Explanation of Statistical Tests for: Summer precipitation pulse response in western Joshua trees

Plant Ecology

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Justification

These analyses are intended to answer our two research questions: 1. Do Joshua trees upregulate photosynthesis in response to summer precipitation pulses. 2. Is there a relationship between antecedent water conditions and the pre-watering level of photosynthetic and water relations parameters.

In order to answer these questions we sampled ten Joshua trees in two years, 2021 and 2023. In 2021, we conducted a water addition experiment wherein each of the five blocks of plants were divided into a treatment and control. The treatment plants were watered with 10 mm of water applied to a one m radius around the tree. In 2023, Tropical Storm Hilary watered all ten plants. Because these designs are not identical (treatment/control vs. before/after), and a lack of statistical power to support a 2-way ANOVA with an interaction term, analyses of response of plants to watering were conducted independently for each year. Due to the large number of statistical tests, a post-hoc Benjamini-Hochberg procedure was applied to correct for possible type I error.

We measured 6 physiological parameters: net photosynthetic assimilation (A_{NET}), stomatal conductance to water vapor (g_s), the light-adapted effective quantum yield of photosystem II (F_v'/Fm'), the light-adapted maximum quantum yield of photosystem II (Φ_{PSII}), the dark-adapted effective quantum yield of photosystem II (F_v/F_m), and leaf water potential (Ψ_{leaf}).

As the plants were either sampled repeatedly or grouped into blocks, a paired-samples t-test was employed to account for the non-independence within blocks or individuals. The samples are independent between blocks and between individuals. In cases where the data were not normally distributed nor able to be transformed to a normal distrubution, a non-parametric Wilcoxon Ranked-Sign analysis was conducted. All statistical tests are one sided and the alternative hypothesis is that the variable of interest increased either after watering (within year comparison) or between the dry (2021) and wet (2023) years.

Here I will explain the r code and rationale for these analyses.

Data Preparation

First, I loaded and prepared the gas exchange data, organizing it by measurement dates and experimental conditions.

```
df2 <- data
df2$year <- year(df2$dateonly)
df2$year <- factor(df2$year)
df2$pulse <- factor(df2$pulse, levels = c("post","pre"))

U23 <- df2 %>% filter(dateonly=="2023-08-16") %>% arrange(Treatment,Plant)
W23 <- df2 %>% filter(dateonly=="2023-08-25") %>% arrange(Treatment,Plant)
U21 <- df2 %>% filter(dateonly=="2021-08-20", pulse=="pre") %>% arrange(Plant)
W21 <- df2 %>% filter(dateonly=="2021-08-20", pulse=="post") %>% arrange(Plant)
```

Note: One measurement is missing from the pre-watering group. I removed this from the post-watering group to maintain paired measurements.

```
U23 <- U23 %>% filter(!Plant=="1W")
all23 <- rbind(U23,W23)
all21 <- rbind(U21,W21)
yearcomp<-rbind(U21,U23[1:5,])
```

Net Photosynthetic Assimilation (A_{NET})

2023

Assumption Testing

First we will check that our data meets the assumptions and best practices for a paired t-test: 1. No extreme outliers and 2. Normal distribution of the difference scores. We have established that samples of different individuals are independent.

First, we will check for outliers.

```
outliers23 <- all23 %>% group_by(pulse) %>% identify_outliers(Photo)
print(outliers23[c(1:8,20,21)])
```

```
## # A tibble: 2 x 10
##   pulse     X Time   dateonly Block Treatment Plant  Photo is.outlier is.extreme
##   <fct> <int> <chr>  <chr>    <int> <chr>     <chr>  <dbl> <lgl>      <lgl>
## 1 post     29 7:53:~ 2023-08~     5 C         5C    -0.782 TRUE       FALSE
## 2 pre      17 7:47:~ 2023-08~     4 W         4W     7.08  TRUE       FALSE
```

There are two outliers, but no extreme outliers. These outliers are biologically reasonable and should not be excluded.

