Experimental conditions

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Contents

General project set-up

```
# Load all libraries and sources required to run the script
    library(tidyverse)
    library(ggthemes)
    library(ggplot2)
    library(plyr)
    library(tidyr)

    library(plotrix)
    library(lme4)
    library(lmerTest)
    library(emmeans)
    library(reshape2)

# Graphs
# Plots
MyTheme<-theme_bw() +
theme(legend.position="top",
          plot.background=element_blank(),
          #axis.text.x = element_text(angle = 90, vjust = 0.5),
          panel.grid.major.y = element_blank(),
```

```
          panel.grid.major.x = element_blank(),
          panel.grid.minor.x = element_blank(),
          panel.grid.minor.y = element_blank(),
          legend.box.background = element_rect(),
          legend.title = element_blank(),
          panel.background =element_rect(fill = NA,
                                         color = "black"))

# Treatment colors
Treat_fill<-scale_fill_manual(values =
                            c("#2b83ba", "#003399",
                              "#d7191c", "#660000"))

Treat_colour<-scale_colour_manual(values =
                            c("#2b83ba", "#003399",
                              "#d7191c", "#660000"))
```

1. Temperature

```
# Data
    Temp<-read.csv("Data/Temperature.csv", header = TRUE)
    #summary(Temp)
    Temp$Time<-as.POSIXct(Temp$Time, format="%m/%d/%Y %H:%M",
                                tz = "America/New_York" )
    Temp<-Temp %>% filter(!between(Time, as.POSIXct('2023-05-18 08:50:00.00'),
                                as.POSIXct('2023-05-18 09:55:00.00')))


    Temp <- gather(Temp, Tank, Temperature,
                   Tank.1:Tank.8, factor_key=TRUE)
    #Temp<-Temp[Temp$Tank!="Tank.6",]
    #Temp<-Temp[Temp$Tank!="Tank.7",]
    Temp<-Temp %>% mutate(Group =
                      case_when(Tank == "Tank.1" ~ "Control Temperature",
                                Tank == "Tank.2" ~ "High Temperature",
                                Tank == "Tank.3" ~ "High Temperature",
                                Tank == "Tank.4" ~ "Control Temperature",
                                Tank == "Tank.5" ~ "Control Temperature",
                                Tank == "Tank.6" ~ "Donor colonies",
                                Tank == "Tank.7" ~ "Donor colonies",
                                Tank == "Tank.8" ~ "High Temperature"))
    Temp$Group<-factor(Temp$Group, levels = c("Control Temperature",
                                              "High Temperature",
                                              "Donor colonies"))

    Temp<-Temp %>% mutate(Group2 =
                      case_when(Group == "Control Temperature" ~ "28 C",
                                Group == "High Temperature" ~ "31 C",
                                Group == "Donor colonies" ~ "Donor colonies"))

    # Remove data from tank that had a sensor failed
```

```
    Temp<-Temp %>% filter((Time<as.POSIXct('2023-06-02 00:50:00.00') |
                           Tank!="Tank.8"))
    Temp$Date<-as.Date(Temp$Time)
    Temp$Day<-as.numeric(difftime(Temp$Date,
                                  as.Date("2023-05-01"), units="days"))
    summary(Temp)
```

```
##       Time                              Tank        Temperature
##  Min.   :2023-05-02 07:00:00.00   Tank.1 :15290   Min.   :20.27
##  1st Qu.:2023-05-14 21:10:00.00   Tank.2 :15290   1st Qu.:28.01
##  Median :2023-05-27 12:05:00.00   Tank.3 :15290   Median :28.04
##  Mean   :2023-05-28 00:45:42.18   Tank.4 :15290   Mean   :28.94
##  3rd Qu.:2023-06-10 01:56:15.00   Tank.5 :15290   3rd Qu.:30.98
##  Max.   :2023-06-24 10:45:00.00   Tank.6 :15290   Max.   :34.78
##                                   (Other):24124
##                  Group          Group2               Date
##  Control Temperature:45870   Length:115864      Min.   :2023-05-02
##  High Temperature   :39414   Class :character   1st Qu.:2023-05-15
##  Donor colonies     :30580   Mode  :character   Median :2023-05-27
##                                                 Mean   :2023-05-27
##                                                 3rd Qu.:2023-06-10
##                                                 Max.   :2023-06-24
##
##       Day
##  Min.   : 1.0
##  1st Qu.:14.0
##  Median :26.0
##  Mean   :26.7
##  3rd Qu.:40.0
##  Max.   :54.0
##
```

Temperature conditions during the whole experiment

```
Temp_summary <- ddply (Temp[Temp$Day>0, ], .(Group2),
                       summarise,
                 meanT = mean (Temperature, na.rm = T),
                 sdT = sd (Temperature, na.rm = T),
                 seT = std.error (Temperature, na.rm = T))
Temp_summary
```

```
##           Group2    meanT       sdT         seT
## 1           28 C 27.98423 0.4356117 0.002033926
## 2           31 C 30.83019 0.7889764 0.003974100
## 3 Donor colonies 27.94829 0.4722004 0.002700273
```

Temperature conditions excluding ramp-up days

```
Temp_summary <- ddply (Temp[Temp$Day>5, ], .(Group2),
                       summarise,
                 meanT = mean (Temperature, na.rm = T),
                 sdT = sd (Temperature, na.rm = T),
```

```
                seT = std.error (Temperature, na.rm = T))
Temp_summary
```

```
##           Group2    meanT       sdT         seT
## 1           28 C 28.00311 0.2793313 0.001363826
## 2           31 C 30.96296 0.4542417 0.002411102
## 3 Donor colonies 27.99300 0.2408386 0.001440161
```

Plot temperature by tanks

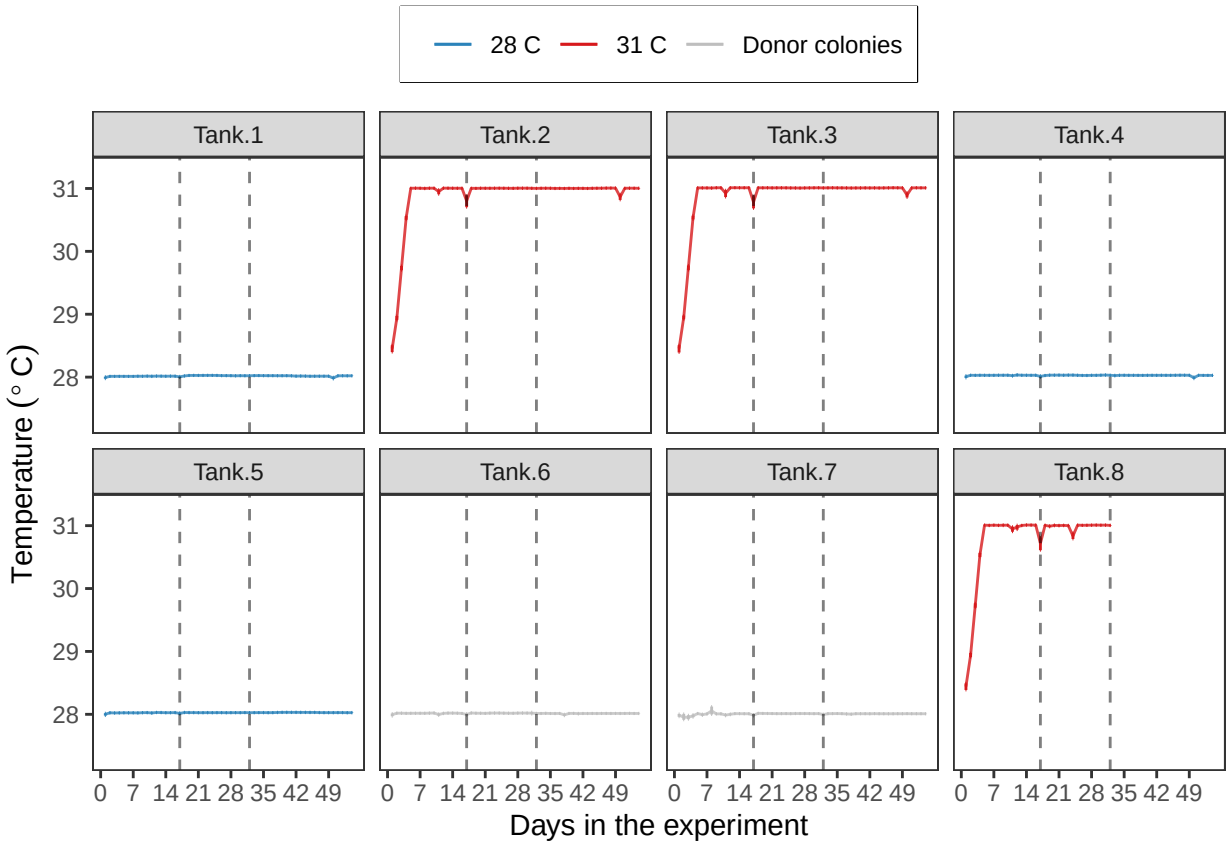
Raw values

```
Temperature<- ggplot(Temp, aes (Time, Temperature)) +
  geom_jitter(aes(colour=Group), alpha=1, size=0.5)+
  # stat_summary(fun.data = "mean_cl_boot",
  #              geom = "errorbar", width = 0.2,  color="black" )+
  # stat_summary(fun=mean, geom="point",  color="black") +

  theme(legend.position = "bottom")+
  scale_y_continuous(limits = c(26, 32),
                     breaks = seq(0,32, 2),
                     #   expand = c(0.01, 0.01),
                     name=("Temperature (C)")) +

    scale_x_datetime(breaks = "7 days",
                     date_labels = "%b %d",
                     limits = c(as.POSIXct("2023-05-02 12:30"),
                                as.POSIXct("2023-06-24 18:00")),
                     #  expand = c(0.01, 0.01),
                     name=("Date")) +

  geom_vline(xintercept = as.POSIXct("2023-05-18"),
             linetype=2)+
  annotate("text", x = c(as.POSIXct("2023-05-13"), as.POSIXct("2023-06-12")), y = 27, label = "*")+

  MyTheme + facet_wrap(~Tank, ncol = 4)+
  #facet_wrap(~(fct_rev(Tank)), ncol = 4)+
  scale_colour_manual(
  values = c("#2b83ba", "#d7191c", "gray"))+
  theme(legend.position = "none")

#Temperature
```

