

Growth Curve Analysis

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Load required packages (and install if necessary).

```
if (!require('tidyverse')) install.packages('tidyverse'); library('tidyverse')
if (!require('modelr')) install.packages('modelr'); library('modelr')
if (!require('growthcurver')) install.packages('growthcurver'); library('growthcurver')
if (!require('here')) install.packages('here'); library('here')

#Load functions in from functions.R
source(here("functions.R"))
```

Load in data. This part is up to you, but the format has to be 'tidy' (i.e. tidyverse style with unique but often long columns)

```
pfas_tidy <- read_csv("pfas_tidy.csv") %>%
  gather(key = "replicate", value = "od",
    -Treatment, -Concentration, -Date, -Hours, -Time) %>% #Make the data frame longer
  group_by(Concentration, Treatment, replicate) %>%
  #standardize starting values and add very small pseudo-count to avoid zeros
  mutate(od_cor = od + abs(min(od)) + 0.00001,
    unique_id = paste(Concentration,replicate,sep = "_")) %>%
  dplyr::select(-od) %>%
  rename(od = "od_cor") %>%
  ungroup()
```

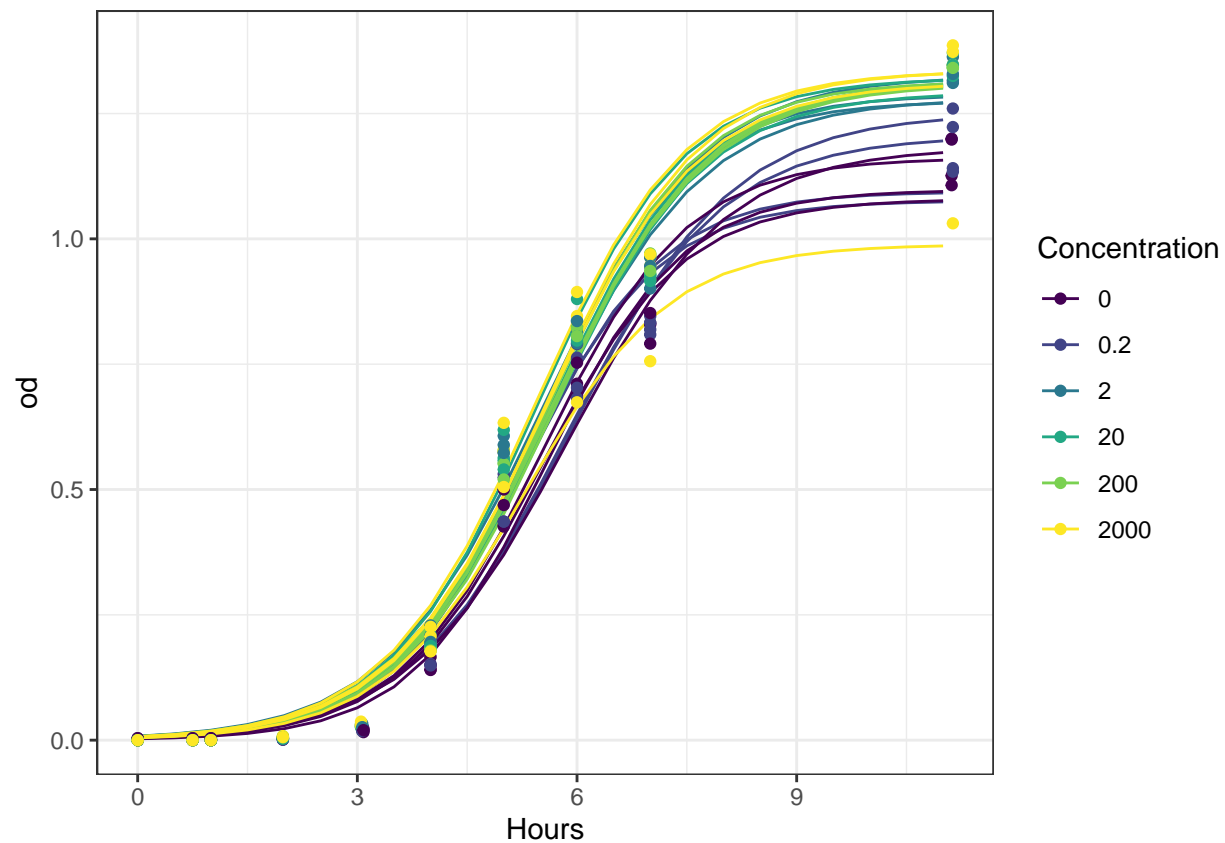
Fit growth curves based on treatment group(s).

```
growth_results <- fit_growth(pfas_tidy,c("Concentration","replicate","unique_id"),
  time_col = "Hours",bg_cor = "none")

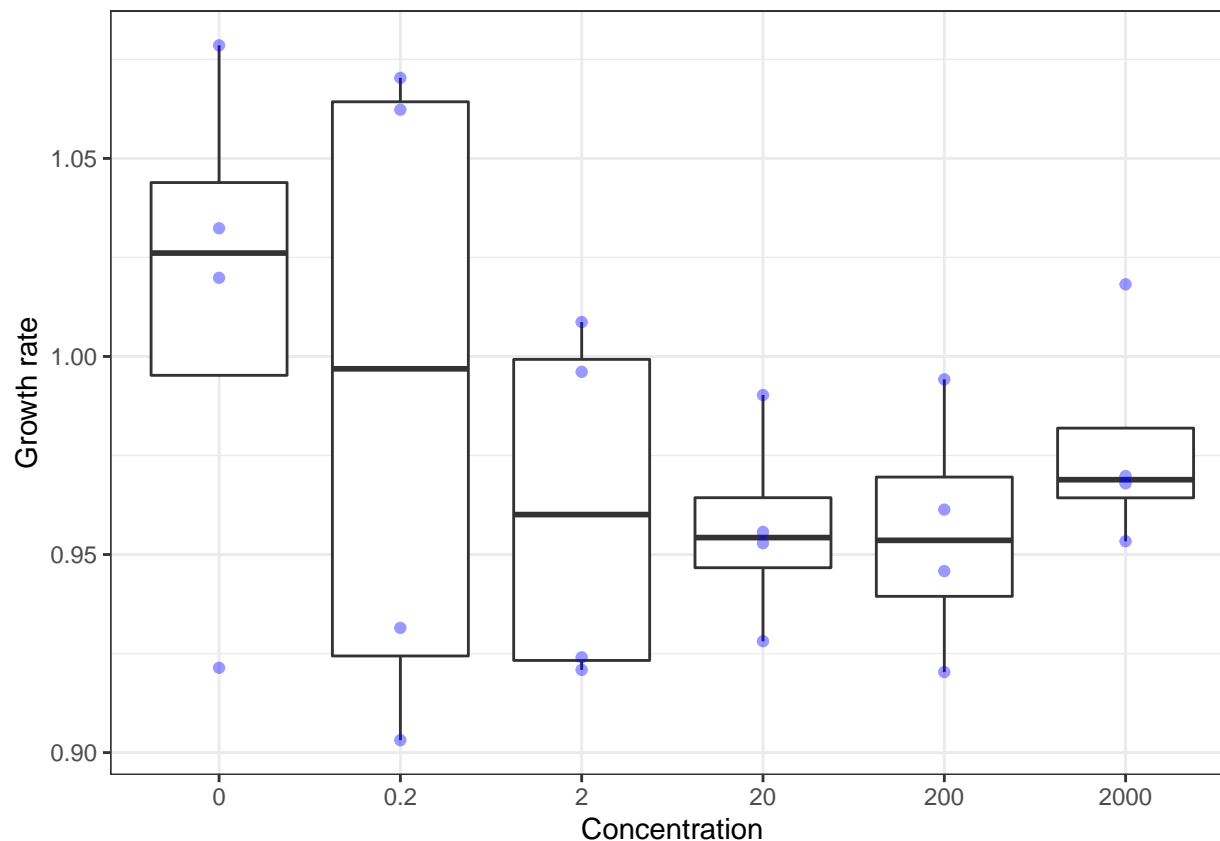
#model results, including columns for each of the growth curve values described below
models <- growth_results$model_data

predicted <- growth_results$predicted_vals #Model predictions for plotting

#Plot predicted OD values based on the fit model, with observed data as points
predicted %>% filter(t < 100) %>%
  ggplot(aes(t,pred, group = unique_id, color = as.factor(Concentration))) +
  geom_line() +
  scale_color_viridis_d() +
  geom_point(data = pfas_tidy %>% rename(t = "Hours", pred = "od"),
