Climate Water Loss Experiment - Plasma Osmolality Data Wrangling

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2021

Contents

Packages

```r
`%nin%` = Negate(`%in%`)
if (!require("tidyverse")) install.packages("tidyverse")
```

```
## Loading required package: tidyverse

## -- Attaching packages --------------------------------------- tidyverse 1.3.0 --

## v ggplot2 3.3.3     v purrr   0.3.4
## v tibble  3.0.6     v dplyr   1.0.2
## v tidyr   1.1.2     v stringr 1.4.0
## v readr   1.4.0     v forcats 0.5.0

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library("tidyverse") # workflow and plots
```

Background and Goals

Blood was drawn from the postorbital sinus of adult male *Sceloporus occidentalis* between June - August 2021. After centrifuging and separating, plasma was run on a VAPRO vapor pressure osmometer in 1-4 replicates, when plasma volume allowed. In this R script, I check the distribution of replicates, omit outliers, and average remaining replicates. The final values will be more precise and accurate estimates of the true plasma osmolality for each lizard, and those values will be used in the capture_analysis and experiment_analysis R script files. Please refer to the published scientific journal article for full details.

Load Data

```
osml_reps <- read.csv("./data/osmolality.csv",
                na.strings = c("","NA"),
                header = TRUE
                ) %>%
    dplyr::mutate(date_blood_drawn = as.Date(date_blood_drawn,
                                             format = "%m/%d/%y"),
                  date_osmom_run = as.Date(date_osmom_run,
                                           format = "%m/%d/%y"),
                  time_osmom_run = as.POSIXct(time_osmom_run,
                                              format = "%H:%M"),
                  individual_ID = as.factor(individual_ID),
                  replicate_no = as.factor(replicate_no),
                  osmolality_mmol_kg = as.numeric(osmolality_mmol_kg)
                  )
summary(osml_reps)
```

```
##  date_blood_drawn     date_osmom_run       time_osmom_run
##  Min.   :2021-06-16   Min.   :2021-06-16   Min.   :2022-10-03 01:00:00
##  1st Qu.:2021-06-30   1st Qu.:2021-06-30   1st Qu.:2022-10-03 10:38:00
##  Median :2021-07-24   Median :2021-07-24   Median :2022-10-03 12:48:00
##  Mean   :2021-07-22   Mean   :2021-07-22   Mean   :2022-10-03 12:58:11
##  3rd Qu.:2021-08-16   3rd Qu.:2021-08-16   3rd Qu.:2022-10-03 15:14:00
##  Max.   :2021-09-01   Max.   :2021-09-02   Max.   :2022-10-03 21:22:00
##                                            NA's   :211
##  individual_ID  replicate_no osmolality_mmol_kg  notes
##  339    :  13   1:544        Min.   :247.0      Mode:logical
##  206    :  12   2:531        1st Qu.:336.0      NA's:1484
##  207    :  12   3:406        Median :351.0
##  208    :  12   4:  3        Mean   :357.3
##  211    :  12                3rd Qu.:370.0
##  214    :  12                Max.   :596.0
##  (Other):1411
```

Check Data

Dates

Blood was drawn on day 0, 4, 8, and 10 of the experiment. Create a list of the dates expected to have blood draw data, then determine whether I have dates outside those.

Trail 1: June 16-24 Trail 2: June 26 - July 4 Trial 3: July 20-28 Trial 4: August 8-16 Trial 5: August 22-30

```
                             # trial 1
expected_dates <- as.Date(c("2021-06-16", "2021-06-20",
                             "2021-06-24", "2021-06-26",
                             # trial 2
                             "2021-06-26", "2021-06-30",
                             "2021-07-04", "2021-07-06",
                             # trial 3
                             "2021-07-20", "2021-07-24",
                             "2021-07-28", "2021-07-30",
                             # trial 4
                             "2021-08-08", "2021-08-12",
                             "2021-08-16", "2021-08-18",
                             # trial 5
                             "2021-08-22", "2021-08-26",
                             "2021-08-30", "2021-09-01"))

# how many dates in our data do not match expected dates (should print zero)
length(osml_reps$date_blood_drawn[osml_reps$date_blood_drawn %nin% expected_dates]
)
```

```
## [1] 0
```

There are zero blood draw dates that are not in our expected list.

Number of Blood Draws

Each lizard should have had their blood drawn on 4 different dates, unless they were taken out of the experiment early.

```
# get ID's of the individuals that completed treatment
individuals <- read.csv("./data/tmt_assignments.csv") %>%
  dplyr::select(conclusion, individual_ID) %>%
  dplyr::filter(conclusion == "complete") %>%
  mutate(individual_ID = as.factor(individual_ID),
         conclusion = as.factor(conclusion))
summary(individuals)
```

```
##     conclusion  individual_ID
##  complete:134   201    :  1
##                 202    :  1
##                 203    :  1
##                 204    :  1
##                 205    :  1
##                 206    :  1
##                 (Other):128
```

```
# calculate the number of dates for each individual
osml_reps %>%
  dplyr::filter(individual_ID %in% individuals$individual_ID) %>%
  group_by(individual_ID, date_blood_drawn) %>%
  summarise(n()) %>%
  group_by(individual_ID) %>%
  summarise(n()) %>%
  arrange(n())
```

```
## `summarise()` regrouping output by 'individual_ID' (override with `.groups` argument)

## `summarise()` ungrouping output (override with `.groups` argument)

## # A tibble: 134 x 2
##    individual_ID `n()`
##    <fct>         <int>
##  1 201               4
##  2 202               4
##  3 203               4
##  4 204               4
##  5 205               4
##  6 206               4
##  7 207               4
##  8 208               4
##  9 209               4
## 10 210               4
## # ... with 124 more rows
```

Wahoo, every lizard has blood draws from 4 different dates.

Replicates

Now, I will try to identify outliers within the replicates for a given individual on a given date. There must be at least 3 replicates to do this, so the first thing I need to do is figure out which individuals/dates have enough replicates, then subset my data to be only those individuals.

Individuals w 3+ Replicates

```
# identify individuals with 3-4 reps
enuf_reps <- osml_reps %>%
  group_by(individual_ID, date_blood_drawn) %>%
  mutate(count = n()) %>%
  dplyr::filter(count > 2) %>%
  arrange(count)
enuf_reps
```

```
## # A tibble: 1,224 x 8
## # Groups:   individual_ID, date_blood_drawn [407]
##    date_blood_drawn date_osmom_run time_osmom_run      individual_ID
##    <date>           <date>         <dttm>              <fct>
##  1 2021-06-16       2021-06-16     2022-10-03 11:00:00 201
##  2 2021-06-16       2021-06-16     2022-10-03 11:00:00 201
##  3 2021-06-16       2021-06-16     2022-10-03 11:00:00 201
##  4 2021-06-16       2021-06-16     2022-10-03 11:00:00 202
##  5 2021-06-16       2021-06-16     2022-10-03 11:00:00 202
##  6 2021-06-16       2021-06-16     2022-10-03 11:00:00 202
##  7 2021-06-16       2021-06-16     2022-10-03 11:10:00 203
##  8 2021-06-16       2021-06-16     2022-10-03 11:10:00 203
##  9 2021-06-16       2021-06-16     2022-10-03 11:10:00 203
## 10 2021-06-16       2021-06-16     2022-10-03 11:25:00 205
## # ... with 1,214 more rows, and 4 more variables: replicate_no <fct>,
## #   osmolality_mmol_kg <dbl>, notes <lgl>, count <int>
```

```
# identify individuals with 1-2 reps
not_reps <- osml_reps %>%
  group_by(individual_ID, date_blood_drawn) %>%
  mutate(count = n()) %>%
  dplyr::filter(count < 3) %>%
  arrange(count)
not_reps
```

```
## # A tibble: 260 x 8
## # Groups:   individual_ID, date_blood_drawn [136]
##    date_blood_drawn date_osmom_run time_osmom_run      individual_ID
##    <date>           <date>         <dttm>              <fct>
##  1 2021-06-24       2021-06-24     2022-10-03 09:26:00 203
##  2 2021-06-24       2021-06-26     NA                  204
##  3 2021-06-24       2021-06-26     NA                  223
##  4 2021-06-26       2021-06-26     2022-10-03 09:47:00 201
##  5 2021-06-26       2021-06-26     2022-10-03 11:40:00 230
##  6 2021-07-04       2021-07-05     2022-10-03 10:03:00 227
##  7 2021-07-06       2021-07-06     2022-10-03 09:59:00 239
##  8 2021-07-28       2021-07-28     2022-10-03 10:32:00 259
##  9 2021-07-28       2021-07-28     2022-10-03 12:07:00 274
## 10 2021-07-30       2021-07-31     2022-10-03 13:55:00 291
## # ... with 250 more rows, and 4 more variables: replicate_no <fct>,
## #   osmolality_mmol_kg <dbl>, notes <lgl>, count <int>
```

```
# check total obs still add to original 1484
nrow(enuf_reps) + nrow(not_reps)
```

```
## [1] 1484
```

```
nrow(enuf_reps) + nrow(not_reps) == nrow(osml_reps)
```

```
## [1] TRUE
```

