Trends in Giant Kelp Nitrogen Concentration and Surge Uptake

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Rationale and Research Questions

Kelp forests along the United States West Coast are impacted by increasing temperatures as a result of climate change. Since the late 19th century, Santa Barbara County in Southern California has had an increase in average temperature by 4.1 degrees Fahrenheit (Wilson, 2019). Kelp not only provides a healthy ecosystem for other flora and fauna but also provides ecosystem services to humans as well. Kelp assists with human-related environmental issues in two ways. First, kelp can sequester a large amount of carbon, aiding in climate change mitigation (Hurlimann, 2019). Secondly, they absorb excess nitrogen, which can lessen the impacts of agricultural runoff (Conroy, 2023).

A recent study has shown that increased nitrogen uptake by kelp can limit the effect that heat has on them. This is an interesting development because nitrogen and eutrophication are generally seen as negative consequences of human interference. However, in this case, it could assist kelp and help them survive increased climate-change-related temperature increases (Fernández, 2020). Interested in this recent research, data about nitrogen concentration amount and uptake were located in the Long-Term Ecological Research database titled "SBC LTER: Reef: Surge uptake capability in Macrocystis pyrifera in response to pulses of three different forms of nitrogen" (Cedeno, 2021) and "SBC LTER: Reef: Macrocystis pyrifera CHN content (carbon, hydrogen, nitrogen), ongoing since 2002" (Santa Barbara, 2021). This data looked simple enough to clean and manipulate, while also providing enough information for a research project.

We are interested in trends in giant kelp nitrogen concentration amount and nitrogen surge uptake. Specific questions include: 1. Has nitrogen concentration in kelp changed over the years (2002-2021)? 2. Does giant kelp nitrogen concentration amount vary by season? 3. Does nitrogen surge uptake vary between the spring and summer seasons? 4. Does giant kelp nitrogen surge uptake vary based on the amount of exposure? 5. Does uptake vary by type of nitrogen (ammonium, nitrate, and urea)?

Github Information: https://github.com/Tani-ValdezRivas/ValdezKuuskvereAnsbro

Dataset Information

Data sets were found on the Environmental Data Initiative. All sets were from the Santa Barbara Coastal Long Term Ecological Research Site (SBC LTER) which is based within the University of California, Santa Barbara (UCSB) Marine Science Institute, and is part of the National Science Foundation's LTER Network.

The first data set focuses on one experiment done that examines nitrogen and biomass uptake by kelp during timed nitrogen blasting. The first data set was used to determine if nitrogen uptake varies by time (minutes), season, or nitrogen type. The second data set has dates and percent nitrogen of kelp over almost a twenty year period. This second data set was used to gain a general sense of nitrogen found in kelp in Santa Barbara, CA and to see if that has changed over time.

Table 1: Data Set Information

Detail	Data Set 1	Data Set 2
Source	Santa Barbara Coastal Long	Santa Barbara Coastal Long
	Term Ecological Research	Term Ecological Research
	(LTER)	(LTER)
Retrieved from	https:	https://doi.org/10.6073/pasta/
	//portal.edirepository.org/nis/	48686fef076a23310a0e48f69d76260d
	mapbrowse?packageid=edi.1022.2	
Variables Used	Nitrogen Types, Season, Day	Year, Month, Date, Nitrogen
	Replicate, Time, Blade Replicate,	Percent
	Uptake Rate, Biomass Uptake	
	Rate	
Date	2021	2002-2021

The data for nitrogen uptake was cleaned by renaming the column names to be better understood by the public. Multiple columns including columns for Blade Replicate, Day Replicate, Time, Season, and Nitrogen Type were changed to factors. Season and Nitrogen Type also had labels added to capitalize the names of seasons and nitrogen types. Data cleaning for the second data set entailed renaming column names as well. Date was changed from a factor to a date data type. Sites with missing values were also removed.

Exploratory Analysis

The first part of the exploratory analysis focused on the dataset from the research study conducted on giant kelp over five days. Box plots were used to explore the relationship of the variables (Nitrogen Type, Season, Length of Exposure, Day Replicate, and Blade Replicate) against nitrogen uptake rate.

The metadata indicates that the giant kelp blades studied were collected during the nitrate replete spring and the nitrate-deplete summer. The five replicate experiments were conducted in both spring and summer of 2019, which allows us to find potential differences in the nitrogen surge uptake of the two populations. The experimenters calculate uptake rate using a modified version of an equation by Legendre and Grosselin (1997) V = (nt - n0)/[t(ds-d0)] where nt is the atom percentage of Nitrogen-15 in the blade after incubation; no is the average concentration of isotope in the control blades; ds is the atom percentage of Nitrogen-15 in the nitrate pool before isotope tracer was added; and t is the length of the incubation time in minutes.

Nitrogen Uptake Rate (V(hr1)) by Nitrogen Type

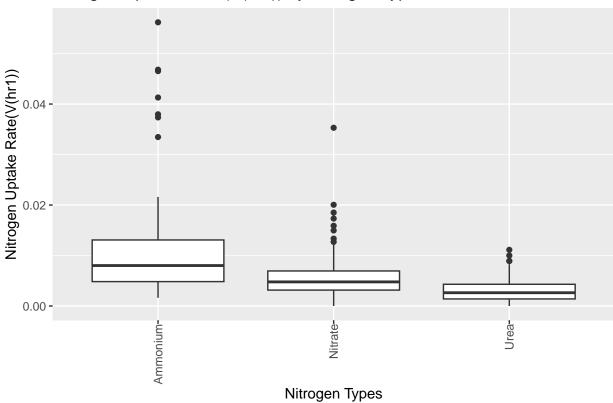


Figure 1: Uptake Rate by Nitrogen Type Box Plot

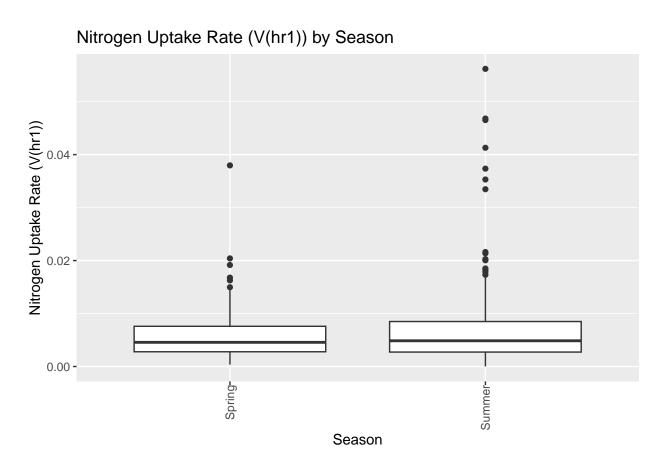


Figure 2: Uptake Rate by Season Box Plot

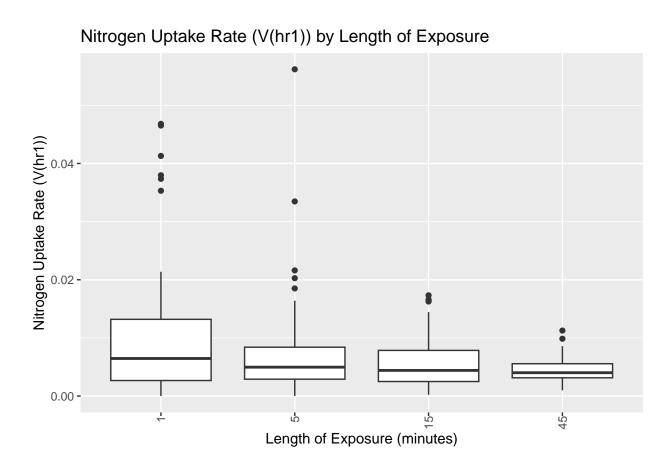


Figure 3: Uptake Rate by Exposure Length Box Plot

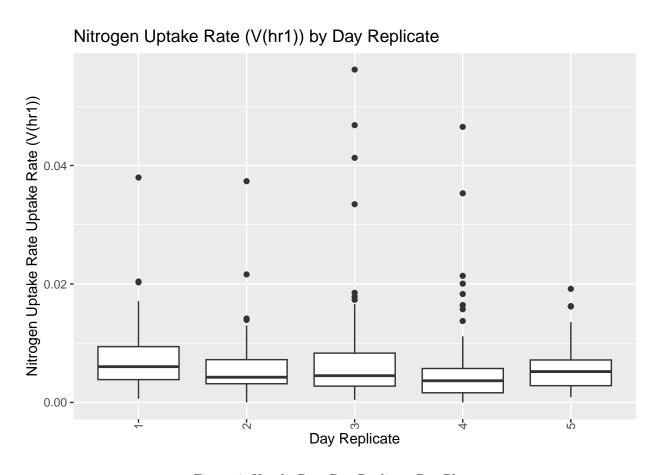
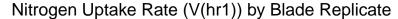
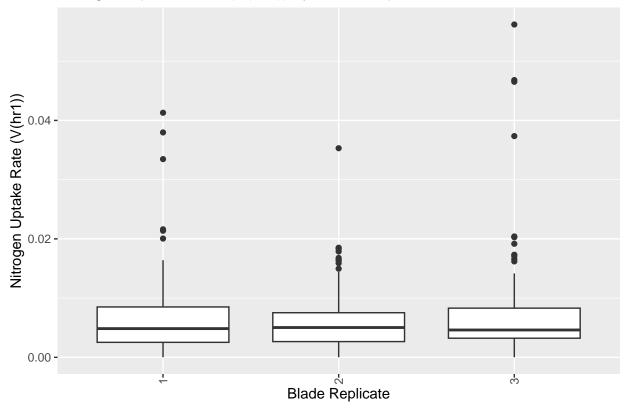


Figure 4: Uptake Rate Day Replicate Box Plot





Violin plots were used to understand the density of the variables when comparing with nitrogen uptake rate. See five violin plots below.

All of the day replicates and blade replicate nitrogen uptake rates were used when comparing the variables of Season, Length of Exposure, and Nitrogen Type were studied further. From both the box plot and violin plot, nitrogen uptake was high in the first minute. Summer also had slightly more and higher nitrogen uptake rates. Ammonium also had a higher range of nitrogen uptake rates than the other two nitrogen types. All three of these variables were studied further to understand if they contributed to a significant different of nitrogen uptake rate.