Next calculate the differences between the measurements of the plants and determine if they are normally distributed.

```
all23 %>%
  pivot_wider(names_from = pulse, values_from = Photo, id_cols = Plant) %>%
  mutate(diff = post - pre) %>%
  shapiro_test(diff)
```

```
## # A tibble: 1 x 3
##   variable statistic     p
##   <chr>        <dbl> <dbl>
## 1 diff         0.934 0.520
```

Because the p-value for the Shapiro-Wilks test is greater than 0.05 we fail to reject the null hypothesis that the difference scores are normally distributed.

Statistical Analysis

```r
(Photo23<-t.test( W23$Photo,U23$Photo,
                  alternative= "greater",
                  paired = TRUE))
```

```
##
##  Paired t-test
##
## data:  W23$Photo and U23$Photo
## t = 3.4935, df = 8, p-value = 0.004078
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  1.07584     Inf
## sample estimates:
## mean difference
##        2.300233
```

The p-value is less than the 0.05 significance level, so we reject the null hypothesis that the difference in mean photosynthetic assimilation between post and pre watering treatment is less than or equal to zero. The mean difference between the post and pre watering data is 2.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2021

Assumption testing

```r
outliers21 <- all21 %>% identify_outliers(Photo)
print(outliers21[c(1:8,20,21)])
```

```
##  [1] X          Time       dateonly   Block      Treatment  Plant
##  [7] Photo      Cond2      is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```r
all21 %>%
  pivot_wider(names_from = pulse, values_from = Photo, id_cols = Block) %>%
  mutate(diff = post - pre) %>%
  shapiro_test(diff)
```

```
## # A tibble: 1 x 3
##   variable statistic     p
##   <chr>        <dbl> <dbl>
## 1 diff         0.889 0.351
```

Differences are normally distributed.

###Statistical Test

```r
(Photo21<-t.test(W21$Photo, U21$Photo,
                 paired = TRUE,
                 alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W21$Photo and U21$Photo
## t = 1.484, df = 4, p-value = 0.106
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.3989833        Inf
## sample estimates:
## mean difference
##       0.9140281
```

We do not find a significant difference in photosynthetic assimilation after watering in 2021.

Interannual Comparison

We compare measurements from the same leaves across years to see if there is a relationship between antecedent water conditions and baseline photosynthetic parameters.

Assumption checking

We already checked these data for outliers. We must test the differences for normality.

```r
Photodiff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = Photo, id_cols = Plant) %>%
  mutate(diff = `2023` - `2021`)
shapiro_test(Photodiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable            statistic p.value
##   <chr>                   <dbl>   <dbl>
## 1 Photodiff_data$diff     0.719  0.0149
```

```r
logPhotodiff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = Photo, id_cols = Plant) %>%
  mutate(diff = log(`2023`+2) - log(`2021`+2))
shapiro_test(logPhotodiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable               statistic p.value
##   <chr>                      <dbl>   <dbl>
## 1 logPhotodiff_data$diff     0.808  0.0947
```

The untransformed data do not have a normal distribution, but the transformed data do. I have transformed the data by adding a constant to ensure positive values and taking the natural logarithm of the sum.

Statistical test

```r
(Photo2y<-t.test(log(U21$Photo+2),log(U23$Photo[1:5]+2),
                 paired = TRUE,
                 alternative="greater"))
```

```
##
##  Paired t-test
##
## data:  log(U21$Photo + 2) and log(U23$Photo[1:5] + 2)
## t = 0.50755, df = 4, p-value = 0.3192
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.4537472        Inf
## sample estimates:
## mean difference
##       0.1417825
```

We find no significant difference in the natural logarithm of the baseline net photosynthetic assimilation rates (A_{net}) between the years.

Stomatal Conductance to water vapor (g_s)

2023

Assumption Testing

Check for outliers.

```r
all23 %>% identify_outliers(Cond2)
```

```
##  [1] X          Time       dateonly   Block      Treatment  Plant
##  [7] Photo      Cond2      Cond       Ci         Trans      PARset
## [13] filename   filename2  WUE        instrument pulsesize  pulse
## [19] year       is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```r
all23 %>%
  pivot_wider(names_from = pulse, values_from = Cond2, id_cols = Plant) %>%
  mutate(diff = post - pre) %>%
  shapiro_test(diff)
```

```
## # A tibble: 1 x 3
##   variable statistic       p
##   <chr>        <dbl>   <dbl>
## 1 diff         0.758 0.00672
```

The differences between paired measurements for the conductance data do not adhere to a normal distribution. Transformation did not improve this. Therefore we will proceed with non-parametric testing using the Wilcoxon Signed-Rank test.

