Timestep Data Analyses

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```
library(here)
library(reshape2)
library(FSA)
library(fGarch)
library(LambertW)
library(patchwork)
library(DHARMa)
library(mgcv)
library(fitdistrplus)
library(goft)
library(gamlss)
library(rstatix)
library(performance)
library(scales)
library(viridis)
library(ggpubr)
library(tidyverse)
```

Load required libraries

Manipulate Raw Tube Growth Data for Plotting and Stats

Load raw data

```
tube_growth = read_csv(here("./data/tube-growth.csv"))
knitr::kable(tube_growth, caption = "Tube growth rates of all marked worms in various treatments.")
```

Table 1: Tube growth rates of all marked worms in various treatments.

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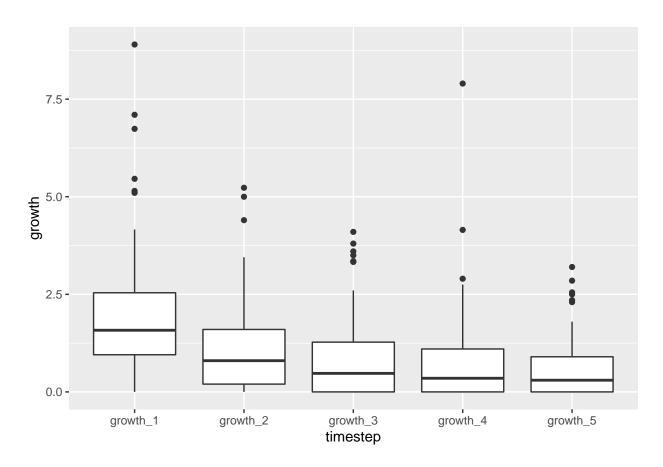
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                       \mathbf{C}
                       3
\mathbf{C}
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\mathbf{C}
             3
                       C 3 controlwhite 13.70 4.050 7.30 12.3016.1017.50 19.1519.403.2505.00 3.80 1.65 0.25 15.350 0
\mathbf{C}
                       C 3 controllade 16.42 4.950 8.00 8.80 9.90 10.50 12.2012.603.0500.80 1.10 1.70 0.40 7.650
```

Wraggle data

Statistical Analyses

Checking Assumptions of a One-way Repeated Measures ANOVA

```
# visualize data
ggplot(clean_tube_growth, aes(x= timestep, y = growth)) +
  geom_boxplot()
```

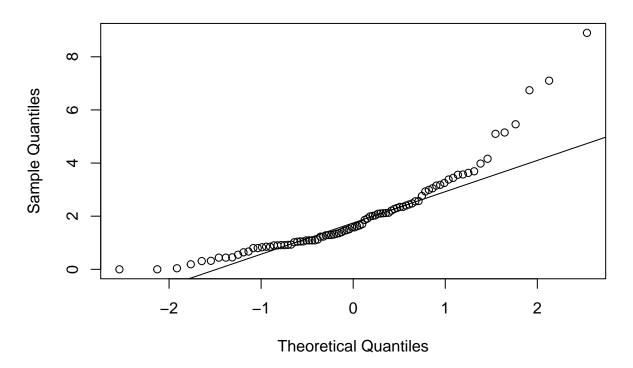


```
# check for normality of the growth at each timestep using Shapiro Wilks test
clean_tube_growth %>%
  group_by(timestep) %>%
  shapiro_test(growth)
```

```
## # A tibble: 5 x 4
     timestep variable statistic
##
             <chr>
##
     <fct>
                           <dbl>
                                    <dbl>
## 1 growth_1 growth
                           0.848 3.67e- 8
                           0.847 3.21e- 8
## 2 growth_2 growth
## 3 growth_3 growth
                           0.811 2.22e- 9
## 4 growth_4 growth
                           0.678 9.56e-13
## 5 growth_5 growth
                           0.782 3.17e-10
```