Daily mean values by tank

```
Temperature<- ggplot(Temp, aes (Day, Temperature, colour=Group2)) +
  #geom_jitter(aes(colour=Group), alpha=1, size=0.5)+
  stat_summary(fun.data = "mean_cl_boot",
               geom = "errorbar", width = 0.2)+
  stat_summary(fun=mean, geom="line", alpha=0.8) +

  theme(legend.position = "bottom")+
```

```
  scale_y_continuous(limits = c(27.3, 31.3),
                     breaks = seq(0,32, 1),
                     #   expand = c(0.01, 0.01),
                     name=expression(Temperature~(degree~C))) +
  scale_x_continuous(breaks = seq(0, 51, 7),
                     name=("Days in the experiment")) +

    # scale_x_date(breaks = "7 days",
    #                   date_labels = "%b %d",
    #                   limits = c(as.Date("2023-05-02"),
    #                              as.Date("2023-06-12")),
    #                   #  expand = c(0.01, 0.01),
    #                   name=("Date")) +

  #geom_vline(xintercept = as.Date(c("2023-05-18", "2023-06-02")),
  #            linetype=2, alpha=0.5)+
  # annotate("text", x = c(as.Date("2023-05-13"),
  #                        as.Date("2023-06-02")), y = 27.5, label = "*")+
  geom_vline(xintercept = c(17, 32),
             linetype=2, alpha=0.5)+

  MyTheme + facet_wrap(~Tank, ncol = 4)+
  #facet_wrap(~(fct_rev(Tank)), ncol = 4)+
  scale_colour_manual(
  values = c("#2b83ba", "#d7191c", "gray"))+
  theme(legend.position = "top")

Temperature
```



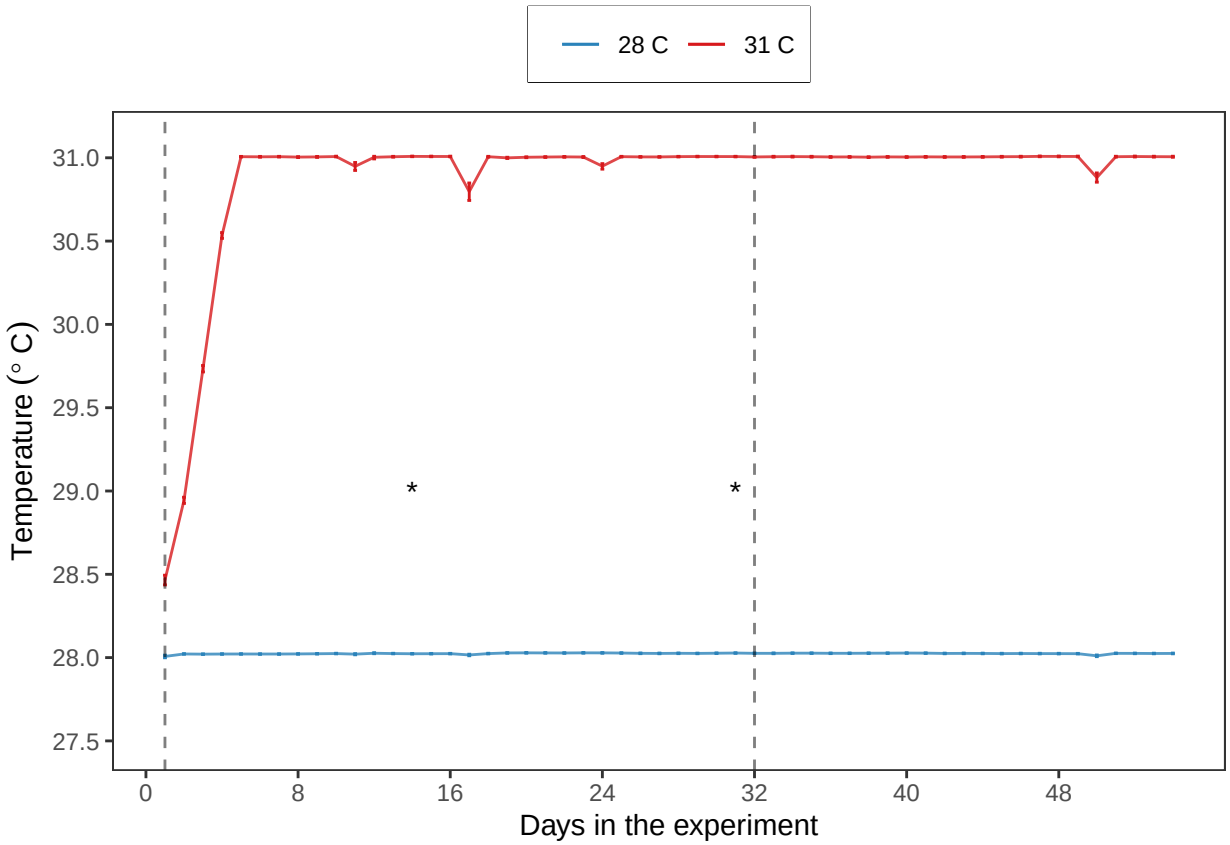

```
#ggsave(file="Outputs/Temperature.svg", plot=Temperature, width=8, height=4)
```

Daily mean values by treatment and experimental timeline

```
TimeLine<- ggplot(Temp, aes (Day, Temperature,
                             colour=Group2)) +
  #geom_jitter(aes(colour=Group), alpha=1, size=0.5)+
  stat_summary(fun.data = "mean_cl_boot",
               geom = "errorbar", width = 0.2)+
  stat_summary(fun=mean, geom="line", alpha=0.8) +

  theme(legend.position = "bottom")+
  scale_y_continuous(limits = c(27.3, 31.3),
                     breaks = seq(0,32, 1),
                     #   expand = c(0.01, 0.01),
                     name=expression(Temperature~(degree~C))) +
  scale_x_continuous(breaks = seq(0, 51, 8),
                     name=("Days in the experiment")) +
  annotate("text", x = c(14, 31), y = 27.5, label = "*")+
  geom_vline(xintercept = c(1, 17, 32),
             linetype=2, alpha=0.5)+

  MyTheme + facet_wrap(~Group2, ncol = 1)+
  scale_colour_manual(
  values = c("#2b83ba", "#d7191c", "gray"))+
```

```
  theme(legend.position = "top")

#TimeLine

#ggsave(file="Outputs/TimeLine.svg", plot=TimeLine, width=4, height=6)
```

```
TimeLine2<- ggplot(Temp[Temp$Group!="Donor colonies", ],
                   aes (Day, Temperature,
                                colour=Group2)) +
  stat_summary(fun.data = "mean_cl_boot",
                geom = "errorbar", width = 0.2)+
  stat_summary(fun=mean, geom="line", alpha=0.8) +

  scale_y_continuous(limits = c(27.5, 31.1),
                     breaks = seq(0,32, 0.5),
                     #   expand = c(0.01, 0.01),
                     name=expression(Temperature~(degree~C))) +
  scale_x_continuous(breaks = seq(0, 51, 8),
                     name=("Days in the experiment")) +
  annotate("text", x = c(14, 31), y = 29, label = "*")+
  geom_vline(xintercept = c(1, 32),
              linetype=2, alpha=0.5)+

  MyTheme +
  scale_colour_manual(
  values = c("#2b83ba", "#d7191c"))+
  theme(legend.position = "top")
TimeLine2
```




```
#ggsave(file="Outputs/TimeLine.svg", plot=TimeLine2, width=4, height=6)
```

