Biehler et al

Tricia

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load libraries

```r
library(ggplot2)
library(dplyr)
```

Import CSV

```r
data <- read.csv("../datafiles/BiehlerDataBKA.csv", header = TRUE, sep = ",")

# Convert 'Sex' and 'Species' to factors
data$Sex <- factor(data$Sex)
data$Species <- factor(data$Species)
```

Mean and Standard Devation for BKA

```r
# Calculate mean and SD for each species
summary_stats <- data %>%
  group_by(Species) %>%
  summarise(
    Mean = mean(Bacterial_Killing_Percentage, na.rm = TRUE),
    SD = sd(Bacterial_Killing_Percentage, na.rm = TRUE)
  )

# Print the summary statistics
print(summary_stats)
```
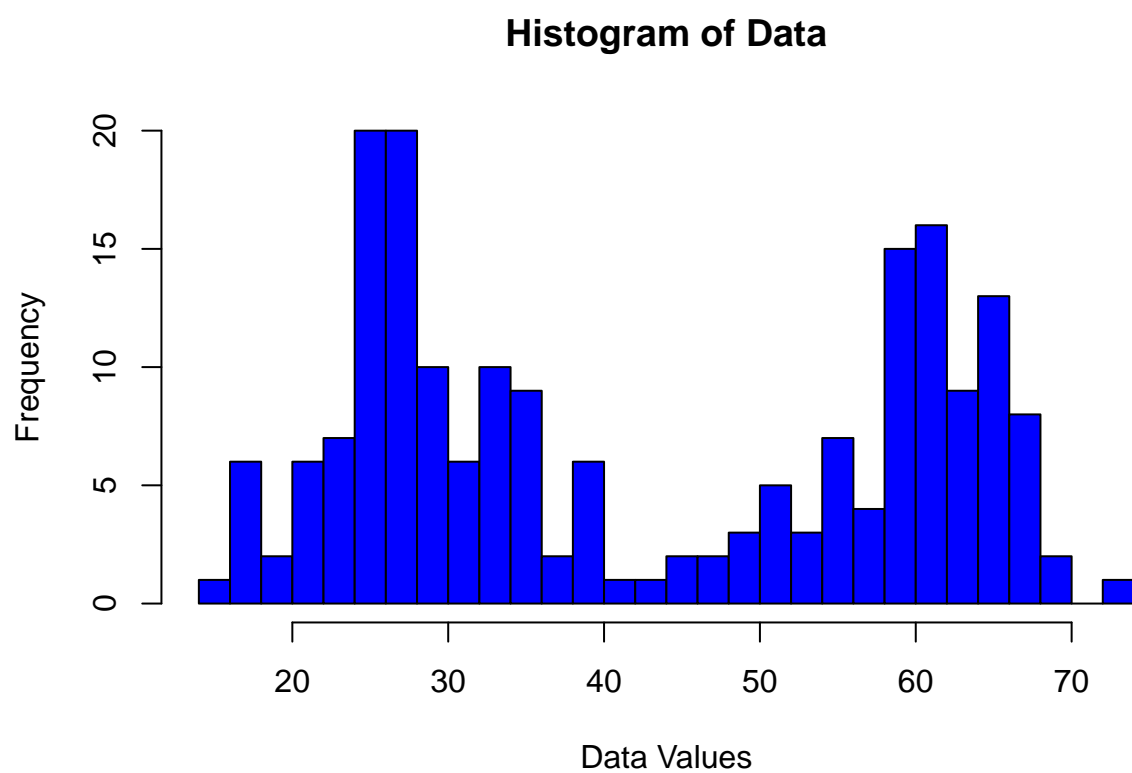
```
## # A tibble: 4 x 3
##   Species  Mean    SD
##   <fct>   <dbl> <dbl>
## 1 AMGO     26.3  4.76
## 2 HOFI     60.1  5.56
## 3 LEGO     27.6  6.12
## 4 PISI     33.4  7.38
```
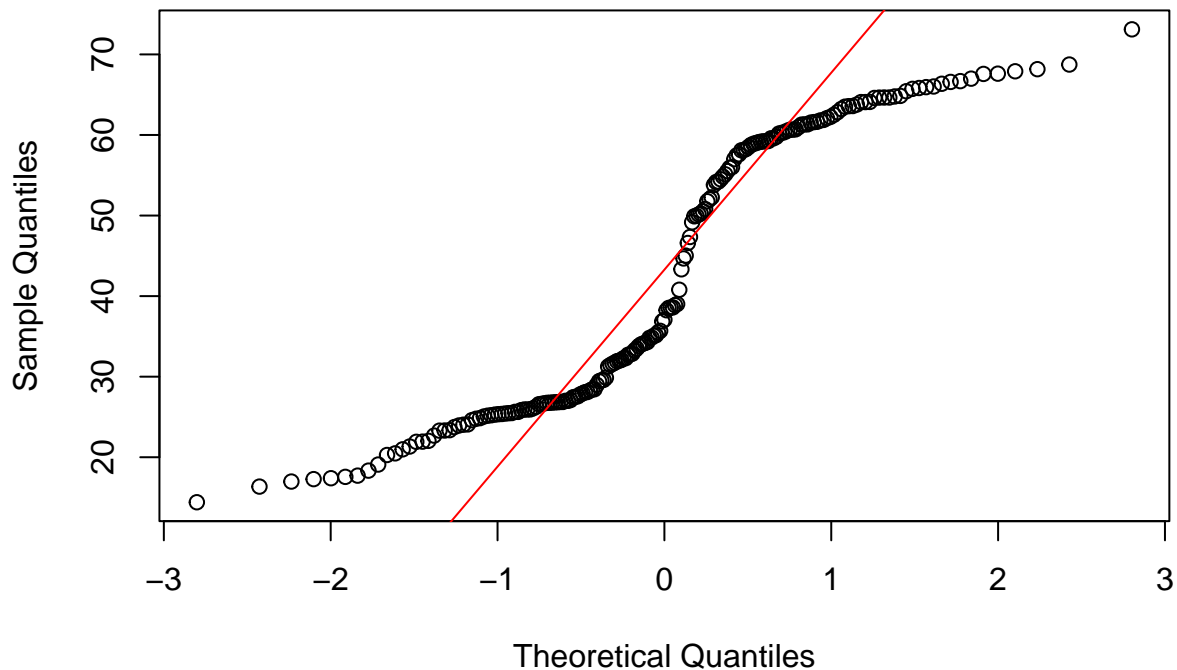
normality for BKA

```r
# histogram
hist(data$Bacterial_Killing_Percentage, main="Histogram of Data", xlab="Data Values",
```

```
                        breaks=30, col="blue")
```

