

```
#Kruskal-Wallis chi-squared = 26.171, df = 3, p-value = 8.781e-06
```

since temperature is the only explanatory variable - thus we can use Kruskal-Wallis Test to see p-values comparing temperatures to controls

```
dunnTest(survival ~ temperature_c, data = tunidata)
```

```
## Dunn (1964) Kruskal-Wallis multiple comparison
```

```
##   p-values adjusted with the Holm method.
```

```
##   Comparison        Z      P.unadj        P.adj
## 1    12 - 50 2.817892 4.834013e-03 1.933605e-02
## 2    12 - 70 4.428115 9.506008e-06 5.703605e-05
## 3    50 - 70 1.610224 1.073490e-01 3.220471e-01
## 4    12 - 90 4.428115 9.506008e-06 4.753004e-05
## 5    50 - 90 1.610224 1.073490e-01 2.146980e-01
## 6    70 - 90 0.000000 1.000000e+00 1.000000e+00
```

```
#Comparison        Z      P.unadj        P.adj
#1    12 - 50 2.817892 4.834013e-03 1.933605e-02*
#2    12 - 70 4.428115 9.506008e-06 5.703605e-05*
#3    50 - 70 1.610224 1.073490e-01 3.220471e-01
#4    12 - 90 4.428115 9.506008e-06 4.753004e-05*
#5    50 - 90 1.610224 1.073490e-01 2.146980e-01
#6    70 - 90 0.000000 1.000000e+00 1.000000e+00

#p-value for 12-90 and 12-70 is significant!
```