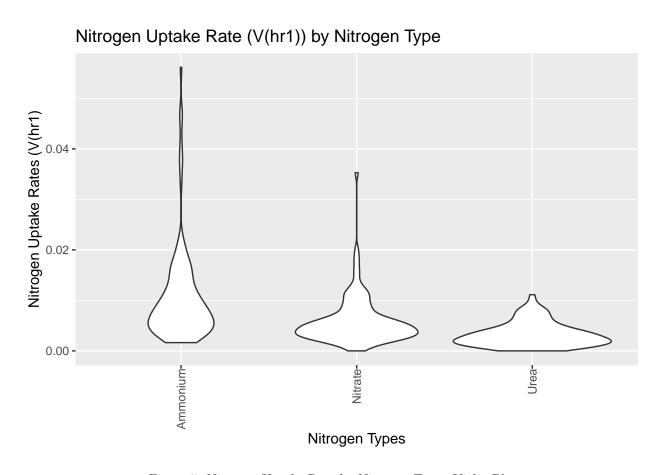


Figure 5: Nitrogen Uptake Rate by Nitrogen Types Violin Plot

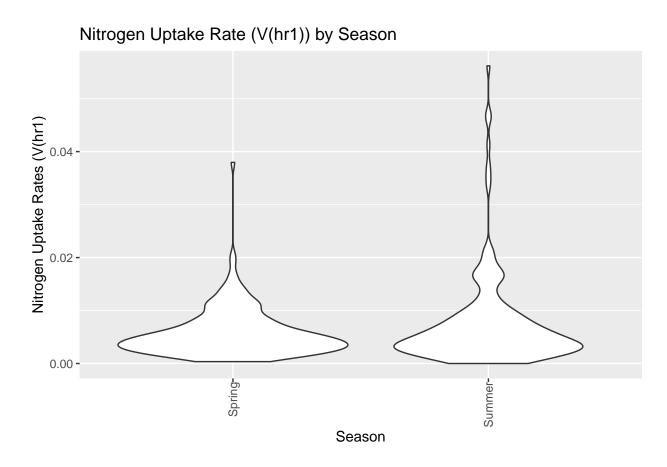


Figure 6: Nitrogen Uptake Rate by Season Violin Plot

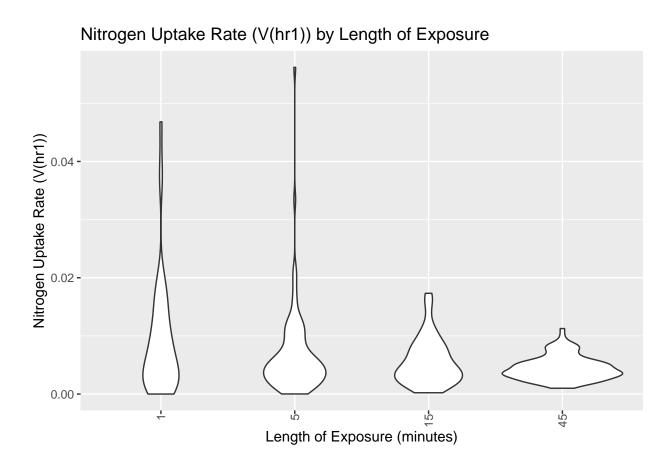


Figure 7: Nitrogen Uptake Rate by Length of Exposure Violin Plot

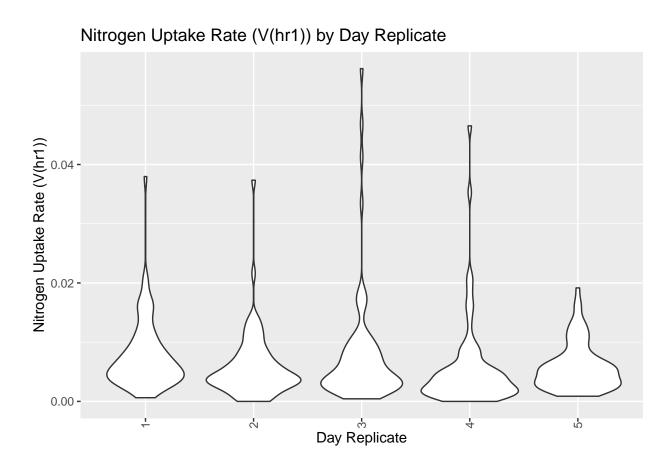


Figure 8: Nitrogen Uptake Rate by Day Replicate Violin Plot

Nitrogen Uptake Rate (V(hr1)) by Blade Replicate

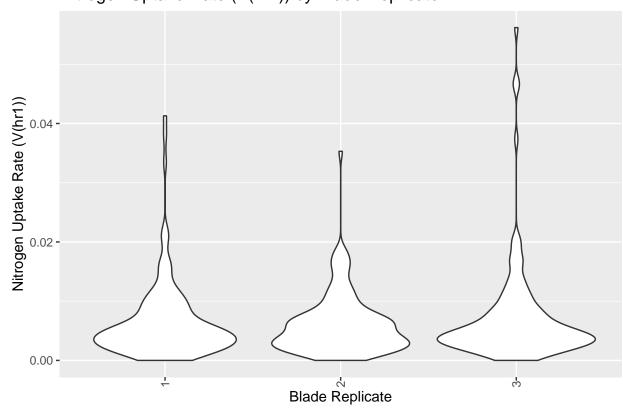


Figure 9: Nitrogen Uptake Rate by Blade Replicate Violin Plot

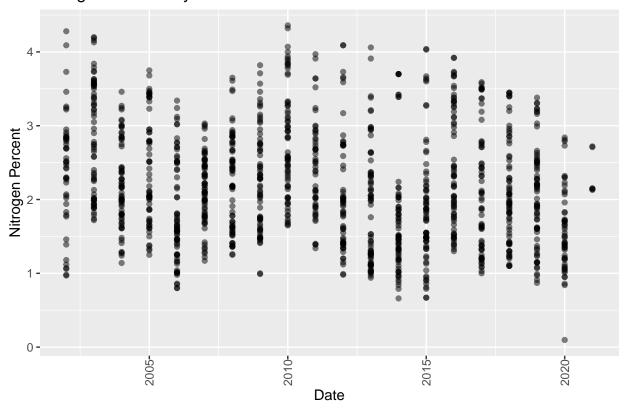
The second part of the exploratory analysis focused on an overview of nitrogen concentration amount over the last twenty years. A summary was used on the entire data set as well as on the nitrogen concentration column to gain a general sense of what amounts were present.

```
##
                      Sample_Number
                                             Year
                                                            Month
##
                3.0
                                               :2002
    Min.
                      Min.
                            : 1.0
                                       Min.
                                                       Min.
                                                               : 1.000
##
    1st Qu.: 337.2
                      1st Qu.:176.0
                                       1st Qu.:2006
                                                       1st Qu.: 4.000
    Median : 665.5
                      Median :352.0
                                       Median:2011
                                                       Median: 7.000
           : 671.5
##
    Mean
                      Mean
                              :352.1
                                       Mean
                                               :2011
                                                       Mean
                                                               : 6.602
##
    3rd Qu.:1007.8
                      3rd Qu.:513.0
                                       3rd Qu.:2016
                                                       3rd Qu.:10.000
##
    Max.
            :1334.0
                              :910.0
                                               :2021
                                                               :12.000
                      Max.
                                       Max.
                                                       Max.
##
##
            Date
                          Site
                                    Number of Samples Sample Replicate
##
    2014-03-12:
                   6
                       ABUR :434
                                    Min.
                                           : 8.00
                                                       Min.
                                                               :1.0
##
    2003-01-16:
                   4
                       ABUR: 2
                                    1st Qu.:15.00
                                                       1st Qu.:1.0
##
    2003-02-18:
                   4
                       AQUE :410
                                    Median :15.00
                                                       Median:1.5
##
    2003-03-05:
                   4
                       MOHK: 436
                                    Mean
                                            :14.67
                                                       Mean
                                                               :1.5
##
    2007-01-03:
                   4
                                    3rd Qu.:15.00
                                                       3rd Qu.:2.0
##
    2007-02-06:
                   4
                                    Max.
                                            :16.00
                                                       Max.
                                                               :2.0
##
    (Other)
               :1256
##
      Wet_Weight
                            Dry_Weight
                                               Ratio_Dry_to_Wet
##
    Min.
           :-99999.00
                         Min.
                                 :-99999.00
                                               Min.
                                                      :-99999.00
                         1st Qu.:
                                       0.48
    1st Qu.:
                  5.88
                                               1st Qu.:
                                                             0.07
##
    Median :
                  7.02
                         Median :
                                               {\tt Median} :
                                       0.66
                                                             0.09
              -616.79
                                    -779.33
                                                         -779.93
##
    Mean
           :
                         Mean
                                               Mean
##
    3rd Qu.:
                  8.53
                         3rd Qu.:
                                       0.86
                                               3rd Qu.:
                                                             0.11
##
    Max.
                 17.19
                         Max.
                                       2.79
                                               Max.
                                                             0.38
##
##
    Analytical_Dry_Weight Carbon_Percent
                                            Hydrogen_Percent
                                                                  Nitrogen_Percent
    Min.
                           Min.
                                   :20.25
                                            Min.
                                                    :-99999.00
                                                                  Min.
                                                                          :0.097
##
            :1797
                                             1st Qu.:
##
    1st Qu.:2155
                            1st Qu.:28.39
                                                           4.01
                                                                  1st Qu.:1.570
    Median:2310
                           Median :31.33
                                                                  Median :2.063
##
                                             Median:
                                                           4.72
                                                    : -4675.62
##
    Mean
            :2394
                           Mean
                                   :32.17
                                                                  Mean
                                                                          :2.174
                                            Mean
    3rd Qu.:2517
##
                            3rd Qu.:35.27
                                             3rd Qu.:
                                                           5.27
                                                                  3rd Qu.:2.710
##
    Max.
            :4770
                                   :44.52
                                                           7.57
                                                                  Max.
                                                                          :4.360
                           Max.
                                            Max.
##
##
    Carbon_Nitrogen_Ratio
##
          : 6.64
##
    1st Qu.:11.43
    Median :14.94
##
           :16.89
##
    Mean
    3rd Qu.:20.86
            :48.16
##
    Max.
##
##
                                                               Notes
##
                                                                  :1247
##
    dry_wet ratio very large, possible processing error
##
    reproducibility and replicate data noisy. Will reanalyze .:
                                                                      6
    missing dry wt
##
                                                                      4
##
    used average of individual plants
                                                                      4
##
    Average of 10 composite samples
                                                                      2
##
    (Other)
                                                                     13
##
               NewSites
##
    Arroyo Burro :436
```

```
##
    Arroyo Quemado:410
##
    Mohawk
                    :436
##
##
##
##
##
      Min. 1st Qu.
                     Median
                                Mean 3rd Qu.
                                                  Max.
     0.097
              1.570
                       2.063
                                2.174
                                        2.710
                                                 4.360
##
```

A scatter plot was used to visualize the relationship between year and nitrogen concentration amount.

Nitrogen Percent by Year



Lastly, a boxplot, see below, was used to visualize the relationship between sites and nitrogen concentration. Looking at this data, it appeared concentration amount was not too different by site. Looking at the scatter plot for year, it looked like nitrogen decreased slightly. This was then explored further in the Data Analysis section.

```
#Data Exploration Part 3

NitrogenYears_Box <- ggplot(NitrogenYears, aes(x=NewSites, y=Nitrogen_Percent))+
    geom_boxplot()+
    labs(title="Nitrogen Percent by Site", x="Site", y="Nitrogen Percent")

ggsave(file=here('Output', 'ExploratoryAnalysis','NitrogenConcentrationBoxbySite.png'), width = 10, hei
print(NitrogenYears_Box)</pre>
```

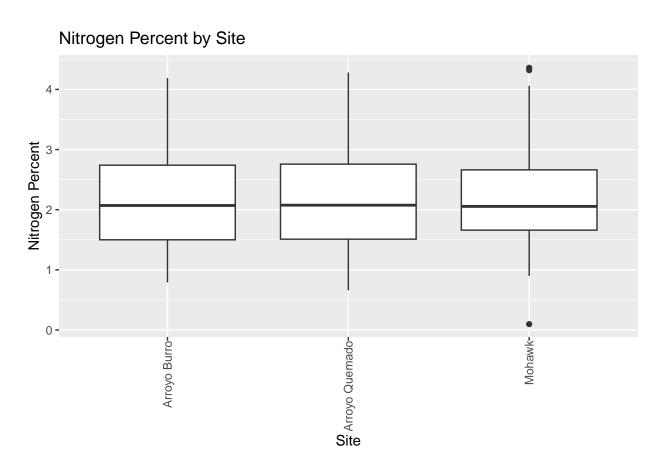


Figure 10: Exploratory Analysis for Nitrogen Concentration by Site

Analysis

Question 1: Does giant kelp nitrogen percent vary over year or season?

