

Chemo.06.ResistanceIndex

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Contents

1	Load complementary data	2
1.1	Abundance and salinity	2
1.2	Bacterial abundance time series	3
2	Loading datasets Community functioning	3
2.1	Functional rates	5
3	Resistance index calculation	7
3.1	Resistance index definition	7
3.2	Estimation of the resistance index	7
3.3	Resistance index plots	8
3.4	Statistical analysis	8
3.5	Exporting Figure 4 ms chemostats	9
3.6	Export figure	9

#Load packages

```
rm(list = ls())
library(readxl) # read excel files
library(dplyr)
library(ggplot2) #Plots
# library(Hmisc) #
library(tidyverse)
# library(ggpubr)
library(rstatix)
# library(emmeans)
library(cowplot) #Arrange plots
library(egg) #Tag in figures
library(olsrr) #Normality test (kolmogorov-smirnov)
library(kableExtra) #Export regular tables
library(nlme) #Mixed linear models
library(lmerTest) #P values mixed linear models
# Color palette
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2",
               "#D55E00", "#CC79A7")
```

1 Load complementary data

1.1 Abundance and salinity

```
Dat=data.frame(read_xls("../data/comm.rates/MicrobialAbundance.xls",sheet="Chemo summary"))
Dat=Dat[Dat$Time<42,] #Only keep the samples until one day 41 for figure
DatS=Dat #Dataframe only for Salinity figure
Dat=Dat[complete.cases(Dat),] #Removing unused columns
Dat$DOM=as.factor(Dat$DOM)
levels(Dat$DOM)= c("HDOM","LDOM")
Dat$id=paste0(Dat$Time ,".",Dat$DOM,".",Dat$Treatment,".",Dat$Replicate)
Dat$id=as.factor(Dat$id)

# Creating the salinity changes and DNA frequency sampling for Figure1 ms chemostats
# DNA sampling frequency plot
pDNA=ggplot()+#
  geom_segment(aes(x=c(4,8,15,18,22,29,36,39,41),y=rep(2,9), xend = c(4,8,15,18,22,29,36,39,41), yend =
    arrow = arrow(length = unit(0.2, "cm")),size=.5)+labs(y="DNA Sampling",x="")+
  annotate("text", x=c(4,8,15,18,22,29,36,39,41)-1, y = rep(1.5,9),
    label=c("T1","T2","T3","T4","T5","T6","T7","T8","T9"),size=3)+
  scale_x_continuous(breaks = c(1:41),expand=c(0.01,0.01),limits = c(1,41))+
  theme_bw()+theme(panel.grid.minor = element_blank(),panel.grid.major = element_blank(),
    axis.text.x = element_blank(),axis.text.y =element_blank())+
  theme(text=element_text(size=10, family="ArialMT"))+
  labs(tag = "")+theme(axis.ticks.y=element_blank(),axis.ticks.x=element_blank(),panel.border = element
  theme(plot.margin=margin(t = -5, r = 5, b = -10, l = 0, unit = "pt"))+
  theme(axis.title.y = element_text(angle = 0, vjust = 0.5))

## Warning: Using 'size' aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use 'linewidth' instead.

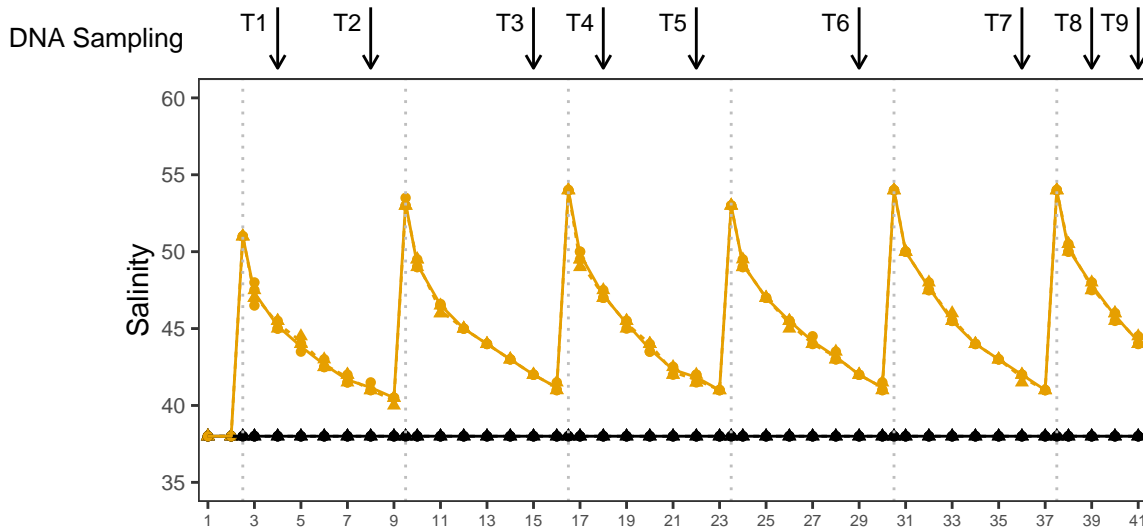
# Salinity time series figure (control vs disturbance)

Salinity_plot=DatS%>%
  ggplot()+geom_point(aes(Time,Salinity,colour=Treatment,shape=DOM))+
  stat_summary(aes(Time,Salinity,colour=Treatment,linetype=DOM),fun= mean,geom = "line")+
  geom_vline(xintercept=c(2.5,9.5,16.5,23.5,30.5,37.5),linetype="dotted",colour="grey")+
  scale_color_manual(values=cbbPalette,name="")+
  xlab("")+ylab("Salinity")+ylim(35,60)+
  labs(tag = "")+scale_x_continuous(breaks = seq(1,41,2),expand=c(0.01,0.01))+
  theme_bw()+theme(panel.grid.minor = element_blank(),panel.grid.major = element_blank(),legend.position
    axis.text.x = element_text(size=6),axis.text.y = element_text(size=8))+
  theme(text=element_text(family="ArialMT"))+
  theme(plot.margin=margin(t = -15, r = 5, b = 0, l = 0, unit = "pt"))+theme(plot.tag=element_text(size=

pdf("../figures/Figure_Salinity.pdf", width=7,height=4)
plot_grid(pDNA,Salinity_plot,
  ncol = 1,axis="l", rel_heights =c(0.1,0.5), hjust=-4, align="v")
dev.off()

## pdf
## 2
```

```
plot_grid(pDNA,Salinity_plot,
          ncol = 1,axis="l", rel_heights =c(0.1,0.5), hjust=-4, align="v")
```