Histogram of Data


```
# qqplot
qqnorm(data$Bacterial_Killing_Percentage)
qqline(data$Bacterial_Killing_Percentage, col = "red")
```

Normal Q–Q Plot


```r
# shapiro-wilk
shapiro.test(data$Bacterial_Killing_Percentage)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  data$Bacterial_Killing_Percentage
## W = 0.88674, p-value = 4.913e-11
```

Kruksal-Wallis of BKA

```r
kruskal.test(data$Bacterial_Killing_Percentage, data$Species)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  data$Bacterial_Killing_Percentage and data$Species
## Kruskal-Wallis chi-squared = 149.93, df = 3, p-value < 2.2e-16
```

Pairwise comparison with Bonferroni correction

```r
bka_pairwise_results <- pairwise.wilcox.test(data$Bacterial_Killing_Percentage,
        data$Species, p.adjust.method = "bonferroni")
print(bka_pairwise_results)
```
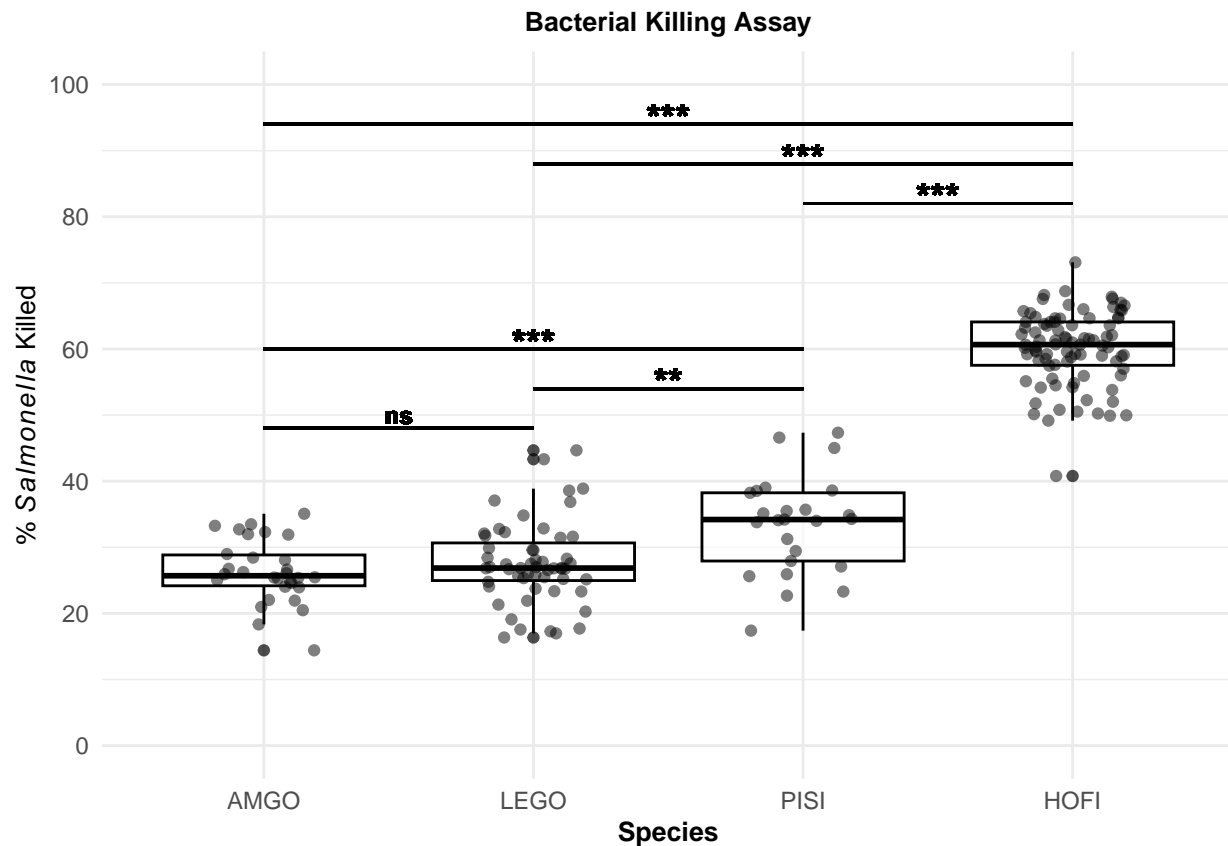
```
## 
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
## 
## data:  data$Bacterial_Killing_Percentage and data$Species
## 
##      AMGO    HOFI    LEGO
## HOFI 2.3e-15 -       -
## LEGO 1.00000 < 2e-16 -
## PISI 0.00039 2.2e-13 0.00462
## 
## P value adjustment method: bonferroni
```

Boxplot of BKA

```r
# Reorder the species factor levels
data$Species <- factor(data$Species, levels = c("AMGO", "LEGO", "PISI", "HOFI"))

# Create box plot with individual points
BKAAssay <- ggplot(data, aes(x=Species, y=Bacterial_Killing_Percentage)) +
  geom_boxplot(color="black", alpha=0.7) +  # Box plot
  geom_jitter(width=0.2, size=1.5, alpha=0.5) +  # Jittered points
  labs(title="Bacterial Killing Assay",
       x="Species",
       y=expression("% " * italic(Salmonella) * " Killed")) +
  scale_y_continuous(limits = c(0, 100), breaks = seq(0, 100, by = 20))  +
  theme_minimal() +
