Jellyfish Experiment Analysis

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Goals

The goal of this analysis is to assess how temperature and water treatments impact various measures of Jellyfish quality, specifically their physical characteristics, ability to reproduce or advance to the next life stage and DNA.

Experimental Design

A sample of 50 Polyps are placed into 18 beakers. Those 18 beakers are evenly distributed across 6 treatments. There are two crossed factors, Water Temperature with three levels: (20 degrees 'low', 28 degrees 'ambient', 33 degrees 'high)' and Microbial Condition with two levels: (ASW 'Regular Artificial Salt Water', sterilized Antibiotic treated ASW 'Antibiotic treated Artificial Salt Water').

Measurements with different methods are performed. I have broken them up as follows.

Count Measurements

On a biweekly basis researchers count the number and stage of jellyfish development for each beaker. There are three stages tracked, Buds, Ephyra and Polyps.

Physical Measurements

On a weekly basis researchers measure each Polyp's width and Ephyra's diameter.

DNA Measurements

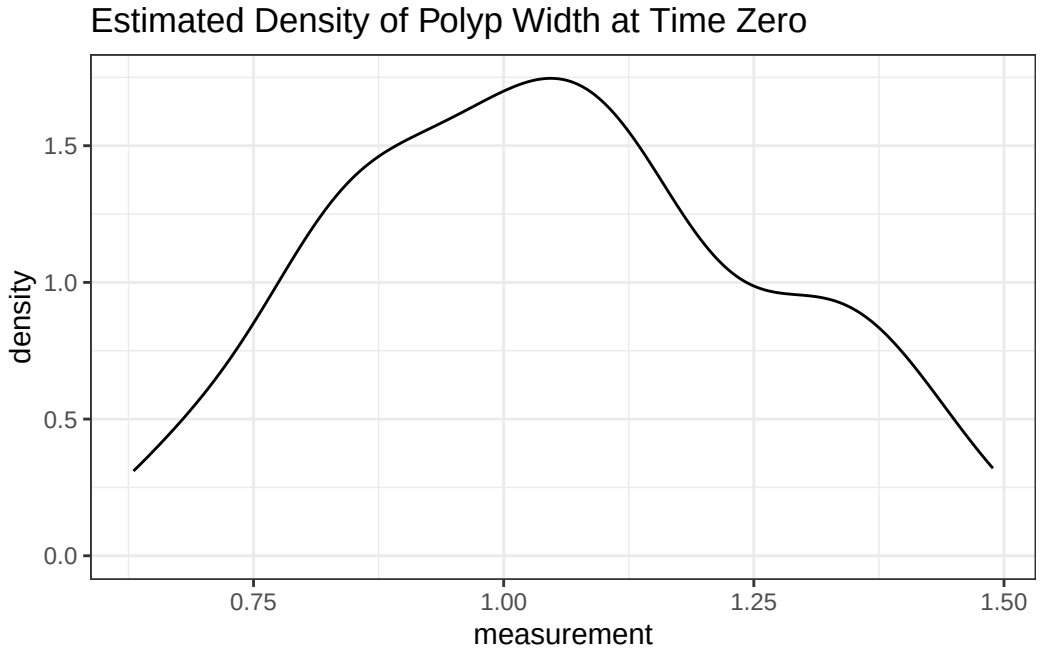
DNA from sampled Polyps are extracted at the start and end of the experiment. DNA is sampled from each beaker's water at the end of the experiment.