1.2 Bacterial abundance time series

```
# Bacterial abundance time series
Abundance_plot = Dat %>%
  ggplot(aes(x = Time, y = Bact/1e+06, group = interaction(Treatment, DOM))) +
  geom_point(aes(colour = Treatment, shape = DOM), size = 1) + scale_colour_manual(values = cbbPalett
name = "Disturbance") + scale_shape_manual(values = c(21, 4), name = "DOM level",
labels = c("HDOM", "LDOM")) + labs(tag = "D") + scale_x_continuous(breaks = seq(1,
41, 2), expand = c(0.01, 0.01)) + xlab("Time (days)") + ylab(expression("BA (x10"^-6 *
"cell mL"^-1 * ")")) + geom_smooth(aes(colour = Treatment), method = "loess",
se = F, span = 0.3) + geom_vline(xintercept = c(2.5, 9.5, 16.5, 23.5, 30.5,
37.5), linetype = "dotted", colour = "grey") + theme_bw() + theme(panel.grid.minor = element_blank(),
panel.grid.major = element_blank(), legend.position = "bottom", legend.box = "vertical",
legend.margin = margin(), legend.spacing.y = unit(0, "cm"), axis.text.x = element_text(size = 10),
axis.text.y = element_text(size = 8)) + theme(text = element_text(family = "ArialMT")) +
theme(plot.margin = margin(t = 0, r = 5, b = 2, l = 0, unit = "pt")) + theme(plot.tag = element_text
face = "bold", vjust = -4))
legend_plot = get_legend(Abundance_plot)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

```
Abundance_plot = Abundance_plot + theme(legend.position = "none")
```

2 Loading datasets Community functioning

Dataset includes microbial community respiration and bacterial production

```
df.comm.funct <- data.frame(read_csv("../data/comm.rates/Functional.response.csv"))
```

```
## Rows: 288 Columns: 8
## -- Column specification -----
## Delimiter: ","
## chr (5): DOM, Treatment, Comment, Variable, Units
## dbl (3): Week, Rep, Value
##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.
```