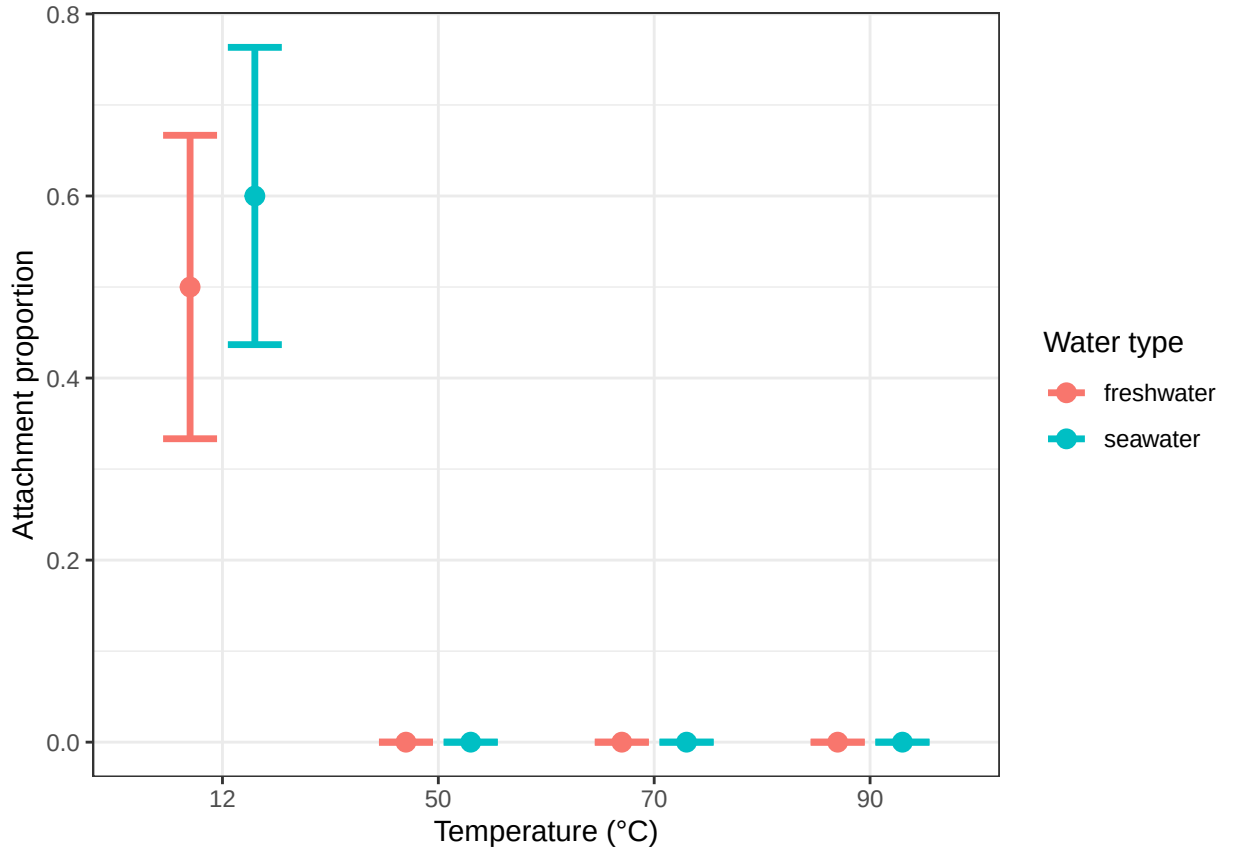
Attachment

Data Visualization

```
#making a column for proportion of samples attached to the container
tuniattach<- tunidata%>%
  group_by(temperature_c, water_type)%>%
  summarize(proportion_attached=sum(attachment)/10, sd=sd(attachment), total= n(), SE = sd(attachment)/s
```

```
## `summarise()` has grouped output by 'temperature_c'. You can override using the `.groups` argument.
```

```
# divided by 10 because each temperature and water type combination has 10 data points

#plotting
ggplot(tuniattach, aes(x=temperature_c, y=proportion_attached, colour=water_type))+
  geom_point(aes(colour=water_type), position=pd, size=3)+
  xlab("Temperature (°C)")+
  ylab("Attachment proportion")+
  geom_errorbar(aes(ymin=proportion_attached-SE, ymax=proportion_attached+SE, width=.5), size=1.2, posi
  theme_bw()+labs(colour="Water type")
```




```
#ggsave("attachmentproportion.jpg")
```

Distribution Fitting

```
fitDist(attachment, data = tuni_data1, type = "binom", try.gamlss = T)
```

```
##   |                                                                      |
##   Lapack routine dgesv: system is exactly singular: U[1,1] = 0
##   |                                                                      |==================
```

```
##
## Family:  c("BI", "Binomial")
## Fitting method: "nlminb"
##
## Call:  gamlssML(formula = y, family = BI)
##
## Mu Coefficients:
## [1]  -1.836
##
##  Degrees of Freedom for the fit: 1 Residual Deg. of Freedom   79
## Global Deviance:     64.0639
##             AIC:     66.0639
##             SBC:     68.4459
```

```
#family = BI (binomial)
```

GAMLSS Model

```
mod <- gamlss(attachment ~ water_type + temperature_c + exposure_time_s + water_type*temperature_c*expos
```

```
## GAMLSS-RS iteration 1: Global Deviance = 26.9209
## GAMLSS-RS iteration 2: Global Deviance = 26.9206
```

```
summary(mod)
```

```
## ******************************************************************
## Family:  c("BI", "Binomial")
##
## Call:  gamlss(formula = attachment ~ water_type + temperature_c +
##     exposure_time_s + water_type * temperature_c *
##     exposure_time_s, family = BI, data = tuni_data1)
##
## Fitting method: RS()
##
## ------------------------------------------------------------------
## Mu link function:  logit
## Mu Coefficients:
##                                                      Estimate Std. Error
## (Intercept)                                           -0.4055     0.9129
## water_typeseawater                                     0.8109     1.2910
## temperature_c50                                      -13.1606   394.7864
## temperature_c70                                      -13.1606   394.7864
## temperature_c90                                      -13.1606   394.7864
## exposure_time_s120                                     0.8109     1.2910
## water_typeseawater:temperature_c50                    -0.8109   558.3026
## water_typeseawater:temperature_c70                    -0.8109   558.3026
## water_typeseawater:temperature_c90                    -0.8109   558.3026
## water_typeseawater:exposure_time_s120                 -0.8109     1.8257
## temperature_c50:exposure_time_s120                    -0.8109   558.3026
## temperature_c70:exposure_time_s120                    -0.8109   558.3026
## temperature_c90:exposure_time_s120                    -0.8109   558.3026
## water_typeseawater:temperature_c50:exposure_time_s120  0.8109   789.5523
## water_typeseawater:temperature_c70:exposure_time_s120  0.8109   789.5523
## water_typeseawater:temperature_c90:exposure_time_s120  0.8109   789.5523
##                                                      t value Pr(>|t|)
## (Intercept)                                           -0.444    0.658
## water_typeseawater                                     0.628    0.532
## temperature_c50                                       -0.033    0.974
## temperature_c70                                       -0.033    0.974
## temperature_c90                                       -0.033    0.974
## exposure_time_s120                                     0.628    0.532
## water_typeseawater:temperature_c50                    -0.001    0.999
## water_typeseawater:temperature_c70                    -0.001    0.999
## water_typeseawater:temperature_c90                    -0.001    0.999
## water_typeseawater:exposure_time_s120                 -0.444    0.658
## temperature_c50:exposure_time_s120                    -0.001    0.999
```

```
## temperature_c70:exposure_time_s120                    -0.001    0.999
## temperature_c90:exposure_time_s120                    -0.001    0.999
## water_typeseawater:temperature_c50:exposure_time_s120  0.001    0.999
## water_typeseawater:temperature_c70:exposure_time_s120  0.001    0.999
## water_typeseawater:temperature_c90:exposure_time_s120  0.001    0.999
##
## ------------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  16
##       Residual Deg. of Freedom:  64
##                       at cycle:  2
##
## Global Deviance:     26.92062
##              AIC:     58.92062
##              SBC:     97.03305
## ******************************************************************
```