For the first question, we wanted to understand the relationship between nitrogen concentration and time. Linear regression and normal distribution tests were run for both the year and the specific date of collection. The box plot below shows the nitrogen concentrations over the years, starting with 2002.

H0: There is no significant difference of nitrogen percent over the past twenty years. HA: There is significant difference of nitrogen percent over the past twenty years.

The interaction between nitrogen concentration and year is significant (p < 2.2e-16). The interaction between nitrogen concentration and collection date is significant (p < 0.001). We can reject the null hypothesis and accept that there is significant difference of nitrogen percent from 2002 to 2021. The R-squared is .974, so a high amount of nitrogen concentration variance is in response to date change. Below is a box plot showing change in nitrogen concentration over time.

```
#2. LM for Date
NitrogenRegressionDate <- lm(NitrogenYears$Nitrogen_Percent ~ NitrogenYears$Date)
#Linear regression on the nitrogen percent amount by year. P-value is less than .05. Which means there
summary(NitrogenRegressionDate)</pre>
```

```
##
## Call:
## lm(formula = NitrogenYears$Nitrogen_Percent ~ NitrogenYears$Date)
##
## Residuals:
##
     Min
              1Q Median
                            3Q
                                 Max
## -0.965 -0.030 0.000 0.030
                               0.985
##
## Coefficients:
##
                                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                 2.800e+00 1.165e-01 24.033 < 2e-16 ***
## NitrogenYears$Date2002-05-10 -2.450e-01 1.648e-01
                                                      -1.487 0.137461
## NitrogenYears$Date2002-05-15 7.950e-01
                                           1.648e-01
                                                       4.825 1.71e-06 ***
## NitrogenYears$Date2002-06-10 -1.020e+00
                                           1.648e-01
                                                      -6.191 1.00e-09 ***
## NitrogenYears$Date2002-06-12 2.150e-01
                                           1.648e-01
                                                       1.305 0.192348
## NitrogenYears$Date2002-06-13 1.385e+00
                                           1.648e-01
                                                       8.406 2.23e-16 ***
## NitrogenYears$Date2002-07-10 -3.100e-01
                                           1.648e-01
                                                      -1.881 0.060310
## NitrogenYears$Date2002-07-11 -1.735e+00
                                           1.648e-01 -10.530 < 2e-16 ***
## NitrogenYears$Date2002-07-12 -6.750e-01
                                           1.648e-01
                                                      -4.097 4.66e-05 ***
## NitrogenYears$Date2002-08-07 -9.150e-01
                                           1.648e-01
                                                      -5.553 3.93e-08 ***
## NitrogenYears$Date2002-08-08 -7.400e-01 1.648e-01
                                                      -4.491 8.23e-06 ***
## NitrogenYears$Date2002-08-09 3.500e-02 1.648e-01
                                                       0.212 0.831838
## NitrogenYears$Date2002-09-04 -1.650e+00
                                           1.648e-01 -10.014 < 2e-16 ***
## NitrogenYears$Date2002-09-05 -5.200e-01
                                           1.648e-01
                                                      -3.156 0.001666 **
## NitrogenYears$Date2002-09-10 -3.050e-01
                                           1.648e-01
                                                      -1.851 0.064560
## NitrogenYears$Date2002-10-09 -1.375e+00 1.648e-01
                                                      -8.345 3.57e-16 ***
## NitrogenYears$Date2002-10-10 -1.825e+00
                                           1.648e-01 -11.076 < 2e-16 ***
## NitrogenYears$Date2002-10-11 5.000e-02
                                           1.648e-01
                                                       0.303 0.761627
## NitrogenYears$Date2002-11-20 4.500e-01
                                           1.648e-01
                                                       2.731 0.006465 **
## NitrogenYears$Date2002-11-21 -7.500e-02
                                           1.648e-01
                                                      -0.455 0.649109
## NitrogenYears$Date2002-11-22 1.000e-01
                                           1.648e-01
                                                       0.607 0.544095
```

```
-1.760 0.078817 .
## NitrogenYears$Date2002-12-06 -2.900e-01
                                             1.648e-01
                                             1.648e-01
## NitrogenYears$Date2002-12-09 -5.050e-01
                                                         -3.065 0.002258 **
                                                          0.121 0.903421
## NitrogenYears$Date2002-12-10
                                 2.000e-02
                                             1.648e-01
## NitrogenYears$Date2003-01-14 -2.200e-01
                                             1.648e-01
                                                         -1.335 0.182222
## NitrogenYears$Date2003-01-16 -4.550e-01
                                             1.427e-01
                                                         -3.189 0.001490 **
  NitrogenYears$Date2003-02-18 -8.475e-01
                                                         -5.939 4.43e-09
                                             1.427e-01
## NitrogenYears$Date2003-02-21 -9.950e-01
                                             1.648e-01
                                                         -6.039 2.47e-09
## NitrogenYears$Date2003-03-05 -6.250e-02
                                             1.427e-01
                                                         -0.438 0.661512
  NitrogenYears$Date2003-03-06 -7.550e-01
                                             1.648e-01
                                                         -4.582 5.41e-06 ***
  NitrogenYears$Date2003-04-08
                                  5.350e-01
                                             1.648e-01
                                                          3.247 0.001220 **
## NitrogenYears$Date2003-04-10
                                  2.900e-01
                                             1.648e-01
                                                          1.760 0.078817
  NitrogenYears$Date2003-04-15
                                  1.380e+00
                                             1.648e-01
                                                          8.375 2.82e-16
  NitrogenYears$Date2003-05-06
                                                          5.037 5.96e-07 ***
                                  8.300e-01
                                             1.648e-01
  NitrogenYears$Date2003-05-12
                                  7.400e-01
                                             1.648e-01
                                                          4.491 8.23e-06 ***
## NitrogenYears$Date2003-05-13
                                  3.950e-01
                                             1.648e-01
                                                          2.397 0.016766
  NitrogenYears$Date2003-06-03
                                             1.648e-01
                                                          5.675 2.01e-08
                                  9.350e-01
  NitrogenYears$Date2003-06-10 -6.000e-01
                                                         -3.642 0.000290 ***
                                             1.648e-01
## NitrogenYears$Date2003-06-11
                                  1.360e+00
                                             1.648e-01
                                                          8.254 7.18e-16
## NitrogenYears$Date2003-07-02
                                                          6.160 1.20e-09 ***
                                  1.015e+00
                                             1.648e-01
                                                         -6.494 1.55e-10
## NitrogenYears$Date2003-07-07 -1.070e+00
                                             1.648e-01
## NitrogenYears$Date2003-07-15 -5.200e-01
                                             1.648e-01
                                                         -3.156 0.001666
## NitrogenYears$Date2003-08-05 -1.200e-01
                                             1.648e-01
                                                         -0.728 0.466663
## NitrogenYears$Date2003-08-06
                                                          3.915 9.90e-05 ***
                                  6.450e-01
                                             1.648e-01
## NitrogenYears$Date2003-08-08
                                  7.800e-01
                                             1.648e-01
                                                          4.734 2.65e-06 ***
## NitrogenYears$Date2003-09-02
                                  7.850e-01
                                             1.648e-01
                                                          4.764 2.29e-06
## NitrogenYears$Date2003-09-04
                                  7.500e-01
                                             1.648e-01
                                                          4.552 6.23e-06 ***
  NitrogenYears$Date2003-09-05
                                  5.700e-01
                                             1.648e-01
                                                          3.459 0.000573 ***
                                  1.300e-01
  NitrogenYears$Date2003-10-01
                                             1.648e-01
                                                          0.789 0.430373
  NitrogenYears$Date2003-11-06
                                  4.600e-01
                                             1.648e-01
                                                          2.792 0.005379 **
## NitrogenYears$Date2003-11-13 -7.900e-01
                                             1.648e-01
                                                         -4.795 1.98e-06 ***
  NitrogenYears$Date2003-11-14 -8.150e-01
                                             1.648e-01
                                                         -4.946 9.40e-07
  NitrogenYears$Date2003-12-03 -9.000e-01
                                             1.648e-01
                                                         -5.462 6.46e-08
  NitrogenYears$Date2003-12-05
                                                          4.036 6.01e-05 ***
                                  6.650e-01
                                             1.648e-01
  NitrogenYears$Date2003-12-08 -9.150e-01
                                                         -5.553 3.93e-08
                                             1.648e-01
  NitrogenYears$Date2004-01-07
                                  7.000e-02
                                             1.648e-01
                                                          0.425 0.671077
## NitrogenYears$Date2004-01-09
                                  4.450e-01
                                             1.648e-01
                                                          2.701 0.007078 **
## NitrogenYears$Date2004-02-02
                                  8.000e-02
                                             1.648e-01
                                                          0.486 0.627444
## NitrogenYears$Date2004-02-06
                                  1.950e-01
                                             1.648e-01
                                                          1.183 0.237001
## NitrogenYears$Date2004-02-12
                                  1.950e-01
                                             1.648e-01
                                                          1.183 0.237001
## NitrogenYears$Date2004-03-08 -3.700e-01
                                             1.648e-01
                                                         -2.246 0.025029 *
## NitrogenYears$Date2004-03-10 -8.200e-01
                                             1.648e-01
                                                         -4.977 8.08e-07
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## NitrogenYears$Date2009-10-07 -5.200e-01
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## NitrogenYears$Date2010-03-02 -6.350e-01
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## NitrogenYears$Date2010-03-05 -3.550e-01
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## NitrogenYears$Date2010-06-04
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## NitrogenYears$Date2010-07-01 -8.500e-01
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## NitrogenYears$Date2010-07-08
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## NitrogenYears$Date2010-11-01 -4.450e-01
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## NitrogenYears$Date2011-02-10 -9.100e-01
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## NitrogenYears$Date2011-03-03
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## NitrogenYears$Date2011-03-04 -4.350e-01
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## NitrogenYears$Date2011-04-01 -8.750e-02
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## NitrogenYears$Date2011-06-07 -3.900e-01
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## NitrogenYears$Date2011-07-07 -8.450e-01
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                                                        -5.922 4.91e-09 ***
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## NitrogenYears$Date2011-09-06 -8.900e-01
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## NitrogenYears$Date2011-10-04 -1.410e+00
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## NitrogenYears$Date2011-11-01 -5.850e-01
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## NitrogenYears$Date2011-12-05
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## NitrogenYears$Date2012-03-05 -4.000e-02
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## NitrogenYears$Date2012-04-10 -3.500e-02
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## NitrogenYears$Date2012-05-02
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## NitrogenYears$Date2012-05-04
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                                                        -4.916 1.09e-06 ***
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## NitrogenYears$Date2012-06-07 -3.650e-01
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## NitrogenYears$Date2012-07-10 -9.900e-01
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## NitrogenYears$Date2012-09-06 -1.335e+00
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## NitrogenYears$Date2012-10-10 -1.540e+00
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## NitrogenYears$Date2013-02-07
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## NitrogenYears$Date2013-03-11 -7.450e-01
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## NitrogenYears$Date2013-03-12
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## NitrogenYears$Date2013-04-02 -1.405e+00
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## NitrogenYears$Date2013-04-04 -7.950e-01
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## NitrogenYears$Date2013-04-10 -1.600e-01
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## NitrogenYears$Date2013-05-01 -5.700e-01
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## NitrogenYears$Date2013-06-04 -4.550e-01
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## NitrogenYears$Date2013-06-05 -1.290e+00
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                                                        -7.829 1.73e-14 ***
## NitrogenYears$Date2013-06-10 -1.425e+00
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## NitrogenYears$Date2013-07-01 -1.535e+00
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## NitrogenYears$Date2013-07-02 -6.650e-01
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## NitrogenYears$Date2013-07-03 -4.550e-01
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## NitrogenYears$Date2013-08-05 -9.300e-01
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## NitrogenYears$Date2013-08-06 -7.700e-01
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## NitrogenYears$Date2013-08-08 -1.530e+00
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## NitrogenYears$Date2013-09-03 -1.705e+00
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## NitrogenYears$Date2013-09-30 -1.560e+00
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## NitrogenYears$Date2013-10-04 -1.500e+00
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## NitrogenYears$Date2013-12-03 -1.768e+00
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## NitrogenYears$Date2013-12-06 -1.785e+00
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## NitrogenYears$Date2014-01-07 -1.295e+00
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## NitrogenYears$Date2014-02-03 -9.000e-01
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                                                        -5.462 6.46e-08 ***
## NitrogenYears$Date2014-02-04 -6.400e-01
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## NitrogenYears$Date2014-02-06 -1.100e+00
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## NitrogenYears$Date2014-03-12 -1.237e+00
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## NitrogenYears$Date2014-04-02
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## NitrogenYears$Date2014-04-08 -7.050e-01
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## NitrogenYears$Date2014-04-09
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                                             1.648e-01
## NitrogenYears$Date2014-05-01
                                 9.000e-01
                                                         5.462 6.46e-08 ***
## NitrogenYears$Date2014-05-02 -7.050e-01
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## NitrogenYears$Date2014-05-09
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## NitrogenYears$Date2014-06-02 -1.800e+00
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## NitrogenYears$Date2014-07-08 -1.410e+00
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## NitrogenYears$Date2014-08-01 -1.375e+00
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## NitrogenYears$Date2014-08-04 -9.450e-01
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## NitrogenYears$Date2015-01-06 -1.415e+00
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## NitrogenYears$Date2015-01-07 -5.750e-01
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## NitrogenYears$Date2015-02-03 -1.320e+00
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## NitrogenYears$Date2015-02-05 -1.215e+00
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## NitrogenYears$Date2015-02-07 -6.400e-01
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## NitrogenYears$Date2015-03-04 -5.000e-03
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## NitrogenYears$Date2015-03-06 -2.130e+00
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## NitrogenYears$Date2015-04-06 -1.075e+00
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## NitrogenYears$Date2015-04-08 -1.375e+00
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## NitrogenYears$Date2015-05-04
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## NitrogenYears$Date2015-05-07
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## NitrogenYears$Date2015-05-12
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```

