

Temperature and Freshwater as Methods for Controlling *D. Vexillum* Biofouling

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Contents

Abstract	1
Libraries	1
Analyses and Graphs	2
Reading in Data	2
Change in mean RGB values	3
Mold Cover	6
Change in weight after 48 hours	12
Change in Weight over 3 Weeks (compare to post-acclimation)	17
Survival	22
Attachment	27

Abstract

Biofouling, the unwanted establishment of organisms on surfaces, impacts aquaculture facilities by decreasing the value of their products and causing expensive damages to their equipment. The biofouling tunicate *Didemnum vexillum* poses a notable threat to aquaculture given that it is an invasive species with strong competition abilities and is rapidly expanding its range. In this study, we seek to determine the impact of combining high-temperatures and freshwater treatments at different immersion times on *D. vexillum* as a method for controlling biofouling. We immersed *D. vexillum* in either freshwater or seawater at one of four different temperatures (12, 50, 70, and 90°C), for 60 or 120 seconds. We then analyzed the survival of the tunicate 3 weeks after treatment. We found that both 70°C and 90°C treatments successfully killed *D. vexillum* regardless of water type and immersion time. Therefore, to maximize the effectiveness of biofouling removal efforts while limiting the amount of time and energy used, we recommend aquaculture facilities should use 60 seconds 70°C seawater dips to control *D. vexillum* on their gear. Using this method to remove *D. vexillum* biofouling will help to decrease aquaculture gear damage, and reduce the spread of an invasive species.

Libraries

```
library(tidyverse)
library(cowplot)
library(patchwork)
library(ggplot2)
library(here)
library(tidyr)
library(performance)
library(DHARMA)
library(fitdistrplus)
library(gamlss)
library(FSA)
library(goft)
library(MASS)
library(ordinal)
library(here)
```

Analyses and Graphs

Reading in Data

```
here::here()

## [1] "/Users/laurengill/Github/tunicate-heat-immersion"

tunidata <- read_csv(here("Data", "tunicate_treatment_rawdata.csv"))

## Registered S3 method overwritten by 'bit':
##   method      from
##   print.ri    gamlss

## New names:
## * id_number -> id_number...2

## Rows: 80 Columns: 19

## -- Column specification -----
## Delimiter: ","
## chr  (4): id_number...2, collection_date, water_type, id_number...16
## dbl  (15): photo_number, acclimation_time_h, temperature_c, exposure_time_s, ...

##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.
```

```
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*temperature_c +
             (1|colony_id), family = gaussian,
             data = tunirgb)
summary(modrgb)
```

```
##
## Call:
## lm(formula = X48hr_rgb ~ exposure_time_s + water_type + temperature_c +
##      exposure_time_s * water_type * temperature_c + (1 | colony_id),
##      data = tunirgb, family = gaussian)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -25.420  -9.929   1.285   8.214  35.806
##
## Coefficients: (1 not defined because of singularities)
##
##              Estimate Std. Error
## (Intercept)    151.174      6.417
## exposure_time_s120    15.362      9.075
## water_type seawater    -1.261      9.075
## temperature_c50      4.087      9.075
## temperature_c70     24.380      9.075
## temperature_c90     14.826      9.075
## 1 | colony_idTRUE           NA           NA
## exposure_time_s120:water_type seawater -16.301     12.835
## exposure_time_s120:temperature_c50    -7.030     12.835
## exposure_time_s120:temperature_c70   -21.306     12.835
```

```
## exposure_time_s120:temperature_c90          -16.871      12.835
## water_type seawater:temperature_c50           2.410      12.835
## water_type seawater:temperature_c70          -5.396      12.835
## water_type seawater:temperature_c90          -4.666      12.835
## exposure_time_s120:water_type seawater:temperature_c50  3.402      18.151
## exposure_time_s120:water_type seawater:temperature_c70 10.765      18.151
## exposure_time_s120:water_type seawater:temperature_c90 26.347      18.151
##                                     t value Pr(>|t|)
## (Intercept)                               23.557 < 2e-16 ***
## exposure_time_s120                           1.693  0.09537 .
## water_type seawater                          -0.139  0.88993
## temperature_c50                             0.450  0.65402
## temperature_c70                             2.686  0.00919 **
## temperature_c90                             1.634  0.10723
## 1 | colony_id TRUE                           NA      NA
## exposure_time_s120:water_type seawater       -1.270  0.20864
## exposure_time_s120:temperature_c50          -0.548  0.58580
## exposure_time_s120:temperature_c70          -1.660  0.10180
## exposure_time_s120:temperature_c90          -1.314  0.19337
## water_type seawater:temperature_c50          0.188  0.85164
## water_type seawater:temperature_c70          -0.420  0.67556
## water_type seawater:temperature_c90          -0.364  0.71739
## exposure_time_s120:water_type seawater:temperature_c50  0.187  0.85191
## exposure_time_s120:water_type seawater:temperature_c70  0.593  0.55522
## exposure_time_s120:water_type seawater:temperature_c90  1.452  0.15150
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 14.35 on 64 degrees of freedom
## Multiple R-squared:  0.2743, Adjusted R-squared:  0.1042
## F-statistic: 1.613 on 15 and 64 DF, p-value: 0.09516
```

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*temperature_c, family = gaussian,
             data = tunirgb)
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

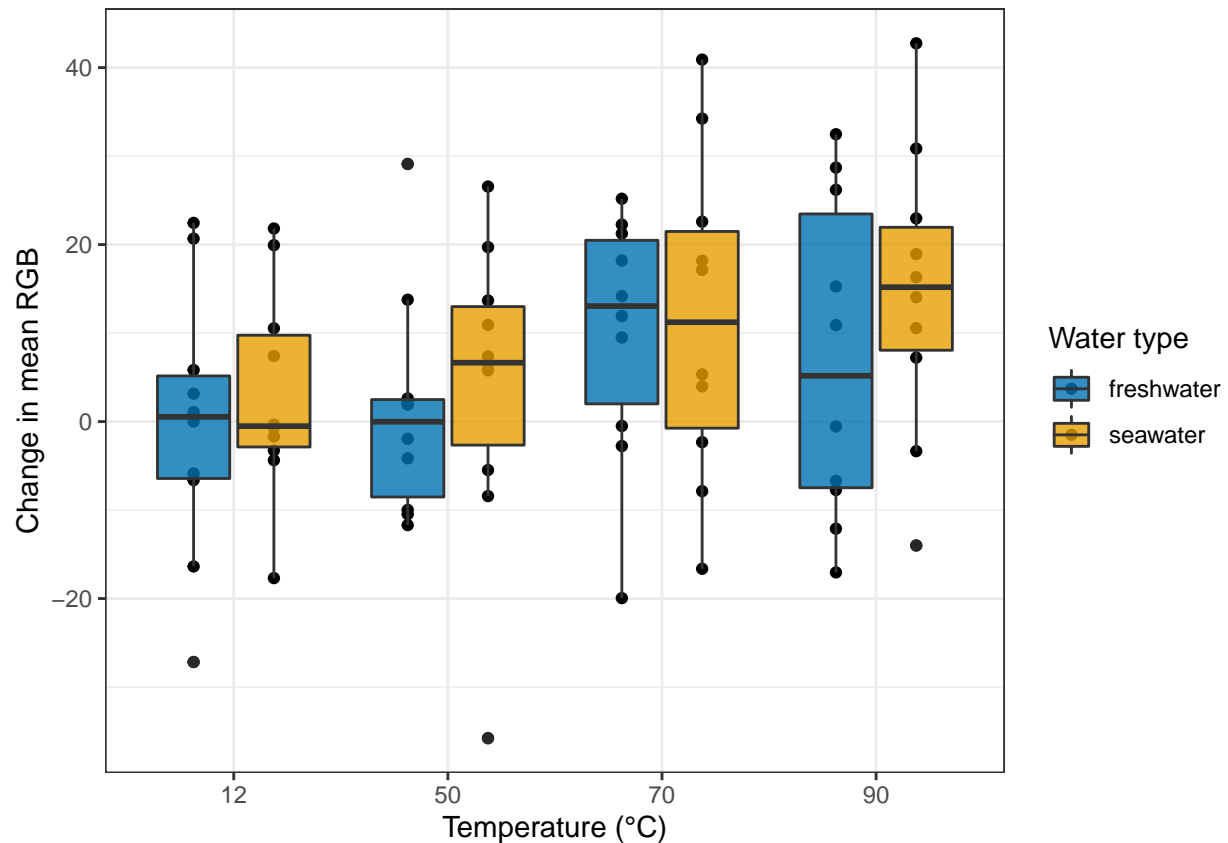
```
newmodrgb <- lm(X48hr_rgb ~ water_type + temperature_c, data = tunirgb)
summary(newmodrgb)
```

```
##
## 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_type seawater    -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)")+
  scale_fill_manual(values = c("#0072B2", "#E69F00"))+
  geom_jitter(position = position_dodge(width=0.75))+
  geom_boxplot(alpha = 0.8)+
  theme_bw()
deltachangergb
```



```
#ggsave("delta-change-rbg.jpg")
```