Assess Variation

We want the Coefficient of Variation (CV) among our technical replicates to be small. We need to calculate it to identify whether there may be outliers.

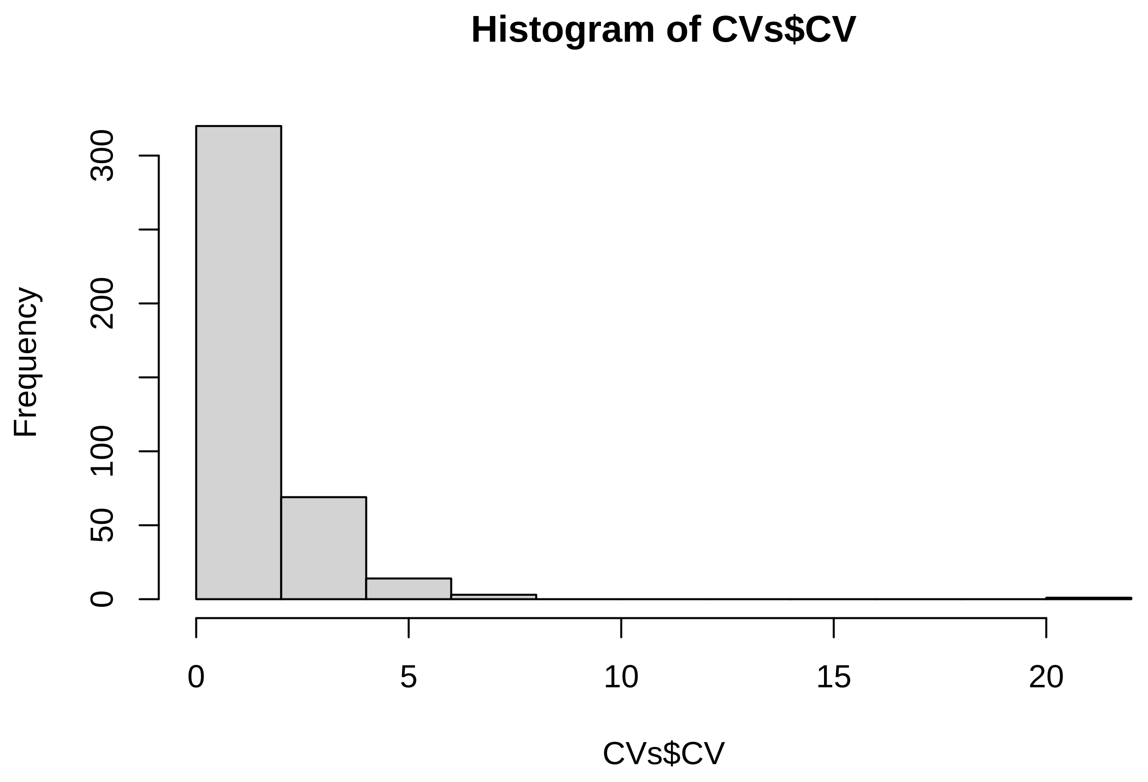
```
CVs <- enuf_reps %>%
  group_by(individual_ID, date_blood_drawn) %>%
  summarise(mean = mean(osmolality_mmol_kg),
            SD = sd(osmolality_mmol_kg),
            CV = (SD/mean) *100,
            min = min(osmolality_mmol_kg),
            max = max(osmolality_mmol_kg),
            osml_range = max - min
            )
```

```
## `summarise()` regrouping output by 'individual_ID' (override with `.groups` argument)
```

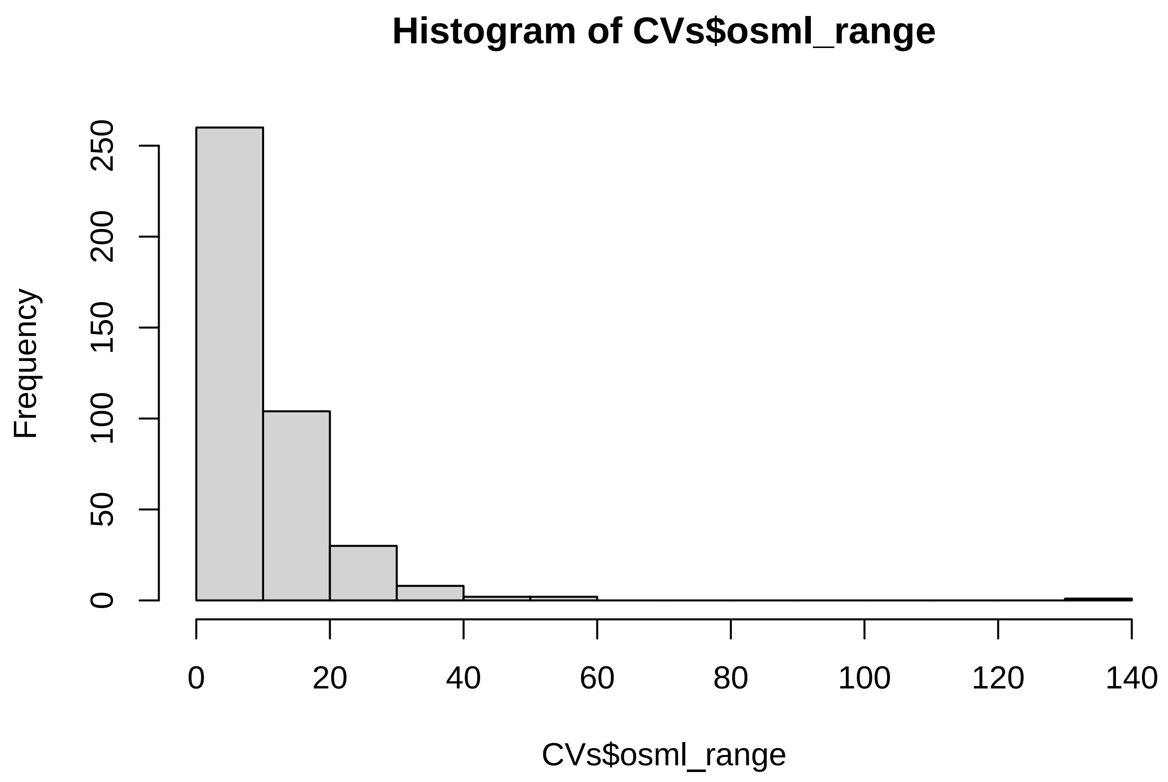
```
summary(CVs)
```

```
##  individual_ID date_blood_drawn          mean              SD
##  206    :  4   Min.   :2021-06-16   Min.   :295.3   Min.   : 0.000
##  207    :  4   1st Qu.:2021-06-30   1st Qu.:335.0   1st Qu.: 2.581
##  208    :  4   Median :2021-07-24   Median :350.0   Median : 4.359
##  211    :  4   Mean   :2021-07-23   Mean   :356.2   Mean   : 5.596
##  214    :  4   3rd Qu.:2021-08-16   3rd Qu.:368.0   3rd Qu.: 6.658
##  220    :  4   Max.   :2021-09-01   Max.   :576.0   Max.   :69.816
##  (Other):383
##        CV               min             max          osml_range
##  Min.   : 0.0000   Min.   :247.0   Min.   :300.0   Min.   :  0.00
##  1st Qu.: 0.7278   1st Qu.:330.5   1st Qu.:339.0   1st Qu.:  5.00
##  Median : 1.2021   Median :345.0   Median :356.0   Median :  8.00
##  Mean   : 1.5627   Mean   :351.1   Mean   :361.7   Mean   : 10.66
##  3rd Qu.: 1.8746   3rd Qu.:364.0   3rd Qu.:374.0   3rd Qu.: 12.50
##  Max.   :21.4600   Max.   :566.0   Max.   :596.0   Max.   :134.00
##
```

```
hist(CVs$CV)
```



```
hist(CVs$osml_range)
```