2. Nutrients

```
# Data
    data<-read.csv("Data/Nutrients.csv", header = TRUE)
    #summary(data)
    data$N<-as.numeric(data$N)
    data$Date<-as.Date(data$Date)

    library(lubridate)
    data$Week<-isoweek(ymd(data$Date))
    data$Week<-as.numeric(data$Week-17)

    data$Target<-factor(data$Target,
                        levels=c("0", "5", "6"))
    # data$Pump<-factor(data$Pump,
    #                     levels=c("1", "2"))

    data<-droplevels(data)
    data$Treatment<-factor(data$Treatment,
                        levels = c ("LN_28", "LN_31", "HN_28", "HN_31",
                                    "Healthy", "Disease",  "Stock", "VAT"))
```

```r
    data$Treatment3<-factor(paste(data$Nutrients,
                                  data$Disease, sep = "_"))
    data<-data[data$Date!="2023-05-18", ]
    data$Day<-as.numeric(difftime(data$Date,
                                  as.Date("2023-05-01"), units="days"))

    summary(data)
```

```
##       Date                Time             Sample.ID       Sample
##  Min.   :2023-05-02   Length:2261        Min.   :   1   Length:2261
##  1st Qu.:2023-05-10   Class :character   1st Qu.: 566   Class :character
##  Median :2023-05-19   Mode  :character   Median :1134   Mode  :character
##  Mean   :2023-05-22                      Mean   :1133
##  3rd Qu.:2023-06-05                      3rd Qu.:1699
##  Max.   :2023-06-22                      Max.   :2264
##
##      Arm                Tank              Beaker              Pump
##  Length:2261        Length:2261        Length:2261        Length:2261
##  Class :character   Class :character   Class :character   Class :character
##  Mode  :character   Mode  :character   Mode  :character   Mode  :character
##
##
##
##
##       TP                Coral             Genotype            Colony
##  Length:2261        Length:2261        Length:2261        Length:2261
##  Class :character   Class :character   Class :character   Class :character
##  Mode  :character   Mode  :character   Mode  :character   Mode  :character
##
##
##
##
##       Tag           Disease            Nutrients              Temp
##  Min.   :  5.0   Length:2261        Length:2261        Min.   :28.00
##  1st Qu.:250.5   Class :character   Class :character   1st Qu.:28.00
##  Median :526.0   Mode  :character   Mode  :character   Median :28.00
##  Mean   :467.5                                         Mean   :29.42
##  3rd Qu.:544.0                                         3rd Qu.:31.00
##  Max.   :873.0                                         Max.   :31.00
##  NA's   :562                                           NA's   :184
##    Treatment    Treatment2         Target   Beaker.conditions
##  LN_28  :575   Length:2261        0:1153   Length:2261
##  HN_28  :521   Class :character   5:1016   Class :character
##  HN_31  :495   Mode  :character   6:  92   Mode  :character
##  LN_31  :486
##  Stock  : 92
##  VAT    : 46
##  (Other): 46
##     Notes                NH4               NO2              N.N
##  Length:2261        Min.   : 0.000   Min.   :0.0000   Min.   : 0.000
##  Class :character   1st Qu.: 0.120   1st Qu.:0.0200   1st Qu.: 0.060
##  Mode  :character   Median : 0.370   Median :0.0700   Median : 0.300
##                     Mean   : 1.430   Mean   :0.3217   Mean   : 1.381
```

```
##                     3rd Qu.: 1.462   3rd Qu.:0.5700   3rd Qu.: 2.250
##                     Max.   :60.030   Max.   :2.7900   Max.   :14.020
##                     NA's   :17       NA's   :560      NA's   :560
##       NO3              PO4               N                Week
##  Min.   :-0.01   Min.   :0.0000   Min.   :  0.000   Min.   :1.000
##  1st Qu.: 0.03   1st Qu.:0.0600   1st Qu.:  0.240   1st Qu.:2.000
##  Median : 0.20   Median :0.1000   Median :  1.025   Median :3.000
##  Mean   : 1.06   Mean   :0.3993   Mean   :  3.184   Mean   :3.825
##  3rd Qu.: 1.53   3rd Qu.:0.2550   3rd Qu.:  4.400   3rd Qu.:6.000
##  Max.   :12.97   Max.   :4.9500   Max.   :212.630   Max.   :8.000
##  NA's   :560     NA's   :560      NA's   :563
##          Treatment3       Day
##  HN_Placebo   :230   Min.   : 1.00
##  HN_SCTLD     :786   1st Qu.: 9.00
##  LN_Placebo   :269   Median :18.00
##  LN_SCTLD     :792   Mean   :21.77
##  Other_Placebo:161   3rd Qu.:35.00
##  Other_SCTLD  : 23   Max.   :52.00
##
```

Exploratory plots

```
NH4_plot<- ggplot(data, aes (Target, NH4)) +
  #geom_boxplot ()+
  geom_jitter(aes(colour=Arm, shape=Pump), alpha=1)+
  # stat_summary(fun.data = "mean_cl_boot",
  #              geom = "errorbar", width = 0.2,  color="black" )+
  # stat_summary(fun=mean, geom="point",  color="black") +
  #geom_point(shape=21)+


  theme(legend.position = "bottom")+
  scale_y_continuous(#limits = c(0, 11),
                     breaks = seq(0,12, 2.5),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  # geom_hline(yintercept = 4.1, linetype=2)+
  # geom_hline(yintercept = 0.3, linetype=2)+
  MyTheme +
  facet_grid(Beaker.conditions~Date, scales = "free_y")

#NH4_plot
```

```
NH4_genotype<- ggplot(data, aes (Date, NH4)) +
  geom_boxplot ()+
  geom_jitter(aes(colour=Genotype, shape=Arm), alpha=1)+
  # stat_summary(fun.data = "mean_cl_boot",
  #              geom = "errorbar", width = 0.2,  color="black" )+
  # stat_summary(fun=mean, geom="point",  color="black") +
  #geom_point(shape=21)+

  theme(legend.position = "bottom")+
```

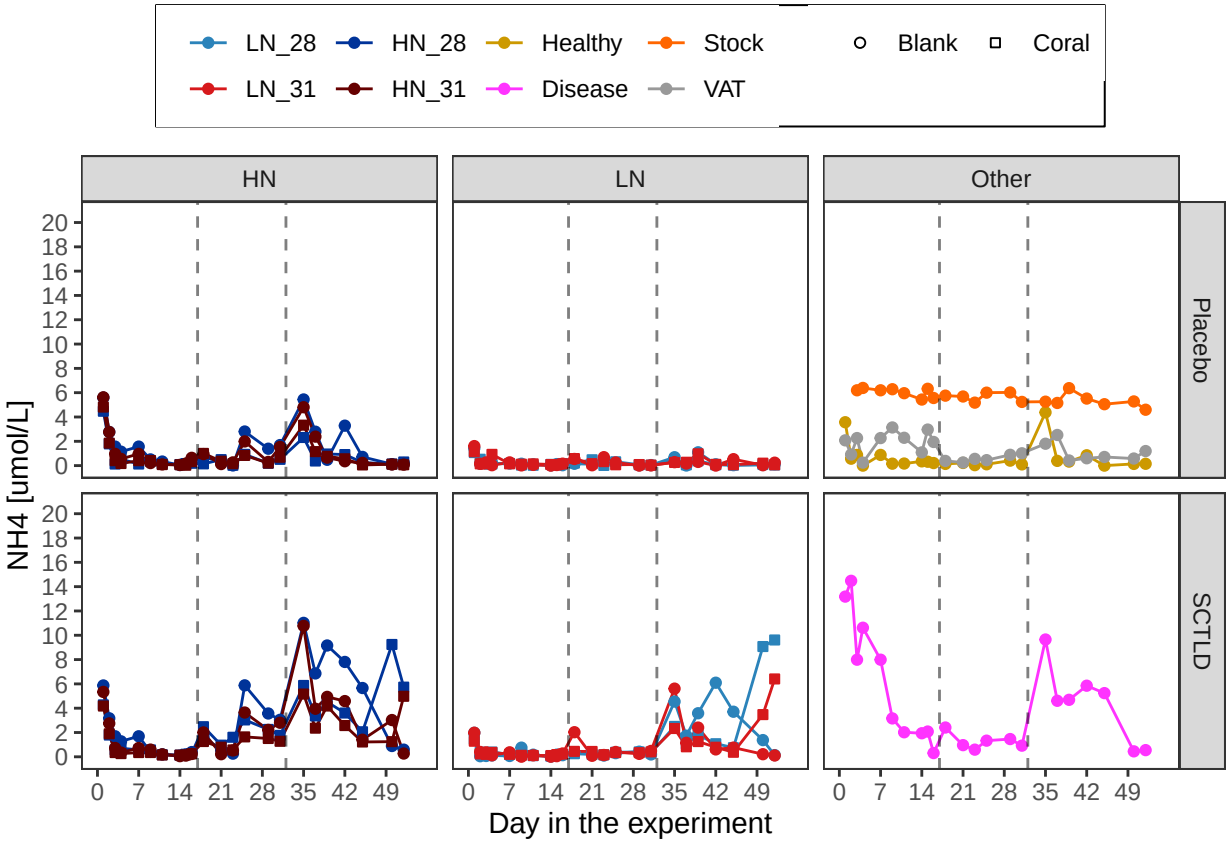
```
  scale_y_continuous(limits = c(0, 12),
                     breaks = seq(0,10, 1),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  # geom_hline(yintercept = 4.1, linetype=2)+
  # geom_hline(yintercept = 0.3, linetype=2)+
  MyTheme +
  facet_wrap(Disease~Nutrients, scales = "free_y")
#NH4_genotype
```

```
NH4_Arms<- ggplot(data, aes (Day, NH4)) +
  #geom_boxplot ()+
  geom_jitter(aes(colour=Arm, shape=Beaker.conditions), alpha=1)+
  stat_summary(fun.data = "mean_cl_boot",
               geom = "errorbar", width = 0.2,  color="black" )+
   stat_summary(fun=mean, geom="point",  color="black") +
  #geom_point(shape=21)+

  theme(legend.position = "bottom")+
  geom_vline(xintercept = c(17, 32),
             linetype=2, alpha=0.5)+
  scale_x_continuous(limits = c(0, 55),
                     breaks = seq(0,55, 7),
                     #   expand = c(0.01, 0.01),
                     name=("Days in the experiment")) +
  scale_y_continuous(limits = c(0, 15),
                     breaks = seq(0,20, 1),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  # geom_hline(yintercept = 4.1, linetype=2)+
  # geom_hline(yintercept = 0.3, linetype=2)+
  MyTheme +
  facet_wrap(Disease~Nutrients)

#NH4_Arms
```