Mold Cover

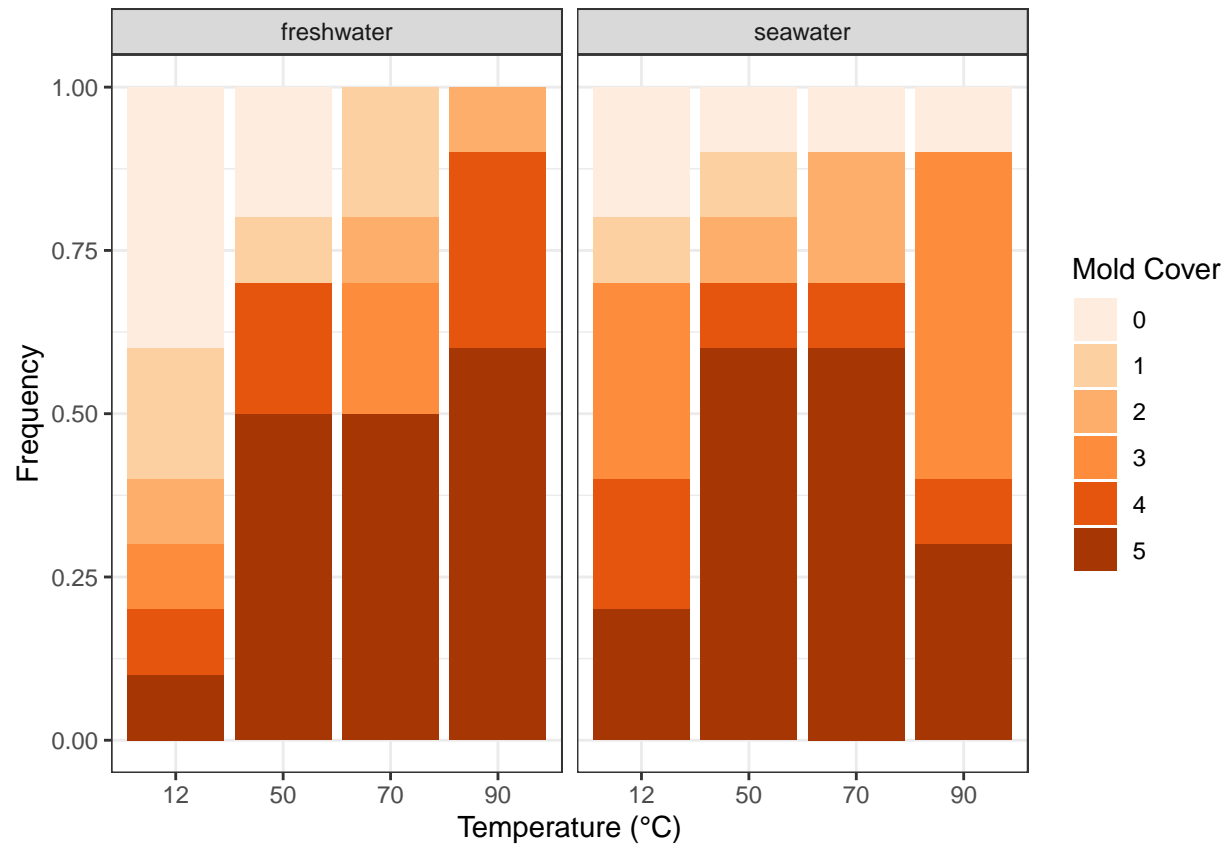
Displaying the data as a proportion of overall score with given mold cover scores

```
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 using .groups = 'drop'.

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()
```

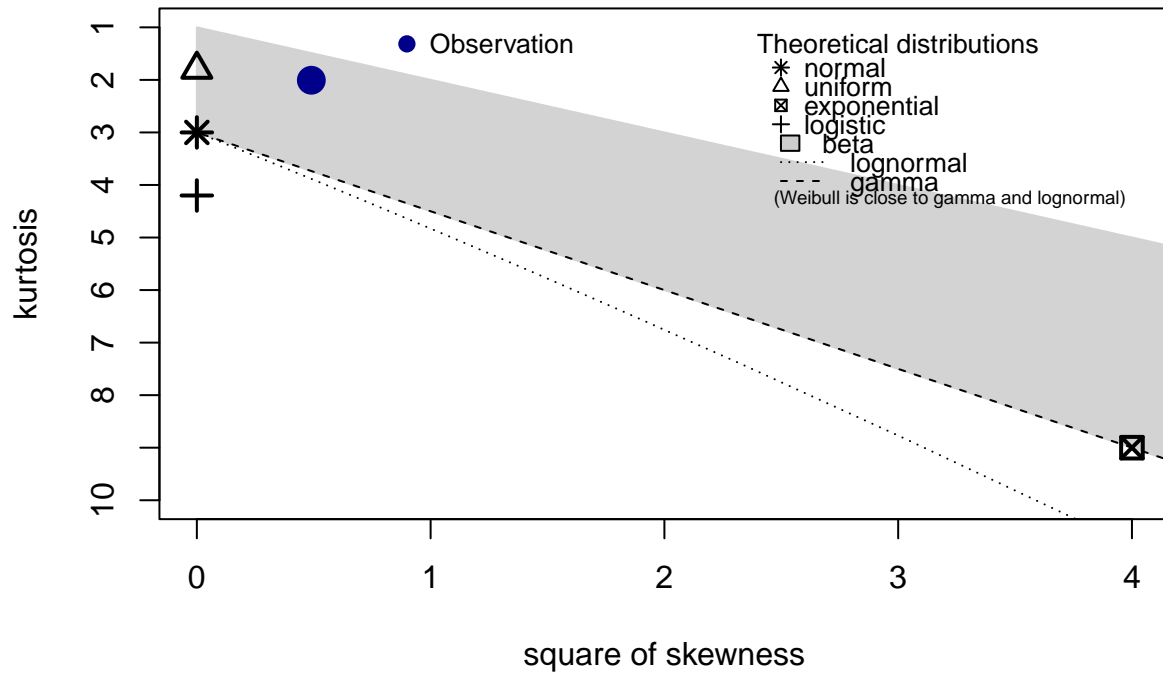


```
#ggsave("mold-cover.jpg")
```

Examining mold cover data to find the best distribution

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

Cullen and Frey graph



```
## 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
```

