## Importing data into R

<http://www.sthda.com/english/wiki/best-practices-in-preparing-data-files-for-importing-into-r>

GC.data <- read.csv("C:/Users/ihear/OneDrive/Desktop/Lab work/R Scripts/GCforRS.csv", header = TRUE, colClasses = c("factor", "numeric"))

## Importing data into R using Excel

library(readxl)

GrowthMalandMDs <- read\_excel("C:/Users/ihear/OneDrive/Desktop/LabWork/RScripts/GrowthMalandMDs.xlsx")

View(GrowthMalandMDs)

## How to do stats in R

https://www.scribbr.com/statistics/anova-in-r/

one.way <- aov(Group ~ AmtGas, data = GC.data)

<http://www.sthda.com/english/wiki/one-way-anova-test-in-r#interpret-the-result-of-one-way-anova-tests>

<http://www.sthda.com/english/wiki/comparing-means-in-r>

<http://www.sthda.com/english/articles/24-ggpubr-publication-ready-plots/76-add-p-values-and-significance-levels-to-ggplots/>

<https://www.datanovia.com/en/blog/how-to-add-p-values-to-ggplot-facets/>

## Add p-vals to ggplot facets with different scales

<https://www.datanovia.com/en/blog/add-p-values-to-ggplot-facets-with-different-scales/>

## Making fancy tables in R

https://rfortherestofus.com/2019/11/how-to-make-beautiful-tables-in-r/

<https://rfortherestofus.com/2019/11/how-to-make-beautiful-tables-in-r/>

## Creating barplots in R using ggplot2

<https://www.datanovia.com/en/lessons/ggplot-barplot/>

Barplots with multiple groups and some other enhancements

<https://www.datanovia.com/en/lessons/highchart-interactive-bar-plot-in-r/>

Install ggpubr – this provides some easy to use functions for creating and customizing ggplot2 to make publication ready plots

Install rstatix – provides pipeline friendly R functions for easy statistical analysis

Install.packages(“ggpubr”)

Install.packages(“rstatix”)

Install.packages(“broom”)

Install.packages(“AICcmodavg”)

Loading R package (install if you do not ggplot):

library(ggplot2)

df <- data.frame(tx=c("Control(PBS)", "CS", "HM", "PS", "VF"), amountgas=c(1.3935, 7.7774, 10.0970, 6.8793, 7.1354))

theme\_set

theme\_classic()

theme(legend.position = "right")

f <- ggplot(df, aes(y = amountgas, x = tx))

View(f)

f + geom\_col()

f + geom\_col(fill = "cornflowerblue")+

geom\_text(aes(label = amountgas), vjust = 1.6, color = "beige")

#Could add text but don’t want to in this case

#puts in alphabetical order, but want control (PBS) first, so change name to control

­­

# Change barplot fill colors by groups­­

f + geom\_col(aes(fill = dose)) +

scale\_fill\_manual(values = c("#00AFBB", "#E7B800", "#FC4E07"))

Change the width of bars using the argument pointWidth (e.g.: width = 15).

# Change bar widths

df %>% hchart(

'column', hcaes(x = dose, y = len),

pointWidth = 15))

# Change barplot fill colors by groups

f + geom\_col(aes(fill = dose)) +

scale\_fill\_manual(values = c("#00AFBB", "#E7B800", "#FC4E07"))

Customizing data bars

<https://kcuilla.github.io/reactablefmtr/articles/data_bars.html>

R Syntax Quirks

<https://www.computerworld.com/article/2497319/business-intelligence-beginner-s-guide-to-r-syntax-quirks-you-ll-want-to-know.html>

June’s R Code

<https://github.com/darrell25/Teichmann_2020/blob/main/R_code/FF2_RS_But_Stacked_Bar.R>