Remove VAT and stock solution values

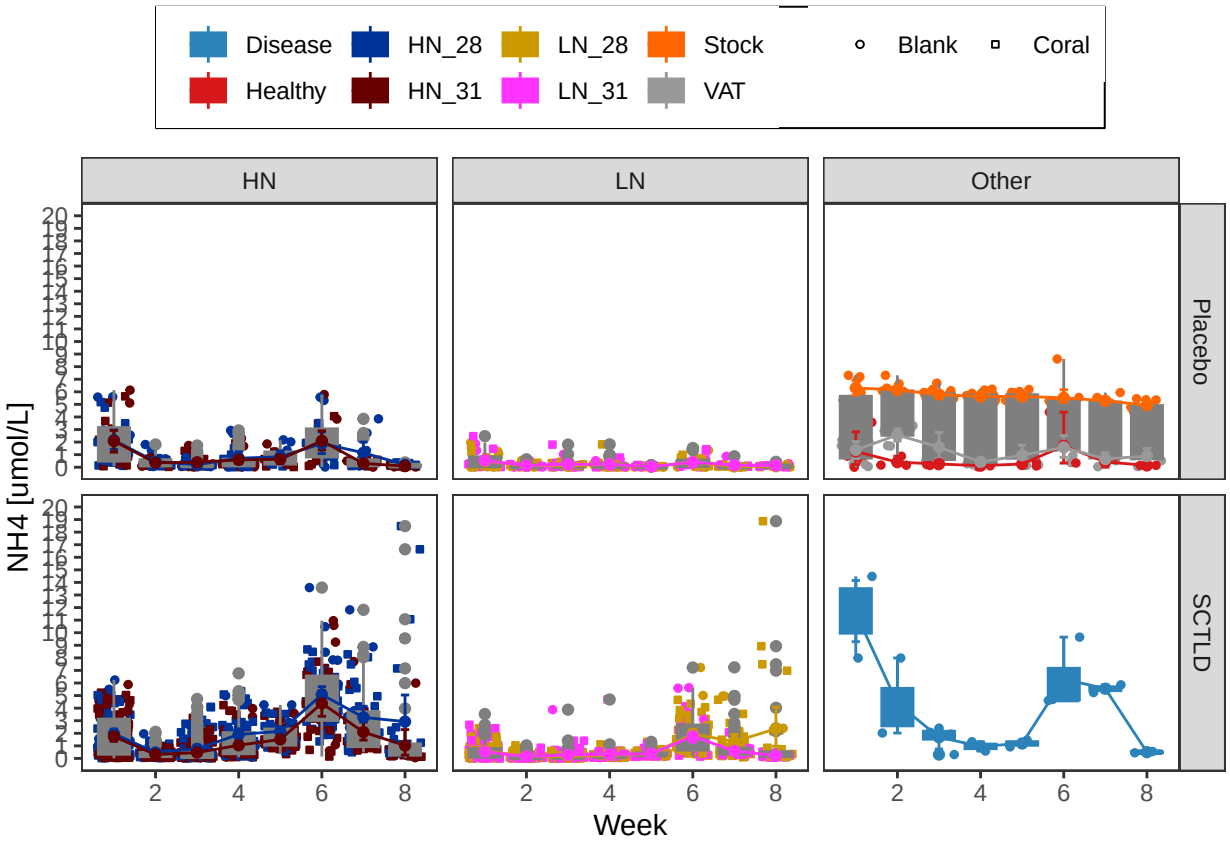
```
NH4_Temp<- ggplot(data, aes (Day, NH4, fill=Treatment,
                             colour=Treatment,
                             shape=Beaker.conditions)) +

  #geom_jitter(aes(colour=Treatment, shape=Beaker.conditions),
  #alpha=1, size =1)+
  #geom_boxplot ()+
  scale_shape_manual(values=c(21, 22))+
  scale_color_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000",
                              '#CC9900','#FF33FF', '#FF6600', "#999999"))+
  scale_fill_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000",
                             '#CC9900','#FF33FF', '#FF6600', "#999999"))+
  # stat_summary(fun.data = "mean_cl_boot",
  #               geom = "errorbar", width = 0.2)+
  stat_summary(fun.data = "mean_cl_boot",
```

```
                geom = "line")+
  stat_summary(fun=mean, geom="point") +
  #geom_point(shape=21)+
   geom_vline(xintercept = c(17, 32),
              linetype=2, alpha=0.5)+
  scale_x_continuous(limits = c(0, 55),
                     breaks = seq(0,55, 7),
                     #   expand = c(0.01, 0.01),
                     name=("Day in the experiment")) +
  scale_y_continuous(#limits = c(0, 60),
                     breaks = seq(0, 20, 2),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  # geom_hline(yintercept = 4.1, linetype=2)+
  # geom_hline(yintercept = 0.3, linetype=2)+
  #ylim()+
  MyTheme
  #NH4_Temp+facet_wrap(~Nutrients)
NH4_Temp+facet_grid(Disease~Nutrients)
```



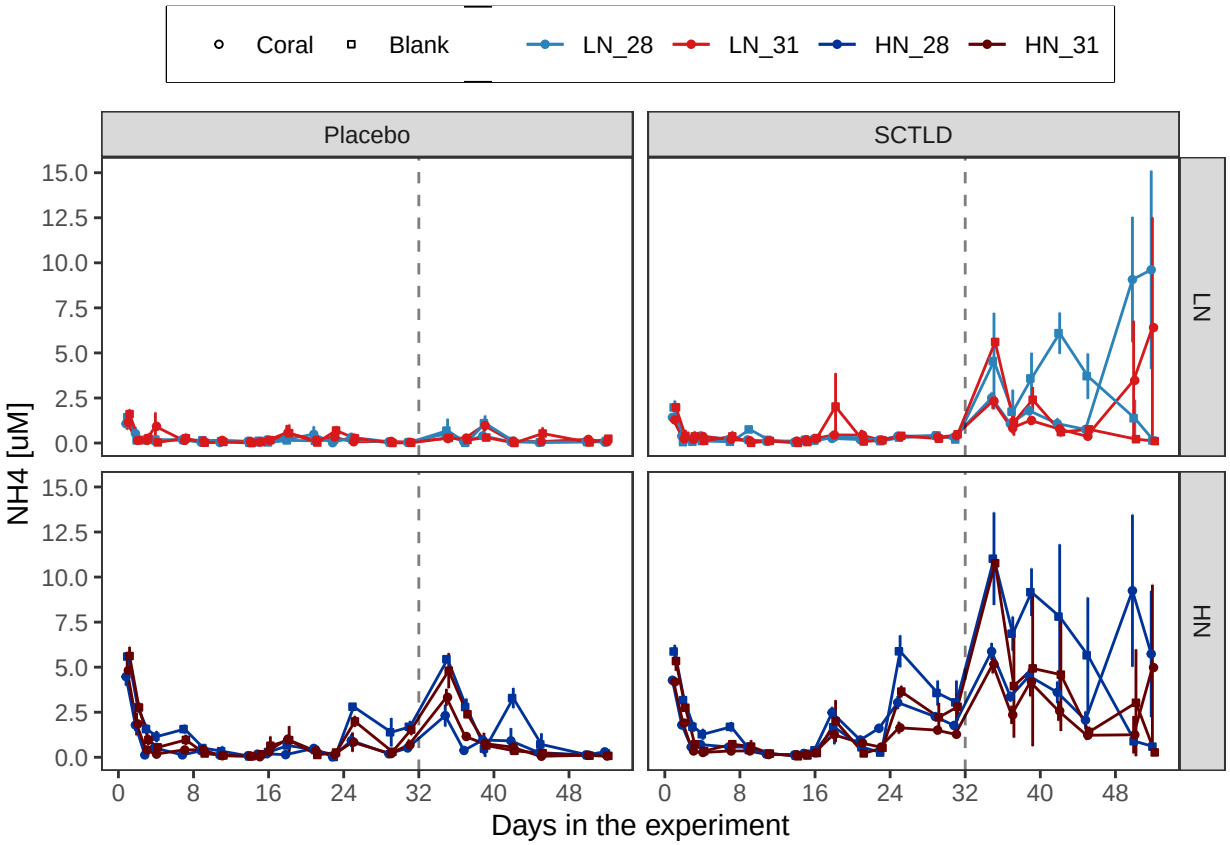

```
NH4_W<- ggplot(data, aes (Week, NH4, fill=Treatment, colour=Treatment)) +

  geom_jitter(aes(colour=Treatment, shape=Beaker.conditions),
              alpha=1, size =1)+
  geom_boxplot(aes (Week, NH4, fill=Treatment, group=Week))+
```

```
  scale_shape_manual(values=c(21, 22))+
  scale_color_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000",
                              '#CC9900','#FF33FF', '#FF6600', "#999999"))+
  scale_fill_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000",
                              '#CC9900','#FF33FF', '#FF6600', "#999999"))+
  stat_summary(fun.data = "mean_cl_boot",
               geom = "errorbar", width = 0.2)+
  stat_summary(fun.data = "mean_cl_boot",
               geom = "line")+
  stat_summary(fun=mean, geom="point") +
  #geom_point(shape=21)+

  theme(legend.position = "bottom")+
  scale_y_continuous(limits = c(0, 20),
                     breaks = seq(0,20, 1),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  # geom_hline(yintercept = 4.1, linetype=2)+
  # geom_hline(yintercept = 0.3, linetype=2)+
  MyTheme
  #NH4_Temp+facet_wrap(~Nutrients)
  NH4_W+facet_grid(Disease~Nutrients)
```



