Tutorial - Plotting data from cultures

Daniel Vaulot

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1 Aim

This tutorial explain how plot growth curves for phytoplankton cultures in different conditions. * Antibiotics treatment : 4 RCC cultures, 8 days, 2 antibiotics, 5 concentrations,

2 Downloads

Install the following software :

- R studio : https://www.rstudio.com/products/rstudio/download/#download
- Download and install the following libraries by running under R studio the following lines

```
install.packages("dplyr")     # To manipulate dataframes
install.packages("tidyr")     # To manipulate dataframes

install.packages("stringr")   # To strings

install.packages("ggplot2")   # for high quality graphics
install.packages("gridExtra") # for grids

install.packages("plotrix" )  # needed for standard error
```

3 Data used

- **cultures antibiotics.txt** contains data obtained by Priscilla Gourvil on antibiotics treatment of RCC strains
- **grazing experiment.xlsx** contains data obtained by Valeria Jimenez on grazing experiment on Micromonas

4 Tutorial description

4.1 Load the libraries

```
library("ggplot2")
library("gridExtra")
library("plotrix" ) # needed for standard error
library("dplyr")
library("tidyr")
library("stringr")
library("readxl")
```

4.2 Antibiotics treatments

4.2.1 Read and reformat the data

Read the data

```
cell<- read.table("cultures antibiotics.txt", header=TRUE, sep="\t", na.strings="NA", dec=".", strip.wh
knitr::kable(head(cell))
```

RCC	Antibio	Well	Concentration	X1	X2	X3	X4	X5	X6	X7
RCC 96	G 418	A01	0.5	35888	17043	3853	593	225	675	591
RCC 96	G 418	A02	0.5	27281	20952	337	450	84	394	562
RCC 96	G 418	A03	0.5	29952	19630	34846	3853	21318	19996	31639
RCC 96	G 418	B01	1.0	33018	17268	6468	337	478	394	253
RCC 96	G 418	B02	1.0	26662	20530	34902	675	22555	8268	14062
RCC 96	G 418	B03	1.0	24946	19040	49011	4725	21599	26746	277217

Change from wide format to long format

```
cell<- gather(cell, X1:X7, key = "day", value = "cell_number")
knitr::kable(head(cell))
```

RCC	Antibio	Well	Concentration	day	cell_number
RCC 96	G 418	A01	0.5	X1	35888
RCC 96	G 418	A02	0.5	X1	27281
RCC 96	G 418	A03	0.5	X1	29952
RCC 96	G 418	B01	1.0	X1	33018
RCC 96	G 418	B02	1.0	X1	26662
RCC 96	G 418	B03	1.0	X1	24946

Reformat day as numeric

```
cell$day<-as.numeric(str_replace(cell$day, "X", ""))
knitr::kable(head(cell))
```

RCC	Antibio	Well	Concentration	day	cell_number
RCC 96	G 418	A01	0.5	1	35888
RCC 96	G 418	A02	0.5	1	27281

RCC	Antibio	Well	Concentration	day	cell_number
RCC 96	G 418	A03	0.5	1	29952
RCC 96	G 418	B01	1.0	1	33018
RCC 96	G 418	B02	1.0	1	26662
RCC 96	G 418	B03	1.0	1	24946

Reformat concentration as character

```
cell$Concentration<-as.character(cell$Concentration)
knitr::kable(head(cell))
```

RCC	Antibio	Well	Concentration	day	cell_number
RCC 96	G 418	A01	0.5	1	35888
RCC 96	G 418	A02	0.5	1	27281
RCC 96	G 418	A03	0.5	1	29952
RCC 96	G 418	B01	1	1	33018
RCC 96	G 418	B02	1	1	26662
RCC 96	G 418	B03	1	1	24946

Compute mean and SD for each RCC, Antibio, Concentration and day using dplyr

```
cell_1<- cell %>% group_by(RCC,Antibio,Concentration, day)%>%
        summarise (cell_mean=mean(cell_number),cell_sd=sd(cell_number), cell_se=std.error(cell_number))
```

```
## Warning: package 'bindrcpp' was built under R version 3.4.4
```

```
knitr::kable(head(cell_1))
```