# Bar plot of mean +/-se

ggbarplot(GrowthMalandMDs, x= “substrates”, y = “LogCFU”, add = "mean\_se")+

stat\_compare\_means() +

stat\_compare\_means(ref.group = "0.5", label = "p.signif",

label.y = c(22, 29)) # compare to ref.group

f3 <- ggplot(GrowthMalandMDs, aes(y = amtgrowth, x = substrate, add = mean\_se))

View(f2)

f2 + geom\_col()

f2 + geom\_col(fill = "cornflowerblue")+

geom\_text(aes(label = amtgrowth), vjust = 1.6, color = "beige")

Script to at least put error bars on in R

https://r-graph-gallery.com/4-barplot-with-error-bar.html

https://r-graph-gallery.com/218-basic-barplots-with-ggplot2.html

*# create dummy data*

data <- **data.frame**(

name=letters[1:5],

value=**sample**(**seq**(4,15),5),

sd=**c**(1,0.2,3,2,4)

)

*# Most basic error bar*

**ggplot**(data) +

**geom\_bar**( **aes**(x=name, y=value), stat="identity", fill="skyblue", alpha=0.7) +

**geom\_errorbar**( **aes**(x=name, ymin=value-sd, ymax=value+sd), width=0.4, colour="orange", alpha=0.9, size=1.3)

This works, could maybe try more to get the colors you want and put spaces in the titles. Try sizing and exporting as TIFF

*# Create data*

Data3 <- data.frame(

Substrate=c("Control(PBS)","Mal","MD4-7","MD16.5-19.5") ,

LogCFUpermL=c(2.004,8.906,7.668,8.906),

sd=c(0.325869033, 0.133575546, 0.03905327, 0.018268127))

*# Most basic error bar*

**ggplot**(Data3) +

**geom\_bar**( **aes**(x=Substrate, y=LogCFUpermL, fill=Substrate), stat="identity", alpha=0.7) +

**geom\_errorbar**( **aes**(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.2) +

theme\_classic()

<https://stackoverflow.com/questions/38907514/saving-a-high-resolution-image-in-r>

Saving as TIFF to set size and res of image:

tiff("test.tiff", units="in", width=5, height=5, res=300)

# insert ggplot code

dev.off()

res=300 specifies that you need a figure with a resolution of 300 dpi. The figure file named 'test.tiff' is saved in your working directory.

Change width and height in the code above depending on the desired output.

Note that this also works for other R plots including plot, image, and pheatmap.

To use tiff command, you have to install.packages(“tiff”)

<https://cran.r-project.org/web/packages/tiff/index.html>

Still, does not work, error message is Error: unexpected input in “tiff(“”

tiff(”Growth\_Mal\_MDs.tiff”, units="in", width=5, height=5, res=300)

**ggplot**(Data3) +

**geom\_bar**(**aes**(x=Substrate, y=LogCFUpermL, fill=Substrate), stat="identity", alpha=0.7) +

**geom\_errorbar**(**aes**(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.2) +

theme\_classic()

dev.off()

To change colors of the bars:

**ggplot**(Data3) +

**geom\_bar**(**aes**(x=Substrate, y=LogCFUpermL, fill= “magenta”, “mediumorchid”, “mistyrose4”, “orange4”), stat="identity", alpha=0.7) +

**geom\_errorbar**(**aes**(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.2) +

theme\_classic()

\*\*Unexpected input, cannot put colors in as above \*\*

**ggplot**(Data3) +

**geom\_bar**(**aes**(x=Substrate, y=LogCFUpermL, fill=Substrate), stat="identity", alpha=0.7) +

scale\_fill\_manual(values=c(“magenta”, “mediumorchid”, “mistyrose4”, “orange4”)) +

**geom\_errorbar**(**aes**(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.2) +

theme\_classic()

\*\*Error in the scale\_fill\_manual, unexpected input

scale\_fill\_manual(values = c("#00AFBB", "#E7B800", "#FC4E07"))

Maybe try another way? This works, makes bars different colors, but can you incorporate the stats?