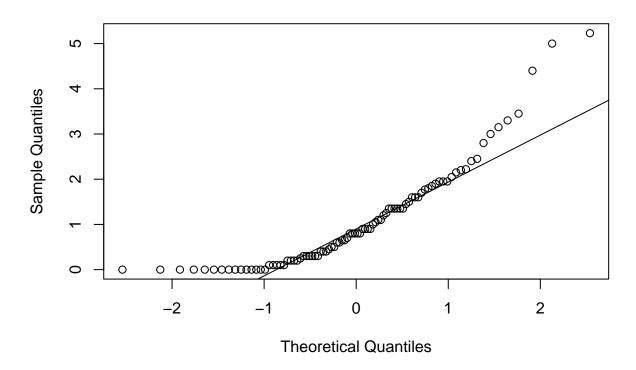
```
# investigate distribution of the growth at each timestep using qqplots since shapiro-wilk test found g
# timestep 1
growth_1 <- clean_tube_growth %>%
    dplyr::filter(timestep == "growth_1")

qqnorm(growth_1$growth)
qqline(growth_1$growth)
```



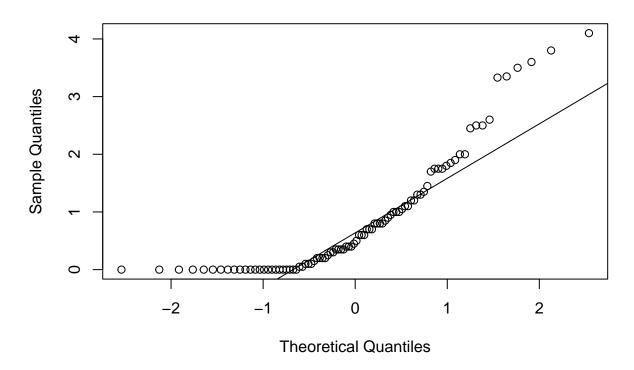
```
# timestep 2
growth_2 <- clean_tube_growth %>%
   dplyr::filter(timestep == "growth_2")

qqnorm(growth_2$growth)
qqline(growth_2$growth)
```



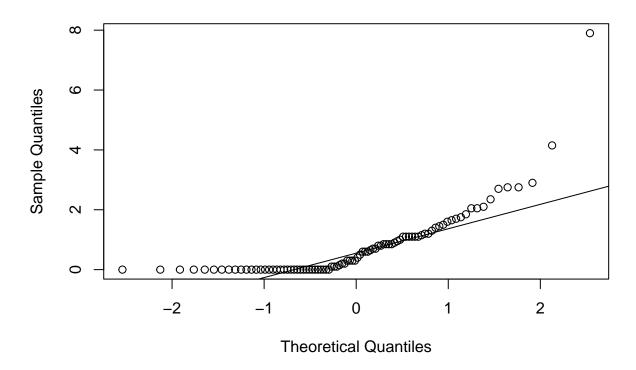
```
# timestep 3
growth_3 <- clean_tube_growth %>%
   dplyr::filter(timestep == "growth_3")

qqnorm(growth_3$growth)
qqline(growth_3$growth)
```