```
tibble(df.comm.funct)
```

```
## # A tibble: 288 x 8
##   Week DOM Treatment Rep Comment Value Variable Units
##   <dbl> <chr> <chr>   <dbl> <chr>   <dbl> <chr>         <chr>
## 1     1   HDOM    C         1 after  0.0195 Community.Respiration umol 02 L-1~
## 2     2   HDOM    C         1 after  0.178  Community.Respiration umol 02 L-1~
## 3     3   HDOM    C         1 after  0.0344 Community.Respiration umol 02 L-1~
## 4     4   HDOM    C         1 after  0.131  Community.Respiration umol 02 L-1~
## 5     5   HDOM    C         1 after  0.213  Community.Respiration umol 02 L-1~
## 6     6   HDOM    C         1 after  0.285  Community.Respiration umol 02 L-1~
## 7     1   LDOM    C         1 after  2.48   Community.Respiration umol 02 L-1~
## 8     2   LDOM    C         1 after  0.360  Community.Respiration umol 02 L-1~
## 9     3   LDOM    C         1 after  0.105  Community.Respiration umol 02 L-1~
## 10    4   LDOM    C         1 after  0.372  Community.Respiration umol 02 L-1~
## # ... with 278 more rows
```

2.0.1 Bacterial growth efficiency (BGE) calculation

```
# RES2= Bulk respiration (uM_h_corr or umol L-1 h-1) need to be scaled to
# daily rate RES3= Bulk production (ugC L-1 d-1) need to be transformed to
# molar by the molecular weight of Carbon
```

```
Ratio = 0.89 #Coefficient to transform O2 consumption to CO2 (Williams and del Giorgio, 2005)
```

```
BGE = df.comm.funct[1:144, 1:5] #Get metadata to store BGE
```

```
BGE$Value = (df.comm.funct$Value[df.comm.funct$Variable == "Bacterial.production"]/12)/((df.comm.funct$
  "Bacterial.production"]/12) + (df.comm.funct$Value[df.comm.funct$Variable ==
  "Community.Respiration"] * Ratio * 24))
```

```
BGE$Units = "Percentage"
```

```
BGE$Variable = "BGE"
```

```
kbl(head(BGE)) %>%
```

```
kable_styling()
```

```
# Combining datasets
```

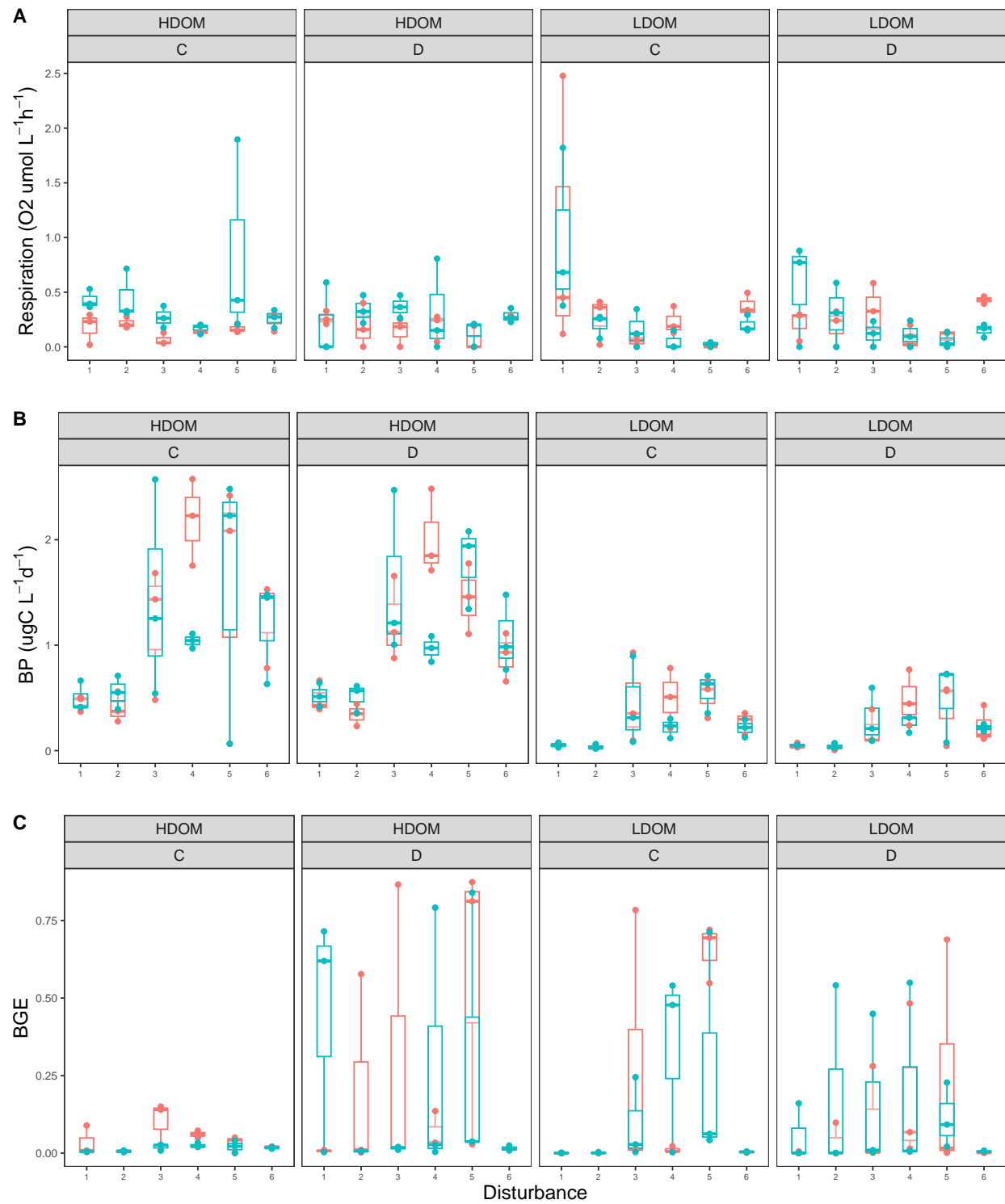
```
df.comm.funct = rbind(df.comm.funct, BGE)
```

Week	DOM	Treatment	Rep	Comment	Value	Units	Variable
1	HDOM	C	1	after	0.0892454	Percentage	BGE
2	HDOM	C	1	after	0.0081200	Percentage	BGE
3	HDOM	C	1	after	0.1400752	Percentage	BGE
4	HDOM	C	1	after	0.0495788	Percentage	BGE
5	HDOM	C	1	after	0.0423676	Percentage	BGE
6	HDOM	C	1	after	0.0205098	Percentage	BGE

2.1 Functional rates

Figures