Analysis Results

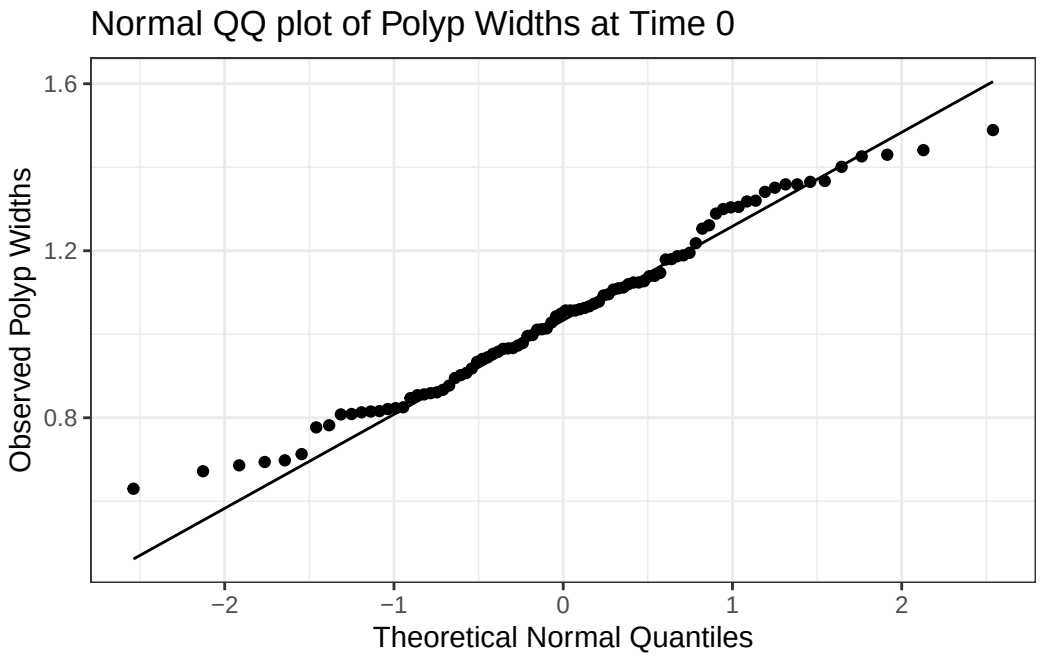
Are all the T0 samples the same (not significantly different)

This question asks whether the polyp samples have widths that are not significantly different than each other.

T0 samples will not have the exact same width, but since they are all subject to the same initial conditions they should be drawn from the same distribution. First we look at the estimated density function of polyp widths at time zero.



This looks close to a normal distribution. I use a normal QQ plot to visually inspect further.



Since the observed data follows the QQ line closely it appears the Polyp width for time 0 samples are from the same normal distribution.

We can formally test whether this sample is from a normal distribution by using a Shapiro-Wilk test. So, we are testing:

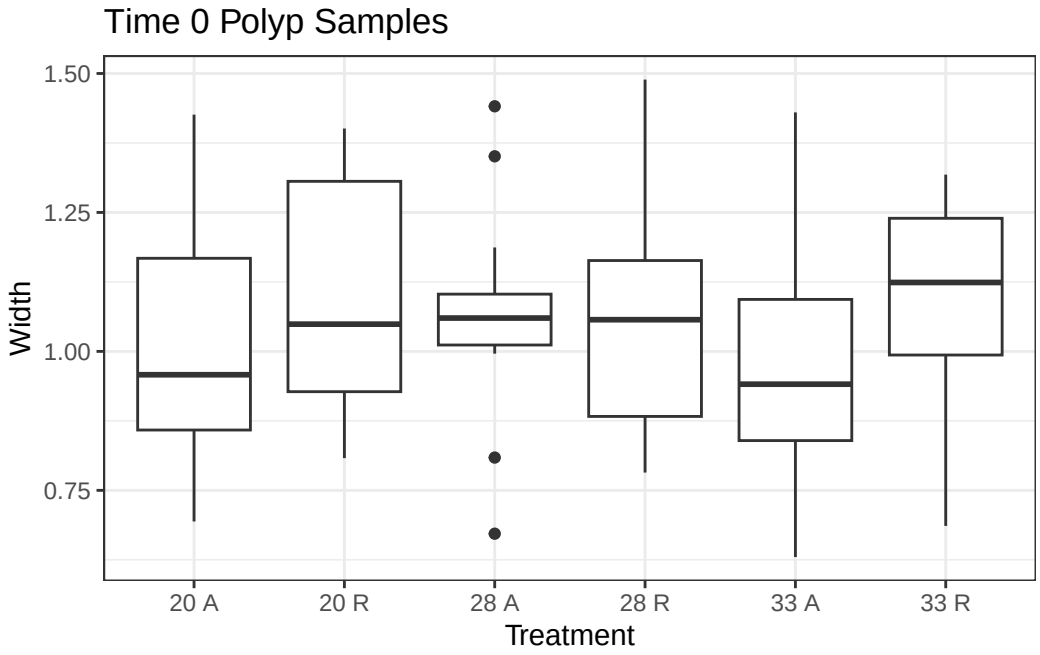
H_0 : Sample Obtained from Normal Distribution, H_a : Sample Not Obtained from a Normal Distribution

```
    Shapiro-Wilk normality test

data:  width_time_0
W = 0.9791, p-value = 0.1562
```

With a p-value of .1562 we cannot reject the null hypothesis that this sample is from a normal distribution.

These results have shown that there is no evidence that time 0 polyp samples are drawn from separate distributions. But it would also be reassuring if we could show that the width of time 0 polyp samples are similar across treatments.



The median of polyp samples at time 0 appears consistent across treatments.