Remove Stock solution, VAT and donnor tank samples

```
Corals<-data[data$Nutrients!="Other", ]

Concentrations <- ddply (Corals, .(Treatment, Disease, Beaker.conditions, Nutrients, Day), summarise,
                meanNH4 = mean (NH4, na.rm = T),
                sdNH4 = sd (NH4, na.rm = T),
                seNH4 = std.error (NH4, na.rm = T))
#Concentrations

Concentrations$Beaker.conditions<-factor(Concentrations$Beaker.conditions,
                                         levels=c("Coral", "Blank"))
Concentrations$Nutrients<-factor(Concentrations$Nutrients,
                                         levels=c("LN", "HN"))

write.csv(Concentrations, "Outputs/Concentrations.csv")

# Standard error of the mean

NH4_day<-ggplot(Concentrations,
                aes(x=Day, y=(meanNH4), shape=Beaker.conditions,
                                    colour=Treatment)) +
    geom_errorbar(aes(ymin=(meanNH4-seNH4), ymax=(meanNH4+seNH4)),
                  width=.1, position=position_dodge(0.5)) +
    geom_line(position=position_dodge(0.5)) +
    geom_point(position=position_dodge(0.5), aes(fill=Treatment), size=1)+
    scale_shape_manual(values=c(21, 22))+
    scale_color_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
    scale_fill_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
    geom_vline(xintercept = c(32),
                linetype=2, alpha=0.5)+
    scale_x_continuous(#limits = c(0, 55),
                        breaks = seq(0,55, 8),
                        #   expand = c(0.01, 0.01),
                        name=("Days in the experiment")) +
    scale_y_continuous(#limits = c(0, 60),
                        breaks = seq(0, 35, 2.5),
                       #   expand = c(0.01, 0.01),
                       name=("NH4 [uM]")) + MyTheme +
  facet_grid(Nutrients~Disease)
NH4_day
```




```
#ggsave(file="Outputs/Nutrients.svg", plot=NH4_day, width=7.5, height=5)
```

Remove empty beakers

```
Concentrations2 <- ddply (Corals[Corals$Beaker.conditions=="Coral", ],
                          .(Treatment, Nutrients, Day),
                         summarise,
                meanNH4 = mean (NH4, na.rm = T),
                sdNH4 = sd (NH4, na.rm = T),
                seNH4 = std.error (NH4, na.rm = T))
#Concentrations2

Concentrations2$Nutrients<-factor(Concentrations2$Nutrients,
                                            levels=c("LN", "HN"))

# Standard error of the mean

NH4_day<-ggplot(Concentrations2,
                aes(x=Day, y=(meanNH4),
                                    colour=Treatment)) +
    geom_errorbar(aes(ymin=(meanNH4-seNH4), ymax=(meanNH4+seNH4)),
                  width=.1, position=position_dodge(0.5)) +
    geom_line(position=position_dodge(0.5)) +
    geom_point(position=position_dodge(0.5), aes(fill=Treatment), size=1)+
    scale_shape_manual(values=c(21, 22))+
```

```
    scale_color_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
    scale_fill_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
    geom_vline(xintercept = c(1, 32),
               linetype=2, alpha=0.5)+
    scale_x_continuous(#limits = c(0, 55),
                       breaks = seq(0,55, 8),
                       #   expand = c(0.01, 0.01),
                       name=("Days in the experiment")) +
    scale_y_continuous(#limits = c(0, 60),
                       breaks = seq(0, 20, 2),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [uM]")) + MyTheme #+
  #facet_grid(~Disease)
NH4_day
```

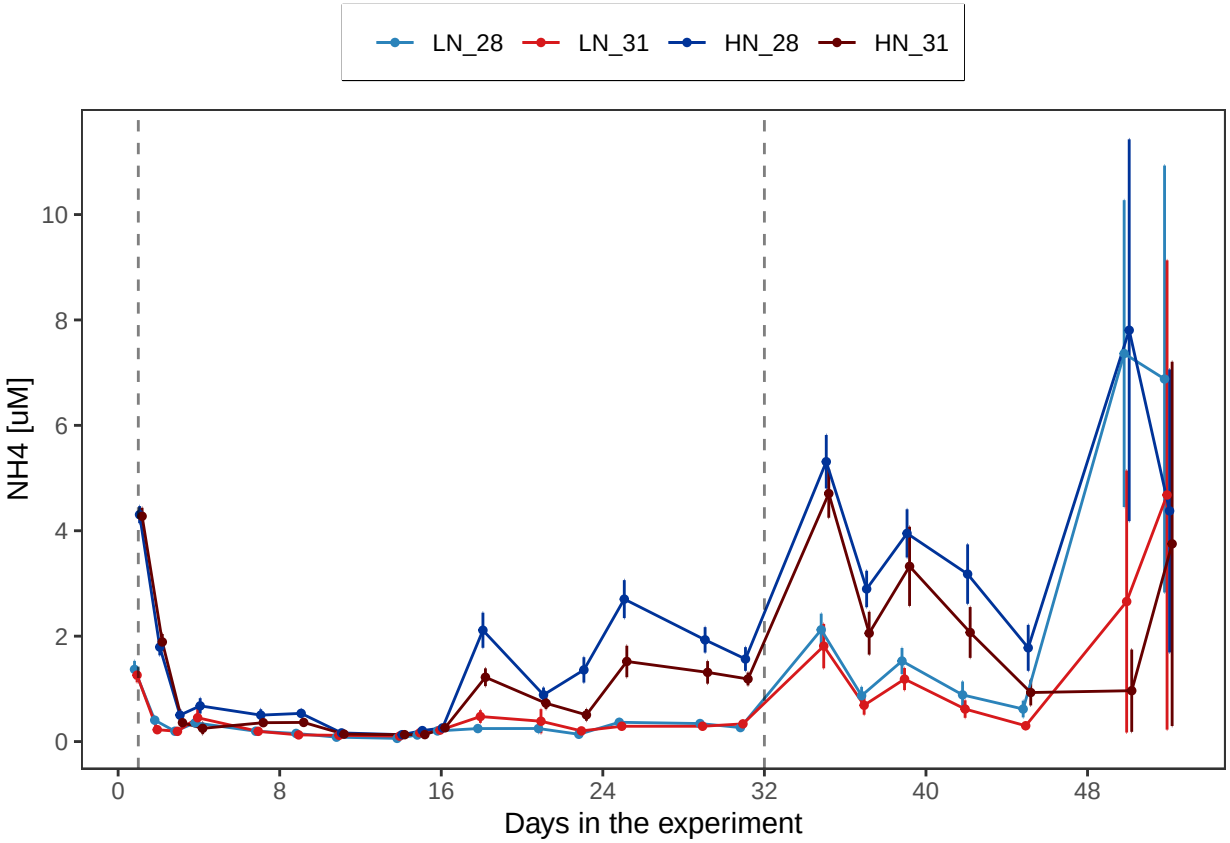
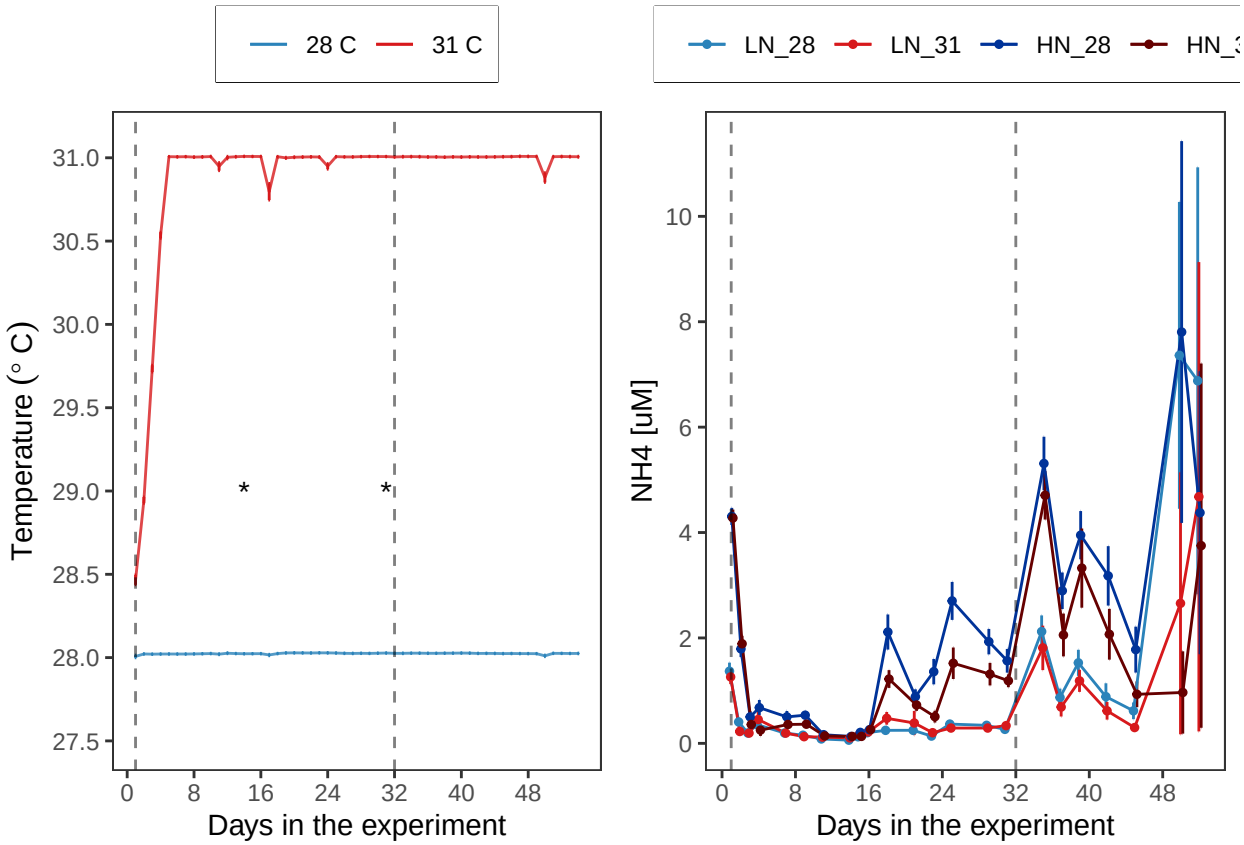


Figure: Experimental conditions

```
library(gridExtra)
experiment<-grid.arrange(TimeLine2, NH4_day, ncol = 2)
```




```
experiment
```