Ideally, CV would be <10-15%. If it's larger, and one of the replicates is very different than the others, we can assume that the replicates that are closer together are more reliable.

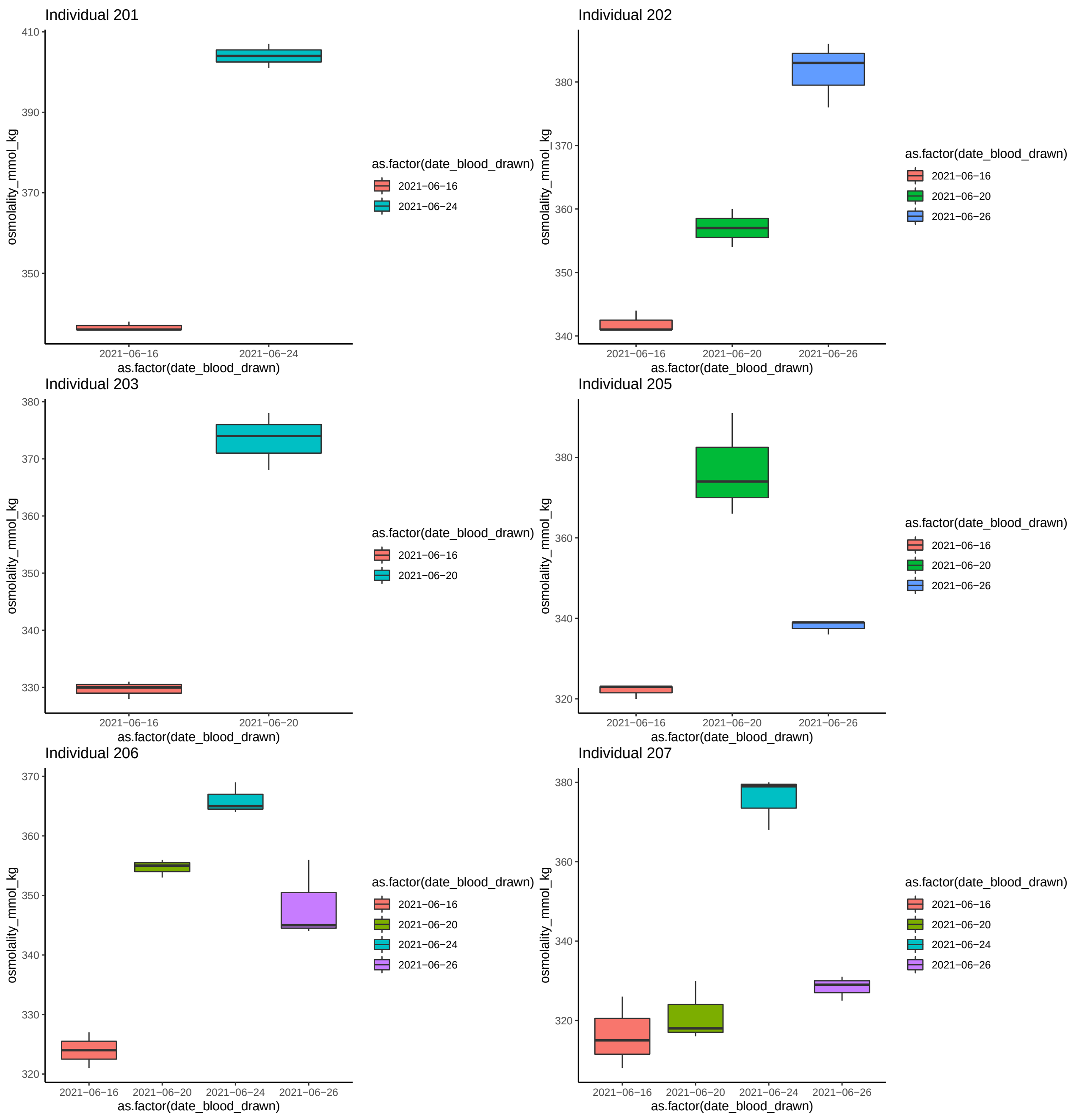
The CV is >10 for only one lizard on one date, so our replicates are already likely to accurately represent the true value. We don't want to lose accuracy by searching for precision, so we will only remove the one point driving the enormous CV value.

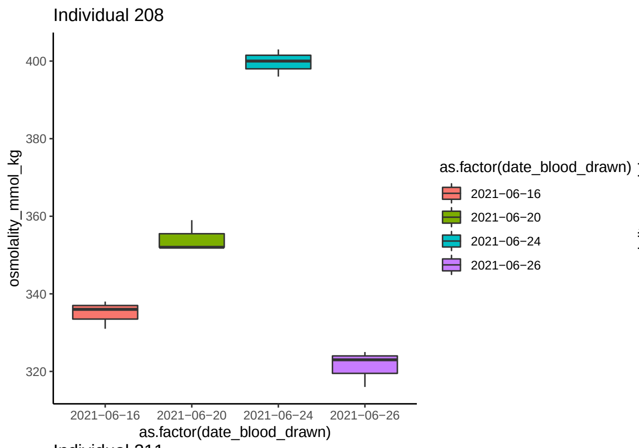
Find Outliers Visually

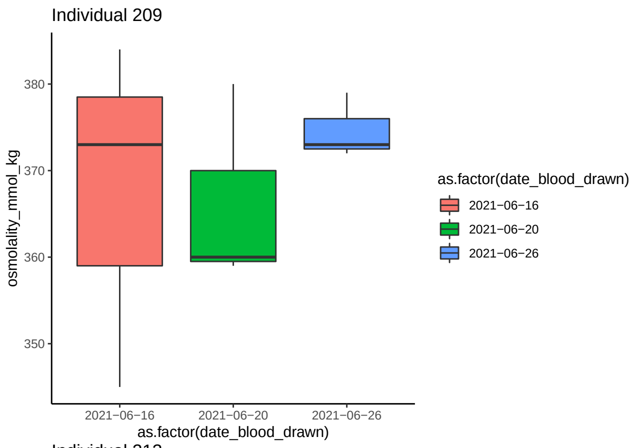
```r
# write function to find outliers for each individual on each date
find_outliers <- function(df) {

  # initiate a for loop to go through every who in df
  for(indiv_ch in unique(df$individual_ID)) {

    # select data for only the individual of interest
    df_sub <- df %>%
      dplyr::filter(individual_ID == as.numeric(indiv_ch))

    # make a boxplot
    ggplot(df_sub) +
      geom_boxplot(aes(x = as.factor(date_blood_drawn),
                       y = osmolality_mmol_kg,
                       fill = as.factor(date_blood_drawn))) +
      ggtitle(paste("Individual", indiv_ch)) +
      theme_classic() -> plot

    # print/save
    print(plot)
  }

}
```