```
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## NitrogenYears$Date2015-06-04 -7.000e-01
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## NitrogenYears$Date2015-06-09 -9.000e-01
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                                                        -5.462 6.46e-08 ***
## NitrogenYears$Date2015-07-02 -1.105e+00
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## NitrogenYears$Date2015-07-08 -1.800e+00
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## NitrogenYears$Date2015-07-13 -1.290e+00
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## NitrogenYears$Date2015-08-03 -1.915e+00
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## NitrogenYears$Date2015-08-05 -1.340e+00
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## NitrogenYears$Date2015-08-06 -1.630e+00
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## NitrogenYears$Date2015-09-02 -1.250e+00
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## NitrogenYears$Date2015-09-03 -1.610e+00
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                                                                < 2e-16 ***
## NitrogenYears$Date2015-09-04 -2.000e+00
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                                                                < 2e-16
  NitrogenYears$Date2015-10-01 -1.305e+00
                                                        -7.920 8.87e-15
                                             1.648e-01
## NitrogenYears$Date2015-10-02 -1.880e+00
                                             1.648e-01 -11.410
                                                                < 2e-16
                                                        -8.588 < 2e-16 ***
## NitrogenYears$Date2015-10-05 -1.415e+00
                                             1.648e-01
## NitrogenYears$Date2015-11-05 -6.800e-01
                                             1.648e-01
                                                        -4.127 4.10e-05 ***
## NitrogenYears$Date2015-11-09 -8.800e-01
                                             1.648e-01
                                                        -5.341 1.24e-07 ***
## NitrogenYears$Date2015-11-11 -1.500e-01
                                                        -0.910 0.362925
                                             1.648e-01
## NitrogenYears$Date2015-12-02 -9.825e-01
                                                        -6.885 1.25e-11 ***
                                             1.427e-01
## NitrogenYears$Date2015-12-03 -5.050e-01
                                             1.648e-01
                                                        -3.065 0.002258
## NitrogenYears$Date2016-01-09
                                 1.200e-01
                                             1.648e-01
                                                         0.728 0.466663
## NitrogenYears$Date2016-01-10
                                 2.750e-01
                                             1.648e-01
                                                         1.669 0.095543
## NitrogenYears$Date2016-01-11
                                                         3.247 0.001220 **
                                 5.350e-01
                                             1.648e-01
## NitrogenYears$Date2016-02-07
                                 8.175e-01
                                             1.427e-01
                                                         5.729 1.48e-08
## NitrogenYears$Date2016-02-09
                                 8.800e-01
                                             1.648e-01
                                                         5.341 1.24e-07 ***
## NitrogenYears$Date2016-03-09 -4.550e-01
                                             1.648e-01
                                                        -2.761 0.005899 **
## NitrogenYears$Date2016-03-10 -1.098e+00
                                             1.427e-01
                                                        -7.691 4.73e-14
## NitrogenYears$Date2016-04-05
                                 9.250e-01
                                             1.648e-01
                                                         5.614 2.81e-08 ***
  NitrogenYears$Date2016-04-07
                                  5.100e-01
                                             1.427e-01
                                                         3.574 0.000375 ***
## NitrogenYears$Date2016-05-03
                                                         4.765 2.27e-06 ***
                                  6.800e-01
                                             1.427e-01
## NitrogenYears$Date2016-05-05
                                  1.650e-01
                                             1.648e-01
                                                          1.001 0.316959
  NitrogenYears$Date2016-06-01
                                 4.800e-01
                                             1.648e-01
                                                         2.913 0.003687 **
## NitrogenYears$Date2016-06-07 -8.250e-01
                                                        -5.007 6.94e-07
                                             1.648e-01
## NitrogenYears$Date2016-06-11 -6.850e-01
                                                        -4.157 3.60e-05
                                             1.648e-01
## NitrogenYears$Date2016-07-07 -1.245e+00
                                             1.648e-01
                                                        -7.556 1.25e-13
## NitrogenYears$Date2016-07-08 -1.375e+00
                                             1.648e-01
                                                        -8.345 3.57e-16
## NitrogenYears$Date2016-07-11 -4.350e-01
                                             1.648e-01
                                                        -2.640 0.008466 **
## NitrogenYears$Date2016-08-02 -1.485e+00
                                                        -9.013 < 2e-16 ***
                                             1.648e-01
## NitrogenYears$Date2016-08-04 -1.235e+00
                                                        -7.495 1.92e-13
                                             1.648e-01
## NitrogenYears$Date2016-08-08 -4.900e-01
                                             1.648e-01
                                                        -2.974 0.003038 **
## NitrogenYears$Date2016-09-01 -6.950e-01
                                             1.648e-01
                                                        -4.218 2.77e-05 ***
## NitrogenYears$Date2016-09-07 -1.035e+00
                                                        -6.282 5.76e-10 ***
                                             1.648e-01
## NitrogenYears$Date2016-09-08 -9.450e-01
                                             1.648e-01
                                                        -5.735 1.43e-08 ***
  NitrogenYears$Date2016-10-03 -8.750e-01
                                                        -5.311 1.45e-07 ***
                                             1.648e-01
## NitrogenYears$Date2016-10-04 -7.550e-01
                                             1.648e-01
                                                        -4.582 5.41e-06 ***
## NitrogenYears$Date2016-10-06 -4.700e-01
                                             1.648e-01
                                                        -2.853 0.004460 **
## NitrogenYears$Date2016-11-01 -4.350e-01
                                             1.648e-01
                                                        -2.640 0.008466 **
## NitrogenYears$Date2016-11-02 -1.210e+00
                                             1.648e-01
                                                        -7.344 5.58e-13 ***
## NitrogenYears$Date2016-11-04 -1.315e+00
                                             1.648e-01
                                                        -7.981 5.65e-15 ***
## NitrogenYears$Date2016-12-02 -1.025e+00
                                             1.648e-01
                                                        -6.221 8.33e-10
## NitrogenYears$Date2016-12-06 -1.385e+00
                                                        -8.406 2.23e-16 ***
                                             1.648e-01
## NitrogenYears$Date2016-12-08 -3.450e-01
                                             1.648e-01
                                                        -2.094 0.036617
## NitrogenYears$Date2017-01-04 -2.100e-01
                                             1.427e-01
                                                        -1.472 0.141535
## NitrogenYears$Date2017-01-10 -1.150e+00
                                             1.648e-01
                                                        -6.980 6.67e-12 ***
```