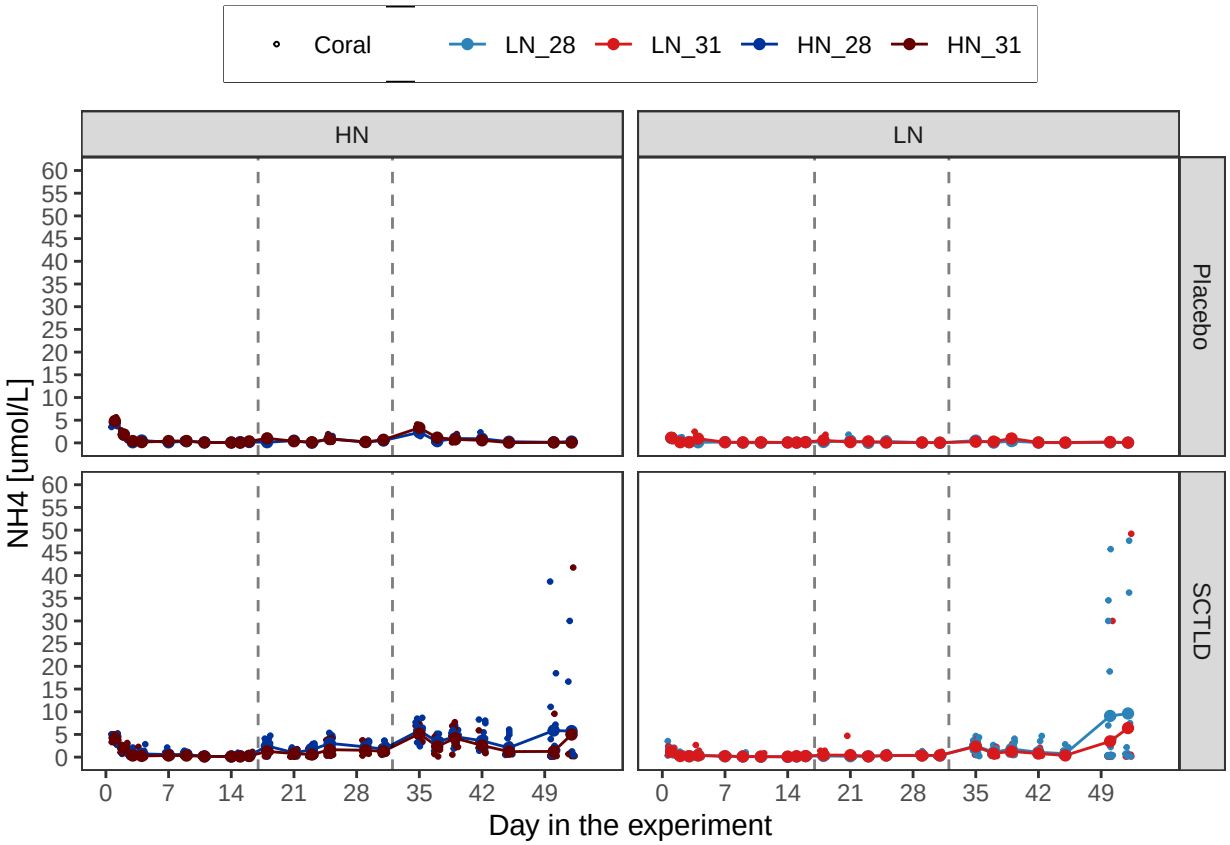
```
## TableGrob (1 x 2) "arrange": 2 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
```

```
#ggsave(file="Outputs/Experiment_conditions.svg", plot=experiment, width=8.5, height=4)
```