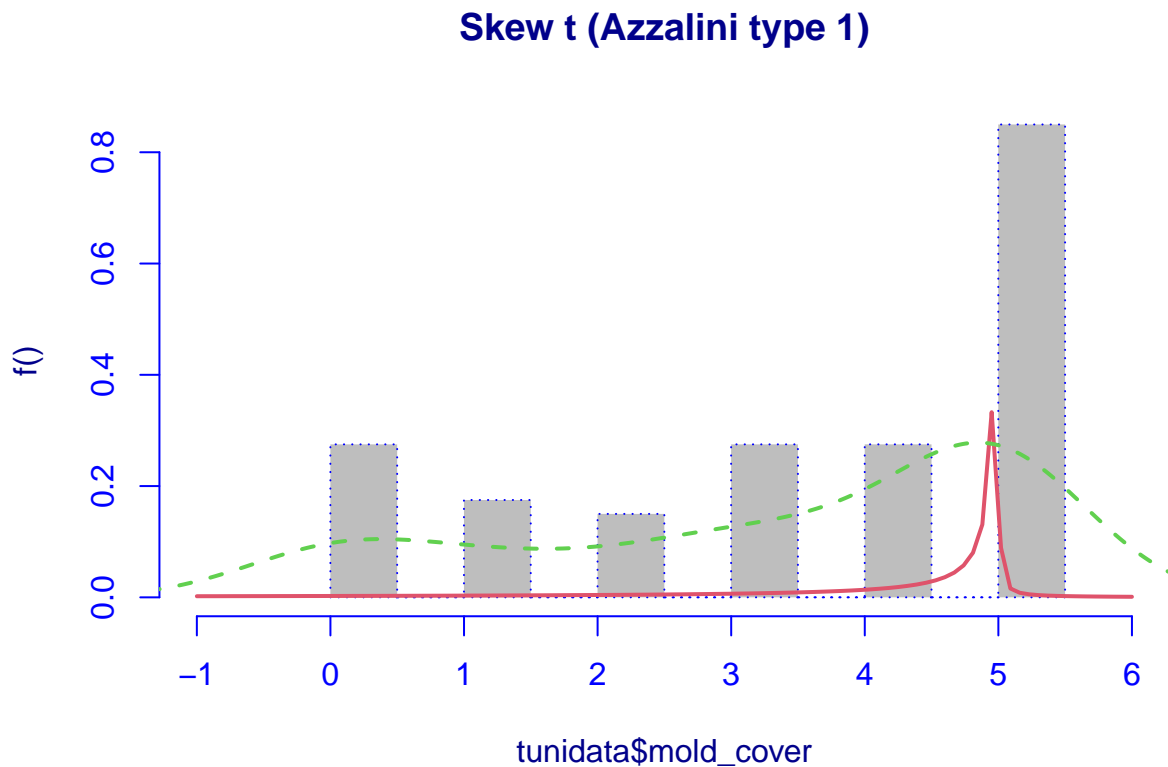
```
##          ST1          SHASHo2          SHASHo          JSUo          JSU
## -7.438242e+02 -7.212029e+02 -5.941774e+02 -5.670796e+02 3.353055e+00
##          ST5          ST3          SN2          SST          ST2
```



```
## 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
```

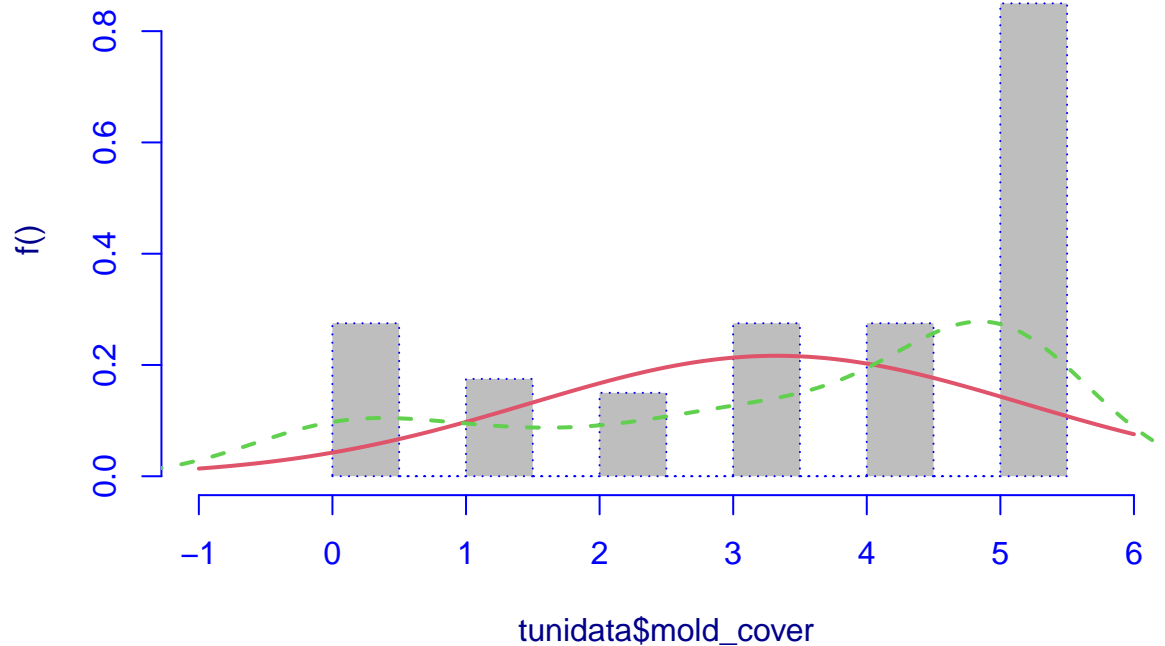
Fitdist determined ST1 to be the best distribution, comparing it against a normal distribution to make sure AIC value is lower. AIC value of ST1 = -743.8 while AIC value of normal = 328