The Wilcoxon Signed-Rank test assumes that the distribution of differences is symmetric.

```
library(lawstat)
all23 %>%
  pivot_wider(names_from = pulse, values_from = Cond2, id_cols = Plant) %>%
  mutate(diff = post - pre) %>%
  pull(diff) %>%
  symmetry.test()
```

```
##
##  m-out-of-n bootstrap symmetry test by Miao, Gel, and Gastwirth (2006)
##
## data:  .
## Test statistic = 0.72564, p-value = 0.62
## alternative hypothesis: the distribution is asymmetric.
## sample estimates:
## bootstrap optimal m
##                   8
```

Since the m-out-of-n bootstrap symmetry test has a p value greater than the significance level of 0.05, we fail to reject the null hypothesis that the differences are symmetric. We may proceed with the ranked-sign analysis.

Statistical Analysis

```
(Cond23<-wilcox_test(all23, Cond2~pulse,
                     paired = TRUE,
                     alternative = "greater"))
```

```
## # A tibble: 1 x 7
##   .y.   group1 group2    n1    n2 statistic       p
## * <chr> <chr>  <chr>  <int> <int>     <dbl>   <dbl>
## 1 Cond2 post   pre        9     9        45 0.00195
```

The p-value is less than 0.05, so we reject the null hypothesis that the difference in medians is less than or equal to zero.

2021

Assumption testing

```
outliers21 <- all21 %>% identify_outliers(Cond2)
print(outliers21[c(1:8,20,21)])
```

```
##   X     Time   dateonly Block Treatment Plant    Photo      Cond2 is.outlier
## 1 7 07:26:08 2021-08-20     4         W    4W 2.967777 0.02195277       TRUE
##   is.extreme
## 1      FALSE
```

One outlier, but it is biologically very reasonable and not extreme.

```r
all21 %>%
  pivot_wider(names_from = pulse, values_from = Cond2, id_cols = Block) %>%
  mutate(diff = post - pre) %>%
  shapiro_test(diff)
```

```
## # A tibble: 1 x 3
##   variable statistic     p
##   <chr>        <dbl> <dbl>
## 1 diff         0.957 0.787
```

The differences in these data are normally distributed.

Statistical Test

```r
(Cond21<-t.test(W21$Cond2, U21$Cond2,
                paired = TRUE,
                alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W21$Cond2 and U21$Cond2
## t = 1.5202, df = 4, p-value = 0.1015
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.002059115          Inf
## sample estimates:
## mean difference
##      0.00511826
```

We find no significant difference in stomatal conductance in 2021 after watering.

Interannual Comparison

We must use a non-parametric test because 2023 data are not normally distributed (as we discovered above). The 2021 data are normally distributed and therefore symmetric, so we do not need to test that assumption.

```r
(Cond2y<-wilcox.test(U23$Cond2[1:5],U21$Cond, paired = TRUE, alternative="greater"))
```

```
##
##  Wilcoxon signed rank exact test
##
## data:  U23$Cond2[1:5] and U21$Cond
## V = 15, p-value = 0.03125
## alternative hypothesis: true location shift is greater than 0
```

The median baseline level of photosynthesis was higher in 2023 than in 2021.

F_v'/Fm'

2023

Data Import for daytime F_n measurements.

```
df<-data
df$pulse<-factor(df$pulse, levels = c("post", "pre"))
df$year<-factor(df$year)

U23<-df %>% filter(pulse=="pre" & year==2023) %>% arrange(Treatment, plant)
W23<-df %>% filter(pulse=="post" & year==2023)%>% arrange(Treatment, plant)

all23<-rbind(U23,W23)

U21<-df %>% filter(pulse=="pre"& year==2021) %>% arrange(plant)
W21<-df %>% filter(pulse=="post"& year==2021) %>% arrange(plant)

all21<-rbind(U21,W21)

yearcomp<-rbind(U21,U23[1:5,])
```

Check assumptions

```
outliers23<-all23 %>% identify_outliers(FVprime)
print(outliers23[c(1,4:8,10:11)])
```

```
##    X     PhiPs2   dateonly pulse plant Block Treatment is.outlier
## 1 30 -0.9910153 2023-08-16   pre    5c     5         c       TRUE
```