Other figures

```
NH4_by_coral<- ggplot(Corals[Corals$Beaker.conditions=="Coral", ],
                aes (Day, NH4, fill=Treatment,
                                colour=Treatment)) +

  geom_jitter(aes(colour=Treatment, shape=Beaker.conditions),
              alpha=1, size =0.5)+
  scale_shape_manual(values=c(21, 22))+
  scale_color_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
  scale_fill_manual(values=c("#2b83ba", "#d7191c", "#003399", "#660000"))+
  # stat_summary(fun.data = "mean_cl_boot",
  #                geom = "errorbar", width = 0.2)+
  stat_summary(fun.data = "mean_cl_boot",
```

```
                geom = "line")+
  stat_summary(fun=mean, geom="point") +
  #geom_point(shape=21)+
   geom_vline(xintercept = c(17, 32),
              linetype=2, alpha=0.5)+
  scale_x_continuous(limits = c(0, 55),
                     breaks = seq(0,55, 7),
                     #   expand = c(0.01, 0.01),
                     name=("Day in the experiment")) +
  scale_y_continuous(limits = c(0, 60),
                     breaks = seq(0, 60, 5),
                     #   expand = c(0.01, 0.01),
                     name=("NH4 [umol/L]")) +
  MyTheme
#NH4_Temp+facet_wrap(~Nutrients)
NH4_by_coral+facet_grid(Disease~Nutrients)
```




```
Concentrations <- ddply (data, .(Nutrients, Disease, Date),
                         summarise,
                  meanNH4 = mean (NH4, na.rm = T),
                  sdNH4 = sd (NH4, na.rm = T))
#Concentrations

Concentrations<-Concentrations %>%
                  mutate_if(is.numeric, round, digits=2)
```

```
Concentrations_Day_Beaker <- ddply (data, .(Nutrients, Day, Beaker.conditions),
                        summarise,
                  meanNH4 = mean (NH4, na.rm = T),
                  sdNH4 = sd (NH4, na.rm = T))
Concentrations_Day_Beaker
```

```
##     Nutrients Day Beaker.conditions   meanNH4       sdNH4
## 1          HN   1             Blank 5.6050000  0.48685580
## 2          HN   1             Coral 4.2902564  0.70357541
## 3          HN   2             Blank 2.8625000  0.29528437
## 4          HN   2             Coral 1.8423077  0.67117909
## 5          HN   3             Blank 1.2362500  0.50036951
## 6          HN   3             Coral 0.4279487  0.37932891
## 7          HN   4             Blank 0.8287500  0.48792381
## 8          HN   4             Coral 0.4587179  0.60078536
## 9          HN   7             Blank 1.2362500  0.50036951
## 10         HN   7             Coral 0.4279487  0.37932891
## 11         HN   9             Blank 0.4300000  0.26316752
## 12         HN   9             Coral 0.4466667  0.29381639
## 13         HN  11             Blank 0.2112500  0.18357073
## 14         HN  11             Coral 0.1521795  0.13800169
## 15         HN  14             Blank 0.0625000  0.02314550
## 16         HN  14             Coral 0.1303846  0.09637772
## 17         HN  15             Blank 0.1487500  0.10020515
## 18         HN  15             Coral 0.1678947  0.19496580
## 19         HN  16             Blank 0.3900000  0.33170555
## 20         HN  16             Coral 0.2602564  0.21599814
## 21         HN  18             Blank 1.3200000  1.07631647
## 22         HN  18             Coral 1.6548718  1.21934597
## 23         HN  21             Blank 0.3250000  0.29587642
## 24         HN  21             Coral 0.8043590  0.54990212
## 25         HN  23             Blank 0.2662500  0.26180350
## 26         HN  23             Coral 0.9221795  0.91281505
## 27         HN  25             Blank 3.5800000  1.64163681
## 28         HN  25             Coral 2.0961538  1.55516350
## 29         HN  29             Blank 1.8687500  1.45688942
## 30         HN  29             Coral 1.6133333  1.03921803
## 31         HN  31             Blank 2.2681250  1.07258678
## 32         HN  31             Coral 1.3721795  0.80108544
## 33         HN  35             Blank 8.0093750  3.42885491
## 34         HN  35             Coral 5.0762903  1.98945050
## 35         HN  37             Blank 3.9975000  2.48364795
## 36         HN  37             Coral 2.5706452  1.50530326
## 37         HN  39             Blank 3.8068750  4.52110048
## 38         HN  39             Coral 3.7082258  2.22852513
## 39         HN  42             Blank 4.0087500  3.95710730
## 40         HN  42             Coral 2.7490323  2.22416892
## 41         HN  45             Blank 2.0012500  2.89665047
## 42         HN  45             Coral 1.4501613  1.61399942
## 43         HN  50             Blank 1.0312500  2.03934470
## 44         HN  50             Coral 5.1574194 12.79517597
## 45         HN  52             Blank 0.2400000  0.19555050
## 46         HN  52             Coral 4.0637500 10.48866596
```

```
## 47         LN   1            Blank 1.6860000  0.42573335
## 48         LN   1            Coral 1.3176316  0.65252290
## 49         LN   2            Blank 0.1800000  0.22715633
## 50         LN   2            Coral 0.3179487  0.28889244
## 51         LN   3            Blank 0.2080000  0.27655821
## 52         LN   3            Coral 0.1951282  0.17187621
## 53         LN   4            Blank 0.1320000  0.18029605
## 54         LN   4            Coral 0.4025641  0.61046021
## 55         LN   7            Blank 0.2080000  0.27655821
## 56         LN   7            Coral 0.1951282  0.17187621
## 57         LN   9            Blank 0.2190000  0.32962774
## 58         LN   9            Coral 0.1405128  0.18085977
## 59         LN  11            Blank 0.1130000  0.07528465
## 60         LN  11            Coral 0.1003846  0.15404367
## 61         LN  14            Blank 0.0125000  0.01388730
## 62         LN  14            Coral 0.0804878  0.09655830
## 63         LN  15            Blank 0.1025000  0.07959720
## 64         LN  15            Coral 0.1439024  0.13947900
## 65         LN  16            Blank 0.1512500  0.12777631
## 66         LN  16            Coral 0.2092683  0.15289523
## 67         LN  18            Blank 0.7637500  1.29792073
## 68         LN  18            Coral 0.3580488  0.40511862
## 69         LN  21            Blank 0.1300000  0.12071217
## 70         LN  21            Coral 0.3150000  0.75952946
## 71         LN  23            Blank 0.2800000  0.30430248
## 72         LN  23            Coral 0.1691463  0.14956563
## 73         LN  25            Blank 0.2868750  0.16972536
## 74         LN  25            Coral 0.3290244  0.23026675
## 75         LN  29            Blank 0.1737500  0.18552532
## 76         LN  29            Coral 0.3167073  0.22899675
## 77         LN  31            Blank 0.1837500  0.21025070
## 78         LN  31            Coral 0.2997561  0.24228328
## 79         LN  35            Blank 2.7825000  2.90042731
## 80         LN  35            Coral 2.0084848  1.41056808
## 81         LN  37            Blank 0.7687500  1.07999256
## 82         LN  37            Coral 0.8048485  0.71313731
## 83         LN  39            Blank 1.8475000  1.60351177
## 84         LN  39            Coral 1.4034848  1.00651742
## 85         LN  42            Blank 1.7062500  2.79341413
## 86         LN  42            Coral 0.7880303  0.98959741
## 87         LN  45            Blank 1.2600000  1.68860889
## 88         LN  45            Coral 0.5019697  0.60870757
## 89         LN  50            Blank 0.4193750  0.81024880
## 90         LN  50            Coral 5.6512121 11.91667923
## 91         LN  52            Blank 0.1616667  0.09174239
## 92         LN  52            Coral 5.9112000 14.71519632
## 93      Other   1            Blank 5.2275000  5.34849745
## 94      Other   2            Blank 4.2200000  6.87828952
## 95      Other   3            Blank 4.5700000  2.78492370
## 96      Other   4            Blank 4.5837500  3.97196223
## 97      Other   7            Blank 4.5700000  2.78492370
## 98      Other   9            Blank 4.3425000  2.30303992
## 99      Other  11            Blank 3.8250000  2.40220732
## 100     Other  14            Blank 2.5566667  2.30242191
```

```
## 101     Other  15             Blank 3.8985714  2.82568829
## 102     Other  16             Blank 3.3325000  2.51078673
## 103     Other  18             Blank 2.9414286  2.74629101
## 104     Other  21             Blank 3.0556250  2.82506566
## 105     Other  23             Blank 2.8081250  2.54535422
## 106     Other  25             Blank 3.2937500  2.92201465
## 107     Other  29             Blank 3.4675000  2.77377386
## 108     Other  31             Blank 3.0012500  2.45654483
## 109     Other  35             Blank 4.8337500  2.51139084
## 110     Other  37             Blank 3.8325000  1.87864503
## 111     Other  39             Blank 3.5692857  3.23125418
## 112     Other  42             Blank 3.7468750  2.54635725
## 113     Other  45             Blank 3.3587500  2.42737623
## 114     Other  50             Blank 2.8593750  2.61078592
## 115     Other  52             Blank 2.6856250  2.10544437
```

```
Concentrations_Nutrients_Day <- ddply (data, .(Nutrients, Day),
                          summarise,
                meanNH4 = mean (NH4, na.rm = T),
                sdNH4 = sd (NH4, na.rm = T))
Concentrations_Nutrients_Day
```

```
##    Nutrients Day    meanNH4       sdNH4
## 1         HN   1 4.51404255  0.83333447
## 2         HN   2 2.01595745  0.73184894
## 3         HN   3 0.56553191  0.50124978
## 4         HN   4 0.52170213  0.59511168
## 5         HN   7 0.56553191  0.50124978
## 6         HN   9 0.44382979  0.28617077
## 7         HN  11 0.16223404  0.14616403
## 8         HN  14 0.11882979  0.09175945
## 9         HN  15 0.16456522  0.18130032
## 10        HN  16 0.28234043  0.24023732
## 11        HN  18 1.59787234  1.19193109
## 12        HN  21 0.72276596  0.54432029
## 13        HN  23 0.81053191  0.87226170
## 14        HN  25 2.34872340  1.65098965
## 15        HN  29 1.65680851  1.10659850
## 16        HN  31 1.52468085  0.90611234
## 17        HN  35 5.67794872  2.59423899
## 18        HN  37 2.86333333  1.80717879
## 19        HN  39 3.72846154  2.77267711
## 20        HN  42 3.00743590  2.65622255
## 21        HN  45 1.56320513  1.91129003
## 22        HN  50 4.31102564 11.52670995
## 23        HN  52 3.29900000  9.46982671
## 24        LN   1 1.39437500  0.62670332
## 25        LN   2 0.28979592  0.28089507
## 26        LN   3 0.19775510  0.19430716
## 27        LN   4 0.34734694  0.55969402
## 28        LN   7 0.19775510  0.19430716
## 29        LN   9 0.15653061  0.21746217
## 30        LN  11 0.10295918  0.14097842
## 31        LN  14 0.06938776  0.09188213
```

```
## 32        LN  15 0.13714286  0.13181426
## 33        LN  16 0.19979592  0.14943630
## 34        LN  18 0.42428571  0.63670375
## 35        LN  21 0.28479592  0.69830894
## 36        LN  23 0.18724490  0.18400934
## 37        LN  25 0.32214286  0.22053203
## 38        LN  29 0.29336735  0.22708843
## 39        LN  31 0.28081633  0.23925089
## 40        LN  35 2.15951220  1.77774745
## 41        LN  37 0.79780488  0.78177926
## 42        LN  39 1.49012195  1.13673518
## 43        LN  42 0.96719512  1.51152863
## 44        LN  45 0.64987805  0.94229141
## 45        LN  50 4.63036585 10.86862120
## 46        LN  52 4.79838710 13.36273926
## 47     Other   1 5.22750000  5.34849745
## 48     Other   2 4.22000000  6.87828952
## 49     Other   3 4.57000000  2.78492370
## 50     Other   4 4.58375000  3.97196223
## 51     Other   7 4.57000000  2.78492370
## 52     Other   9 4.34250000  2.30303992
## 53     Other  11 3.82500000  2.40220732
## 54     Other  14 2.55666667  2.30242191
## 55     Other  15 3.89857143  2.82568829
## 56     Other  16 3.33250000  2.51078673
## 57     Other  18 2.94142857  2.74629101
## 58     Other  21 3.05562500  2.82506566
## 59     Other  23 2.80812500  2.54535422
## 60     Other  25 3.29375000  2.92201465
## 61     Other  29 3.46750000  2.77377386
## 62     Other  31 3.00125000  2.45654483
## 63     Other  35 4.83375000  2.51139084
## 64     Other  37 3.83250000  1.87864503
## 65     Other  39 3.56928571  3.23125418
## 66     Other  42 3.74687500  2.54635725
## 67     Other  45 3.35875000  2.42737623
## 68     Other  50 2.85937500  2.61078592
## 69     Other  52 2.68562500  2.10544437
```

```
Concentrations_Nutrients_Week <- ddply (data, .(Nutrients, Week),
                        summarise,
                  meanNH4 = mean (NH4, na.rm = T),
                  sdNH4 = sd (NH4, na.rm = T))
Concentrations_Nutrients_Week
```

```
##    Nutrients Week   meanNH4      sdNH4
## 1         HN    1 1.9043085  1.7599295
## 2         HN    2 0.3905319  0.3810693
## 3         HN    3 0.5429144  0.8688467
## 4         HN    4 1.2940071  1.3432905
## 5         HN    5 1.5907447  1.0080741
## 6         HN    6 4.0899145  2.6815205
## 7         HN    7 2.2853205  2.4110082
## 8         HN    8 3.8710145 10.6183037
```

```
## 9         LN    1 0.5530256  0.6608567
## 10        LN    2 0.1524150  0.1897650
## 11        LN    3 0.2076531  0.3598637
## 12        LN    4 0.2647279  0.4366877
## 13        LN    5 0.2870918  0.2321292
## 14        LN    6 1.4824797  1.4042235
## 15        LN    7 0.8085366  1.2618301
## 16        LN    8 4.7027083 11.9166468
## 17     Other    1 4.6282609  4.1934187
## 18     Other    2 4.2317391  2.3958253
## 19     Other    3 3.2100000  2.5114464
## 20     Other    4 3.0525000  2.6533768
## 21     Other    5 3.2343750  2.5425524
## 22     Other    6 4.1006522  2.5077327
## 23     Other    7 3.5528125  2.4115721
## 24     Other    8 2.7725000  2.2929522
```

```
Concentrations<-Concentrations %>%
 mutate_if(is.numeric, round, digits=2)

# write.csv(Concentrations, "Outputs/weekly_NH4.csv", row.names = F)
```