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



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

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
```

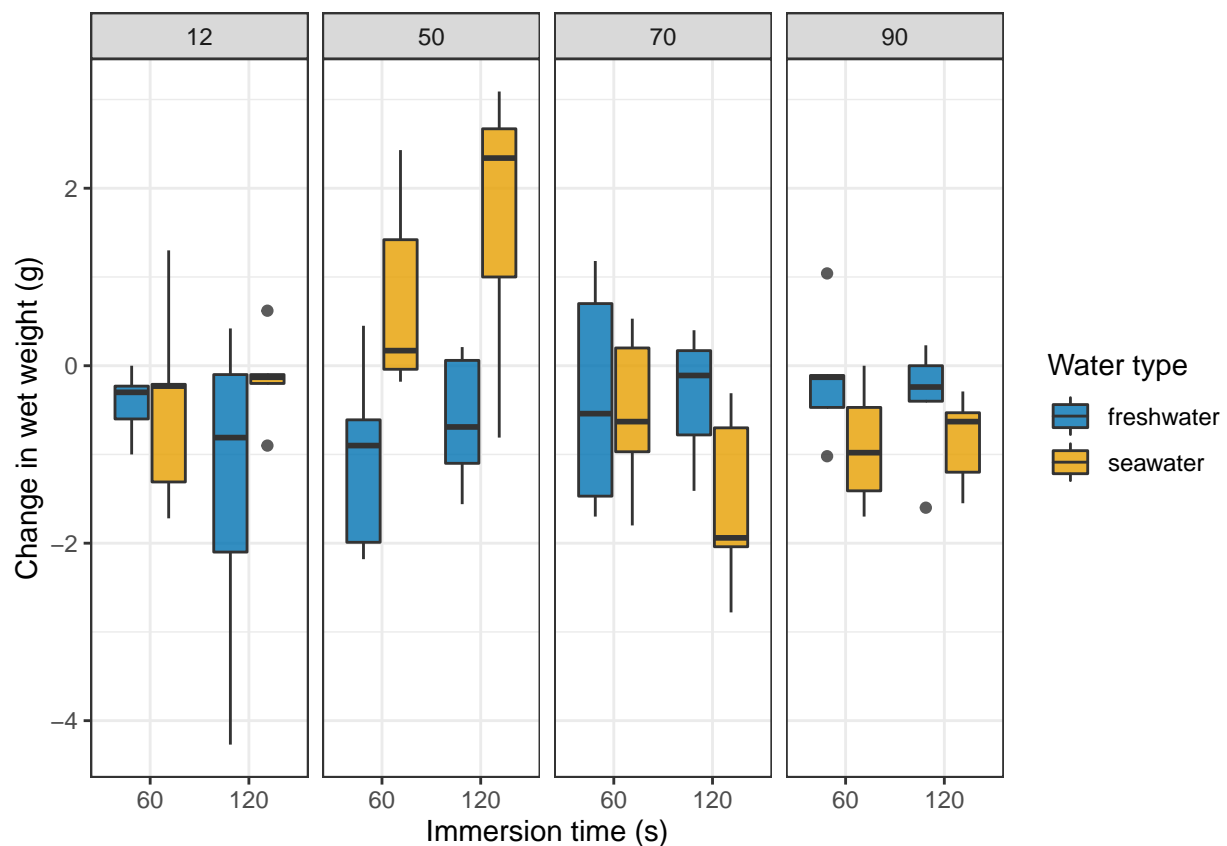
Change in weight after 48 hours

Data Visualization

```
#Making new columns for changes in weight
tuni_data1 <- tunidata %>%
  mutate(change_wet_weight_3wk = final_weight_g-post_acclimation_weight_g)
tuni_data1 <- tuni_data1 %>%
  mutate(change_wet_weight_48 = `X48hr_weight`-post_acclimation_weight_g)

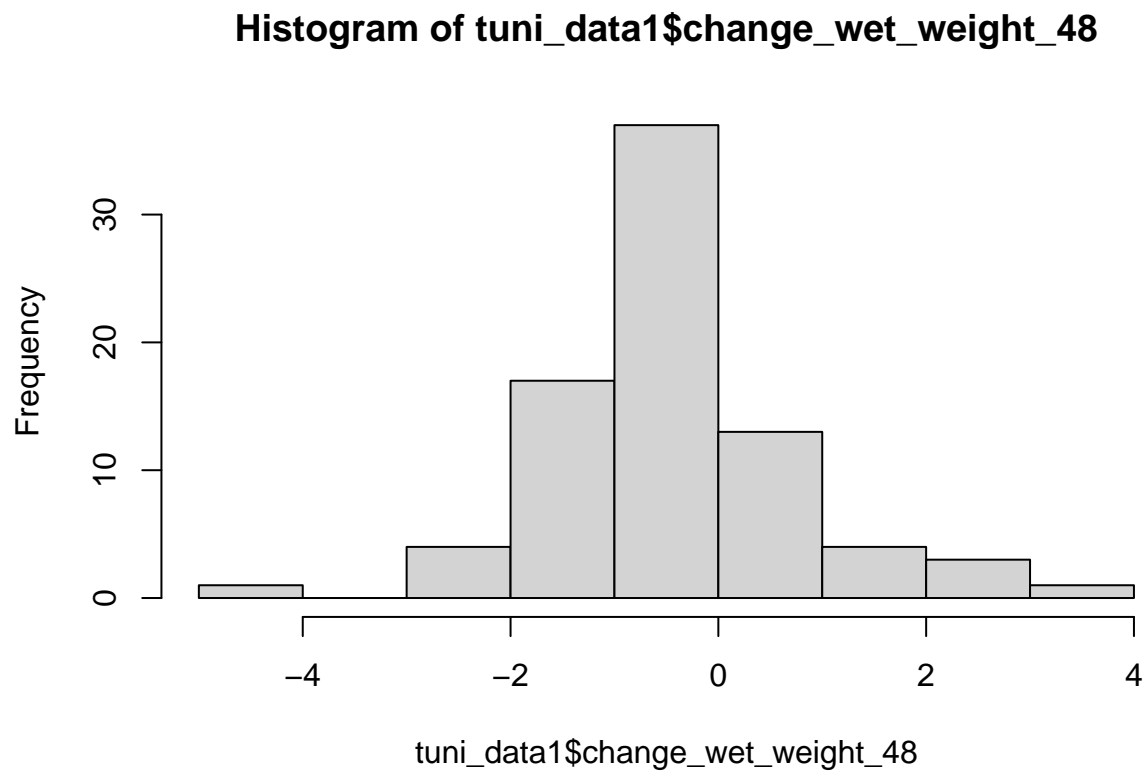
p2 <- ggplot(tuni_data1, aes(x = exposure_time_s, y = change_wet_weight_48,
                             fill = water_type, ))+
  labs(x = "Immersion time (s)", y = "Change in wet weight (g)",
       fill = "Water type")+
  geom_boxplot(alpha = 0.8)+
  scale_fill_manual(values = c("#0072B2", "#E69F00"))+
  theme_bw()

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



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

```
hist(tuni_data1$change_wet_weight_48)
```