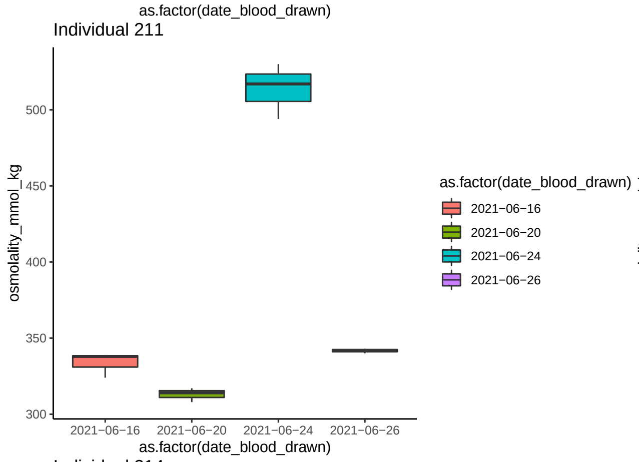
Now apply the function to the data:

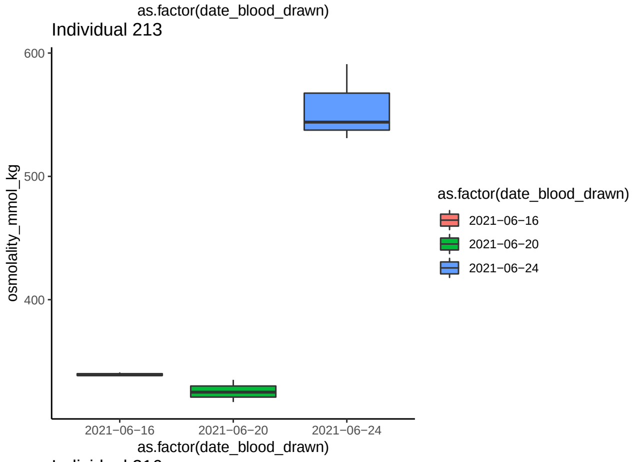
```
par(mfrow = c(71, 2))
find_outliers(enuf_reps)
par(mfrow = c(1, 1))
```

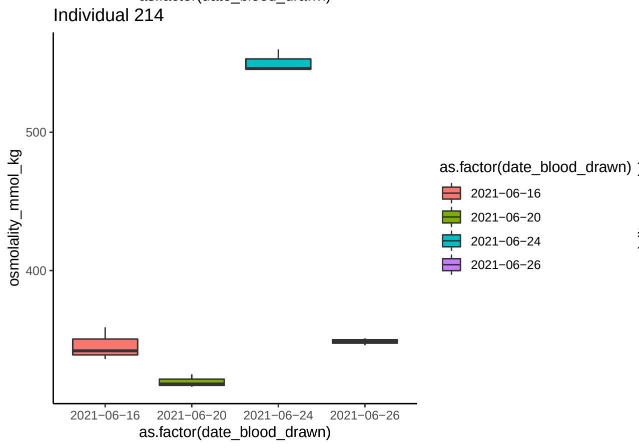


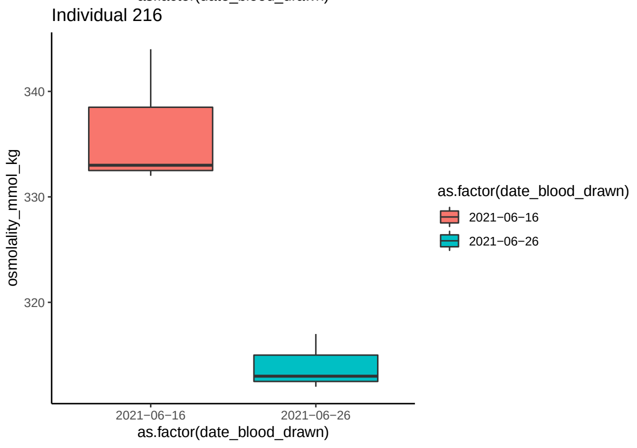

Individual 208
osmolality_mmol_kg
400
380
360
340
320
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

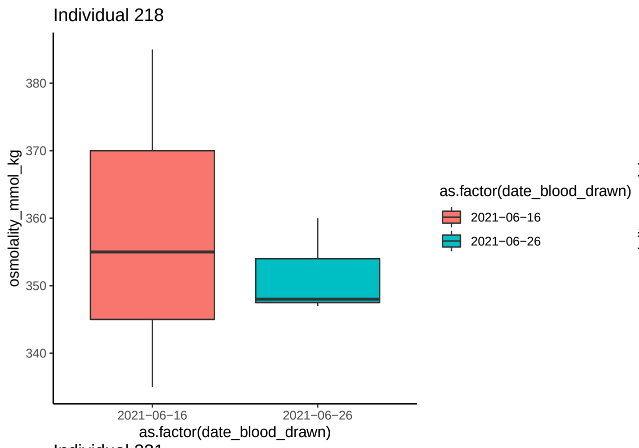

Individual 209
osmolality_mmol_kg
380
370
360
350
2021−06−16
2021−06−20
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−26

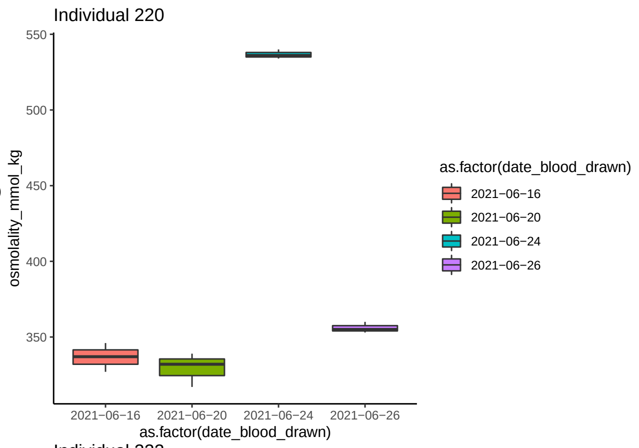

Individual 211
osmolality_mmol_kg
500
450
400
350
300
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

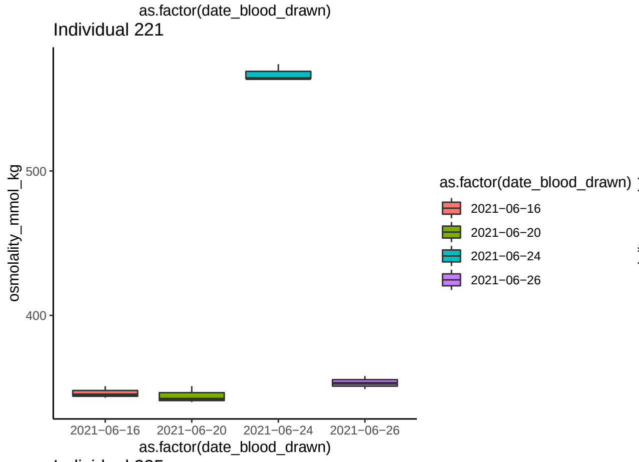

Individual 213
osmolality_mmol_kg
600
500
400
2021−06−16
2021−06−20
2021−06−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24

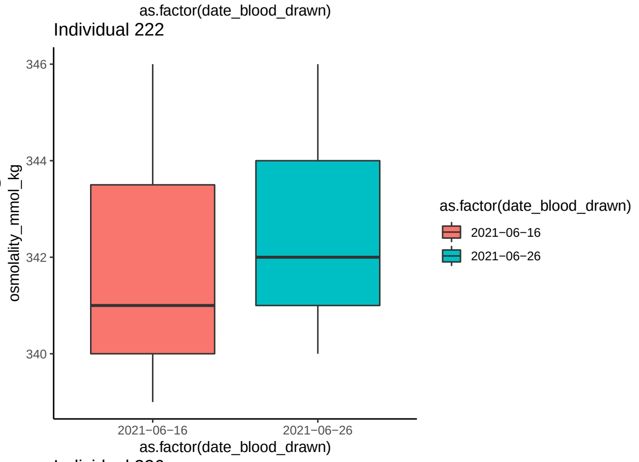

Individual 214
osmolality_mmol_kg
500
400
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