An extreme outlier with a value of -4.95 is present in the data. For this measurement Fo' = -3.7, Fm' = 0.6, Fs = -1.25. There must have been an error in data collection as these values are impossible. This value and its pair from the post watering measurement will be excluded from the analysis.

```
all23<-all23 %>% filter(!plant=="5c",)

U23<-U23 %>% filter(!plant=="5c")
W23<-W23 %>% filter(!plant=="5c")
yearcomp<-yearcomp %>% filter(!plant=="5c")

FVprimediff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = FVprime, id_cols = plant) %>%
  mutate(diff = post - pre)

shapiro_test(FVprimediff_data$diff)
```

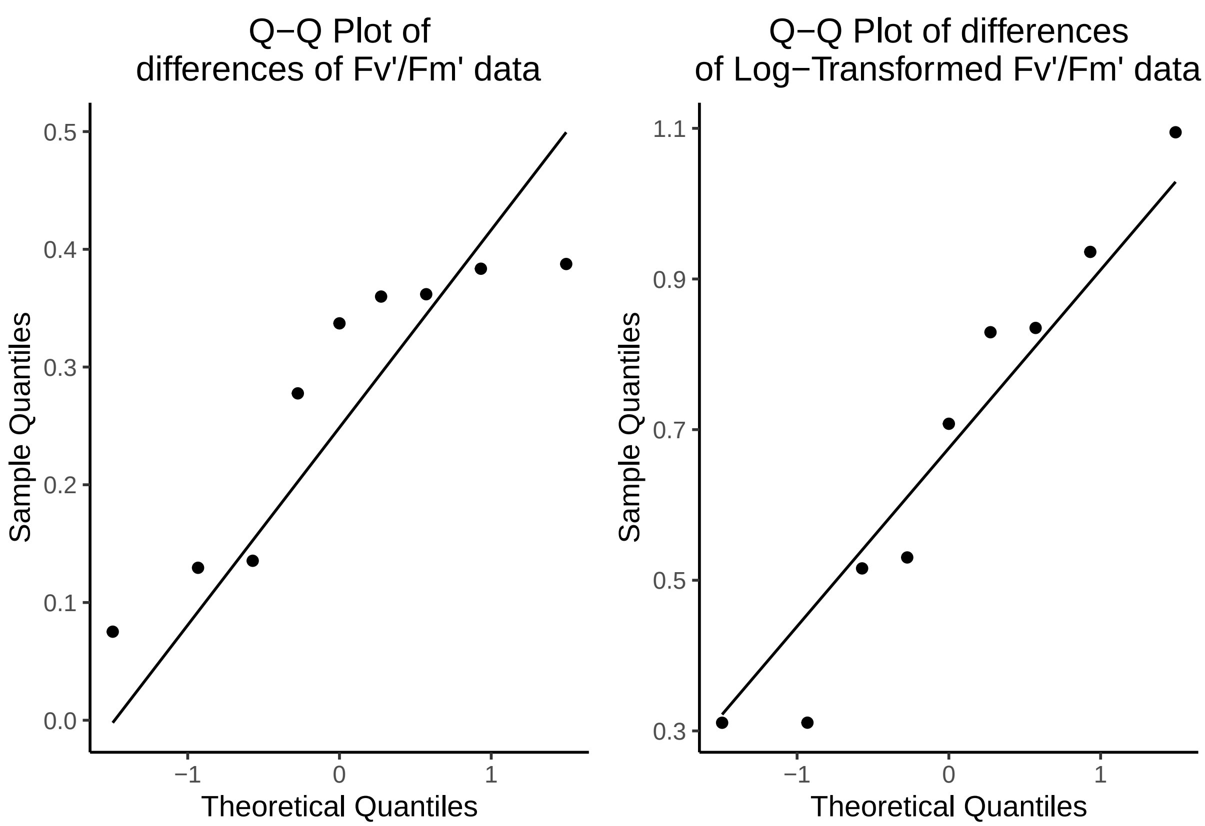
```
## # A tibble: 1 x 3
##   variable                statistic p.value
##   <chr>                       <dbl>   <dbl>
## 1 FVprimediff_data$diff      0.823  0.0375
```

The untransformed data are not normally distributed.

```
logFVprimediff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = FVprime, id_cols = plant) %>%
  mutate(diff = log(post) - log(pre))

shapiro_test(logFVprimediff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable                   statistic p.value
##   <chr>                          <dbl>   <dbl>
## 1 logFVprimediff_data$diff      0.942   0.607
```



the log-transformed data are normally distributed.