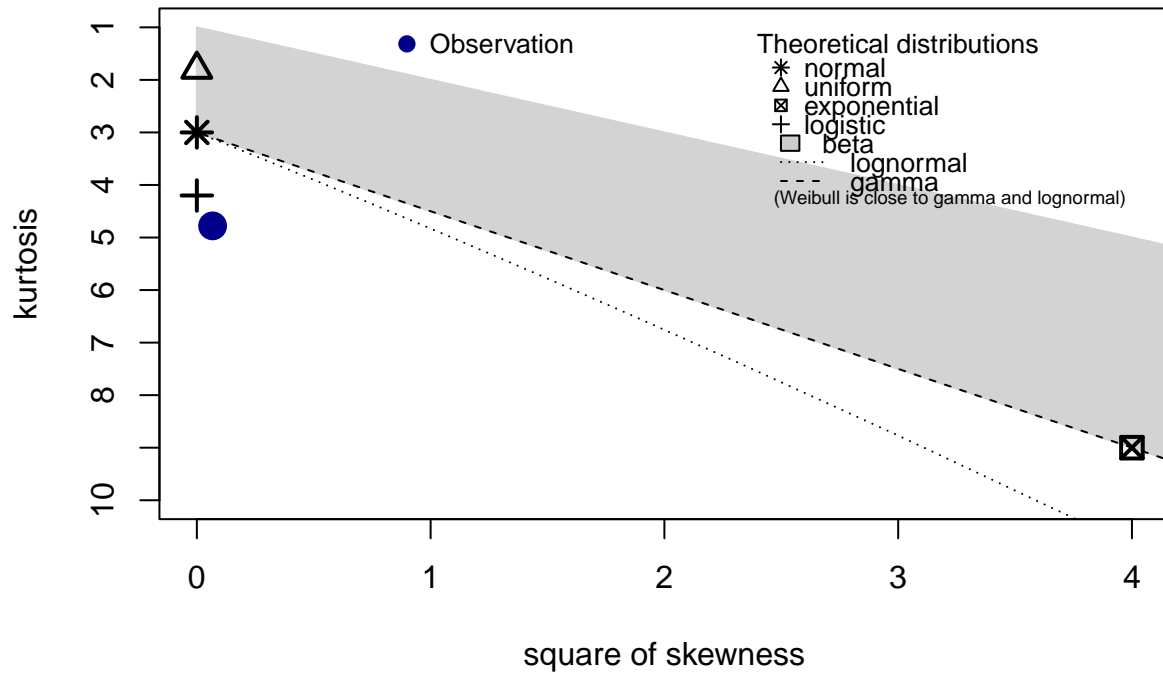


```
shapiro.test(tuni_data1$change_wet_weight_48)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data:  tuni_data1$change_wet_weight_48  
## W = 0.96216, p-value = 0.01824
```

```
#p-value = 0.01824, not normal  
descdist(tuni_data1$change_wet_weight_48)
```

Cullen and Frey graph



```
## 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(change_wet_weight_48, data=tuni_data1, type="realAll", try.gamlss = T)
```

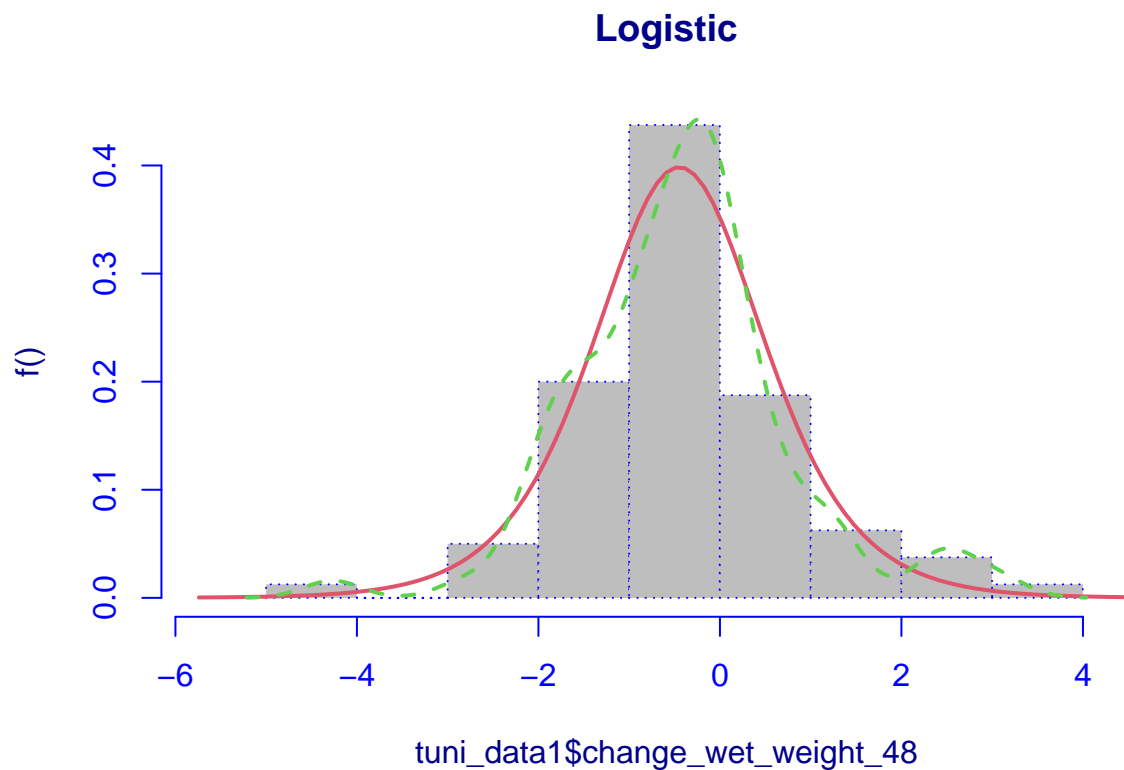
```
## |
```

|

```
## [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=L0, logistic
```

```
mLOG_weight <- histDist(tuni_data1$change_wet_weight_48, "L0", density = T,
                        main = "Logistic")
```



```
GAIC(mLOG_weight)
```

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

```
GAMLSS Model
```

```
mod_weight <- gamlss(change_wet_weight_48 ~ water_type + temperature_c +
                    exposure_time_s + water_type*temperature_c*exposure_time_s,
                    family = L0, data = tuni_data1)
```

```
Model Selection - final formula for change
```

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

```
## Start: AIC= 246.79
## change_wet_weight_48 ~ water_type + temperature_c + exposure_time_s +
## water_type * temperature_c * exposure_time_s
```

```
summary(stepmodweight48)
```

```
## *****
## Family: c("L0", "Logistic")
##
## Call: gamlss(formula = change_wet_weight_48 ~ water_type +
## temperature_c + water_type:temperature_c, family = L0,
## 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_type seawater     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_type seawater:temperature_c50  1.7036     0.6363   2.677  0.00922 **
## water_type seawater:temperature_c70 -1.0045     0.6109  -1.644  0.10453
## water_type seawater: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_type:seawater:temperature_c50 p-value = 0.00922 significant
```

```
formula(stepmodweight48)
```

```
## change_wet_weight_48 ~ water_type + temperature_c + water_type:temperature_c
```

```
#change_wet_weight_48 ~ water_type + temperature_c + water_type:temperature_c
```