```
# setting up graphic setting for the figures
my_theme = theme_bw() + theme(text = element_text(size = 14, family = "ArialMT")) +
  theme(legend.position = "none", panel.grid.minor = element_blank(), panel.grid.major = element_blank(),
        axis.text.x = element_text(size = 6), axis.text.y = element_text(size = 8))
```



3 Resistance index calculation

3.1 Resistance index definition

Here we defined the resistance index as the difference between the natural log of the fold change of the disturbance treatment against the control

```
# Option 1
resistance1 <- function(control, disturbance) {
  abs(log(control) - log(disturbance))
}
```

3.2 Estimation of the resistance index

Here we defined calculation step by step for the resistance index for all the functional parameters.

```
# Pooling data into a list to perform all the calculation at one by
# applying a loop for each element (functional measurement) of the list.

# Set up levels as factors
df.comm.funct$Variable = factor(df.comm.funct$Variable)
df.comm.funct$Variable = factor(df.comm.funct$Variable, c("Bacterial.production",
  "Community.Respiration", "BGE"))
# Empty list for storing results
List.ratio = list()
for (i in 1:3) {
  # Splitting the data between before and after the salt pulse
  # disturbance. Here the function aggregate allow us to retrieve the
  # data sorted always in the same way.
  index.level = levels(df.comm.funct$Variable)[i]

  # Before disturbance
  T_bef = aggregate(Value ~ Week + Treatment + DOM + Rep + Comment, data = df.comm.funct[df.comm.funct$Variable == index.level & df.comm.funct$Comment == "before", ], FUN = "mean")
  # After disturbance
  T_aft = aggregate(Value ~ Week + Treatment + DOM + Rep + Comment, data = df.comm.funct[df.comm.funct$Variable == index.level & df.comm.funct$Comment == "after", ], FUN = "mean")

  # Pooling data for calculations
  T_aft$Before = T_bef$Value

  # Calculating the response ratio (RR) as F_after/F_before
  T_aft$RR = (T_aft$Value/T_aft$Before)

  # Extracting RR for Control and Disturbed treatments
  dist = T_aft[T_aft$Treatment == "D", ] #Disturbance treatment
  cont = T_aft[T_aft$Treatment == "C", ] #Control

  # Calculate the mean of the control RR. To represent the overall
  # variability of the control we used the mean value of the triplicated
  # measurements.
```

```

T0 <- cont %>%
  group_by(interaction(DOM, Treatment, Week)) %>%
  mutate(mControl_RR = mean(RR)) # Calculate the mean of the controls
T0 <- data.frame(T0)

# State column as factors
T0$Week = as.factor(T0$Week)
T0$DOM = as.factor(T0$DOM)
T0$DOM = factor(T0$DOM, c("LDOM", "HDOM"))
# Calculate the absolute difference between the LRRs
# (meanControl-Disturbed_replicates)
T0$res.index = resistance1(T0$mControl_RR, dist$RR) * -1 #option
T0$Variable = index.level
List.ratio[[i]] = T0
}

```

3.3 Resistance index plots

3.4 Statistical analysis