```
## NitrogenYears$Date2017-02-01 -4.850e-01
                                             1.427e-01
                                                        -3.399 0.000713 ***
                                             1.648e-01
## NitrogenYears$Date2017-02-09 -5.500e-02
                                                        -0.334 0.738623
                                                         3.364 0.000809 ***
## NitrogenYears$Date2017-03-07
                                 4.800e-01
                                             1.427e-01
## NitrogenYears$Date2017-03-10 -3.700e-01
                                             1.648e-01
                                                        -2.246 0.025029
## NitrogenYears$Date2017-04-04
                                 7.550e-01
                                             1.648e-01
                                                         4.582 5.41e-06
## NitrogenYears$Date2017-04-11
                                 7.050e-01
                                                         4.279 2.13e-05
                                             1.648e-01
## NitrogenYears$Date2017-05-03
                                 7.300e-01
                                             1.648e-01
                                                         4.431 1.08e-05 ***
## NitrogenYears$Date2017-05-04
                                 6.950e-01
                                             1.648e-01
                                                         4.218 2.77e-05 ***
## NitrogenYears$Date2017-06-01
                                 4.000e-02
                                             1.648e-01
                                                         0.243 0.808254
## NitrogenYears$Date2017-06-06 -2.500e-02
                                             1.648e-01
                                                        -0.152 0.879442
## NitrogenYears$Date2017-07-07 -1.220e+00
                                             1.427e-01
                                                        -8.550
                                                                < 2e-16 ***
## NitrogenYears$Date2017-07-11 -6.900e-01
                                             1.648e-01
                                                        -4.188 3.16e-05
                                             1.427e-01 -10.740
## NitrogenYears$Date2017-08-01 -1.533e+00
                                                                < 2e-16
## NitrogenYears$Date2017-08-03 -1.445e+00
                                             1.648e-01
                                                        -8.770
                                                                < 2e-16
## NitrogenYears$Date2017-09-07 -1.735e+00
                                             1.427e-01 -12.159
                                                                < 2e-16 ***
## NitrogenYears$Date2017-09-10 -1.425e+00
                                             1.648e-01
                                                        -8.649
                                                                 < 2e-16
## NitrogenYears$Date2017-10-02 -1.610e+00
                                             1.648e-01
                                                        -9.771
                                                                < 2e-16 ***
## NitrogenYears$Date2017-10-03 -1.245e+00
                                                        -8.725
                                             1.427e-01
                                                                < 2e-16
                                             1.648e-01
                                                        -9.650
## NitrogenYears$Date2017-11-02 -1.590e+00
                                                                < 2e-16 ***
## NitrogenYears$Date2017-11-03 -1.223e+00
                                             1.427e-01
                                                        -8.567
                                                                < 2e-16
## NitrogenYears$Date2017-12-06 -1.185e+00
                                             1.648e-01
                                                        -7.192 1.59e-12
## NitrogenYears$Date2017-12-07 -8.225e-01
                                             1.427e-01
                                                        -5.764 1.21e-08 ***
                                             1.648e-01 -10.257
## NitrogenYears$Date2018-01-23 -1.690e+00
                                                                < 2e-16 ***
## NitrogenYears$Date2018-01-24 -6.600e-01
                                             1.648e-01
                                                        -4.006 6.82e-05 ***
                                             1.648e-01
## NitrogenYears$Date2018-01-25 -9.850e-01
                                                        -5.978 3.53e-09 ***
## NitrogenYears$Date2018-02-15 -1.450e+00
                                             1.648e-01
                                                        -8.800
                                                                < 2e-16 ***
## NitrogenYears$Date2018-02-16 -1.575e+00
                                             1.427e-01 -11.038
                                                                < 2e-16
## NitrogenYears$Date2018-03-07
                                             1.427e-01
                                                         3.662 0.000269
                                 5.225e-01
## NitrogenYears$Date2018-03-12 -2.000e-02
                                             1.648e-01
                                                        -0.121 0.903421
## NitrogenYears$Date2018-04-03
                                 6.300e-01
                                             1.648e-01
                                                         3.824 0.000143 ***
## NitrogenYears$Date2018-04-05
                                 1.800e-01
                                             1.648e-01
                                                         1.092 0.274996
  NitrogenYears$Date2018-04-06 -2.400e-01
                                             1.648e-01
                                                        -1.457 0.145657
## NitrogenYears$Date2018-05-02 -5.883e-01
                                                        -3.570 0.000380 ***
                                             1.648e-01
## NitrogenYears$Date2018-05-04 -8.295e-01
                                             1.427e-01
                                                        -5.813 9.16e-09
## NitrogenYears$Date2018-06-04 -1.041e-01
                                             1.427e-01
                                                        -0.730 0.465777
## NitrogenYears$Date2018-06-06 6.209e-01
                                             1.648e-01
                                                         3.768 0.000178 ***
## NitrogenYears$Date2018-07-05 -1.287e+00
                                             1.648e-01
                                                        -7.813 1.95e-14 ***
## NitrogenYears$Date2018-07-06 -1.453e+00
                                                        -8.819 < 2e-16 ***
                                             1.648e-01
## NitrogenYears$Date2018-07-10
                                 9.197e-02
                                             1.648e-01
                                                         0.558 0.576896
## NitrogenYears$Date2018-08-06 -9.366e-01
                                             1.648e-01
                                                        -5.684 1.90e-08 ***
## NitrogenYears$Date2018-08-13 -8.452e-01
                                             1.427e-01
                                                        -5.923 4.86e-09
## NitrogenYears$Date2018-09-06 -5.088e-01
                                                        -3.088 0.002091
                                             1.648e-01
## NitrogenYears$Date2018-09-07 -6.591e-01
                                             1.427e-01
                                                        -4.619 4.55e-06
## NitrogenYears$Date2018-10-04 -1.030e+00
                                                        -7.219 1.32e-12 ***
                                             1.427e-01
## NitrogenYears$Date2018-10-05 -1.400e+00
                                             1.648e-01
                                                        -8.496
                                                                < 2e-16 ***
## NitrogenYears$Date2018-11-01 -1.565e+00
                                             1.648e-01
                                                        -9.498
                                                                < 2e-16
                                             1.427e-01 -10.074
## NitrogenYears$Date2018-11-02 -1.438e+00
                                                                < 2e-16
## NitrogenYears$Date2018-12-04 -7.850e-01
                                             1.648e-01
                                                        -4.764 2.29e-06
## NitrogenYears$Date2018-12-11 -1.055e+00
                                             1.427e-01
                                                        -7.394 3.94e-13 ***
## NitrogenYears$Date2019-01-03 -1.290e+00
                                             1.648e-01
                                                        -7.829 1.73e-14
## NitrogenYears$Date2019-01-04 -1.158e+00
                                                        -8.112 2.12e-15 ***
                                             1.427e-01
## NitrogenYears$Date2019-02-07 -5.500e-01
                                             1.427e-01
                                                        -3.854 0.000126
## NitrogenYears$Date2019-02-08 -1.850e-01
                                                        -1.123 0.261893
                                             1.648e-01
## NitrogenYears$Date2019-03-04 -7.550e-01
                                             1.427e-01
                                                        -5.291 1.61e-07 ***
```

```
## NitrogenYears$Date2019-03-05 -2.750e-01
                                            1.648e-01
                                                       -1.669 0.095543 .
## NitrogenYears$Date2019-04-01 -7.250e-02
                                            1.427e-01
                                                      -0.508 0.611547
                                                        2.640 0.008466 **
## NitrogenYears$Date2019-04-04
                                4.350e-01
                                            1.648e-01
## NitrogenYears$Date2019-05-02 5.400e-01
                                                        3.277 0.001098 **
                                            1.648e-01
## NitrogenYears$Date2019-05-03 -5.450e-01
                                            1.427e-01
                                                       -3.819 0.000145
## NitrogenYears$Date2019-06-04 3.250e-02
                                            1.427e-01
                                                        0.228 0.819895
## NitrogenYears$Date2019-06-06 4.100e-01
                                            1.648e-01
                                                        2.488 0.013056 *
## NitrogenYears$Date2019-07-02 -1.445e+00
                                            1.648e-01
                                                       -8.770
                                                              < 2e-16 ***
## NitrogenYears$Date2019-07-08 -1.540e+00
                                            1.648e-01
                                                      -9.347
                                                               < 2e-16 ***
## NitrogenYears$Date2019-07-10 -1.655e+00
                                            1.648e-01 -10.044
                                                               < 2e-16 ***
## NitrogenYears$Date2019-08-02 -1.910e+00
                                            1.648e-01 -11.592
                                                               < 2e-16 ***
## NitrogenYears$Date2019-08-05 -1.820e+00
                                            1.648e-01 -11.046
                                                               < 2e-16
                                                      -6.342 3.97e-10 ***
## NitrogenYears$Date2019-08-09 -1.045e+00
                                            1.648e-01
## NitrogenYears$Date2019-09-06 -6.650e-01
                                            1.648e-01
                                                      -4.036 6.01e-05 ***
## NitrogenYears$Date2019-09-12 -1.588e+00
                                            1.427e-01 -11.125 < 2e-16 ***
## NitrogenYears$Date2019-10-02 -2.050e-01
                                            1.648e-01
                                                       -1.244 0.213833
## NitrogenYears$Date2019-10-03 -8.400e-01
                                            1.648e-01
                                                       -5.098 4.38e-07 ***
## NitrogenYears$Date2019-10-04 -9.200e-01
                                                      -5.584 3.33e-08
                                            1.648e-01
## NitrogenYears$Date2019-11-01 -1.725e+00
                                            1.648e-01 -10.469 < 2e-16
                                                       -7.253 1.05e-12
## NitrogenYears$Date2019-11-04 -1.195e+00
                                            1.648e-01
## NitrogenYears$Date2019-11-06 -8.800e-01
                                            1.648e-01
                                                       -5.341 1.24e-07 ***
## NitrogenYears$Date2019-12-03 -6.000e-01
                                            1.648e-01
                                                       -3.642 0.000290 ***
## NitrogenYears$Date2019-12-05 -2.700e-01
                                            1.648e-01
                                                       -1.639 0.101712
## NitrogenYears$Date2019-12-06 -8.700e-01
                                            1.648e-01
                                                       -5.280 1.71e-07 ***
                                            1.648e-01
## NitrogenYears$Date2020-01-07 -5.350e-01
                                                      -3.247 0.001220 **
## NitrogenYears$Date2020-01-14 -1.478e+00
                                            1.427e-01 -10.354 < 2e-16 ***
## NitrogenYears$Date2020-02-04 -1.410e+00
                                                      -8.558 < 2e-16 ***
                                            1.648e-01
                                                      -6.973 6.97e-12 ***
## NitrogenYears$Date2020-02-06 -9.950e-01
                                            1.427e-01
## NitrogenYears$Date2020-03-02 -1.780e+00
                                            1.648e-01 -10.803 < 2e-16 ***
## NitrogenYears$Date2020-03-03 -1.590e+00
                                                      -9.650 < 2e-16 ***
                                            1.648e-01
## NitrogenYears$Date2020-03-05 -2.227e+00
                                            1.648e-01 -13.513
                                                               < 2e-16 ***
## NitrogenYears$Date2020-07-08 -4.900e-01
                                            1.648e-01
                                                       -2.974 0.003038 **
## NitrogenYears$Date2020-07-10 2.500e-02
                                            1.648e-01
                                                        0.152 0.879442
## NitrogenYears$Date2020-07-13 -1.765e+00
                                            1.648e-01 -10.712
                                                               < 2e-16 ***
## NitrogenYears$Date2020-08-04 -1.415e+00
                                            1.648e-01
                                                       -8.588
                                                               < 2e-16
                                                       -7.738 3.37e-14 ***
## NitrogenYears$Date2020-08-06 -1.275e+00
                                            1.648e-01
## NitrogenYears$Date2020-08-07 -1.075e+00
                                            1.648e-01
                                                       -6.524 1.28e-10 ***
## NitrogenYears$Date2020-09-01 -1.140e+00
                                                       -6.919 9.99e-12 ***
                                            1.648e-01
## NitrogenYears$Date2020-09-02 -1.353e+00
                                                       -9.478
                                            1.427e-01
                                                               < 2e-16 ***
## NitrogenYears$Date2020-10-05 -1.483e+00
                                            1.427e-01 -10.389 < 2e-16 ***
## NitrogenYears$Date2020-10-06 -1.095e+00
                                            1.648e-01
                                                      -6.646 5.91e-11 ***
## NitrogenYears$Date2020-11-02 -1.715e+00
                                            1.648e-01 -10.409 < 2e-16 ***
## NitrogenYears$Date2020-11-04 -1.200e+00
                                            1.427e-01
                                                       -8.410 < 2e-16 ***
## NitrogenYears$Date2020-12-03 -7.050e-01
                                                       -4.279 2.13e-05 ***
                                            1.648e-01
## NitrogenYears$Date2020-12-04 -9.200e-01
                                            1.427e-01
                                                       -6.447 2.07e-10 ***
## NitrogenYears$Date2021-01-06 -6.550e-01
                                                       -3.975 7.73e-05 ***
                                            1.648e-01
## NitrogenYears$Date2021-01-12 -3.725e-01
                                            1.427e-01
                                                       -2.611 0.009227 **
##
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.1648 on 728 degrees of freedom
## Multiple R-squared: 0.974, Adjusted R-squared: 0.9542
## F-statistic: 49.28 on 553 and 728 DF, p-value: < 2.2e-16
```