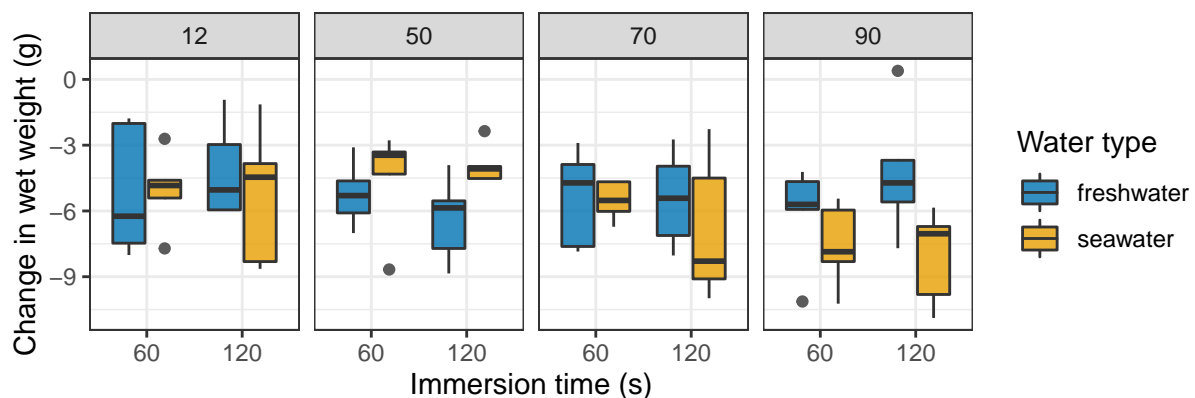
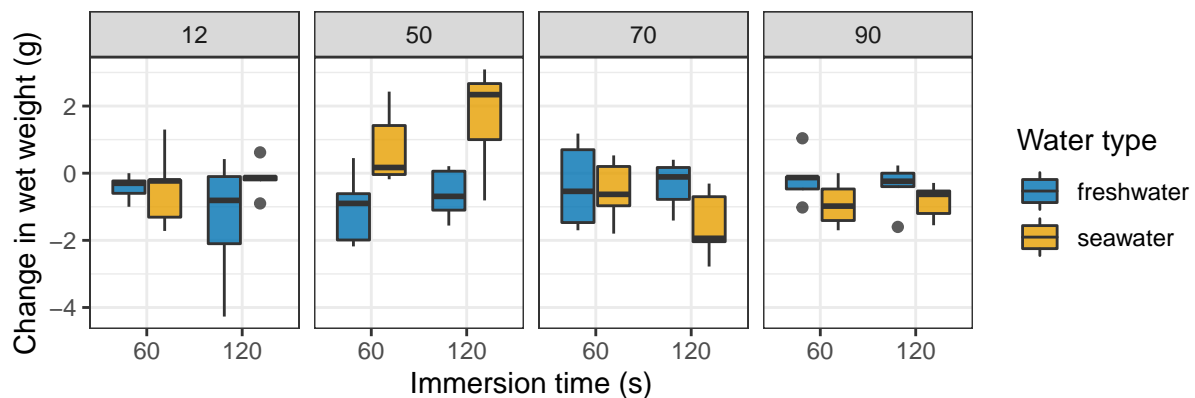
Change in Weight over 3 Weeks (compare to post-acclimation)

Data Visualization

```
p <- ggplot(tuni_data1, aes(x = exposure_time_s, y = change_wet_weight_3wk,
                           fill = water_type))+
  labs(x = "Immersion time (s)", y = "Change in wet weight (g)",
       fill = "Water type")+
  #labs(title = "Temperature (°C)")+
  geom_boxplot(alpha = 0.8)+
  scale_fill_manual(values = c("#0072B2", "#E69F00"))+
  theme_bw()

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

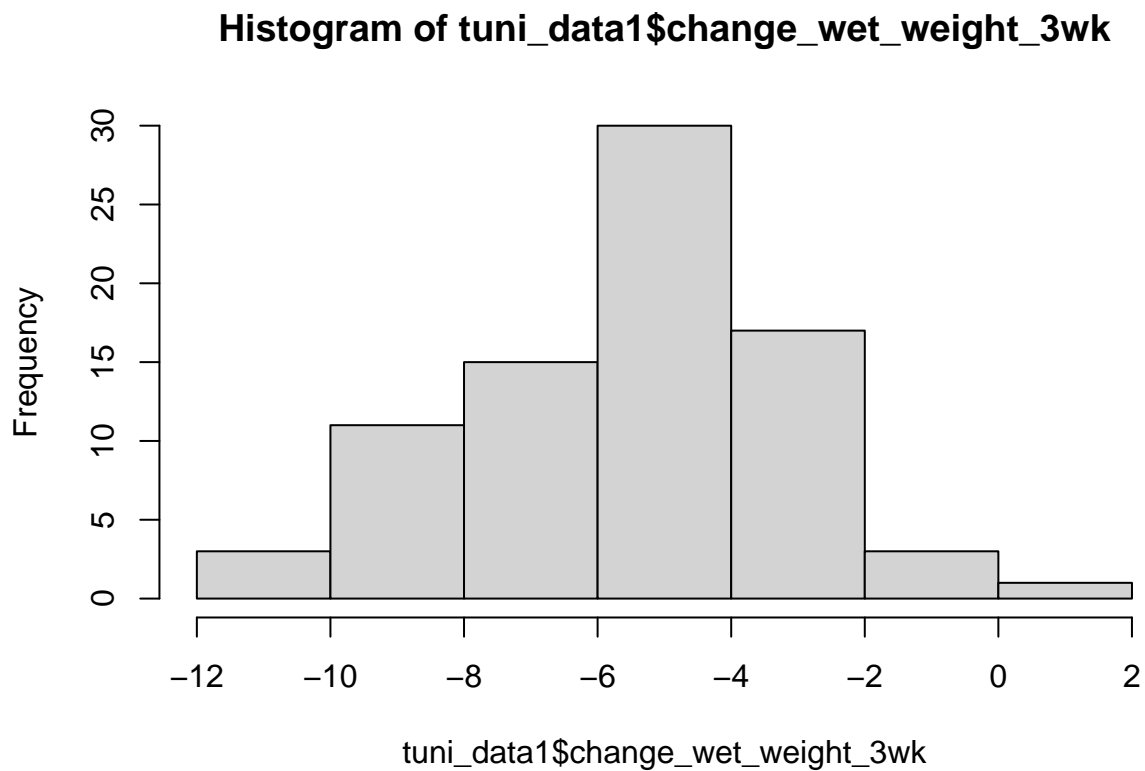
p3 + p4 + plot_layout(ncol=1)
```