\*\* alpha command here seems like it is making the bars lighter in color the lower it is

This works!!!

perf <-ggplot(data=Data3, aes(x=Substrate, y=LogCFUpermL,fill=Substrate))+

  geom\_bar(stat="identity", alpha=0.8) +

  scale\_fill\_manual(values=c("thistle1",

                             "paleturquoise3",

                             "mediumorchid",

                             "orchid1")) +

**geom\_errorbar**(**aes**(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.4) +

theme\_classic()

perf

OK – now to export the file as a PNG, maintain aspect ratio, select width = 513 and height = 482 (pixels?)

Set directory as the Figs for Paper, then save

Try to make both fig 1 (growth) as one with a shared legend:

<https://www.geeksforgeeks.org/how-to-join-multiple-ggplot2-plots-with-cowplot/?ref=rp>

# Load ggplot2 and cowplot

library("ggplot2")

library("cowplot")

##will not work in this case because the legends need to be the same, does not combine the legends into one

##if you were going to, the code is below

To produce the barplot as above, but with no legend, to create a shared legend with fig1b

#make sure you Load ggplot2 and cowplot

library("ggplot2")

library("cowplot")

perfNOleg <-ggplot(data=Data3, aes(x=Substrate, y=LogCFUpermL,fill=Substrate))+

geom\_bar(stat="identity", alpha=0.8) +

scale\_fill\_manual(values=c("thistle1",

"paleturquoise3",

"mediumorchid",

"orchid1")) +

geom\_errorbar(aes(x=Substrate, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.4) +

theme\_classic()+

theme(legend.position="none")

perfNOleg

#To make figure as above, with no legend, to create a shared legend w Fig1a

perf3NOleg <- ggplot(data=DataRSGrow, aes(x=Substrates, y=LogCFUpermL, fill=Substrates)) +

geom\_bar(stat="identity", alpha=0.8) +

scale\_fill\_manual(values=c("thistle1", "darkseagreen2", "coral", "coral4", "cornflowerblue")) +

geom\_errorbar(aes(x=Substrates, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.4) +

theme\_classic()+

theme(legend.position="none")

perf3NOleg

##TO MAKE BOTH PLOTS INTO ONE FIGURE WITH A SHARED LEGEND##

#Create both plots as above and store in variable without the legends

#combine plots into one using plot\_grid()

combined\_plot<-plot\_grid(perfNOleg, perf3NOleg,ncol=2)

#extract legend from plot2

legend <- get\_legend(

perf + perf3

guides(color = guide\_legend(nrow = 1)) +

theme(legend.position = "bottom")

)

## Combine combined plot and legend using plot\_grid()

plot\_grid(combined\_plot, legend,ncol=1,rel\_heights = c(1, .1))

###To make a stacked barplot###

*# library*

library(ggplot2)

*# create a dataset*

Substrate <- c(rep("Mal" , 3) , rep("MD16.5-19.5" , 3) , rep("MD4-7" , 3))

SCFA <- rep(c("Formate" , "Acetate" , "Butyrate") , 3)

value <- abs(rnorm(12 , 0 , 15))

data <- data.frame(specie,condition,value)

*# Stacked*

ggplot(data, aes(fill=condition, y=value, x=specie)) +

geom\_bar(position="stack", stat="identity")

perf3NOleg <- ggplot(data=DataRSGrow, aes(x=Substrates, y=LogCFUpermL, fill=Substrates)) +

geom\_bar(stat="identity", alpha=0.8) +

scale\_fill\_manual(values=c("thistle1", "darkseagreen2", "coral", "coral4", "cornflowerblue")) +

geom\_errorbar(aes(x=Substrates, ymin=LogCFUpermL-sd, ymax=LogCFUpermL+sd), width=0.1, colour="black", alpha=0.9, linewidth=0.4) +

theme\_classic()+

theme(legend.position="none")

perf3NOleg

geom\_errorbar(aes(ymin=col3-temp\_col, ymax=col3+temp\_col),

                width=.2, colour="red",

                position=position\_dodge(.9))