  theme( plot.title = element_text(hjust = 0.5, face = "bold", size = 10),
    axis.title.x = element_text(face = "bold", size = 10),
    axis.title.y = element_text(face = "bold", size = 10)
  )  +
  # Add line for significance between AMGO and HOFI
  geom_segment(aes(x=1, xend=4, y=94, yend=94), color="black", linewidth=.5) +
  # Add text for significance label "***"
  geom_text(aes(x=2.5, y=95, label="***"), color="black", size=5, fontface="bold") +
  # Add line for significance between LEGO and HOFI
  geom_segment(aes(x=2, xend=4, y=88, yend=88), color="black", linewidth=.5) +
  # Add text for significance label "***"
  geom_text(aes(x=3, y=89, label="***"), color="black", size=5, fontface="bold") +
  # Add line for significance between PISI and HOFI
  geom_segment(aes(x=3, xend=4, y=82, yend=82), color="black", linewidth=.5) +
  # Add text for significance label "***"
  geom_text(aes(x=3.5, y=83, label="***"), color="black", size=5, fontface="bold") +
  # Add line for significance between PISI and AMGO
  geom_segment(aes(x=1, xend=3, y=60, yend=60), color="black", linewidth=.5) +
  # Add text for significance label "***"
  geom_text(aes(x=2, y=61, label="***"), color="black", size=5, fontface="bold") +
  # Add line for significance between PISI and LEGO
  geom_segment(aes(x=2, xend=3, y=54, yend=54), color="black", linewidth=.5) +
  # Add text for significance label "***"
  geom_text(aes(x=2.5, y=55, label="**"), color="black", size=5, fontface="bold") +
  # Add line for significance between AMGO and LEGO
  geom_segment(aes(x=1, xend=2, y=48, yend=48), color="black", linewidth=.5) +
  # Add text for significance label "***"
```

```
    geom_text(aes(x=1.5, y=50, label="ns"), color="black", size=3, fontface="bold")

print(BKAAssay)
```