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

Testing for Normality

```
hist(tuni_data1$change_wet_weight_3wk)
```

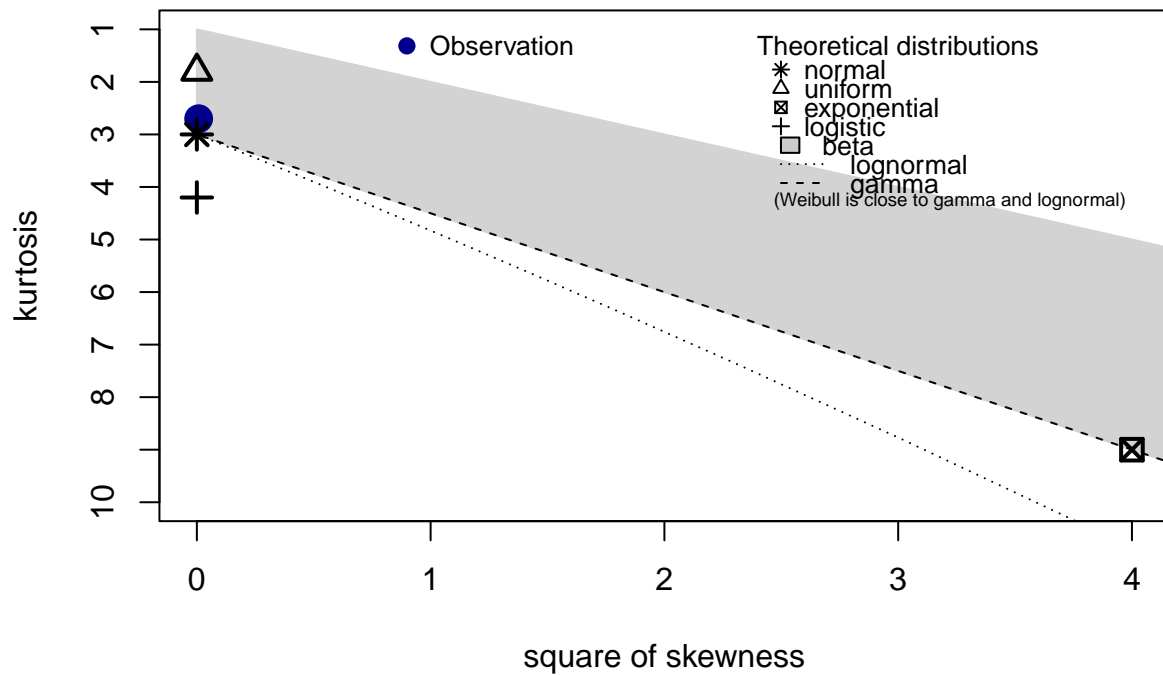


```
shapiro.test(tuni_data1$change_wet_weight_3wk)
```

```
##  
##  Shapiro-Wilk normality test  
##  
## data:  tuni_data1$change_wet_weight_3wk  
## W = 0.98952, p-value = 0.7646
```

```
#p-value = 0.7646 thus follows a normal distribution!  
descdist(tuni_data1$change_wet_weight_3wk)
```

Cullen and Frey graph



```
## 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(change_wet_weight_3wk, 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(change_wet_weight_3wk ~
  exposure_time_s + water_type +
  temperature_c +
  exposure_time_s*water_type*temperature_c,
  family = gaussian, data = tuni_data1)
```

Model Selection

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

```
##
## Call:
## lm(formula = change_wet_weight_3wk ~ 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_typedseawater    -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_type:seawater:temperature_c50 2.1290 1.4050 1.515 0.134
## water_type:seawater:temperature_c70 -0.2180 1.4050 -0.155 0.877
## water_type:seawater: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)
```

```
## change_wet_weight_3wk ~ water_type + temperature_c + water_type:temperature_c
```

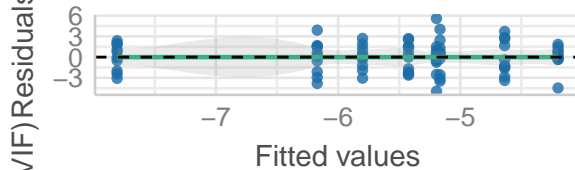
```
#change_wet_weight_3wk ~ water_type + temperature_c + water_type:temperature_c
```

Check Model

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

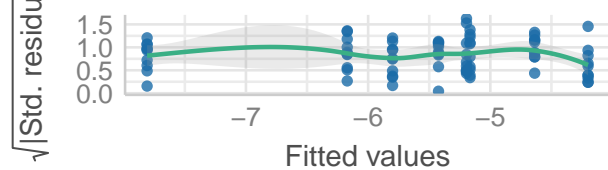
Linearity

Reference line should be flat and horizontal



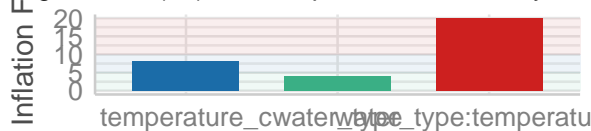
Homogeneity of Variance

Reference line should be flat and horizontal



Collinearity

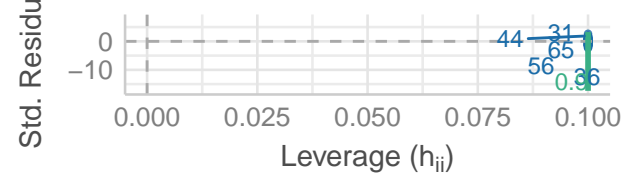
Higher bars (>5) indicate potential collinearity issues



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

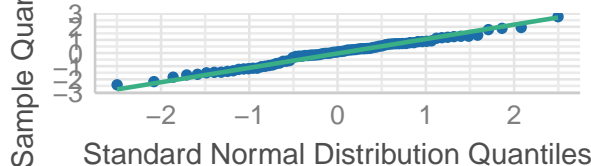
Influential Observations

Points should be inside the contour lines



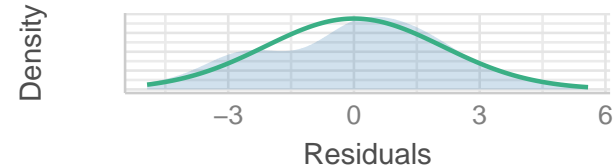
Normality of Residuals

Points should fall along the line



Normality of Residuals

Distribution should be close to the normal curve



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

New Model

```
mod_changeweightv2 <- lm(change_wet_weight_3wk ~ water_type + temperature_c,  
                          family = gaussian, data = tuni_data1)  
summary(mod_changeweightv2)
```

```
##  
## Call:  
## lm(formula = change_wet_weight_3wk ~ 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(total))  
# divided by 10 because each combination of  
#temperature and water type has 10 data points
```