```
NitrogenYears$Year <- as.factor(NitrogenYears$Year)
NitrogenPlot <- ggplot(NitrogenYears, aes(x=Year, y=Nitrogen_Percent))+
    geom_boxplot()+
    labs(title="Nitrogen Concentration Percent by Year", x="Year", y="Nitrogen Concentration Percent")
print(NitrogenPlot)</pre>
```

Nitrogen Concentration Percent by Year

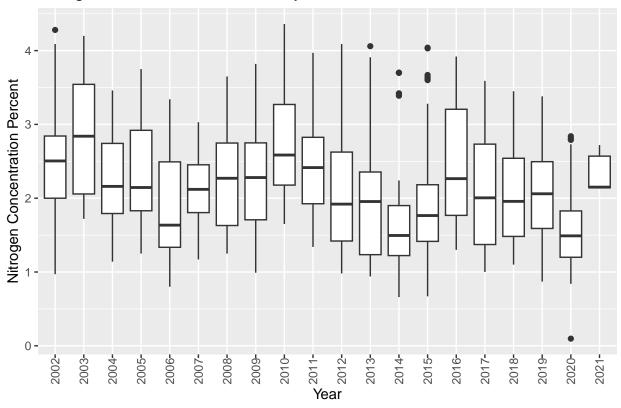


Figure 11: Nitrogen Concentration over the Years

```
ggsave(file=here('Output', 'Analysis','NitrogenConcentrationBoxbyDate.png'), width = 10, height = 10, u
```

Below are the normal distribution residuals charts for the linear regression of nitrogen concentration by date.

```
par(mfrow = c(2,2), mar=c(2,2,2,2))
plot(NitrogenRegressionDate)
```

```
par(mfrow = c(1,1))
```

A time series analysis was also completed to see how nitrogen concentration changes over time. The following plot displays the decomposed nitrogen concentration components of the time series run. As you can see, there is seasonality, which confirms our rejecting of the null hypothesis.

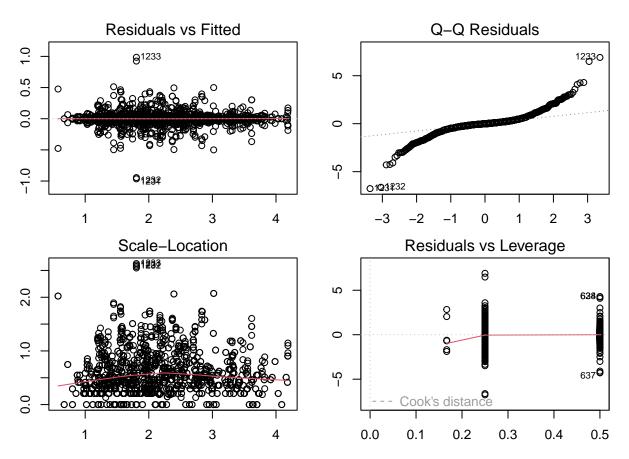


Figure 12: Nitrogen Concentration Resdiual Plots by Date

```
#6. Season Plot #average the months
Nitrogen.monthly.preliminary <- NitrogenYears %>%
  mutate(CleanDate = my(pasteO(Month,"-",Year)))
Nitrogen.monthly <-aggregate(Nitrogen.monthly.preliminary$Nitrogen_Percent,</pre>
                                    by=list(Nitrogen.monthly.preliminary$CleanDate),
                                    FUN=mean)
colnames(Nitrogen.monthly) <- c("Date", "Mean_NitrogenPercent")</pre>
#7. Generate time series monthly
f_monthmonthly <- month(first(Nitrogen.monthly$Date))</pre>
f_yearmonthly <- year(first(Nitrogen.monthly$Date))</pre>
Nitrogen.monthly.ts <- ts(Nitrogen.monthly$Mean_NitrogenPercent,</pre>
                    start=c(f_yearmonthly,f_monthmonthly),
                    frequency=12)
#Decompose monthly
Nitrogen.monthly.ts.decomp <- stl(Nitrogen.monthly.ts,s.window = "periodic")</pre>
plot(Nitrogen.monthly.ts.decomp)
```

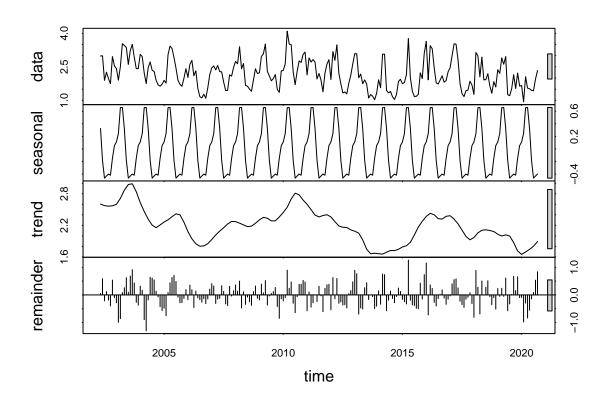


Figure 13: Nitrogen Concentration Residual Plots

The Mann-Kendall analysis performed on the nitrogen concentration non-seasonal time series produced a

p-value of less than 0.05 (<.001, tau value of -0.19). As a result, the data displays a significant, decreasing trend for nitrogen concentration. The plot below displays the non-seasonal nitrogen concentration amount in the time series which was produced by removing the seasonal component from the time series.

```
#12 Run SMK test monthly
Nitrogen_Monthly_SMK <- Kendall::SeasonalMannKendall(Nitrogen.monthly.ts)
Nitrogen_Monthly_SMK
## tau = -0.19, 2-sided pvalue =0.00011555
summary(Nitrogen_Monthly_SMK)
## Score = -365, Var(Score) = 8963
## denominator = 1925.499
## tau = -0.19, 2-sided pvalue =0.00011555
# 13 Visualization
Nitrogen_Plot <-</pre>
ggplot(Nitrogen.monthly, aes(x = Date, y = Mean_NitrogenPercent)) +
  geom_point() +
  geom_line() +
  labs("Mean Nitrogen (percent)", title="Nitrogen Concentration Percent over Time") +
  geom_smooth( method = lm )
print(Nitrogen_Plot)
ggsave(file=here('Output', 'Analysis','NitrogenConcentrationPointbySeason.png'), width = 10, height = 1
#taking out seasonal
NitrogenComponents <- as.data.frame(Nitrogen.monthly.ts.decomp$time.series[,1:3])</pre>
Nitrogen_NoSeasonal <-NitrogenComponents$trend+NitrogenComponents$remainder
#16 Run MK test monthly
Nitrogen_Monthly_MK <- Kendall::MannKendall(Nitrogen_NoSeasonal)</pre>
Nitrogen_Monthly_MK
## tau = -0.188, 2-sided pvalue =3.0712e-05
summary(Nitrogen_Monthly_MK)
## Score = -4581 , Var(Score) = 1207396
## denominator = 24309.5
## tau = -0.188, 2-sided pvalue =3.0712e-05
```

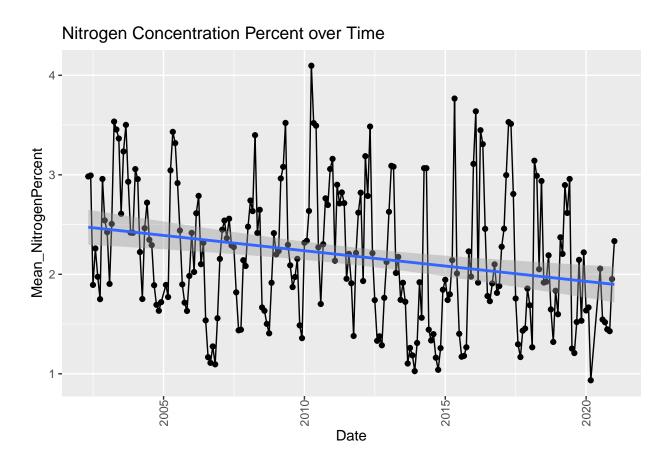


Figure 14: Time Series Analysis for Nitrgoen Concentration

Question 2: Does giant kelp nitrogen concentration amount vary by season?

For the second question, we wanted to know the relationship between nitrogen concentration and season. Winter is defined as beggining of December through February, Spring is beginning of March through May, Summer is June through August, and Fall is September through November.

H0: There is no significant difference of nitrogen concentration by season. HA: There is significant difference of nitrogen concentration by season.

The interaction between nitrogen concentration and season is significant because the p-value is < .05 (p < 2.2e-16). We can reject the null hypothesis and accept that there is significant difference of nitrogen concentration by season. The box plot below shows the change in nitrogen concentration over seasons.

```
##
## Call:
## lm(formula = NitrogenYears$Nitrogen_Percent ~ NitrogenYears$SeasonNames)
## Residuals:
                                    3Q
##
       Min
                  1Q
                      Median
                                            Max
## -2.64975 -0.49742 -0.05865 0.43835
##
## Coefficients:
##
                                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                    2.18584
                                               0.03784 57.764
                                                                 <2e-16 ***
## NitrogenYears$SeasonNamesSpring 0.56091
                                               0.05430 10.330
                                                                 <2e-16 ***
## NitrogenYears$SeasonNamesSummer -0.09819
                                               0.05335 - 1.840
                                                                 0.0659 .
## NitrogenYears$SeasonNamesFall
                                   -0.46618
                                               0.05319 - 8.765
                                                                 <2e-16 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 0.679 on 1278 degrees of freedom
## Multiple R-squared: 0.2242, Adjusted R-squared: 0.2224
## F-statistic: 123.1 on 3 and 1278 DF, p-value: < 2.2e-16
```

par(mfrow = c(1,1))

Below are the normal distribution residuals charts for the linear regression of nitrogen concentration

```
#3. Normal Distribution

par(mfrow = c(2,2), mar=c(2,2,2,2))

plot(NitrogenRegressionSeason)
```

```
NitrogenSeasonPlot <- ggplot(NitrogenYears, aes(x=SeasonNames, y=Nitrogen_Percent))+
    geom_boxplot()+
    labs(title="Nitrogen Concentration Percent by Season", x="Season", y="Nitrogen Concentration Percent"
print(NitrogenSeasonPlot)</pre>
```