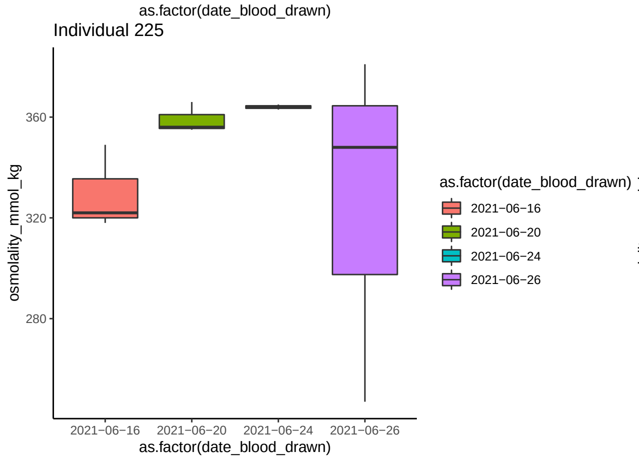

Individual 216
osmolality_mmol_kg
340
330
320
2021−06−16
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−26

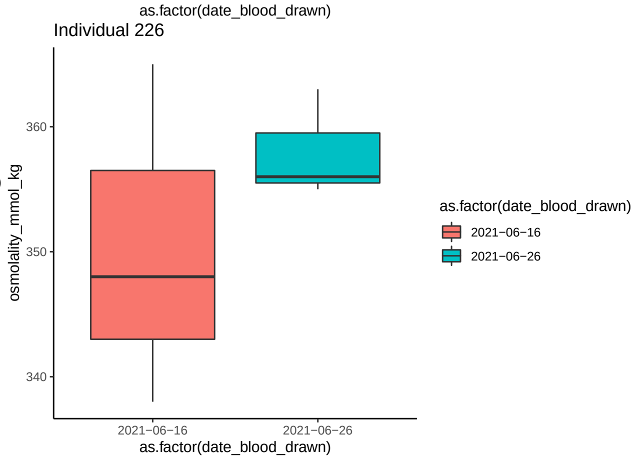

Individual 218
osmolality_mmol_kg
340
350
360
370
380
2021−06−16
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−26

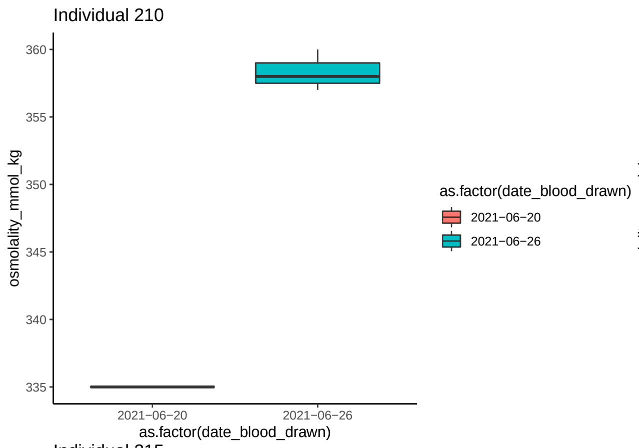

Individual 220
osmolality_mmol_kg
350
400
450
500
550
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

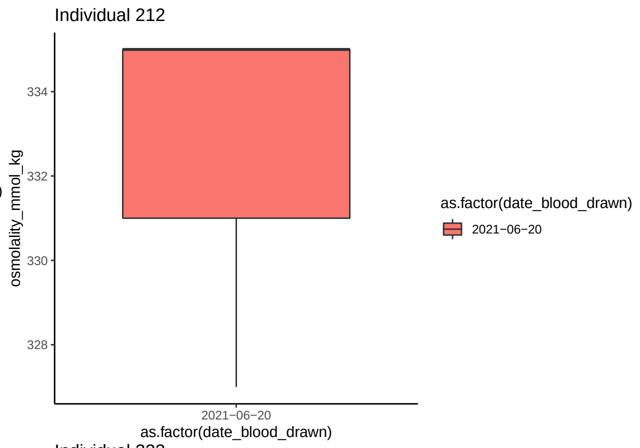

Individual 221
osmolality_mmol_kg
400
500
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

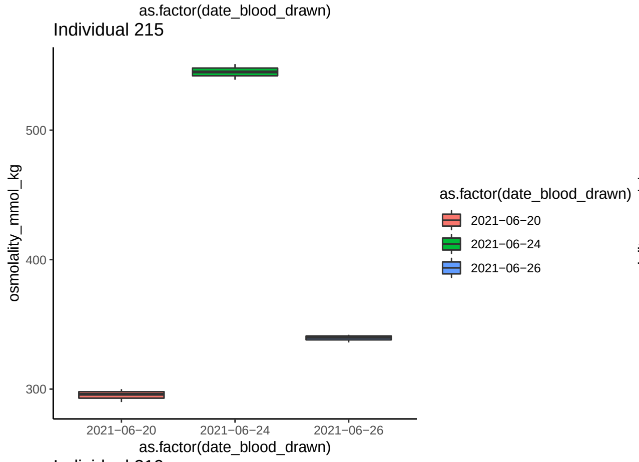

Individual 222
osmolality_mmol_kg
340
342
344
346
2021−06−16
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−26

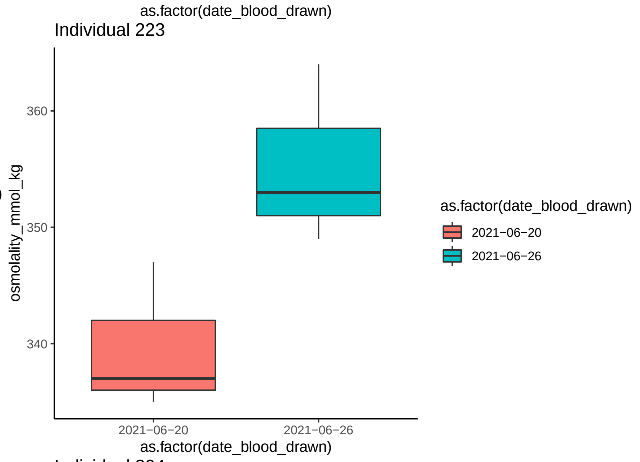

Individual 225
osmolality_mmol_kg
280
320
360
2021−06−16
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−20
2021−06−24
2021−06−26

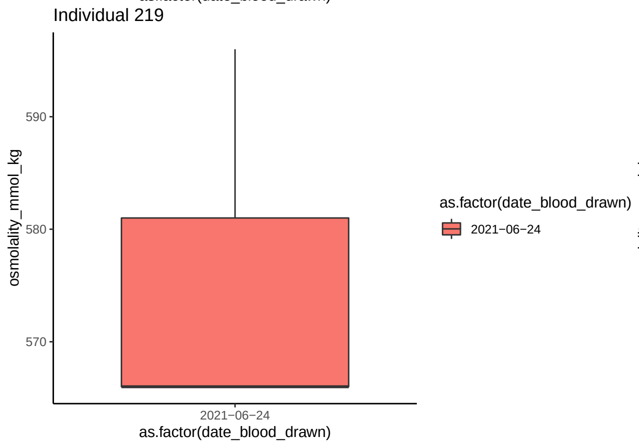

Individual 226
osmolality_mmol_kg
340
350
360
2021−06−16
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−16
2021−06−26