#3. Treatment effects on NH4 concentrations

```
## model 1: LMER for both species
  fit1<-lmerTest::lmer(NH4 ~Nutrients * Temp * Disease * Week +
                         (1|Beaker.conditions), data=data[data$Nutrients!="Other", ])
  isSingular(fit1)
```

```
## [1] FALSE
```

```
  anova(fit1)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                              Sum Sq Mean Sq NumDF  DenDF F value   Pr(>F)
## Nutrients                     1.354   1.354     1 2058.4  0.1328 0.715615
## Temp                         14.267  14.267     1 2058.7  1.3989 0.237050
## Disease                      12.826  12.826     1 2058.6  1.2576 0.262243
## Week                         78.526  78.526     1 2058.9  7.6996 0.005573 **
## Nutrients:Temp                0.527   0.527     1 2058.4  0.0517 0.820141
## Nutrients:Disease             0.475   0.475     1 2058.6  0.0466 0.829173
## Temp:Disease                  9.238   9.238     1 2058.3  0.9058 0.341337
## Nutrients:Week                0.637   0.637     1 2058.5  0.0625 0.802686
## Temp:Week                    62.996  62.996     1 2058.9  6.1768 0.013022 *
## Disease:Week                 65.411  65.411     1 2058.1  6.4136 0.011399 *
## Nutrients:Temp:Disease        0.508   0.508     1 2058.5  0.0498 0.823436
## Nutrients:Temp:Week           0.658   0.658     1 2058.4  0.0646 0.799445
## Nutrients:Disease:Week        0.080   0.080     1 2058.5  0.0078 0.929571
## Temp:Disease:Week            47.866  47.866     1 2058.1  4.6933 0.030395 *
## Nutrients:Temp:Disease:Week   0.028   0.028     1 2058.5  0.0028 0.958040
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
step(fit1)
```

```
## Backward reduced random-effect table:
##
##                          Eliminated npar  logLik   AIC   LRT Df Pr(>Chisq)
## <none>                                18 -5371.2 10778
## (1 | Beaker.conditions)          1    17 -5371.8 10778 1.142  1     0.2852
##
## Backward reduced fixed-effect table:
##                              Eliminated Df Sum of Sq   RSS    AIC F value
## Nutrients:Temp:Disease:Week          1  1      0.01 21023 4835.0  0.0006
## Nutrients:Temp:Week                  2  1      1.14 21024 4833.1  0.1121
## Nutrients:Temp:Disease               3  1      3.01 21027 4831.4  0.2955
## Nutrients:Disease:Week               4  1      4.84 21032 4829.9  0.4751
## Nutrients:Week                       5  1      1.34 21034 4828.0  0.1311
## Nutrients:Disease                    6  1      9.33 21043 4826.9  0.9156
## Nutrients:Temp                       7  1     21.20 21064 4827.0  2.0809
## Nutrients                            0  1    479.21 21543 4871.7 47.0018
## Temp:Disease:Week                    0  1     47.84 21112 4829.7  4.6923
##                                Pr(>F)
## Nutrients:Temp:Disease:Week   0.97967
## Nutrients:Temp:Week           0.73785
## Nutrients:Temp:Disease        0.58675
## Nutrients:Disease:Week        0.49075
## Nutrients:Week                0.71733
## Nutrients:Disease             0.33874
## Nutrients:Temp                0.14931
## Nutrients                   9.332e-12 ***
## Temp:Disease:Week             0.03041 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Model found:
## NH4 ~ Nutrients + Temp + Disease + Week + Temp:Disease + Temp:Week + Disease:Week + Temp:Disease:Week
```

```
ranova(fit1)
```

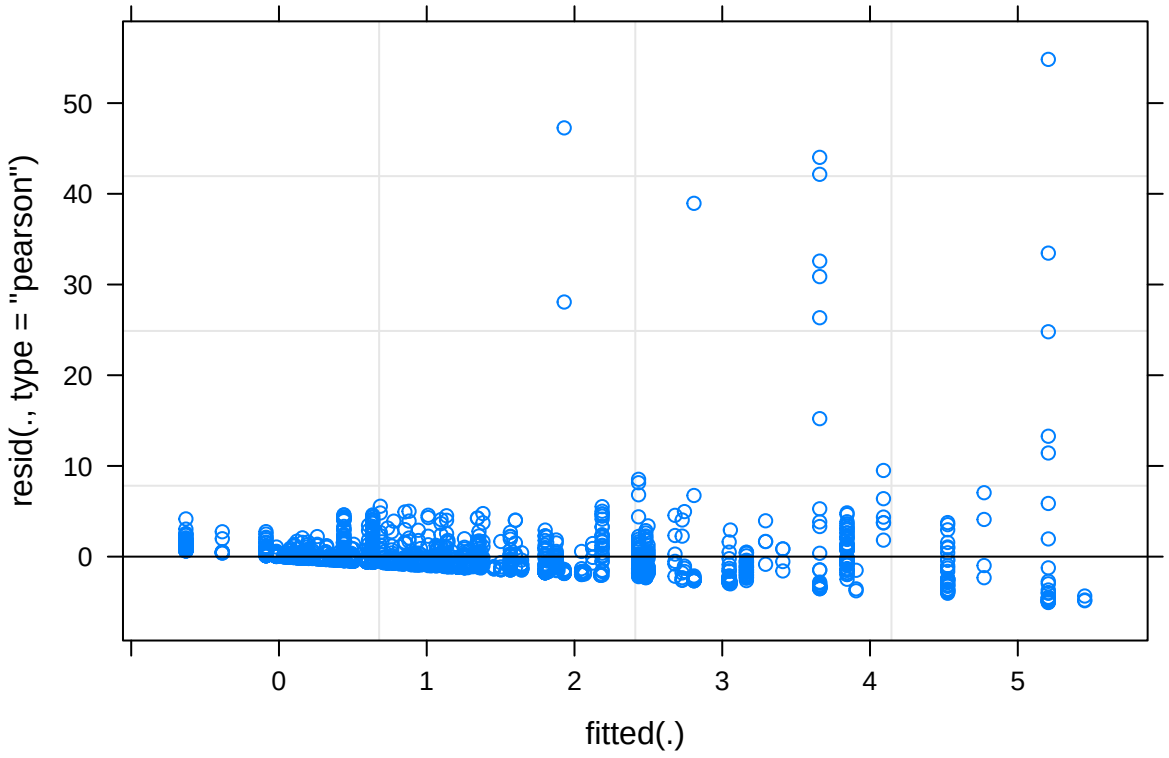
```
## ANOVA-like table for random-effects: Single term deletions
##
## Model:
## NH4 ~ Nutrients + Temp + Disease + Week + (1 | Beaker.conditions) + Nutrients:Temp + Nutrients:Disease
##                          npar  logLik   AIC   LRT Df Pr(>Chisq)
## <none>                     18 -5371.2 10778
## (1 | Beaker.conditions)    17 -5371.8 10778 1.142  1     0.2852
```

```
summary(fit1)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: NH4 ~ Nutrients * Temp * Disease * Week + (1 | Beaker.conditions)
##    Data: data[data$Nutrients != "Other", ]
##
```

```
## REML criterion at convergence: 10742.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.5834 -0.2982 -0.1176  0.0647 17.1669
##
## Random effects:
##  Groups            Name        Variance Std.Dev.
##  Beaker.conditions (Intercept)  0.04324 0.2079
##  Residual                      10.19870 3.1935
## Number of obs: 2075, groups:  Beaker.conditions, 2
##
## Fixed effects:
##                                     Estimate Std. Error         df t value
## (Intercept)                         5.766e-01  8.471e+00  2.059e+03   0.068
## NutrientsLN                        -9.787e-01  1.151e+01  2.059e+03  -0.085
## Temp                                2.537e-02  2.868e-01  2.058e+03   0.088
## DiseaseSCTLD                       -5.966e+00  9.608e+00  2.058e+03  -0.621
## Week                                2.967e-01  1.846e+00  2.058e+03   0.161
## NutrientsLN:Temp                    9.441e-04  3.908e-01  2.059e+03   0.002
## NutrientsLN:DiseaseSCTLD           -2.843e+00  1.318e+01  2.059e+03  -0.216
## Temp:DiseaseSCTLD                   1.629e-01  3.253e-01  2.058e+03   0.501
## NutrientsLN:Week                   -2.367e-01  2.528e+00  2.059e+03  -0.094
## Temp:Week                          -1.298e-02  6.251e-02  2.058e+03  -0.208
## DiseaseSCTLD:Week                   3.841e+00  2.125e+00  2.058e+03   1.808
## NutrientsLN:Temp:DiseaseSCTLD       9.979e-02  4.472e-01  2.059e+03   0.223
## NutrientsLN:Temp:Week               1.003e-02  8.581e-02  2.059e+03   0.117
## NutrientsLN:DiseaseSCTLD:Week      -2.591e-01  2.931e+00  2.059e+03  -0.088
## Temp:DiseaseSCTLD:Week             -1.105e-01  7.201e-02  2.058e+03  -1.534
## NutrientsLN:Temp:DiseaseSCTLD:Week  5.240e-03  9.958e-02  2.058e+03   0.053
##                                    Pr(>|t|)
## (Intercept)                          0.9457
## NutrientsLN                          0.9323
## Temp                                 0.9295
## DiseaseSCTLD                         0.5347
## Week                                 0.8724
## NutrientsLN:Temp                     0.9981
## NutrientsLN:DiseaseSCTLD             0.8292
## Temp:DiseaseSCTLD                    0.6166
## NutrientsLN:Week                     0.9254
## Temp:Week                            0.8356
## DiseaseSCTLD:Week                    0.0708 .
## NutrientsLN:Temp:DiseaseSCTLD        0.8234
## NutrientsLN:Temp:Week                0.9069
## NutrientsLN:DiseaseSCTLD:Week        0.9296
## Temp:DiseaseSCTLD:Week               0.1252
## NutrientsLN:Temp:DiseaseSCTLD:Week   0.9580
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
par(mfrow=c(2,2))
plot(fit1)
```




```
  par(mfrow=c(1,1))
```

```
#Pairwise comparisons
Sw.emmc<-multcomp::cld(emmeans(fit1, specs=c("Nutrients", "Disease")))
Sw.emmc
```

```
##  Nutrients Disease emmean    SE   df lower.CL upper.CL .group
##  LN        Placebo  0.272 0.246 3.79   -0.428    0.971  1
##  HN        Placebo  0.997 0.259 4.63    0.315    1.678  12
##  LN        SCTLD    1.031 0.212 1.41   -0.365    2.427   2
##  HN        SCTLD    2.054 0.212 1.41    0.661    3.447    3
##
## Results are averaged over the levels of: Temp
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## P value adjustment: tukey method for comparing a family of 4 estimates
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
##       then we cannot show them to be different.
##       But we also did not show them to be the same.
```

Packages used

```
# Creates bibliography
#knitr::write_bib(c(.packages()), "packages.bib")
```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