To formally test whether sample widths are different depending on treatments we fit the model:

$$y_{ijk} = \mu + w_i + t_j + w_i t_j + e_{ijk}, \quad i = 1, 2, \quad j = 1, 2, 3 \quad k = 1, ..., 15$$

Where y_{ijk} is the width of the polyp sample, w_i is the water treatment i , t_j is temperature treatment j.

We obtain an ANOVA table to test:

$$H_0 : w_i = 0, t_j = 0, w_i t_j = 0 \quad H_a : w_i \neq 0, t_j \neq 0, w_i t_j \neq 0$$

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Analysis of Variance Table

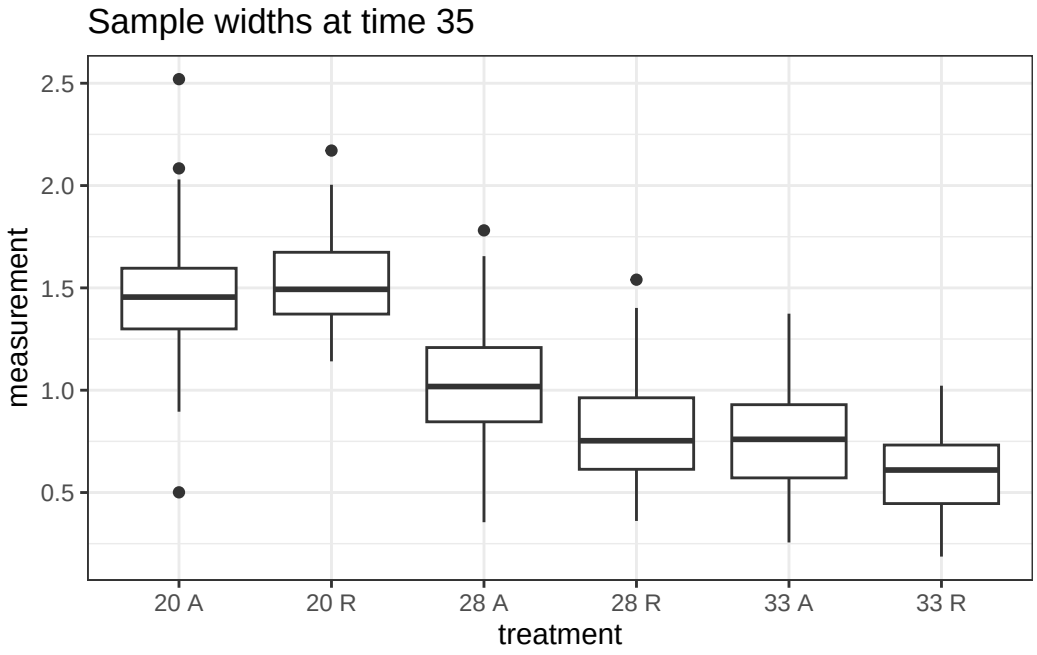
Response: measurement
                  Df Sum Sq  Mean Sq F value Pr(>F)
water              1 0.0794 0.079388  1.8172 0.1813
temperature        2 0.0109 0.005434  0.1244 0.8832
water:temperature  2 0.0579 0.028967  0.6631 0.5179
Residuals         84 3.6696 0.043686
```

We cannot reject the null hypothesis that there is no difference in sample means across treatments.

All evidence points in the direction of time 0 polyp samples being the same (not significantly different).

Are the T35 samples significantly different from one another across treatments?

After treatments are applied, samples are drawn again.



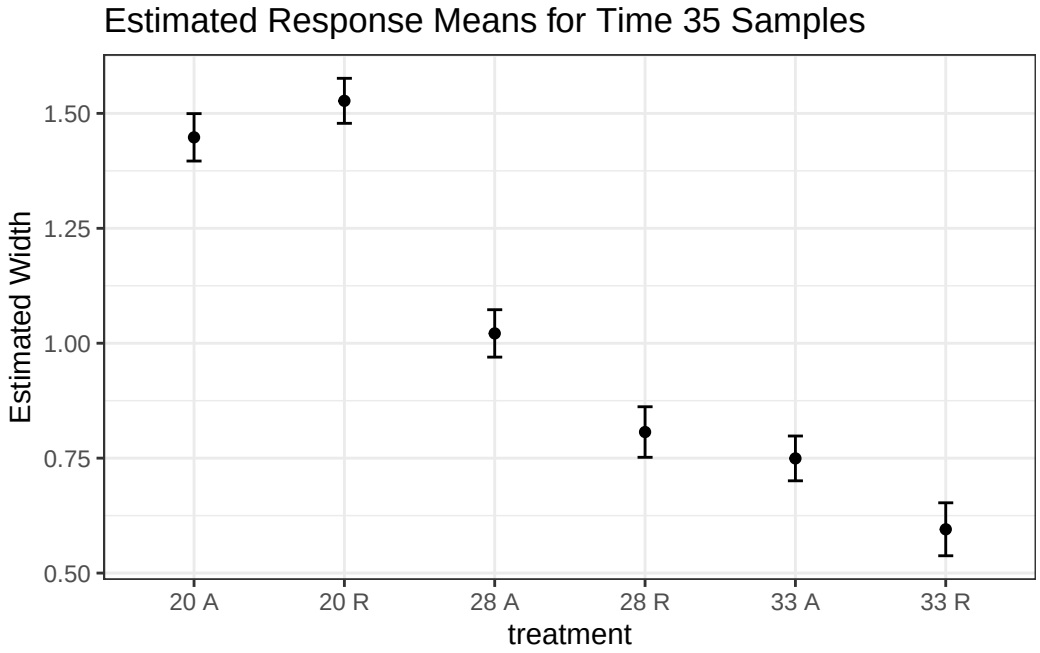
A box-plot indicates that there does appear to be treatment effects.

We formally test this using the same model as before, but on the time 35 samples.