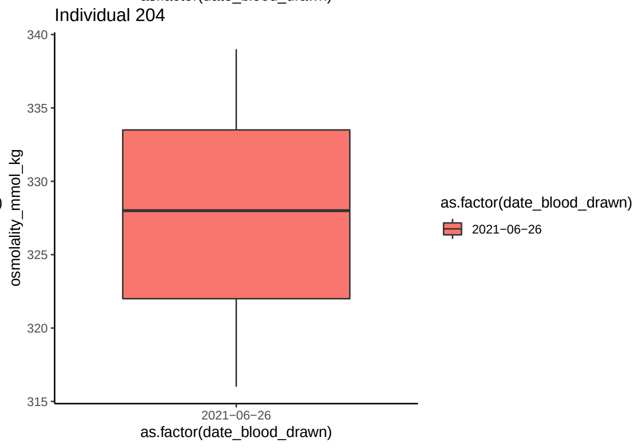

Individual 210
360
355
350
345
340
335
osmolality_mmol_kg
2021−06−20
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−20
🟦 2021−06−26

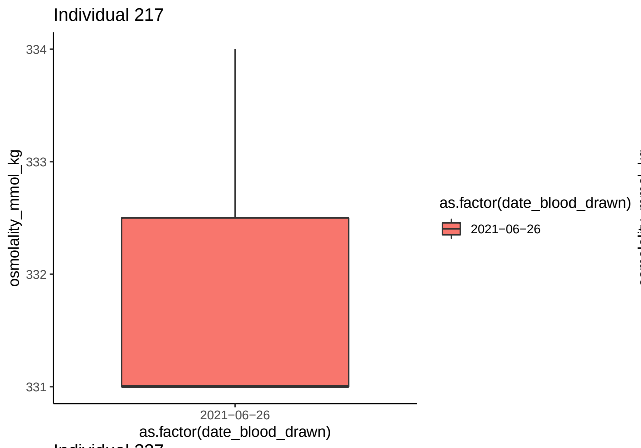

Individual 212
334
332
330
328
osmolality_mmol_kg
2021−06−20
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−20

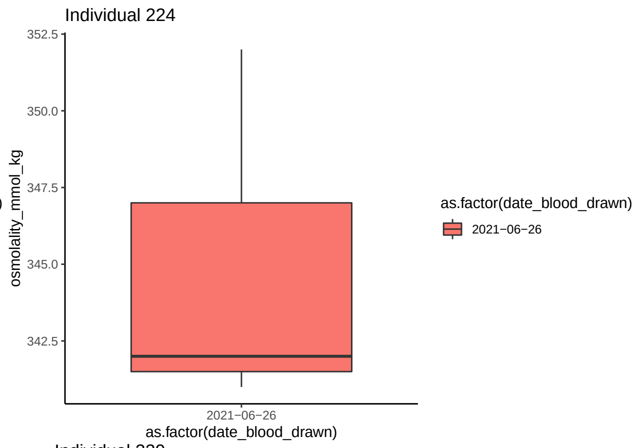

Individual 215
500
400
300
osmolality_mmol_kg
2021−06−20
2021−06−24
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−20
🟩 2021−06−24
🟦 2021−06−26

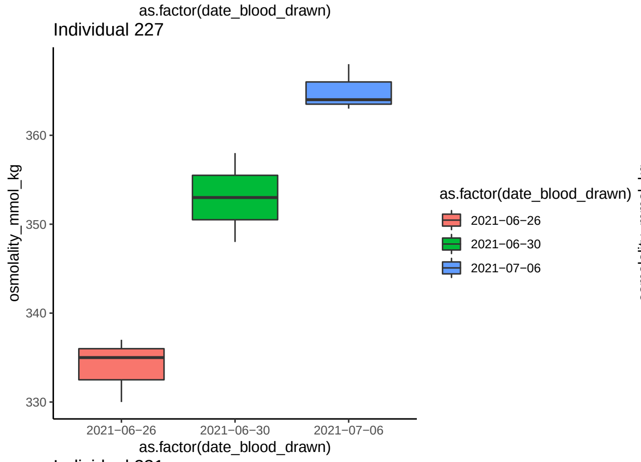

Individual 223
360
350
340
osmolality_mmol_kg
2021−06−20
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−20
🟦 2021−06−26

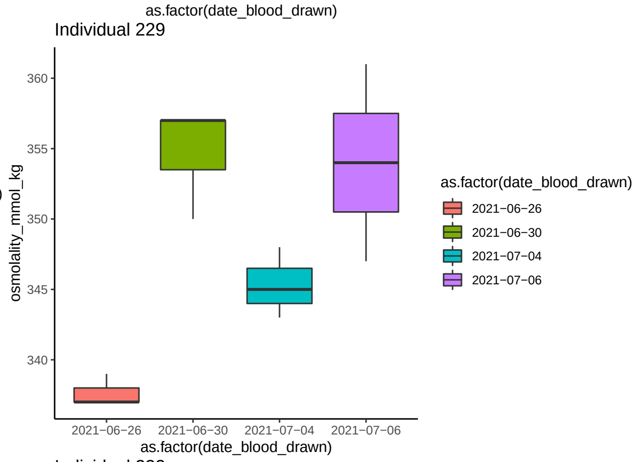

Individual 219
590
580
570
osmolality_mmol_kg
2021−06−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−24


Individual 204
340
335
330
325
320
315
osmolality_mmol_kg
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
🟥 2021−06−26


Individual 217
osmolality_mmol_kg
334
333
332
331
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26


Individual 224
osmolality_mmol_kg
352.5
350.0
347.5
345.0
342.5
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26


Individual 227
osmolality_mmol_kg
360
350
340
330
2021−06−26
2021−06−30
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−06−30
2021−07−06


Individual 229
osmolality_mmol_kg
360
355
350
345
340
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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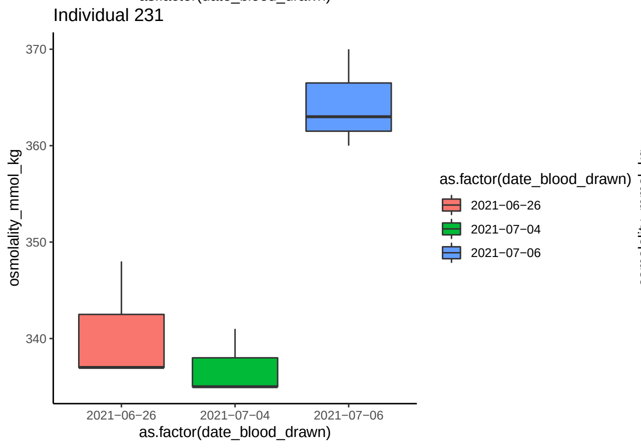
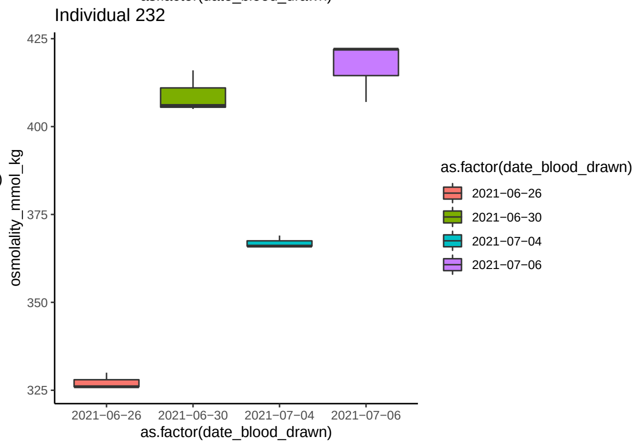
2021−07−04
2021−07−06


Individual 231
osmolality_mmol_kg
370
360
350
340
2021−06−26
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−07−04
2021−07−06