Statistical test

```r
(FVprime23<-t.test(log(W23$FVprime), log(U23$FVprime),
                   paired = TRUE,
                   alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  log(W23$FVprime) and log(U23$FVprime)
## t = 7.3504, df = 8, p-value = 3.995e-05
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  0.5038664       Inf
## sample estimates:
## mean difference
##       0.6745068
```

F_v'/Fm' was significantly higher following Tropical Storm Hilary in 2023.

2021

Assumption testing

```r
outliers21<-all21 %>% identify_outliers(FVprime)

print(outliers21)
```

```
##  [1] X           ETR         FVprime     PhiPs2      dateonly    pulse
##  [7] plant       Block       year        Treatment   is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```r
FVprimediff_data <- all21 %>%
  pivot_wider(names_from = pulse, values_from = FVprime, id_cols = Block) %>%
  mutate(diff = post - pre)
shapiro_test(FVprimediff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable              statistic p.value
##   <chr>                     <dbl>   <dbl>
## 1 FVprimediff_data$diff     0.859   0.223
```

Normal Distribution of differences.

Statistical test

```r
(FVprime21<-t.test(W21$FVprime,U21$FVprime,
                   paired = T,
                   alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W21$FVprime and U21$FVprime
## t = 2.9184, df = 4, p-value = 0.02166
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  0.02145723        Inf
## sample estimates:
## mean difference
##      0.07961283
```

F_v'/Fm' did increase signifiicantly but not by a meaningful amount.

##Interannual Comparison

###Assumption testing

Already tested for outliers. Test differences between years.

```r
FVprimediff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = FVprime, id_cols = plant) %>%
  mutate(diff = `2023` - `2021`)
shapiro_test(FVprimediff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable              statistic p.value
##   <chr>                     <dbl>   <dbl>
## 1 FVprimediff_data$diff     0.666 0.00427
```

```r
logFVprimediff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = FVprime, id_cols = plant) %>%
  mutate(diff = log(`2023`) - log(`2021`))
shapiro_test(logFVprimediff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable                 statistic p.value
##   <chr>                        <dbl>   <dbl>
## 1 logFVprimediff_data$diff     0.741  0.0320
```

The differences are not normally distributed. Log transformation improves normality but not beyond the threshold. We would proceed with non parametric testing, but with only 4 differences between pairs we do not meet the minimum sample size for symmetry testing. These data cannot be analyzed.

Φ_{PSII}

2023

Assumption testing

```
outliers23<-all23 %>% identify_outliers(PhiPs2)
print(outliers23[c(1,4:8,10:11)])
```

```
## [1] X           PhiPs2      dateonly    pulse       plant       Block       Treatment
## [8] is.outlier
## <0 rows> (or 0-length row.names)
```

No outliers.

```
PhiPS2diff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = PhiPs2, id_cols = plant) %>%
  mutate(diff = post - pre)
shapiro_test(PhiPS2diff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable             statistic p.value
##   <chr>                    <dbl>   <dbl>
## 1 PhiPS2diff_data$diff     0.707 0.00171
```

```
logPhiPS2diff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = PhiPs2, id_cols = plant) %>%
  mutate(diff = log(post) - log(pre))
shapiro_test(logPhiPS2diff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable                statistic p.value
##   <chr>                       <dbl>   <dbl>
## 1 logPhiPS2diff_data$diff     0.938   0.564
```

The differences are not normal but are normal after log-transformation.

Statistical test

```
(PhiPs223<- t.test(log(W23$PhiPs2), log(U23$PhiPs2),
                   paired = TRUE,
                   alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  log(W23$PhiPs2) and log(U23$PhiPs2)
## t = 6.9637, df = 8, p-value = 5.84e-05
```

```
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  1.095082      Inf
## sample estimates:
## mean difference
##        1.494044
```

PhiPS2 is significantly elevated following watering.