    aes(t, pred, color = as.factor(Concentration)),inherit.aes = FALSE) +
  theme_bw() +
  labs(y = "od", x = "Hours", color = "Concentration")
```



```
#Boxplot of growth rates by concentration
models %>% ggplot(aes(as.factor(Concentration), r)) +
  geom_boxplot(outlier.shape = NA) +
  geom_point(alpha = 0.4, color = "blue") +
  theme_bw() +
  labs(y = "Growth rate", x = "Concentration")
```



```
#Run ANOVA for concentration
aov(r ~ Concentration , data = models)
```

```
## Call:
##   aov(formula = r ~ Concentration, data = models)
##
## Terms:
##              Concentration  Residuals
## Sum of Squares    0.00000571 0.05983572
## Deg. of Freedom           1          22
##
## Residual standard error: 0.05215176
## Estimated effects may be unbalanced
```

```
#Run Kruskal-wallis test for concentration
kruskal.test(r ~ Concentration, data=models)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  r by Concentration
## Kruskal-Wallis chi-squared = 3.4, df = 5, p-value = 0.6386
```

The growth curve values `help("gcvals")` are:

- k: The carrying capacity parameter
- k_se: The standard error of the carrying capacity parameter
- k_p: The p value of the carrying capacity parameter
- n0: The initial population size
- n0_se: The standard error of the initial population size
- n0_p: The p value of the initial population size
- r: The growth rate
- r_se: The standard error of the growth rate
- r_p: The p value of the growthrate
- sigma: Residual standard error from non-linear least squares fit of the model to the data
- df: Degrees of freedom
- t_mid: The time at the inflection point of the logistic curve (occurs at half of the carrying capacity)
- dt: The maximum doubling time, obtained by evaluating the the unrestrained growth of the population with growth rate r
- auc_l: The area under the curve of the fitted logistic equation from time 0 to time t
- auc_e: The area under the curve of the measurements.