RCC	Antibio	Concentration	day	cell_mean	cell_sd	cell_se
RCC 4094	G 418	0.5	1	188562.333	46983.373	27125.863
RCC 4094	G 418	0.5	2	100846.333	48010.521	27718.887
RCC 4094	G 418	0.5	3	4087.667	3401.215	1963.692
RCC 4094	G 418	0.5	4	42195.333	18511.363	10687.541
RCC 4094	G 418	0.5	5	8727.667	14751.465	8516.763
RCC 4094	G 418	0.5	6	3806.000	6105.543	3525.037

4.2.2 Define graphics options

Define the color, line type and symbol shape

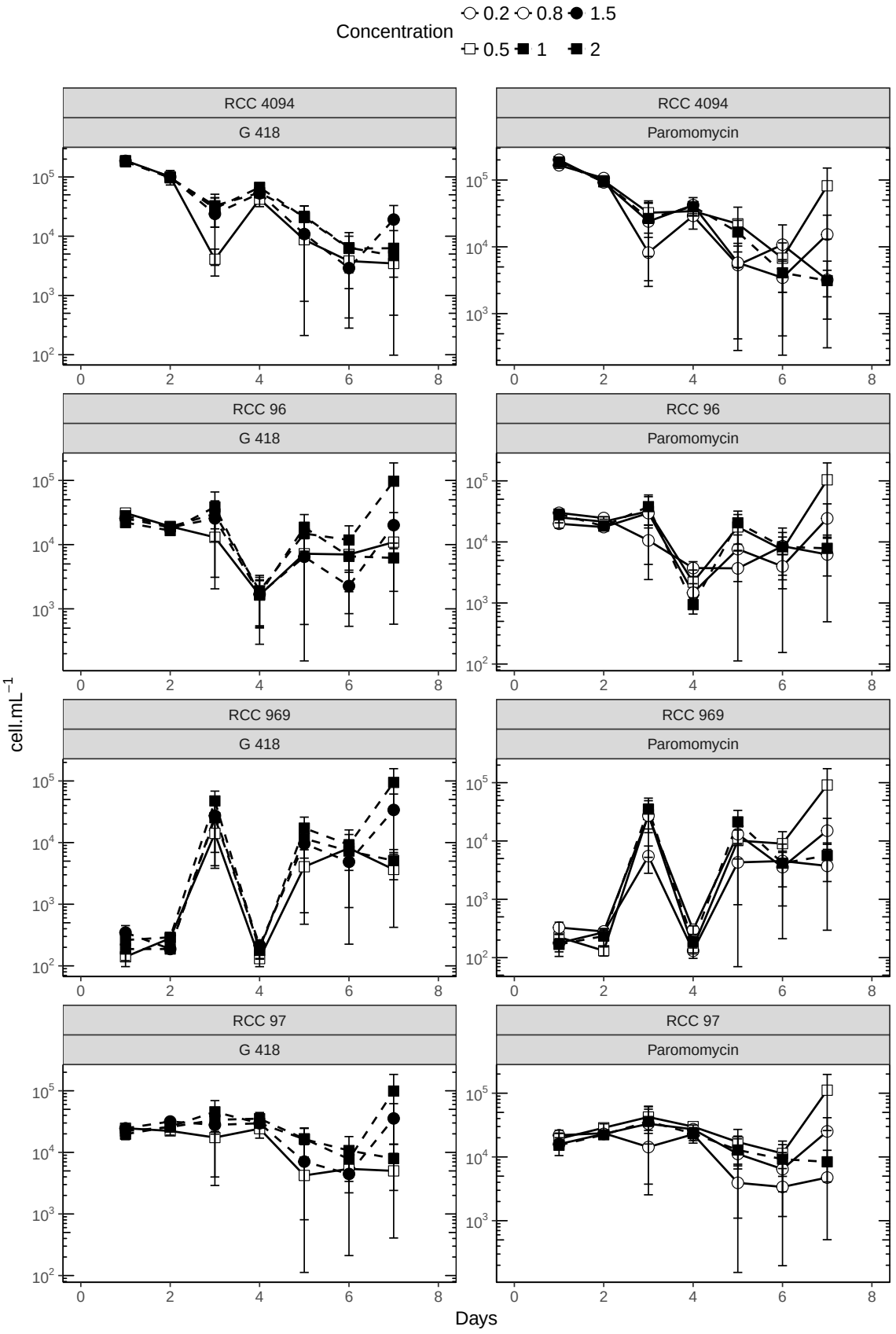
```
Concentration_color<-c("0.2"="white","0.5"="white", "0.8"="white", "1"="black", "1.5"="black", "2"="bla
Concentration_linetype<-c("0.2"=1,"0.5"=1, "0.8"=1, "1"=2, "1.5"=2, "2"=2)
Concentration_shape<-c("0.2"=21,"0.5"=22, "0.8"=21, "1"=22, "1.5"=21, "2"=22)
```