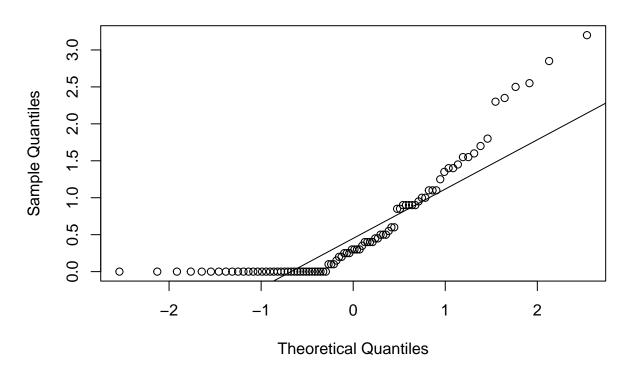
```
# timestep 4
growth_4 <- clean_tube_growth %>%
   dplyr::filter(timestep == "growth_4")

qqnorm(growth_4$growth)
qqline(growth_4$growth)
```



```
# timestep 5
growth_5 <- clean_tube_growth %>%
   dplyr::filter(timestep == "growth_5")

qqnorm(growth_5$growth)
qqline(growth_5$growth)
```



```
# Since sample size is N=90 overall and n= 30 for each treatment, sample size is large enough to ignore
# Checking for extreme outliers
clean_tube_growth %>%
  group_by(timestep) %>%
  identify_outliers(growth)
```

```
## # A tibble: 24 x 8
      timestep replicate worm_id amt_cut_mm treatment
##
                                                         growth is.outlier is.extreme
##
      <fct>
               <chr>>
                          <fct>
                                       <dbl> <fct>
                                                          <dbl> <lgl>
                                                                            <1g1>
##
    1 growth_1 B_1
                          B_1_2
                                        16.1 high_temp
                                                           5.46 TRUE
                                                                            FALSE
##
    2 growth_1 B_1
                         B_1_6
                                        15.4 high_temp
                                                           6.74 TRUE
                                                                            FALSE
                                                           5.1 TRUE
##
   3 growth_1 B_1
                          B_1_8
                                             high_temp
                                                                            FALSE
##
   4 growth_1 C_2
                          C_2_1
                                        18.4 control
                                                           8.9 TRUE
                                                                            TRUE
##
   5 growth_1 C_2
                          C 2 5
                                        13.8 control
                                                           5.15 TRUE
                                                                            FALSE
##
   6 growth_1 C_2
                          C_2_9
                                        17.2 control
                                                           7.1 TRUE
                                                                            FALSE
   7 growth_2 B_2
                          B_2_6
                                        19.5 high_temp
                                                           4.4 TRUE
                                                                            FALSE
    8 growth_2 B_2
                          B_2_8
                                        15.0 high_temp
                                                           5.23 TRUE
                                                                            FALSE
##
   9 growth_2 C_3
                          C_3_9
                                        13.7 control
                                                           5
                                                                TRUE
                                                                            FALSE
                                                           3.33 TRUE
                                                                            FALSE
## 10 growth_3 A_1
                          A_1_1
                                        17.0 low_salin~
## # ... with 14 more rows
```

```
# Since two extreme outliers were found (B_2_6, C_2_1) remove extreme outliers so that results of ANOVA no_exouts <- clean_tube_growth %>% dplyr::filter(growth < 7.90)
```

```
#check that extreme outliers were successfully removed
no_exouts %>%
 group_by(timestep) %>%
 identify outliers(growth)
## # A tibble: 22 x 8
##
     timestep replicate worm_id amt_cut_mm treatment growth is.outlier is.extreme
##
                                   <dbl> <fct>
                                                    <dbl> <lgl>
     <fct>
             <chr>
                       <fct>
                                                                     <1g1>
## 1 growth_1 B_1
                       B_1_2
                                     16.1 high_temp 5.46 TRUE
                                                                     FALSE
                                                                     FALSE
                                                      6.74 TRUE
## 2 growth 1 B 1
                       B_1_6
                                     15.4 high_temp
## 3 growth 1 B 1
                       B_1_8
                                        high_temp
                                                     5.1 TRUE
                                                                     FALSE
## 4 growth_1 C_2
                                     13.8 control
                                                      5.15 TRUE
                       C_2_5
                                                                     FALSE
                       C_2_9
## 5 growth 1 C 2
                                     17.2 control
                                                     7.1 TRUE
                                                                     FALSE
## 6 growth_2 B_2
                                     19.5 high_temp
                                                      4.4 TRUE
                       B_2_6
                                                                     FALSE
                                     15.0 high_temp
## 7 growth_2 B_2
                                                      5.23 TRUE
                       B_2_8
                                                                     FALSE
## 8 growth_2 C_3
                       C_3_9
                                    13.7 control
                                                     5
                                                          TRUE
                                                                     FALSE
## 9 growth_3 A_1
                       A_1_1
                                    17.0 low_salin~
                                                      3.33 TRUE
                                                                     FALSE
## 10 growth_3 A_2
                                     15.6 low_salin~
                                                      4.1 TRUE
                                                                     FALSE
                       A_2_9
## # ... with 12 more rows
#assumption of sphericity automatically checked by ANOVA test
```

One-way Repeated Measures Analysis of Variance (ANOVA)

```
# run one-way repeated measures ANOVA and obtain ANOVA table
tube.aov <-anova_test(data = clean_tube_growth, dv = growth,</pre>
                       wid = worm_id,
                       within = timestep)
get_anova_table(tube.aov)
## ANOVA Table (type III tests)
##
##
       Effect DFn
                      DFd
                              F
                                        p p<.05
## 1 timestep 3.01 268.12 23.846 9.41e-14
# run one-way repeated measures ANOVA for data with extreme outliers removed
noexouts.aov <- anova_test(data = no_exouts, dv = growth,</pre>
                       wid = worm_id,
                       within = timestep)
get_anova_table(noexouts.aov)
## ANOVA Table (type III tests)
##
                                        p p<.05
##
       Effect DFn
                      DFd
                               F
## 1 timestep 3.13 272.12 25.107 8.18e-15
                                               * 0.174
```