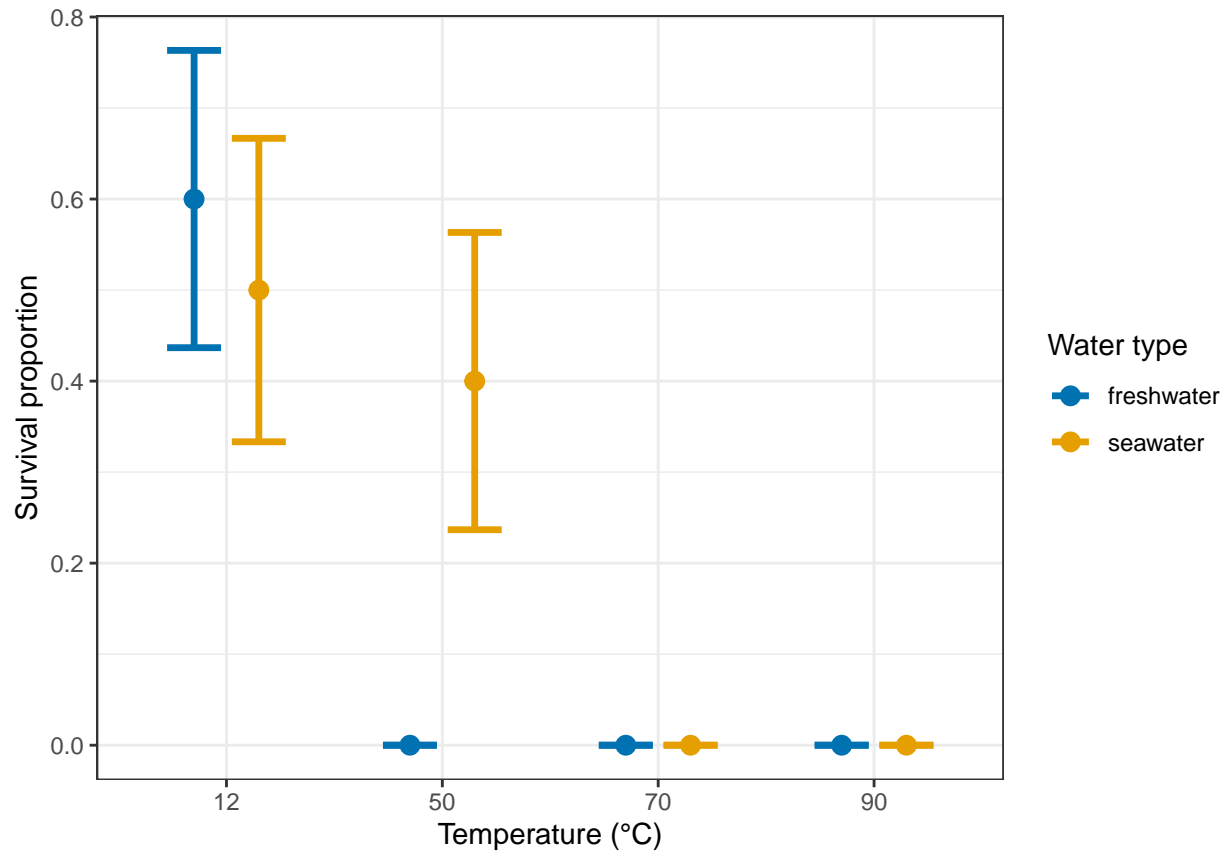
```
my_cols = c("freshwater" = "#0072B2", "seawater" = "#E69F00")
```

```
#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, position = pd)+
theme_bw()+labs(colour="Water type")+
  scale_color_manual(values = my_cols)+
  scale_fill_manual(values = my_cols)

```



pd

```

## <ggproto object: Class PositionDodge, Position, gg>
##   compute_layer: function
##   compute_panel: function
##   preserve: total
##   required_aes:
##   setup_data: function
##   setup_params: function
##   width: 0.6
##   super: <ggproto object: Class PositionDodge, Position, gg>

```

```
#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_c*exposure_time_s +
                        random(as.factor(colony_id)),
                        family = BI, data = tunidata)
```

```
## 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
```

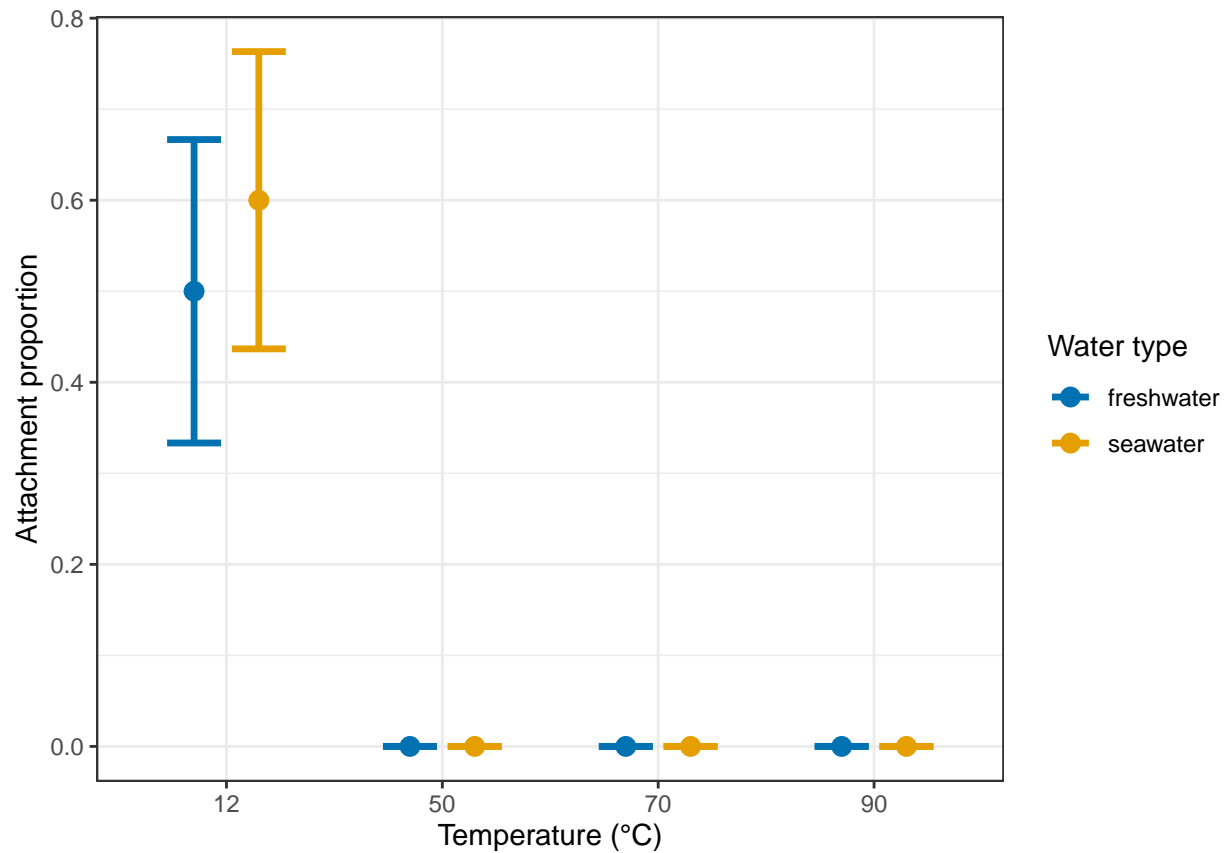
```
#p-value for 12-90 and 12-70 is significant!
```

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)/sqrt(total))
# 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, position=pd)+
  theme_bw()+labs(colour="Water type")+
  scale_color_manual(values = my_cols)+
  scale_fill_manual(values = my_cols)
```



```
#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*exposure_time_s,
               family = BI, data = tuni_data1)
```

```
## 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_type seawater                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_type seawater:temperature_c50 -0.8109     558.3026
## water_type seawater:temperature_c70 -0.8109     558.3026
## water_type seawater:temperature_c90 -0.8109     558.3026
## water_type seawater: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_type seawater:temperature_c50:exposure_time_s120 0.8109     789.5523
```

```
## water_type:seawater:temperature_c70:exposure_time_s120 0.8109 789.5523
## water_type:seawater:temperature_c90:exposure_time_s120 0.8109 789.5523
## t value Pr(>|t|)
## (Intercept) -0.444 0.658
## water_type:seawater 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_type:seawater:temperature_c50 -0.001 0.999
## water_type:seawater:temperature_c70 -0.001 0.999
## water_type:seawater:temperature_c90 -0.001 0.999
## water_type:seawater: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_type:seawater:temperature_c50:exposure_time_s120 0.001 0.999
## water_type:seawater:temperature_c70:exposure_time_s120 0.001 0.999
## water_type:seawater: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
```