export tiff with 300dpi
```
ggsave(
filename="../figures/BKAAssay.tiff",
plot = BKAAssay,
width = 200,
height = 200,
units = c("mm"),
dpi = 300,
bg = "white"
)
```

Test effect of sex

```
clean_data <- data[!is.na(data$Species) & !is.na(data$Sex), ]
wilcox.test(data$Bacterial_Killing_Percentage ~ data$Sex)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  data$Bacterial_Killing_Percentage by data$Sex
## W = 5033.5, p-value = 0.1569
```

```
## alternative hypothesis: true location shift is not equal to 0
```

Kruskal-Wallis test for interaction

```
kw_test <- kruskal.test(Bacterial_Killing_Percentage ~ interaction(Species, Sex), data = data)
print(kw_test)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Bacterial_Killing_Percentage by interaction(Species, Sex)
## Kruskal-Wallis chi-squared = 149.79, df = 7, p-value < 2.2e-16
```

Pairwise Results

```
pairwise_results <- pairwise.wilcox.test(data$Bacterial_Killing_Percentage,
                interaction(data$Species, data$Sex),
                p.adjust.method = "bonferroni")
  print(pairwise_results)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum exact test
##
## data:  data$Bacterial_Killing_Percentage and interaction(data$Species, data$Sex)
##
##             AMGO.Female LEGO.Female PISI.Female HOFI.Female AMGO.Male LEGO.Male
## LEGO.Female 1.000       -           -           -           -         -
## PISI.Female 0.196       1.000       -           -           -         -
## HOFI.Female 2.5e-09     1.6e-10     6.1e-10     -           -         -
## AMGO.Male   1.000       1.000       0.119       1.3e-13     -         -
## LEGO.Male   1.000       1.000       0.074       < 2e-16     1.000     -
## PISI.Male   0.157       1.000       1.000       6.1e-10     0.076     0.190
## HOFI.Male   7.3e-06     1.1e-06     3.6e-06     1.000       5.2e-09   5.8e-15
##             PISI.Male
## LEGO.Female -
## PISI.Female -
## HOFI.Female -
## AMGO.Male   -
## LEGO.Male   -
## PISI.Male   -
## HOFI.Male   2.7e-06
##
## P value adjustment method: bonferroni
```

Import Growth Curve Data

```
growthcurvedata <- read.csv("../datafiles/GrowthCurve.csv", header = TRUE, sep = ",")
```

Growth Curve

```r
# Calculate mean absorbance for each time point
average_data <- growthcurvedata %>%
  group_by(Time.h.) %>%
  summarise(
    Mean = mean(A600),
    SD = sd(A600),
    Min = min(A600),
    Max = max(A600)
  )

# Plot with smoothed line and shaded area representing the standard deviation
growthcurve <- ggplot() +
  geom_ribbon(data = average_data, aes(x = Time.h., y = Mean, ymin = Min, ymax = Max),
              fill = "grey80", alpha = 0.5) +
  geom_smooth(data = average_data, aes(x = Time.h., y = Mean), method = "loess",
              formula = y ~ x, color = "black", se = FALSE,
              size = 0.5) +
  geom_point(data = growthcurvedata, aes(x = Time.h., y = A600), color = "black", size = 0.5) +
  labs(title = "Bacterial Growth Curve", x = "Time of Incubation (h)",
       y = "Absorbance (600nm)") +
  theme_minimal() +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold", size = 10),
    axis.title.x = element_text(face = "bold", size = 10),
    axis.title.y = element_text(face = "bold", size = 10)
  )

print(growthcurve)
```