Pairwise Comparison

```
# performing pairwise comparison between timesteps
pwc <- clean_tube_growth %>%
 pairwise_t_test(growth ~ timestep, paired = TRUE,
                  p.adjust.method = "bonferroni")
pwc <- pwc %>% add_xy_position(x = "timestep")
pwc
## # A tibble: 10 x 14
##
      .у.
            group1
                     group2
                                  n1
                                       n2 statistic
                                                        df
                                                                       p.adj
##
      <chr> <chr>
                     <chr>
                               <int> <int>
                                               <dbl> <dbl>
                                                              <dbl>
                                                                       <dbl>
## 1 growth growth_1 growth_2
                                               4.50
                                                        89 2.08e- 5 2.08e- 4
                                  90
                                       90
                                                       89 2.09e- 7 2.09e- 6
## 2 growth growth_1 growth_3
                                  90
                                       90
                                               5.63
                                                       89 1.26e- 8 1.26e- 7
## 3 growth growth 1 growth 4
                                               6.27
                                  90
                                       90
                                                        89 1.54e-12 1.54e-11
## 4 growth growth_1 growth_5
                                  90
                                       90
                                               8.22
## 5 growth growth_2 growth_3
                                  90
                                       90
                                              1.72
                                                       89 8.8 e- 2 8.83e- 1
## 6 growth growth_2 growth_4
                                  90
                                       90
                                               2.22
                                                       89 2.9 e- 2 2.88e- 1
## 7 growth growth_2 growth_5
                                                        89 4.18e- 4 4
                                  90
                                        90
                                               3.67
                                                                        e- 3
## 8 growth growth_3 growth_4
                                  90
                                        90
                                               0.555
                                                        89 5.8 e- 1 1
                                                                        e+ 0
## 9 growth growth_3 growth_5
                                  90
                                        90
                                               2.10
                                                        89 3.8 e- 2 3.83e- 1
## 10 growth growth_4 growth_5
                                  90
                                        90
                                               1.62
                                                        89 1.1 e- 1 1
                                                                        e+ 0
## # ... with 5 more variables: p.adj.signif <chr>, y.position <dbl>,
    groups <named list>, xmin <dbl>, xmax <dbl>
```

Finding the Mean and Standard Error of Tube Growth in each Timestep

```
# finding the mean and standard error of tube growth in timestep 1
summary(tube_growth$growth_1)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                             Max.
  0.0000 0.9525 1.5800 2.0116 2.5400 8.9000
se(tube_growth$growth_1)
## [1] 0.1663338
# finding the mean and standard error of tube growth in timestep 2
summary(tube_growth$growth_2)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                             Max.
    0.000
           0.200
                   0.800
                            1.089
                                   1.600
                                            5.230
##
```

```
se(tube_growth$growth_2)
## [1] 0.1190655
# finding the mean and standard error of tube growth in timestep 3
summary(tube_growth$growth_3)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
   0.0000 0.0000 0.4750 0.8648 1.2750 4.1000
se(tube_growth$growth_3)
## [1] 0.1078754
# finding the mean and standard error of tube growth in timestep 4
summary(tube_growth$growth_4)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                             Max.
## 0.0000 0.0000 0.3500 0.7856 1.1000 7.9000
se(tube_growth$growth_4)
## [1] 0.1211917
# finding the mean and standard error of tube growth in timestep 5
summary(tube_growth$growth_5)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                             Max.
   0.0000 0.0000 0.3000 0.5794 0.9000 3.2000
se(tube_growth$growth_5)
## [1] 0.07921231
```