```

# Setup elements for loop
List.aov = list()
M.stats = matrix(NA, 3, 8)
MLM_LDOM = list()
MLM_HDOM = list()
# Define variable names
rownames(M.stats) = c("BP", "Respiration", "BGE")
# Define stats names
colnames(M.stats) = c("F", "P-value", "F", "P-value", "Slope", "P-value", "Slope",
  "P-value")

# Loop for functional data
for (i in 1:3) {
  # Normality
  List.ratio[[i]] %>%
    group_by(DOM, Week) %>%
    shapiro_test(res.index)

  # Homogeneity of variances
  List.ratio[[i]] %>%
    group_by(Week) %>%
    levene_test(res.index ~ DOM)

  # ANOVA Repeated measurement ANOVA
  # (https://stats.idre.ucla.edu/r/seminars/repeated-measures-analysis-with-r/)
  # https://m-clark.github.io/docs/mixedModels/anovamixed.html#introduction

  summary(aov(res.index ~ DOM * Week + Error(Rep), data = List.ratio[[i]]))
  tmp = aov(res.index ~ DOM * Week + Error(Rep), data = List.ratio[[i]])

  # Retrieving stats from results
}

```


Function	RM-ANOVA				MLM			
Variable	DOM		Week		LDOM		HDOM	
	F	P-value	F	P-value	Slope	P-value	Slope	P-value
BP	10.828	0.003	0.564	0.726	0.035	0.515	-0.026	0.061
Respiration	0.413	0.527	2.130	0.098	0.170	0.623	0.629	0.029
BGE	0.924	0.347	1.719	0.170	0.241	0.404	0.655	0.009

```

M.stats[i, 1] = as.numeric(unlist(summary(tmp))["Error: Within.F value1"])
M.stats[i, 2] = as.numeric(unlist(summary(tmp))["Error: Within.Pr(>F)1"])
M.stats[i, 3] = as.numeric(unlist(summary(tmp))["Error: Within.F value2"])
M.stats[i, 4] = as.numeric(unlist(summary(tmp))["Error: Within.Pr(>F)2"])

# Mixed linear model for LDOM
MLM_LDOM[[i]] = lme(res.index ~ as.numeric(Week), random = ~1 | Rep, data = List.ratio[[i]][List.ra
  "LDOM", ])
# Retrieving stats from results
M.stats[i, 5] = as.numeric(unlist(summary(MLM_LDOM[[i]]))$`coefficients.fixed.as.numeric(Week)` ) #
M.stats[i, 6] = as.numeric(unlist(summary(MLM_LDOM[[i]]))$tTable10) #get P-value

# Mixed linear model for HDOM
MLM_HDOM[[i]] = lme(res.index ~ as.numeric(Week), random = ~1 | Rep, data = List.ratio[[i]][List.ra
  "HDOM", ])
# Retrieving stats from results
M.stats[i, 7] = as.numeric(unlist(summary(MLM_HDOM[[i]]))$`coefficients.fixed.as.numeric(Week)` ) #
M.stats[i, 8] = as.numeric(unlist(summary(MLM_HDOM[[i]]))$tTable10) #get P-value
}

```

Table summary statistical analyses Statistical results for the repeated measurement ANOVA applied to the resistance index. Results from mixed model to screen for time trend are also included in the table.

```

kable(M.stats, digits = 3, booktabs = TRUE, format = "latex") %>%
  kable_classic() %>%
  add_header_above(c(Variable = 1, DOM = 2, Week = 2, LDOM = 2, HDOM = 2)) %>%
  add_header_above(c(Function = 1, `RM-ANOVA` = 4, MLM = 4)) %>%
  kable_styling(latex_options = c("striped", "condensed", "scale_down"), position = "center",
    full_width = FALSE)

```

3.5 Exporting Figure 4 ms chemostats

3.6 Export figure

```

pdf("../figures/Figure4_ResistanceIndex.pdf", width = 7, height = 9)
plot_grid(pBP, pR, pBGE, Abundance_plot, legend_plot, ncol = 1, axis = "l",
  rel_heights = c(0.4, 0.4, 0.4, 0.65, 0.1), hjust = -4, align = "v")

```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

```
dev.off()

## pdf
## 2

plot_grid(pBP, pR, pBGE, Abundance_plot, legend_plot, ncol = 1, axis = "l",
          rel_heights = c(0.4, 0.4, 0.4, 0.65, 0.1), hjust = -4, align = "v")

## 'geom_smooth()' using formula = 'y ~ x'
```