Individual 232
osmolality_mmol_kg
425
400
375
350
325
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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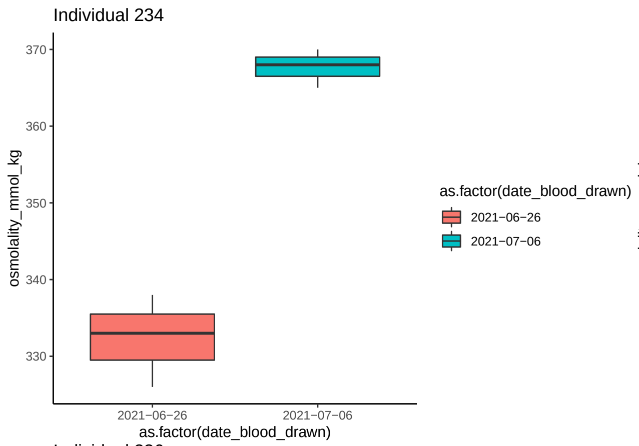
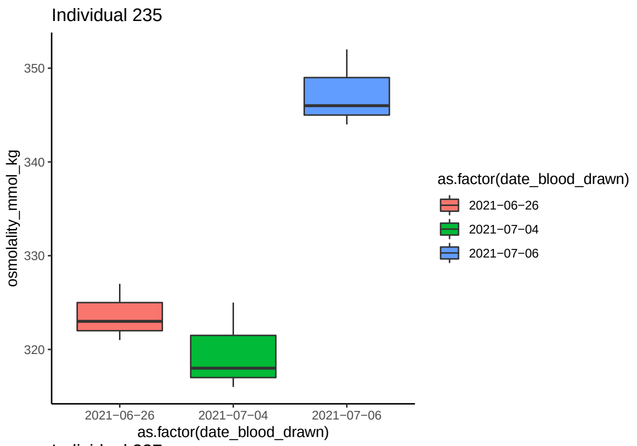
2021−07−04
2021−07−06


Individual 234
osmolality_mmol_kg
370
360
350
340
330
2021−06−26
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−07−06


Individual 235
osmolality_mmol_kg
350
340
330
320
2021−06−26
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
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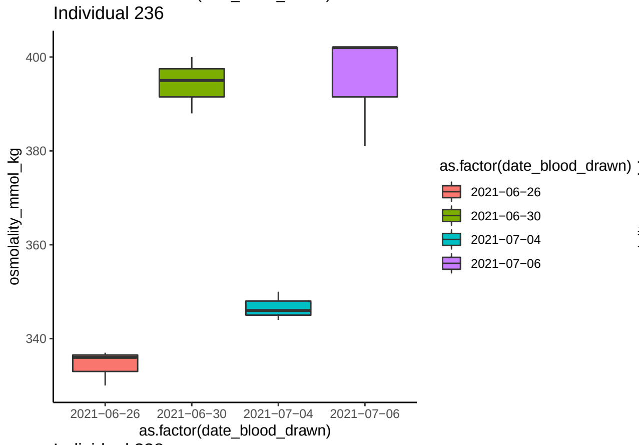
2021−07−06


Individual 236
osmolality_mmol_kg
400
380
360
340
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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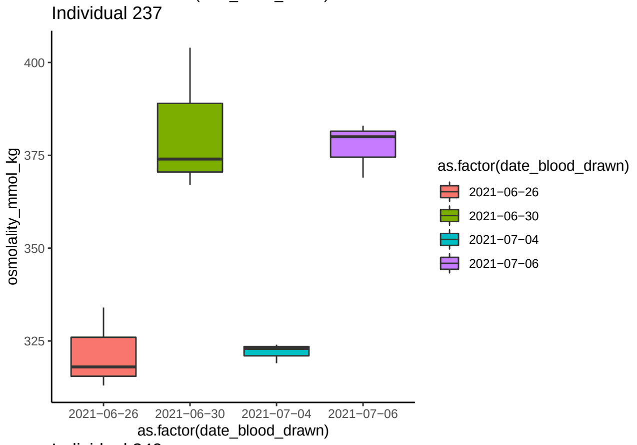
2021−07−04
2021−07−06


Individual 237
osmolality_mmol_kg
400
375
350
325
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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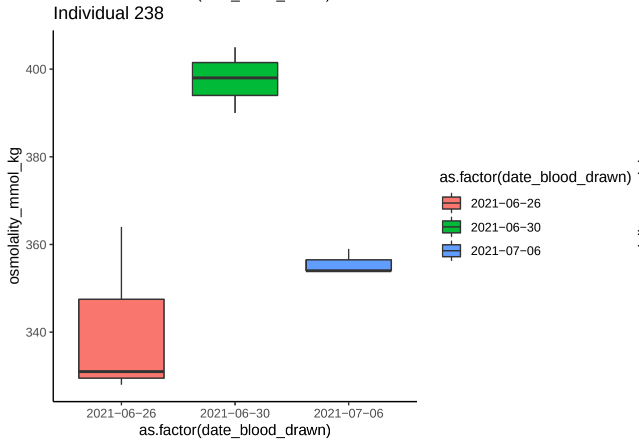
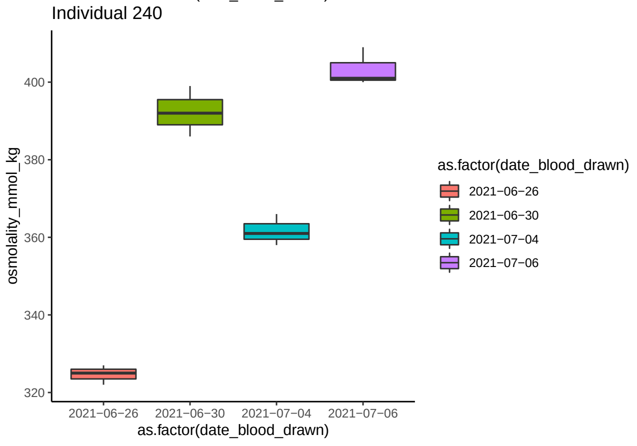
2021−07−06


Individual 238
osmolality_mmol_kg
400
380
360
340
2021−06−26
2021−06−30
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−06−30
2021−07−06


Individual 240
osmolality_mmol_kg
400
380
360
340
320
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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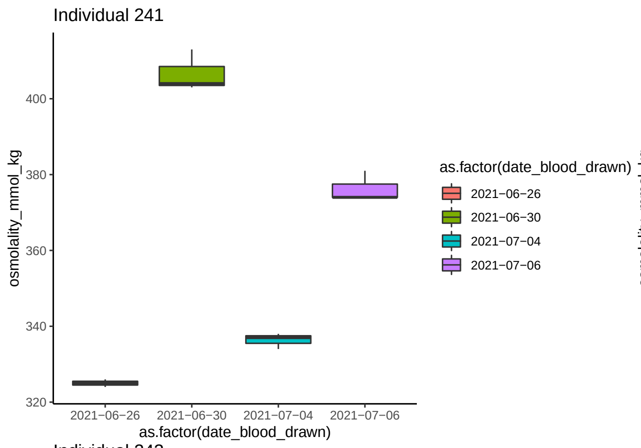
2021−07−04
2021−07−06


Individual 241
osmolality_mmol_kg
320
340
360
380
400
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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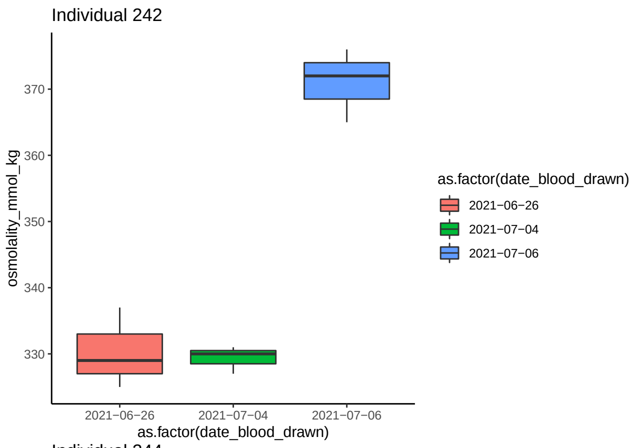
2021−07−04
2021−07−06