```
ggsave(file=here('Output', 'Analysis','NitrogenConcentrationBoxbySeason.png'), width = 10, height = 10,
```

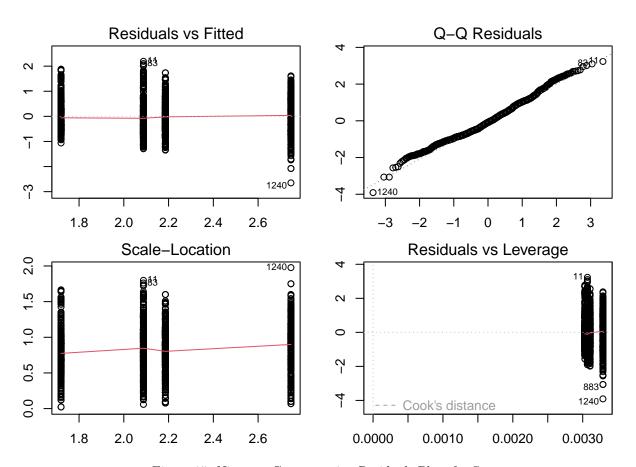


Figure 15: Nitrogen Concentration Residuals Plots for Season

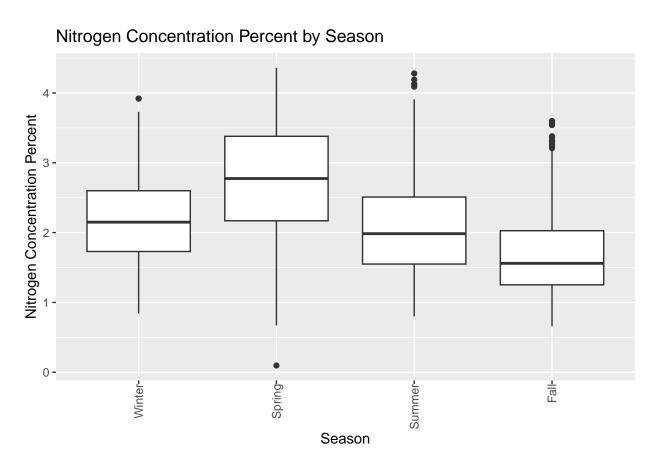


Figure 16: Nitrgoen Concentration over Seasons

Question 3: Does nitrogen surge uptake vary between the spring and summer seasons?

The last three questions focus on the experiment completed over five days in the spring and summer of 2019. Nitrogen was blasted for 45 minutes and data on nitrogen and biomass update was recorded.

H0: There is no significant difference of nitrogen surge uptake between spring and summer seasons. HA: There is significant difference of nitrogen surge uptake between spring and summer seasons.

The interaction between nitrogen uptake and season is significant because the p-value is <.05 (p <.0216). We can reject the null hypothesis and accept that there is significant difference of nitrogen surge uptake between spring and summer seasons. The R-squared is .014, so a low amount of nitrogen uptake variance is in response to data being collected in the spring or summer. The box plot below shows the change in nitrogen concentration over seasons.

```
NitrogenSeasonBox <-
ggplot(SurgeUptakeNitrogen, aes(x = Season, y = Uptake_Rate)) +
geom_boxplot()+
labs(title="Nitrogen Uptake Rate by Season", x="Season", y="Nitrogen Uptake Rate (V(hr1))")
print(NitrogenSeasonBox)</pre>
```

Nitrogen Uptake Rate by Season

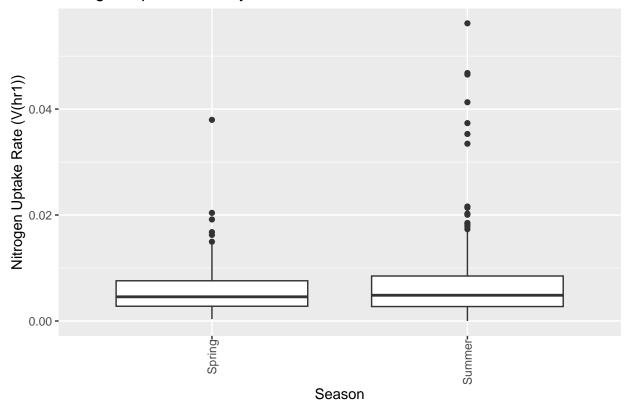


Figure 17: Nitrogen Uptake Rate by Season Box Plot

```
#ggsave(file=here('Output', 'Analysis','NitrogenBoxSeason.png'), width = 10, height = 10, units = #"cm"
```

The scatter plot below shows the change in nitrogen concentration over seasons by testing day replicate.

```
NitrogenSeasonPoint <-
    ggplot(SurgeUptakeNitrogen, aes(x = Day_Replicate, y = Uptake_Rate, shape=Season)) +
    geom_point(alpha=.3)+
    labs(title="Nitrogen Uptake Rate by Day and Season", x="Days", y="Nitrogen Uptake Rate (V(hr1))")
print(NitrogenSeasonPoint)</pre>
```

Nitrogen Uptake Rate by Day and Season

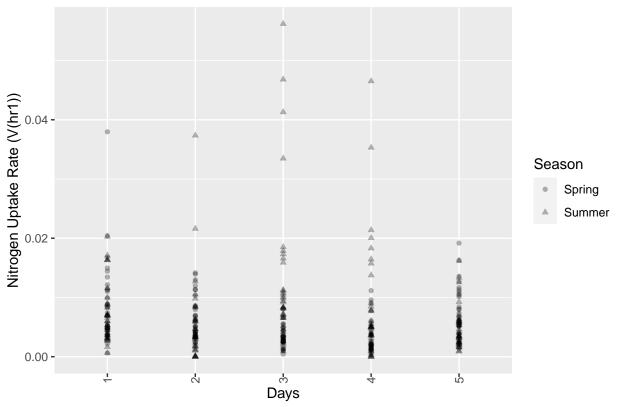


Figure 18: Nitrogen Uptake by Season Scatter Plot

```
#ggsave(file=here('Output', 'Analysis','NitrogenPointSeason.png'), width = 10, height = 10, units = #"c
#linear regression
NitrogenRegressionSeason <- lm(SurgeUptakeNitrogen$Uptake_Rate ~ SurgeUptakeNitrogen$Season)
summary(NitrogenRegressionSeason)
##</pre>
```

Call:

```
## lm(formula = SurgeUptakeNitrogen$Uptake_Rate ~ SurgeUptakeNitrogen$Season)
##
## Residuals:
##
                   1Q
                         Median
                                       ЗQ
        \mathtt{Min}
                                               Max
## -0.007419 -0.003936 -0.001798 0.001434 0.048773
##
## Coefficients:
##
                                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                   0.0057806 0.0005195 11.127
                                                                 <2e-16 ***
## SurgeUptakeNitrogen$SeasonSummer 0.0016387 0.0007336 2.234
                                                                 0.0261 *
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 0.00695 on 357 degrees of freedom
## Multiple R-squared: 0.01378,
                                  Adjusted R-squared: 0.01102
## F-statistic: 4.989 on 1 and 357 DF, p-value: 0.02612
```

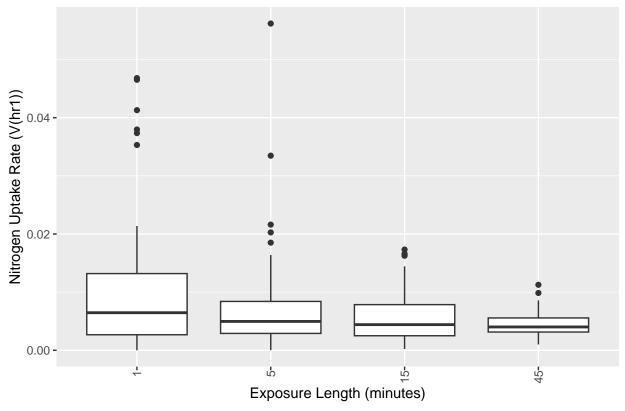
Question 4: Does giant kelp nitrogen surge uptake vary based on the amount of exposure?

H0: There is no significant difference of nitrogen surge uptake between different exposure lengths. HA: There is significant difference of nitrogen surge uptake between different exposure lengths.

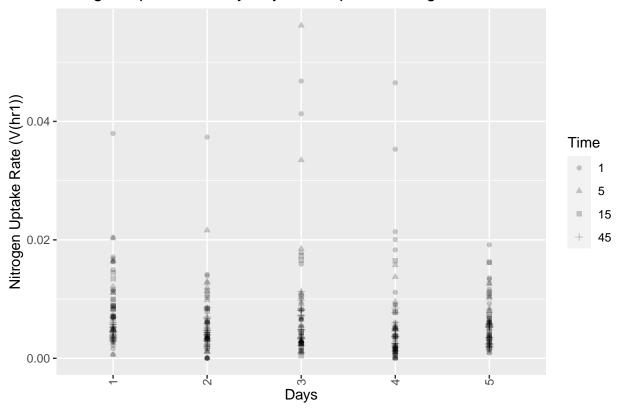
The interaction between nitrogen uptake and season is significant because the p-value is <.05 (p = 8.394e-06). We can reject the null hypothesis and accept that there is significant difference of nitrogen surge uptake between different exposure lengths. The R-squared is .014, so a low amount of nitrogen uptake variance is in response to data being collected in the spring or summer. The box plot below shows the change in nitrogen concentration over seasons.

The Tukey HSD test shows that there is significant difference between the means of time interval 1 and 45 (p=value = 0.0000068), intervals 1 and 15 (p-value=0.0076), and intervals 1 and 5 (p-value=0.04). There is not significant difference between the means of time interval 15 and 45 (p-value=0.04), 5 and 45 (p-value=0.04), and 5 and 15 (p-value=0.04) because their p-value is 0.040.

Nitrogen Uptake Rate by Exposure Length



Nitrogen Uptake Rate by Day and Exposure Length



```
TimeLM <-lm(data = ExposureLength, Uptake_Rate~Time)
summary(TimeLM)</pre>
```

```
##
## Call:
## lm(formula = Uptake_Rate ~ Time, data = ExposureLength)
##
## Residuals:
##
        Min
                   1Q
                         Median
## -0.009494 -0.003410 -0.001144 0.001705 0.049377
##
## Coefficients:
               Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) 0.009494
                         0.000713 13.316 < 2e-16 ***
## Time5
              -0.002679
                          0.001008 -2.657 0.008238 **
              -0.003909
                          0.001011 -3.866 0.000131 ***
## Time15
## Time45
                          0.001008 -4.950 1.15e-06 ***
              -0.004991
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.006764 on 355 degrees of freedom
## Multiple R-squared: 0.07122, Adjusted R-squared: 0.06337
## F-statistic: 9.074 on 3 and 355 DF, p-value: 8.394e-06
```

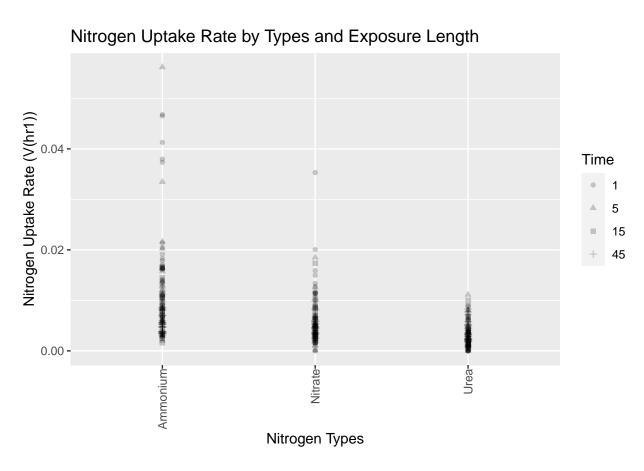


Figure 19: Nitrogen Uptake Rates by Type with Time Scatter Plot