2021

Assumption Testing

```
outliers21<-all21 %>% identify_outliers(PhiPs2)
print(outliers21[c(1,4:8,10:11)])
```

```
## [1] X          PhiPs2     dateonly   pulse      plant      Block      Treatment
## [8] is.outlier
## <0 rows> (or 0-length row.names)
```

No outliers

```
PhiPS2diff_data <- all21 %>%
  pivot_wider(names_from = pulse, values_from = PhiPs2, id_cols = Block) %>%
  mutate(diff = post - pre)
shapiro_test(PhiPS2diff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable             statistic p.value
##   <chr>                    <dbl>   <dbl>
## 1 PhiPS2diff_data$diff     0.917   0.513
```

The differences are normally distributed.

Statistical test

```
(PhiPs221<-t.test(log(W21$PhiPs2), log(U21$PhiPs2),
                  paired = TRUE,
                  alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  log(W21$PhiPs2) and log(U21$PhiPs2)
## t = 0.32683, df = 4, p-value = 0.3801
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.2936844        Inf
## sample estimates:
## mean difference
##      0.05317634
```

There is not a significant increase in Phi_{PSII} in 2021.

Interannual Comparison

Assumption testing

```
PhiPs2diff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = PhiPs2, id_cols = plant) %>%
  mutate(diff = `2023` - `2021`)
shapiro_test(PhiPs2diff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable             statistic p.value
##   <chr>                    <dbl>   <dbl>
## 1 PhiPs2diff_data$diff     0.950   0.715
```

Statistical Test

```
(PhiPs22y<-t.test(U23$PhiPs2[1:4],U21$PhiPs2[1:4],
                  paired = TRUE,
                  alternative="greater"))
```

```
##
##  Paired t-test
##
## data:  U23$PhiPs2[1:4] and U21$PhiPs2[1:4]
## t = -7.8244, df = 3, p-value = 0.9978
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.3816204        Inf
## sample estimates:
## mean difference
##      -0.2933798
```

Φ_{PSII} did not get larger from 2021 to 2023. It may have decreased, but only very slightly.

F_v/F_m

```
df<-data
df$pulse<-factor(df$pulse, levels = c("post", "pre"))
df$year<-factor(df$year)

U23<-df %>% filter(pulse=="pre" & year==2023) %>% arrange(Treatment, Plant)
W23<-df %>% filter(pulse=="post" & year==2023)%>% arrange(Treatment, Plant)

all23<-rbind(U23,W23)
```

```r
U21<-df %>% filter(Treatment=="c"& year==2021) %>% arrange(Plant)
W21<-df %>% filter(Treatment=="w"& year==2021) %>% arrange(Plant)

all21<-rbind(U21,W21)

yearcomp<-rbind(U21,U23[1:5,])
```

2023

Check Assumptions

```r
outliers23<-all23 %>% identify_outliers(FvFm)
print(outliers23)
```

```
##  [1] X          FvFm       dateonly   year       Block      Plant
##  [7] Treatment  pulse      is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```r
FvFmdiff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = FvFm, id_cols = Plant) %>%
  mutate(diff = post - pre)
shapiro_test(FvFmdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable           statistic p.value
##   <chr>                  <dbl>   <dbl>
## 1 FvFmdiff_data$diff     0.936   0.505
```

These data are normally distributed.

Statistical Test

```r
(FvFm23<-t.test(W23$FvFm, U23$FvFm,
                paired = TRUE,
                alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W23$FvFm and U23$FvFm
## t = -6.1515, df = 9, p-value = 0.9999
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.1147923        Inf
## sample estimates:
## mean difference
##     -0.08843836
```

F_v/F_m actually decreased following watering.

2021

Check Assumptions

```
outliers21<-all21 %>% identify_outliers(FvFm)

print(outliers21)
```

```
##  [1] X          FvFm       dateonly   year       Block      Plant
##  [7] Treatment  pulse      is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```
FvFmdiff_data <- all21 %>%
  pivot_wider(names_from = Treatment, values_from = FvFm, id_cols = Block) %>%
  mutate(diff = w - c)
shapiro_test(FvFmdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable           statistic p.value
##   <chr>                  <dbl>   <dbl>
## 1 FvFmdiff_data$diff     0.938   0.654
```

Normal distribution.