Define graphics options

```
scaling_factor=15
cell_label <- expression (paste("cell.",mL^-1))
cell_breaks=c(100,1000,10000,100000,1000000)
x_max=8
x_breaks=c(0, 2,4,6,8)
x_labels=c("0", "2","4","6","8")
```

4.2.3 Plot the data

```
plot1<- ggplot(cell_1, aes(x=day, y=cell_mean, group = Concentration, xmin=0, xmax=x_max,
                shape=Concentration, fill=Concentration, linetype=Concentration)) +
               facet_wrap(~ RCC + Antibio, nrow=4, ncol=2, scales="free") +
                 geom_line (size=0.8, colour="black") +
               geom_point(size = 4) +
               geom_errorbar(aes(ymin=cell_mean-cell_se, ymax=cell_mean+cell_se),width=0.2, linetype=1) +
               theme_bw(scaling_factor) +
               theme(panel.border = element_rect(colour = "black"), panel.grid.major = element_blank(), p
               axis.line = element_line(colour = "black"),
                   legend.title=element_text(size=scaling_factor), legend.key=element_blank(),
                   axis.title = element_text(size=scaling_factor),
                   legend.text=element_text(size=scaling_factor), legend.key.height = unit(1, "cm"),
                   axis.text = element_text(size=0.8*scaling_factor), panel.background = element_rect(fi
                 theme(legend.position = "top", legend.box = "horizontal")  +
                 labs(x = "Days", y = cell_label ) +
               scale_x_continuous(breaks=x_breaks, labels=x_labels) +
                 scale_y_log10(breaks = cell_breaks ,labels = scales::trans_format("log10", scales::math_
               annotation_logticks(sides = "lr") +
                 scale_fill_manual(values=Concentration_color) +
               scale_shape_manual(values=Concentration_shape) +
               scale_linetype_manual(values=Concentration_linetype)

# Add next line to zoom
# + coord_cartesian(ylim=c(100, 10000000))

plot1
```


Concentration
0.2
0.8
1.5
0.5
1
2
RCC 4094
G 418
RCC 4094
Paromomycin
RCC 96
G 418
RCC 96
Paromomycin
RCC 969
G 418
RCC 969
Paromomycin
RCC 97
G 418
RCC 97
Paromomycin
cell.mL^{-1}
Days

```
# Next can be used to save the plot as pdf
# ggsave(file="Fig 1 version 2.0.pdf", plot=plot1, scale=5, width = 7, height = 10, units = "cm", useDi
```

4.3 Grazing experiment

Micromonas are fed with fluorescent labelled beads and one looks at the % of cells that have beads The idea is to do a plot with 2 different scales for the y axis.