```
Analysis of Variance Table

Response: measurement
                   Df Sum Sq Mean Sq  F value    Pr(>F)
water               1  0.104   0.104   1.6592    0.1983
temperature         2 64.222  32.111 511.7473 < 2.2e-16 ***
water:temperature   2  2.209   1.105  17.6053 3.949e-08 ***
Residuals         532 33.382   0.063
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

We reject the null hypothesis of $w_i t_j = 0$ at any reasonable choice of a p-value. There is a treatment effect and an interaction effect between water and temperature.



A plot of the estimated response means shows that, as reported in our anova table, the interaction between temperature and water is significant. For temperature 20 regular water and artificial water have similar widths, in the other two temperatures artificial water leads to higher widths.

The biggest takeaway is the impact of temperature, as temperature gets higher the widths of polyps get smaller.

T35 samples are significantly different from one another across treatments.

Are there differences between T0 and T35 between temperatures and microbe treatments

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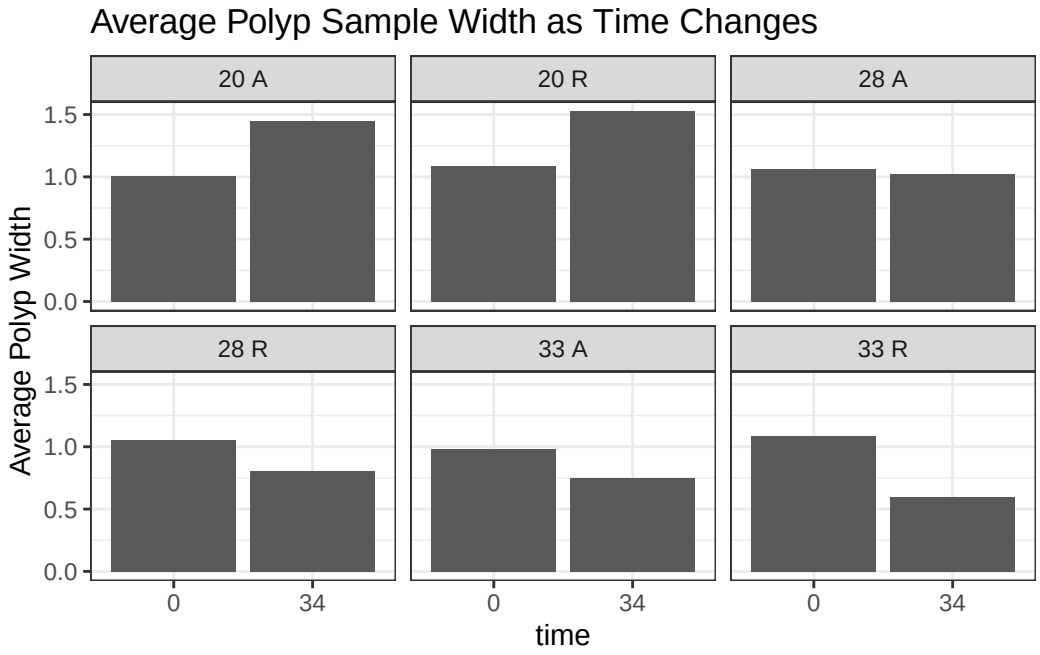


There is initial evidence pointing towards differences between treatments, for low temperatures widths seem to increase for high temperatures widths seem to decrease. The impact of water treatment seems to depend on the temperature of the water.