Model Selection

```
step.mod <- stepAIC(mod, direction = "backward", trace = F)
```

```
## GAMLSS-RS iteration 1: Global Deviance = 26.9209
## GAMLSS-RS iteration 2: Global Deviance = 26.9206
## GAMLSS-RS iteration 1: Global Deviance = 26.9209
## GAMLSS-RS iteration 2: Global Deviance = 26.9206
## GAMLSS-RS iteration 1: Global Deviance = 26.9209
## GAMLSS-RS iteration 2: Global Deviance = 26.9206
## GAMLSS-RS iteration 1: Global Deviance = 27.1191
## GAMLSS-RS iteration 2: Global Deviance = 27.1189
## GAMLSS-RS iteration 1: Global Deviance = 27.3236
## GAMLSS-RS iteration 2: Global Deviance = 27.3233
## GAMLSS-RS iteration 1: Global Deviance = 27.526
## GAMLSS-RS iteration 2: Global Deviance = 27.5257
```

```
summary(step.mod)
```

```
## ******************************************************************
## Family:  c("BI", "Binomial")
##
## Call:  gamlss(formula = attachment ~ temperature_c, family = BI,
##     data = tuni_data1)
##
## Fitting method: RS()
##
## ------------------------------------------------------------------
## Mu link function:  logit
## Mu Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.2007     0.4495   0.446    0.657
## temperature_c50 -13.7667   197.3881  -0.070    0.945
## temperature_c70 -13.7667   197.3881  -0.070    0.945
## temperature_c90 -13.7667   197.3881  -0.070    0.945
```

```
##
## ----------------------------------------------------------------
## No. of observations in the fit:  80
## Degrees of Freedom for the fit:  4
##       Residual Deg. of Freedom:  76
##                       at cycle:  2
##
## Global Deviance:     27.52571
##             AIC:     35.52571
##             SBC:     45.05381
## ****************************************************************
```

```
formula(step.mod)
```

```
## attachment ~ temperature_c
```

```
#attachment ~ temperature_c
```

Kruskie

```
kruskal.test(attachment ~ temperature_c, data = tunidata)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  attachment by temperature_c
## Kruskal-Wallis chi-squared = 37.783, df = 3, p-value = 3.142e-08
```

```
#Kruskal-Wallis chi-squared = 37.783, df = 3, p-value = 3.142e-08
```

since temperature is the only explanatory variable - thus we can use Kruskal-Wallis Test to see p-values comparing temperatures to controls

```
dunnTest(attachment ~ temperature_c, data = tuni_data1)
```

```
## Dunn (1964) Kruskal-Wallis multiple comparison
```

```
##   p-values adjusted with the Holm method.
```

```
##   Comparison        Z      P.unadj        P.adj
## 1    12 - 50 5.018805 5.199384e-07 3.119630e-06
## 2    12 - 70 5.018805 5.199384e-07 2.599692e-06
## 3    50 - 70 0.000000 1.000000e+00 1.000000e+00
## 4    12 - 90 5.018805 5.199384e-07 2.079753e-06
## 5    50 - 90 0.000000 1.000000e+00 1.000000e+00
## 6    70 - 90 0.000000 1.000000e+00 1.000000e+00
```

```
#Comparison        Z      P.unadj        P.adj
#1    12 - 50 5.018805 5.199384e-07 3.119630e-06*
#2    12 - 70 5.018805 5.199384e-07 2.599692e-06*
#3    50 - 70 0.000000 1.000000e+00 1.000000e+00
#4    12 - 90 5.018805 5.199384e-07 2.079753e-06*
#5    50 - 90 0.000000 1.000000e+00 1.000000e+00
#6    70 - 90 0.000000 1.000000e+00 1.000000e+00

#p-value for 12-90, 12-70 and 12-50 are significant
```