4.3.0.1 Read the data

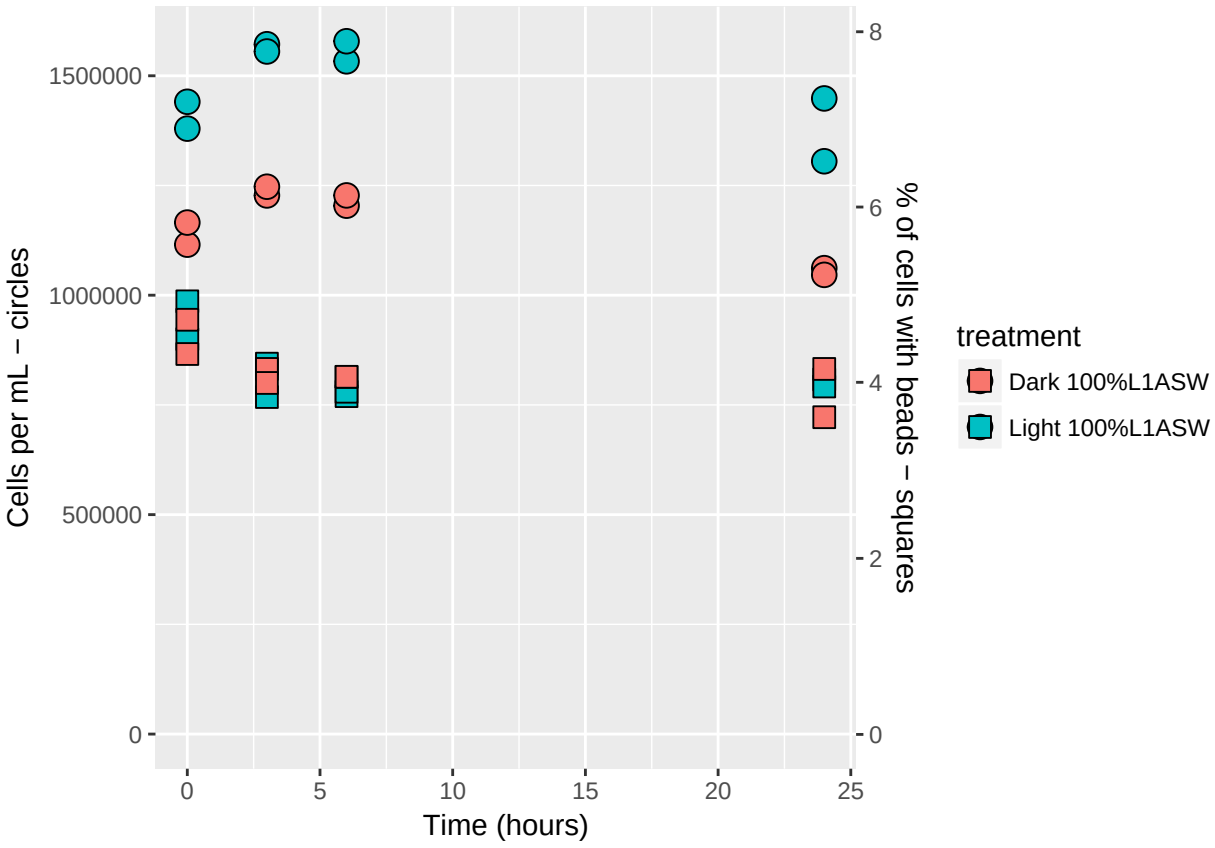
```
grazing <- read_xlsx("grazing experiment.xlsx", sheet="RCC2306")

# Compute a new variable with the same scale as the cell concentration to be able to plot on the same g
grazing$cell_beads_pct_scaled <- grazing$cell_beads_pct*200000

 knitr::kable(head(grazing))
```

species	RCC#	exp	treatment	time pnt	time	rep	Well	Date	allPhyto.Count	PhytoBead
M. polaris	2306	4	Light 100%L1ASW	T0	0	A	A09	02.23.2018	17192	
M. polaris	2306	4	Light 100%L1ASW	T0	0	B	A10	02.23.2018	17955	
M. polaris	2306	4	Dark 100%L1ASW	T0	0	A	A11	02.23.2018	13898	
M. polaris	2306	4	Dark 100%L1ASW	T0	0	B	A12	02.23.2018	14525	
M. polaris	2306	4	Light 100%L1ASW	T3	3	A	C09	02.23.2018	19589	
M. polaris	2306	4	Light 100%L1ASW	T3	3	B	C10	02.23.2018	19383	

4.3.0.2 Plot the data

Demonstrate the use of sec_axis.

```
plot <- ggplot(data=grazing, aes(x=time, y=cell_ml, ymin=0, fill=treatment)) +
              geom_point(size = 4, shape = 21) +
                labs(x = "Time (hours)", y = "Cells per mL - circles" ) +
              geom_point(size = 4, shape = 22, aes(x=time, y=cell_beads_pct_scaled, fill=treatment)) +
              scale_y_continuous(sec.axis = sec_axis(~./200000, name="% of cells with beads - squares"))
print(plot)
```


Cells per mL – circles
1500000
1000000
500000
0
% of cells with beads – squares
8
6
4
2
0
0
5
10
15
20
25
Time (hours)
treatment
Dark 100%L1ASW
Light 100%L1ASW