With these findings, we fit the model:

$$y_{ijkl} = \mu + w_i + t_j + w_i t_j + s_k + s_k w_i + s_k t_j + e_{ijkl}, \quad i = 1, 2, \quad j = 1, 2, 3 \quad k = 1, 2, \quad l = 1, ..., n_{ijk}$$

Where y_{ijk} is the width of the polyp sample, w_i is the water treatment i , t_j is temperature treatment j, s_k is an indicator variable for whether the sample was performed at the start (time 0) or end (time 35) of the treatment. Of note n_{ijk}, the number of observations in each treatment, time group is uneven as there as more samples are conducted at the end of the experiment.

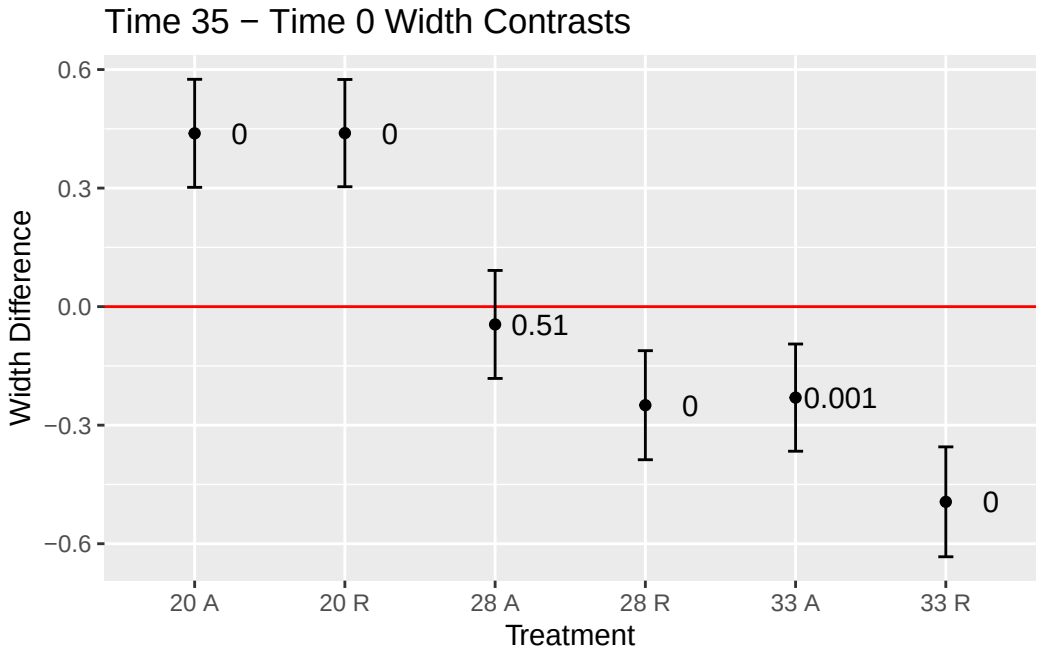
We will first test the significance of $s_k, s_k w_i, s_k t_j$ and then conduct contrasts between each treatment at time 0 and time 35.

```
Anova Table (Type III tests)

Response: measurement
                     Sum Sq  Df  F value    Pr(>F)
(Intercept)          17.761   1 295.2798 < 2.2e-16 ***
temperature           0.011   2   0.0873    0.9164
```

```
water                    0.047   1   0.7743    0.3792
time                     2.519   1  41.8837 1.978e-10 ***
temperature:water        0.058   2   0.4816    0.6180
temperature:time         6.009   2  49.9502 < 2.2e-16 ***
water:time               0.000   1   0.0000    0.9950
temperature:water:time   0.248   2   2.0611    0.1282
Residuals               37.051 616
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

We see that the two-way interaction between the sample time s_k and temperature t_j is significant. This indicates that the effect depends on temperature. The two-way interaction between the sample time s_k and water treatment w_i is not significant.



We have plotted the contrasts of time 35 - time 0 polyp widths. To the right of each confidence interval is the adjusted p-value of the test of the contrast being equal to 0.

The only treatment that does not differ over time is the treatment where temperature is 28 and water is antibiotic treated. All other treatments have differences between time 0 and time 35 between treatments.

For most treatments there is a difference between time 0 and time 35, but the nature of that difference depends primarily on the temperature of the water. For low temperatures the width of polyps are larger, for high temperatures the widths are smaller.