Bacterial Growth Curve
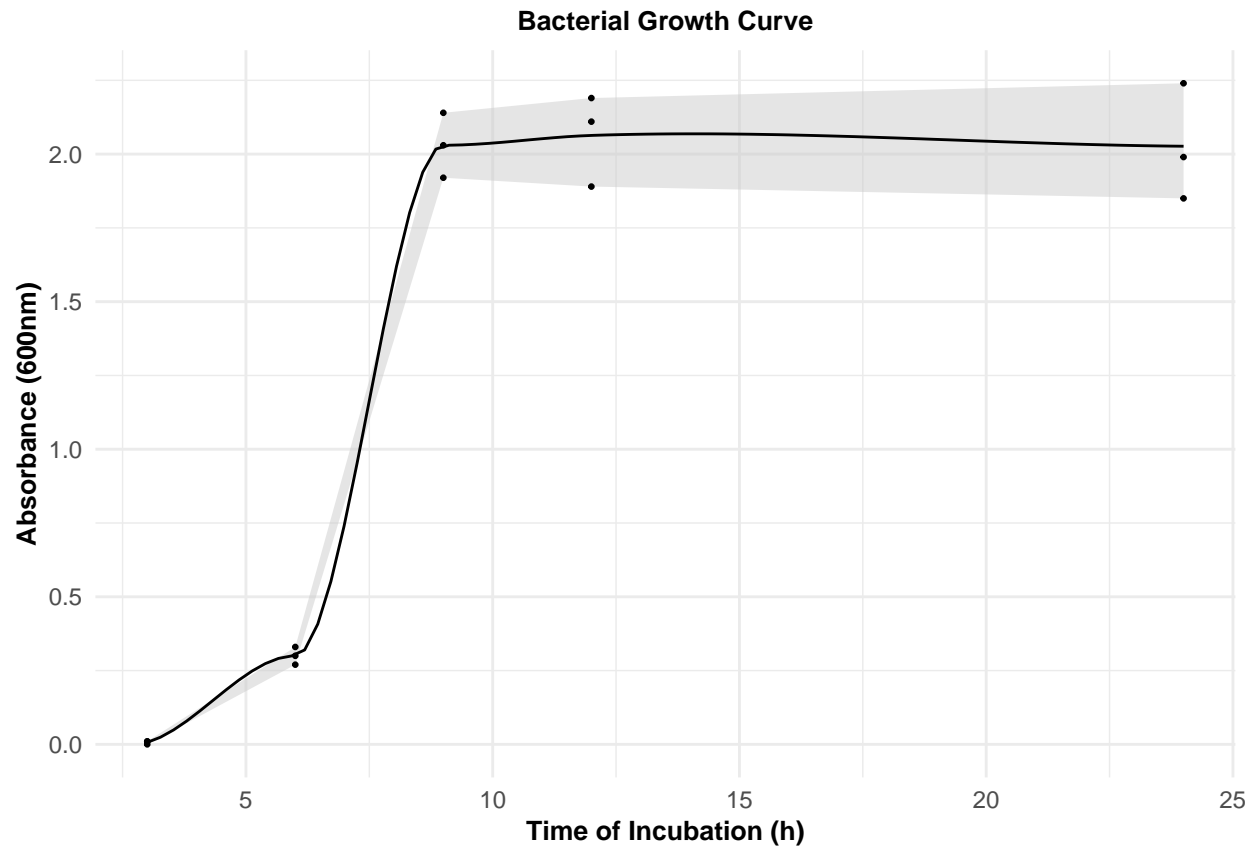
export tiff with 300dpi

```
ggsave(
filename="../figures/growthcurve.tiff",
plot = growthcurve,
width = 200,
height = 100,
units = c("mm"),
dpi = 300,
bg = "white"
)
```