```
TimeAOV <- aov(data=ExposureLength, Uptake_Rate~Time)</pre>
summary(TimeAOV)
##
                              Mean Sq F value
                     Sum Sq
                                                Pr(>F)
## Time
                 3 0.001245 0.0004151
                                        9.074 8.39e-06 ***
              355 0.016241 0.0000457
## Residuals
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
#TimeNitrogenAIC <- lm(data=ExposureLength, Uptake_Rate~Time+Nitrogen_Types+Season)
#step(TimeNitrogenAIC)
TimeDurationAOV <- aov(data=ExposureLength, Uptake_Rate~Time)</pre>
summary(TimeDurationAOV)
##
                              Mean Sq F value
                     Sum Sq
                                                Pr(>F)
## Time
                 3 0.001245 0.0004151
                                        9.074 8.39e-06 ***
## Residuals
              355 0.016241 0.0000457
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
TukeyHSD(TimeDurationAOV)
##
     Tukey multiple comparisons of means
##
      95% family-wise confidence level
##
## Fit: aov(formula = Uptake_Rate ~ Time, data = ExposureLength)
##
## $Time
##
                 diff
                               lwr
                                             upr
                                                     p adj
         -0.002679140 -0.005281795 -7.648551e-05 0.0408781
## 5-1
## 15-1 -0.003909204 -0.006519159 -1.299248e-03 0.0007557
## 45-1 -0.004990860 -0.007593515 -2.388205e-03 0.0000068
## 15-5 -0.001230063 -0.003840019 1.379892e-03 0.6167476
## 45-5 -0.002311720 -0.004914375 2.909349e-04 0.1016435
## 45-15 -0.001081656 -0.003691612 1.528299e-03 0.7081966
```

Question 5: Does uptake vary by type of nitrogen (ammonium, nitrate, and urea)?

H0: There is no significant difference of nitrogen surge uptake of different nitrogen types. HA: There is significant difference of nitrogen surge uptake between different nitrogen types.

The interaction between nitrogen uptake and season is significant because the p-value is < .05 (p < 2.2e-16). We can reject the null hypothesis and accept that there is significant difference of nitrogen surge uptake between spring and summer seasons.. The R-squared is .192, so a moderate amount of nitrogen uptake variance is in response to the nitrogen type being blasted. The box plot below shows the change in nitrogen uptake rate by nitrogen type.

The Tukey HSD test shows that there is significant difference between the means of time interval 1 and 45 (p=value = 0.0000068), intervals 1 and 15 (p-value=0.0076), and intervals 1 and 5 (p-value=0.04). There is not significant difference between the means of time interval 15 and 45 (p-value=0.04), 5 and 45 (p-value=0.04), and 5 and 15 (p-value=0.04) because their p-value is 0.040.

```
# Creating a ggplot to compare uptake of various types of nitrogen
NitrogenUptaketype <-
    ggplot(SurgeUptakeNitrogen, aes(x = Nitrogen_Types, y = Uptake_Rate)) +
    geom_boxplot() +
    labs(title="Uptake Rate by Nitrogen Type", x="Nitrogen Types", y="Nitrogen Uptake Rate (V(hr1))")
print(NitrogenUptaketype)</pre>
```

Uptake Rate by Nitrogen Type

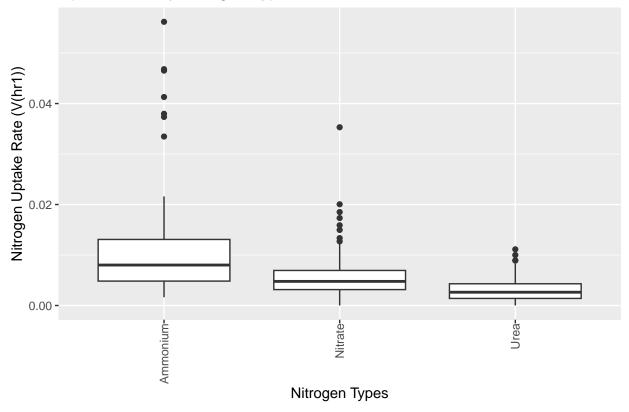


Figure 20: Nitrogen Uptake by Type Box Plot

```
ggsave(file=here('Output', 'Analysis','NitrogenUptakeBoxtype.png'), width = 10, height = 10, units = "creating a ggplot to compare biomass uptake of various types of nitrogen
```

The jitter plot below shows the change in nitrogen uptake rate by nitrogen type with day replicate added.

Nitrogen Uptake per Day for Each Nitrogen Type

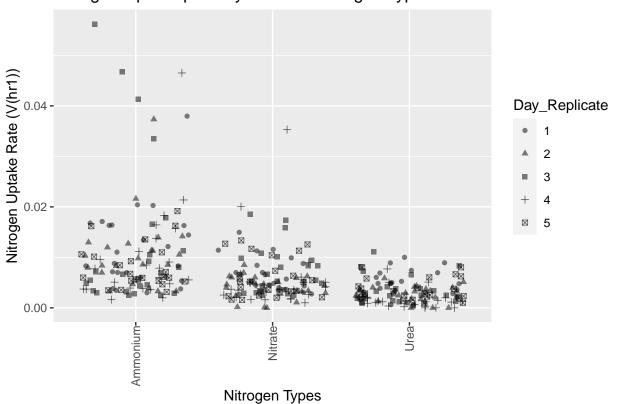


Figure 21: Nitrogen Uptake by Type Jigger Plot

```
ggsave(file=here('Output', 'Analysis','NitrogenUptakeJitterType.png'), width = 10, height = 10, units =
# Generalized Linear Model (GLM)
NitorgenLM <-lm(data = SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types)
summary(NitorgenLM)</pre>
```

```
##
## Call:
## lm(formula = Uptake_Rate ~ Nitrogen_Types, data = SurgeUptakeNitrogen)
## Residuals:
                         Median
##
        Min
                   1Q
                                       3Q
                                                Max
## -0.009010 -0.002975 -0.001082 0.001395 0.045547
##
## Coefficients:
##
                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                         0.0106449 0.0005750 18.512 < 2e-16 ***
## Nitrogen_TypesNitrate -0.0047592  0.0008132  -5.852  1.1e-08 ***
## Nitrogen_TypesUrea
                        -0.0073967 0.0008149 -9.077 < 2e-16 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
##
## Residual standard error: 0.006299 on 356 degrees of freedom
## Multiple R-squared: 0.1922, Adjusted R-squared: 0.1877
## F-statistic: 42.36 on 2 and 356 DF, p-value: < 2.2e-16
# One-way ANOVA
NitrogenAOV <- aov(data = SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types)
summary(NitrogenAOV)
                               Mean Sq F value Pr(>F)
                   Df
                       Sum Sq
                   2 0.003361 0.0016807
## Nitrogen_Types
                                          42.36 <2e-16 ***
                 356 0.014125 0.0000397
## Residuals
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
TukeyHSD(NitrogenAOV)
##
     Tukey multiple comparisons of means
       95% family-wise confidence level
##
## Fit: aov(formula = Uptake_Rate ~ Nitrogen_Types, data = SurgeUptakeNitrogen)
##
## $Nitrogen_Types
##
                           diff
                                         lwr
                                                               p adj
                                                        upr
## Nitrate-Ammonium -0.004759165 -0.006673064 -0.0028452667 0.0000000
## Urea-Ammonium -0.007396726 -0.009314641 -0.0054788108 0.0000000
## Urea-Nitrate
                   -0.002637561 -0.004555476 -0.0007196454 0.0037754
# One-way ANOVA
NitrogenAOV <- aov(data = SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types)</pre>
summary(NitrogenAOV)
                       Sum Sq
                                Mean Sq F value Pr(>F)
                   2 0.003361 0.0016807
                                          42.36 <2e-16 ***
## Nitrogen_Types
## Residuals
                 356 0.014125 0.0000397
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

TukeyHSD(NitrogenAOV)

```
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = Uptake_Rate ~ Nitrogen_Types, data = SurgeUptakeNitrogen)
##
## $Nitrogen_Types
                            diff
                                          lwr
                                                         upr
## Nitrate-Ammonium -0.004759165 -0.006673064 -0.0028452667 0.0000000
## Urea-Ammonium -0.007396726 -0.009314641 -0.0054788108 0.0000000
## Urea-Nitrate -0.002637561 -0.004555476 -0.0007196454 0.0037754
# AIC-based Model Selection:
NitrogenTypeAIC <- lm(data=SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types+Season+Time)
step(NitrogenTypeAIC)
## Start: AIC=-3667.7
## Uptake_Rate ~ Nitrogen_Types + Season + Time
##
##
                    Df Sum of Sq
                                      RSS
                                              AIC
                                 0.012625 -3667.7
## <none>
## - Season
                     1 0.0002483 0.012873 -3662.7
## - Time
                     3 0.0012540 0.013879 -3639.7
## - Nitrogen_Types 2 0.0033740 0.015998 -3586.7
##
## Call:
## lm(formula = Uptake_Rate ~ Nitrogen_Types + Season + Time, data = SurgeUptakeNitrogen)
##
## Coefficients:
##
            (Intercept)
                          Nitrogen_TypesNitrate
                                                     Nitrogen_TypesUrea
                0.012720
                                      -0.004759
                                                              -0.007413
##
            {\tt SeasonSummer}
##
                                          Time5
                                                                 Time15
                                      -0.002679
                                                              -0.003956
##
                0.001663
##
                  Time45
##
               -0.004991
summary(NitrogenTypeAIC)
##
## Call:
## lm(formula = Uptake_Rate ~ Nitrogen_Types + Season + Time, data = SurgeUptakeNitrogen)
##
## Residuals:
        Min
                    1Q
                          Median
                                        3Q
                                                  Max
## -0.009974 -0.003295 -0.000467 0.001954 0.044488
##
## Coefficients:
##
                           Estimate Std. Error t value Pr(>|t|)
                          0.0127198  0.0008351  15.232  < 2e-16 ***
## (Intercept)
```

```
## Nitrogen_TypesNitrate -0.0047592  0.0007731  -6.156  2.04e-09 ***
## Nitrogen_TypesUrea -0.0074125 0.0007748 -9.567 < 2e-16 ***
## SeasonSummer
                      0.0016633  0.0006322  2.631  0.00888 **
## Time5
                     -0.0026791 0.0008927 -3.001 0.00288 **
## Time15
                      ## Time45
                      -0.0049909 0.0008927 -5.590 4.55e-08 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
##
## Residual standard error: 0.005989 on 352 degrees of freedom
## Multiple R-squared: 0.278, Adjusted R-squared: 0.2657
## F-statistic: 22.59 on 6 and 352 DF, p-value: < 2.2e-16
```

For biomass

#BiomassTypeAIC <- lm(data=SurgeUptakeNitrogen, Biomass_Uptake~Nitrogen_Types+Season+Time)
#step(NitrogenTypeAIC)
#summary(NitrogenTypeAIC)

Summary and Conclusions

Akaike information criterion (AIC) was performed for the final three questions to see if those three variables all contributed to the variation of nitrogen uptake. All three variables (season, nitrogen exposure lengths, and nitrogen type), contributed to the variance of nitrogen uptake rate.

What does these p-values about the first data set tell us about the intro

What does the second set tell us about the intro as well as the data set (aka, more testing needs to be done, was a short experiment) Table 2: Significance Values

Research Question	P-Value
Question 1	< 2.2e-16
Question 2	< 1.16e-4
Question 3	.0216
Question 4	
Question 5	< 2.2e-16

References

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