Statistical test

```
(FvFm21<-t.test(W21$FvFm, U21$FvFm, paired = T, alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W21$FvFm and U21$FvFm
## t = 1.6762, df = 4, p-value = 0.08451
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.02128716         Inf
## sample estimates:
## mean difference
##      0.07829929
```

The difference is not significant, but marginal.

Interannual comparison

Assumption Check

```
FvFmdiff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = FvFm, id_cols = Plant) %>%
  mutate(diff = `2023` - `2021`)
shapiro_test(FvFmdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable           statistic p.value
##   <chr>                  <dbl>   <dbl>
## 1 FvFmdiff_data$diff     0.939   0.656
```

Normal distribution

Statistical Test

```
(FvFm2y<-t.test(U23$FvFm[1:5],U21$FvFm,
                paired = TRUE,
                alternative="greater"))
```

```
##
##  Paired t-test
##
## data:  U23$FvFm[1:5] and U21$FvFm
## t = 4.2565, df = 4, p-value = 0.006545
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  0.05533952        Inf
## sample estimates:
## mean difference
##       0.1108671
```

F_v/F_m was higher in 2023.

Water Potential

```
df<-data

df$pulse<-factor(df$pulse, levels = c("post", "pre"))
df$year<-factor(df$year)

U23<-df %>% filter(pulse=="pre" & year==2023) %>% arrange(treatment, plant)
W23<-df %>% filter(pulse=="post" & year==2023)%>% arrange(treatment, plant)

all23<-rbind(U23,W23)
```

```
U21<-df %>% filter(treatment=="c"& year==2021 & pulse=="post") %>% arrange(plant)
W21<-df %>% filter(treatment=="w"& year==2021&pulse=="post") %>% arrange(plant)

all21<-rbind(U21,W21)

yearcomp<-rbind(U21,U23[1:5,])
```

2023

Check Assumptions

```
outliers23<-all23 %>% identify_outliers(wp)

print(outliers23)
```

```
##  [1] X          wp         plant      Date       pulse      treatment
##  [7] Block      year       is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

No outliers.

```
wpdiff_data <- all23 %>%
  pivot_wider(names_from = pulse, values_from = wp, id_cols = plant) %>%
  mutate(diff = post - pre)
shapiro_test(wpdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable          statistic p.value
##   <chr>                 <dbl>   <dbl>
## 1 wpdiff_data$diff      0.939   0.546
```

Normal distribution of differences.

Statistical Test

```
wp23<-t.test(W23$wp, U23$wp, paired = T, alternative = "greater")
```

No significant change in water potential attributable to TS Hilary.

2021

Assumption check

```
outliers21<-all21 %>% identify_outliers(wp)
print(outliers21)
```

```
##  [1] X          wp         plant      Date       pulse      treatment
##  [7] Block      year       is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

no outliers.

```
wpdiff_data <- all21 %>%
  pivot_wider(names_from = treatment, values_from = wp, id_cols = Block) %>%
  mutate(diff = w - c)
shapiro_test(wpdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable         statistic p.value
##   <chr>                <dbl>   <dbl>
## 1 wpdiff_data$diff     0.916   0.507
```

Normal distribution.

Statistical test

```
(wp21<-t.test(W21$wp, U21$wp,
              paired = T,
              alternative = "greater"))
```

```
##
##  Paired t-test
##
## data:  W21$wp and U21$wp
## t = -1.2044, df = 4, p-value = 0.8526
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  -0.7202025        Inf
## sample estimates:
## mean difference
##           -0.26
```

No significant change in water potential due to watering.

Interannual comparison

Assumption check

```
wpdiff_data <- yearcomp %>%
  pivot_wider(names_from = year, values_from = wp, id_cols = plant) %>%
  mutate(diff = `2023` - `2021`)
shapiro_test(wpdiff_data$diff)
```

```
## # A tibble: 1 x 3
##   variable          statistic  p.value
##   <chr>                 <dbl>    <dbl>
## 1 wpdiff_data$diff     0.603 0.000683
```

Normal distribution ###Statistical Test

```
(wp2y<-t.test(U23$wp[1:5],U21$wp, paired = TRUE, alternative="greater"))
```

```
##
##  Paired t-test
##
## data:  U23$wp[1:5] and U21$wp
## t = 3.8801, df = 4, p-value = 0.00892
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
##  0.2428017       Inf
## sample estimates:
## mean difference
##       0.5388785
```

The leaf water potential of plants was significantly higher in 2023 by about 0.8 MPa.