Finding the Mean and Standard Error of Tube Growth for Each Treatment Within all Timesteps

```
# Making a dataset containing only control growth values

control <- tube_growth %>%
   dplyr::filter(treatment == "control")

# making a dataset containing only low salinity treatment growth values

salinity <- tube_growth %>%
   dplyr::filter(treatment == "low_salinity")
```

```
# making a dataset containing only high temperature treatment growth values
temperature <- tube_growth %>%
 dplyr::filter(treatment == "high_temp")
# finding the mean and standard error of tube growth of all treatments for timestep 1
summary(control$growth_1)
##
     Min. 1st Qu. Median
                           Mean 3rd Qu.
                                            Max.
                  1.745
##
    0.440
          1.028
                            2.345 2.978
                                           8.900
se(control$growth_1)
## [1] 0.3538703
summary(salinity$growth_1)
##
     Min. 1st Qu. Median
                            Mean 3rd Qu.
                                            Max.
    0.000 0.950
                   1.350
##
                          1.684
                                    2.118
                                           3.980
se(salinity$growth_1)
## [1] 0.196041
summary(temperature$growth_1)
     Min. 1st Qu. Median
                             Mean 3rd Qu.
## 0.0000 0.9475 1.6350 2.0060 2.5150 6.7400
se(temperature$growth_1)
## [1] 0.2890319
\# finding the mean and standard error of tube growth of all treatments for timestep 2
summary(control$growth_2)
     Min. 1st Qu. Median
                             Mean 3rd Qu.
##
## 0.0000 0.3000 0.6500 0.9633 1.3125 5.0000
se(control$growth_2)
```

[1] 0.1942408

```
summary(salinity$growth_2)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
    0.000
           0.125
                   0.600
                            0.753
                                    1.212
                                            2.800
##
se(salinity$growth_2)
## [1] 0.1356171
summary(temperature$growth_2)
##
     Min. 1st Qu. Median
                            Mean 3rd Qu.
                                            Max.
##
    0.000 0.325 1.425 1.551
                                    2.188
                                           5.230
se(temperature$growth_2)
## [1] 0.2502065
\# finding the mean and standard error of tube growth of all treatments for timestep 3
summary(control$growth_3)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
    0.000 0.100 0.700
                                    1.087
                                            3.800
##
                            0.765
se(control$growth_3)
## [1] 0.1563622
summary(salinity$growth_3)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
    0.000 0.000 0.350
                            0.946
##
                                  1.600
                                           4.100
se(salinity$growth_3)
## [1] 0.2195579
summary(temperature$growth_3)
     Min. 1st Qu. Median
                             Mean 3rd Qu.
##
                                            Max.
  0.0000 0.1625 0.5500 0.8833 1.5625 3.5000
se(temperature$growth_3)
```

[1] 0.184131

```
# finding the mean and standard error of tube growth of all treatments for timestep 4
summary(control$growth_4)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
## 0.0000 0.0000 0.8250 0.8117 1.3500 2.7500
se(control$growth_4)
## [1] 0.1536095
summary(salinity$growth_4)
     Min. 1st Qu. Median
                             Mean 3rd Qu.
##
## 0.0000 0.0000 0.1000 0.3683 0.6000 2.0500
se(salinity$growth_4)
## [1] 0.09329484
summary(temperature$growth_4)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
    0.000
           0.000
                   0.850
                                           7.900
##
                            1.177
                                    1.575
se(temperature$growth_4)
## [1] 0.3026961
\# finding the mean and standard error of tube growth of all treatments for timestep 5
summary(control$growth_5)
##
     Min. 1st Qu. Median
                             Mean 3rd Qu.
                                            Max.
    0.000
           0.050 0.400
##
                            0.915
                                  1.525
                                           3.200
se(control$growth_5)
## [1] 0.1764944
summary(salinity$growth_5)
##
     Min. 1st Qu. Median
                            Mean 3rd Qu.
                                            Max.
    0.000 0.000 0.100
                            0.335 0.500
                                           1.800
```

```
se(salinity$growth_5)

## [1] 0.08228595

summary(temperature$growth_5)

## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 0.0000 0.0000 0.2250 0.4883 0.8875 2.5500

se(temperature$growth_5)
```

