

JoVE PH3 quantitation – Hagen et al., 2022

#All code is in grey boxes. As this is a simple plot comparing two means, use the GUI web interface listed below to get starter code for the plot: <https://shiny.gmw.rug.nl/ggplotgui/>, then perform the statistical analysis.

1. Load libraries to be able to make plots and perform statistical comparisons.

```
library(readxl)
library(ggplot2)
library(tidyverse)
library(ggsci)
library(ggsignif)
library(ggpubr)
library(rmarkdown)
```

- 1.1. Note that the first line of code reads the data file in.

- 1.2. Set up the comparison that will ultimately be made with the statistics.

- 1.3. Note that the final bit of code in this chunk makes a “Figure” of the plot. It generates a boxplot overlaid by a scatter plot of all points with jitter.

- 1.4. Note that the last line of code prints the plot.

```
PH3_data <- read_excel("/filepath/PH3 quantitation_9dpf.xlsx")
```

2. Raw data table:

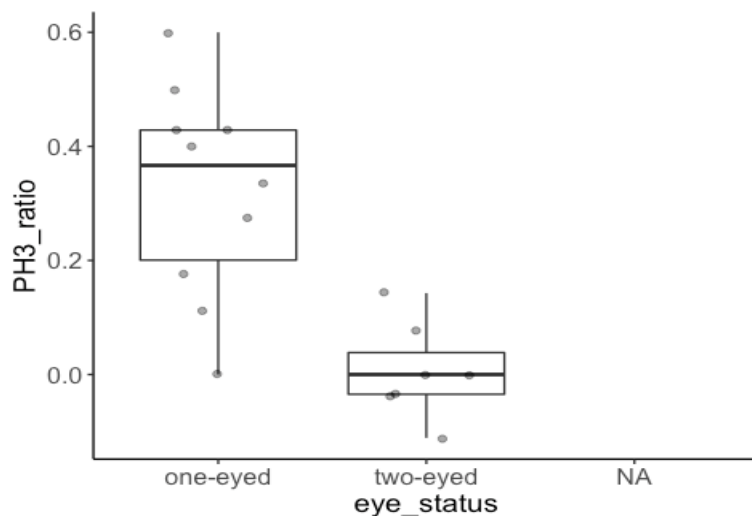
image	eye_status	innervated or left PH3 count	denervated or right PH3 count	PH3_ratio (L-R/total)
1	one-eyed	5	2	0.42857143
2	one-eyed	10	7	0.17647059
3	one-eyed	3	3	0
4	one-eyed	8	4	0.33333333
5	one-eyed	4	1	0.6
6	one-eyed	5	4	0.11111111
7	one-eyed	5	2	0.42857143
8	one-eyed	7	4	0.27272727
11	one-eyed	7	3	0.4
12	one-eyed	3	1	0.5
1 WT	two-eyed	3	3	0
3 WT	two-eyed	3	3	0
4 WT	two-eyed	13	14	-0.037037

5 WT	two-eyed	14	12	0.07692308
6 WT	two-eyed	15	16	-0.0322581
7 WT	two-eyed	4	3	0.14285714
8 WT	two-eyed	8	10	-0.11111111

```
mycomparison <- list(c("one-eyed", "two-eyed"))
```

```
Figure <- ggplot(PH3_data, aes(x = eye_status, y = PH3_ratio)) +
  geom_boxplot(notch = FALSE) +
  geom_jitter(size = 1.5, alpha = 0.4, width = 0.25, colour = 'black') +
  theme_classic() +
  theme(
    axis.title = element_text(size = 14),
    axis.text = element_text(size = 12),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank())
```

Figure



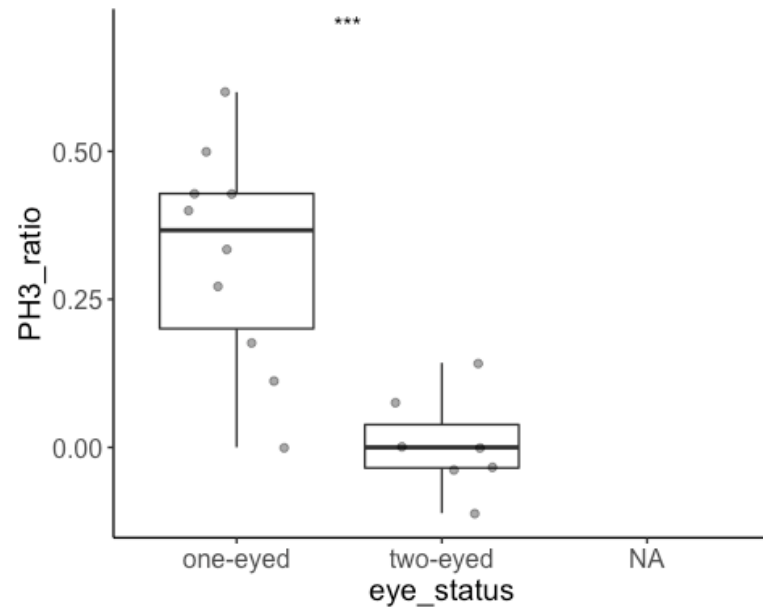
2.1. To see the statistical comparison results as a table, use the `compare_means` function.

```
compare_means(PH3_ratio ~ eye_status, data = PH3_data, method = "t.test", paired = FALSE)
## # A tibble: 1 x 8
##   .y.      group1  group2      p    p.adj p.format p.signif method
##   <chr>    <chr>   <chr>    <dbl>  <dbl> <chr>    <chr>   <chr>
## 1 PH3_ratio one-eyed two-eyed 0.000325 0.00033 0.00033 ***     T-test
```

2.2. Create a plot that has a statistical comparison on it. Name the next plot something easy (e.g., A) and use `stat_compare_means` to perform the same analysis as above (Welch's *t*-test) but label the significance according to * = $p < 0.05$, ** = $p < 0.005$, and *** = $p < 0.0005$

```
A <- Figure + stat_compare_means(method = "t.test", paired = FALSE, label = "p.signif", label.
y = 0.7, label.x = 1.5)
```

A



2.3. Save the figure so that it is of a specific size.

```
ggsave("PH3_compare_plot_stat_final1_signif.png", A, width = 9, height = 10, units = "cm")
```