Correction for multiple comparisons: Benjamini-Hochberg Procedure

```
test_list<-list(
  Photo21=Photo21,
  Photo23=Photo23,
  Photo2y=Photo2y,
  Cond21=Cond21,
  Cond23=Cond23,
  Cond2y=Cond2y,
  FVprime21=FVprime21,
  FVprime23=FVprime23,
  PhiPs221=PhiPs221,
  PhiPs223=PhiPs223,
  PhiPs22y=PhiPs22y,
  FvFm21=FvFm21,
  FvFm23=FvFm23,
  FvFm2y=FvFm2y,
  wp21=wp21,
  wp23=wp23,
  wp2y=wp2y)


get_pvalue <- function(x) {
  if ("p.value" %in% names(x)) {
    return(x$p.value)
  } else if ("p" %in% names(x)) {
```

```r
    return(x$p)
  }
}


pvalue_df <- data.frame(
  test_name = names(test_list),
  p_value = sapply(test_list, get_pvalue)
)

pvalue_df<-pvalue_df %>% arrange(p_value)

pvalue_df$rank<-seq(1,17,1)
Benjamini<-pvalue_df %>%
  mutate(criticalvalue=(rank/17)*0.05) %>%
  mutate(CorrSig=sign(criticalvalue - p_value), UncorrSig=sign(0.05-p_value))
rownames(Benjamini)<-NULL

print(Benjamini)
```

```
##    test_name      p_value rank criticalvalue CorrSig UncorrSig
## 1  FVprime23 3.995355e-05    1   0.002941176       1         1
## 2   PhiPs223 5.840255e-05    2   0.005882353       1         1
## 3     Cond23 1.950000e-03    3   0.008823529       1         1
## 4    Photo23 4.078338e-03    4   0.011764706       1         1
## 5     FvFm2y 6.545160e-03    5   0.014705882       1         1
## 6       wp2y 8.919909e-03    6   0.017647059       1         1
## 7  FVprime21 2.165595e-02    7   0.020588235      -1         1
## 8     Cond2y 3.125000e-02    8   0.023529412      -1         1
## 9     FvFm21 8.450810e-02    9   0.026470588      -1        -1
## 10    Cond21 1.015427e-01   10   0.029411765      -1        -1
## 11   Photo21 1.059794e-01   11   0.032352941      -1        -1
## 12   Photo2y 3.192387e-01   12   0.035294118      -1        -1
## 13  PhiPs221 3.800925e-01   13   0.038235294      -1        -1
## 14      wp21 8.525920e-01   14   0.041176471      -1        -1
## 15      wp23 9.063850e-01   15   0.044117647      -1        -1
## 16  PhiPs22y 9.978267e-01   16   0.047058824      -1        -1
## 17    FvFm23 9.999158e-01   17   0.050000000      -1        -1
```

In the above table, the "Critical Value" column represent the threshold for significance for each test. Tests that were significant in the uncorrected analysis have a positive 1 in the "UncorrSig" column and the "CorrSig" column shows tests that found significant differences after correction in the same way.

The Benjamini-Hochberg procedure for controlling the false discovery rate corrects three of the eight previously significant tests as non-significant. These parameters are the baseline water potential and stomatal conductance between the years, and the F_v'/Fm' in 2021.

5 tests are statistically significant, and four of them are responses of physiological variables to tropical storm Hilary. The two light-adapted fluoresence measurements, F_v'/Fm' and Φ_{PSII}, and the two gas exchange measurements, A_{NET} and g_s, were significantly different after the precipitation in 2023. F_v/F_m was significantly higher in unwatered plants in 2023 than in 2021.

In addition to these 5 significant tests, we had two tests that were likely significant opposite the direction of the hypothesis. Baseline Φ_{PSII} likely was lower in 2023 than in 2021 and F_v/F_m looks to have decreased following watering in 2023.