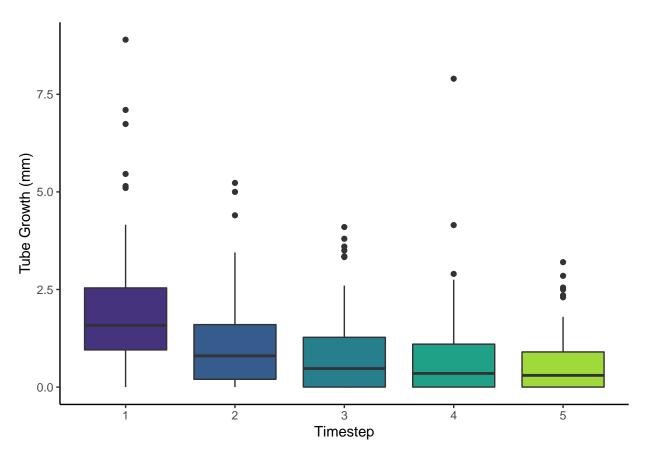
[1] 0.1166999

Plotting the Timestep Data

```
# choosing colours for one.way plot

one.way.cols = c("growth_1" = "#46337EFF", "growth_2" = "#365C8DFF", "growth_3" = "#277F8EFF", "growth_4"
# Making a plot of the mean growth of all treatments over all timesteps

one.way =
    ggplot(clean_tube_growth, aes(x = timestep, y = growth)) +
    geom_boxplot(aes(fill = timestep)) +
    theme_classic() +
    theme(legend.position = "none") +
    xlab("Timestep") +
    ylab("Tube Growth (mm)") +
    scale_x_discrete(labels=c("growth_1" = "1", "growth_2" = "2", "growth_3" = "3", "growth_4" = "4", "groscale_color_manual(values = one.way.cols) +
    scale_fill_manual(values = one.way.cols)
```



```
# saving one.way plot

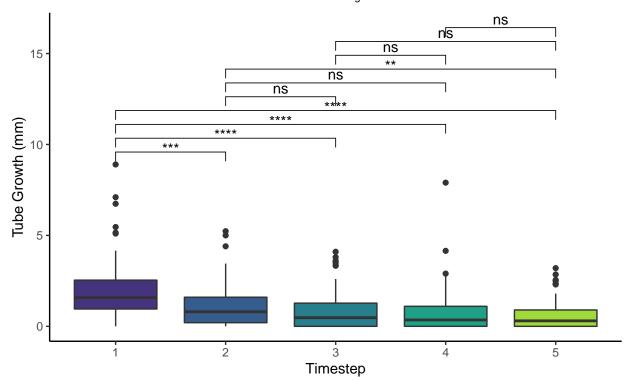
ggsave(plot = one.way, filename = "timestep-growth.jpg")

#adding significance bars from pairwise comparisons to one.way plot (significant bars labelled ns were

p.one.way <- one.way +
    stat_pvalue_manual(pwc) +
    labs(subtitle = get_test_label(tube.aov, detailed = TRUE),
        caption = get_pwc_label(pwc))

p.one.way</pre>
```

Anova, F(3.01,268.12) = 23.85, p = <0.0001, $\eta_{q}^{2} = 0.16$



pwc: T test; p.adjust: Bonferroni

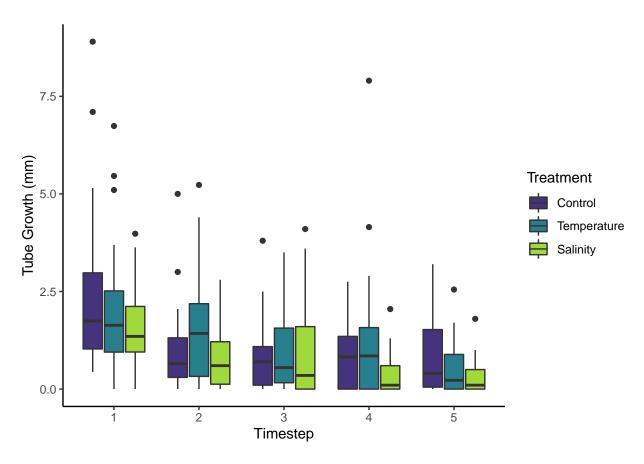
```
# saving p.one.way plot

ggsave(plot = p.one.way, filename = "p-value-timestep-growth.jpg", width = 6, height = 7, dpi = 200, um

# Making a plot of the mean tube growth per treatment over all timesteps

treat.growth =
    ggplot(clean_tube_growth, aes(x = timestep, y = growth, fill = treatment)) +
        geom_boxplot() +
        scale_fill_manual(name = "Treatment", labels = c("Control", "Temperature", "Salinity"), values = c(
        theme_classic() +
        xlab("Timestep") +
        ylab("Tube Growth (mm)") +
        scale_x_discrete(labels=c("growth_1" = "1", "growth_2" = "2", "growth_3" = "3", "growth_4" = "4", "g

treat.growth
```



```
# saving treat.growth plot
ggsave(plot = treat.growth, filename = "treatment-timestep-growth.jpg")
```