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This R-script is part of the manuscript "The ectoparasitic seal louse, *Echinophthirius horridus*, relies on a sealed tracheal system and spiracle closing apparatus for underwater respiration"

```
+++++++ ANOVA Buoyancy  
+++++++  
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```
# Clear workspace
```

```
rm(list = ls())
```

```
library(ggplot2)
```

```
library(tidyverse)
```

```
library(ggpubr)
```

```
library(rstatix)
```

```
# Import dataset
```

```
library(readxl)
```

```
Supplementary_Data1 <- read_excel("F:/Manuskripte/Buoyancy/Datens?tze/  
Supplementary_Data1",
```

```
    sheet = "ANOVA")
```

```
View(Supplementary_Data1)
```

```
attach(Supplementary_Data1)
```

```
# Normality assumption
```

```
shapiro.test(S3_Buoyancy$Respiration)
```

```
# Data preparation
```

```
Supplementary_Data1 <- Supplementary_Data1 %>%
```

```
  gather(key = "Treatment", value = "Respiration", Natural, Triton, Washed) %>%
```

```
  convert_as_factor(Species_shortcut, Iteration)
```

```
data.frame(head(Supplementary_Data1))
```

```
# Summary statistics
```

```
Supplementary_Data1 %>%
```

```
  group_by(Treatment) %>%
```

```
  get_summary_stats(Respiration, type = "mean_sd")
```

```
# Check for outliers
```

```
Supplementary_Data1 %>%
```

```
  group_by(Treatment) %>%
```

```
  identify_outliers(Respiration)
```

```
# Normality assumption
```

```
Supplementary_Data1 %>%
```

```
  group_by(Treatment) %>%
```

```
  shapiro_test(Respiration) # normally distributed -> One-way ANOVA
```

```
# One-way ANOVA
```

```
one.way <- aov(Respiration ~ Treatment, data = Supplementary_Data1)
```

```
summary(one.way)
```

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```
+++++++ Boxplot Buoyancy
```

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```
# Clear workspace
```

```
rm(list = ls())
```

```
library(ggplot2)
```

```
# Import dataset
```

```
library(readxl)
```

```
Supplementary_Data1 <- read_excel("F:/Manuskripte/Buoyancy/Datens?tze/  
Supplementary_Data1.xlsx",
```

```
    sheet= "Calculations")
```

```
View(Supplementary_Data1)
```

```
attach(Supplementary_Data1)
```

```
# Make boxplot
```

```
pd = position_dodge(width = 1.1)
```

```
p <- ggplot(Supplementary_Data1, aes(x=Iteration_of_measurement, y=Vresp_in_mm3_calibrated))  
+  
  stat_boxplot(geom='errorbar', position = pd, width=0.1) +  
  geom_boxplot(width = 0.7, position=position_dodge(width = 1.1)) +  
  stat_summary(fun = mean, geom = "point", color = "firebrick", shape = 17, size = 2, position =  
position_dodge(width = 1.1)) +  
  scale_fill_manual(values=c("#999999", "#E69F00")) +  
  theme_classic() +  
  labs(y = "Vresp in mm3") +  
  theme(plot.title = element_text(hjust = 0.5, size = 16, face = "bold"), plot.caption =  
element_text(hjust = 0, size = 10), plot.tag = element_text(size = 16, face = "bold")) + # adjust title  
and caption position  
  scale_y_continuous(expand = c(0, 0), limits = c(0, 0.1))  
p
```

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```
+++++++ Lineplot Buoyancy  
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+++++++
```

```
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```

```
# Clear workspace
```

```
rm(list = ls())
```

```
library(ggplot2)
```

```
# Import dataset
```

```
library(readxl)
```

```
Supplementary_Data1 <- read_excel("F:/Manuskripte/Buoyancy/Datens?tze/  
Supplementary_Data1",
```

```
    sheet = "Progression_curve")
```

```
View(Supplementary_Data1)
```

```
attach(Supplementary_Data1)
```

```
# Make plot
```

```
p <- ggplot(Supplementary_Data1, aes(x = time, y = Vresp_in_mm3_calibrated, colour=Iteration)) +  
  geom_point(size=2) +  
  stat_smooth(se=F) +  
  theme_classic()
```

```
p
```

```
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```
+++++++ Scatterplot comparison of tracheal volume
```

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```

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```

```
# Clear workspace
```

```
rm(list = ls())
```

```
library(ggplot2)
```

```

# Import dataset

library(readxl)

Supplementary_Data3 <- read_excel("F:/Manuskripte/Buoyancy/ Supplementary_Data3")

View(Supplementary_Data3)


attach(Supplementary_Data3)


# Make barplot


tracheal.data <- data.frame(

  tracheal_ID <- c(1:10),

  Species_Name <-
c("Tribolium_castaneum", "Pediculus_humanus_capitis", "Echinophthirius_horridus", "Tenebrio_molit
or", "Calliphora_vicina", "Carausius_morosus", "Schistocerca_americana", "Blaptica_duplica", "Eleodes_
obscura", "Manduca_sexta"),

  Volume <- c(0.5, 0.27, 0.22, 2.76, 49.15, 1.3, 40, 12, 4.8, 7.5))


# Plot the bar chart

barplot(Volume, names.arg=Species_Name, xlab="Species",
        ylab="Tracheal volume", col="black")

```