Individual 242
osmolality_mmol_kg
330
340
350
360
370
2021−06−26
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
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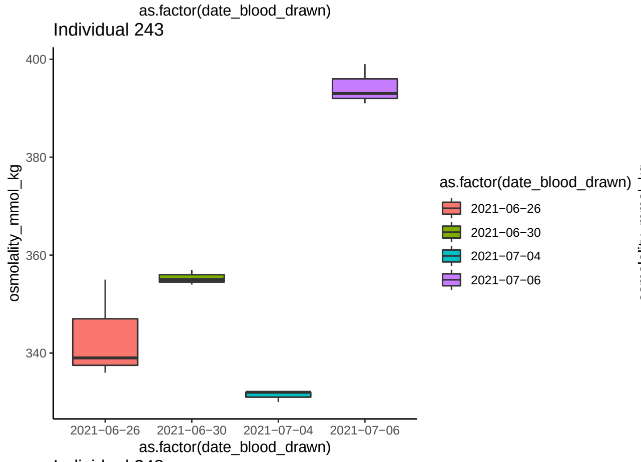
2021−07−04
2021−07−06


Individual 243
osmolality_mmol_kg
340
360
380
400
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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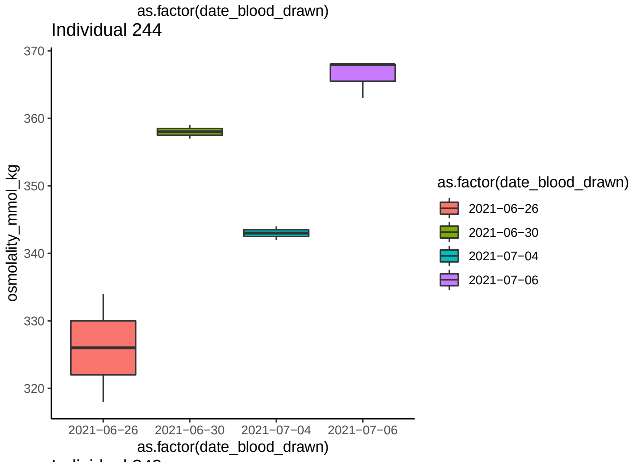
2021−07−04
2021−07−06


Individual 244
osmolality_mmol_kg
320
330
340
350
360
370
2021−06−26
2021−06−30
2021−07−04
2021−07−06
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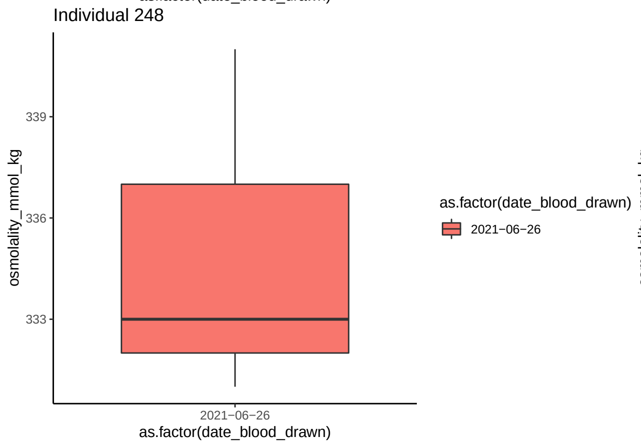
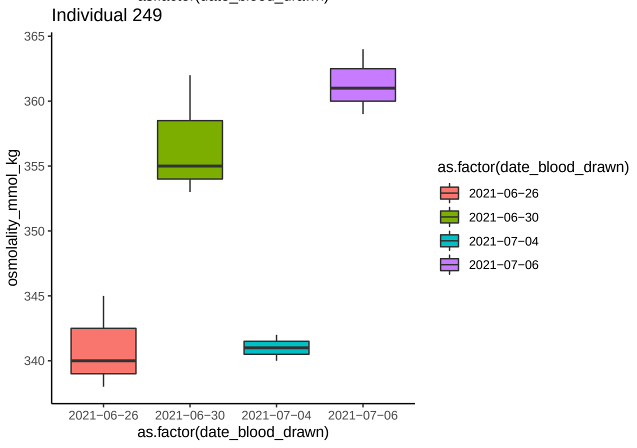
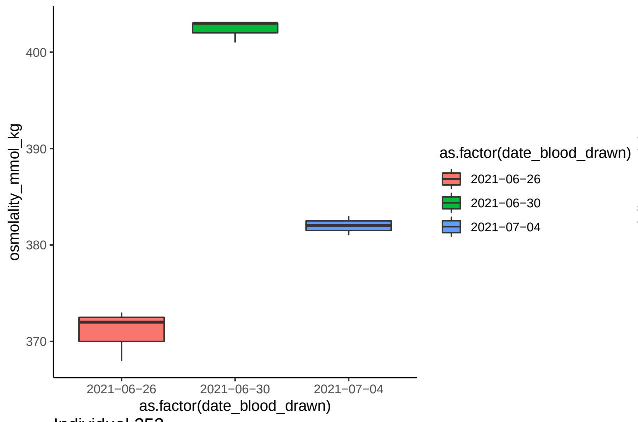
2021−07−04
2021−07−06

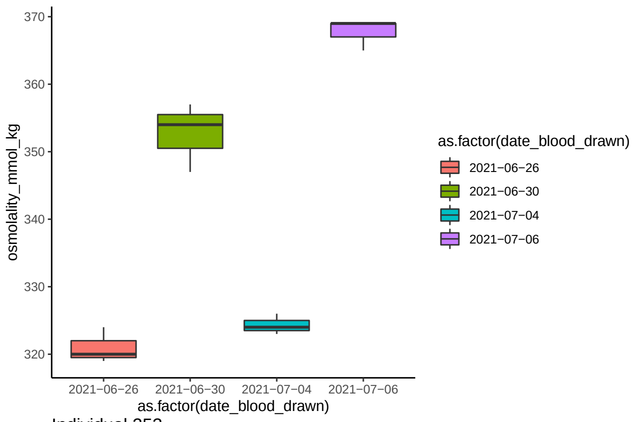

Individual 248
osmolality_mmol_kg
333
336
339
2021−06−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26


Individual 249
osmolality_mmol_kg
340
345
350
355
360
365
2021−06−26
2021−06−30
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−06−30
2021−07−04
2021−07−06

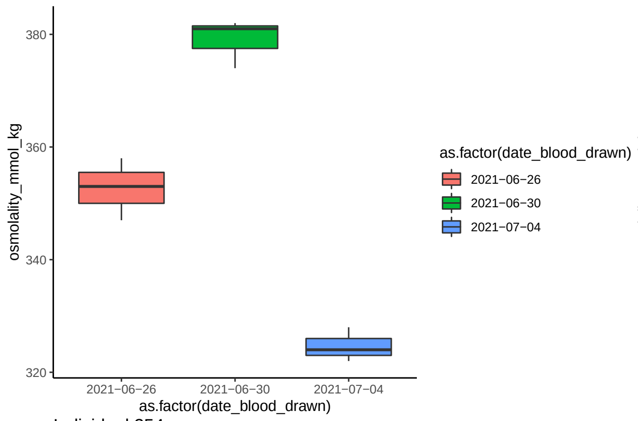
Individual 250



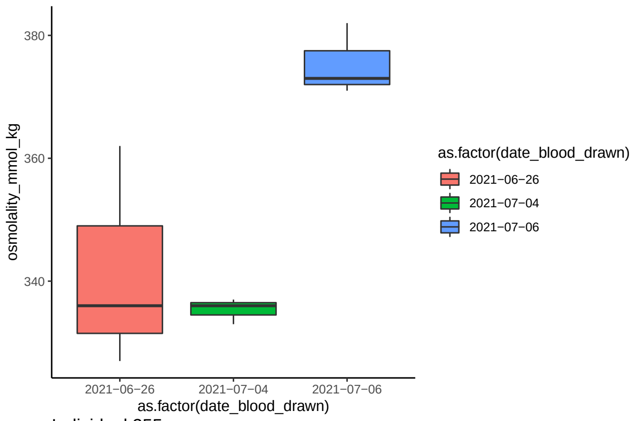
Individual 251



Individual 252



Individual 253



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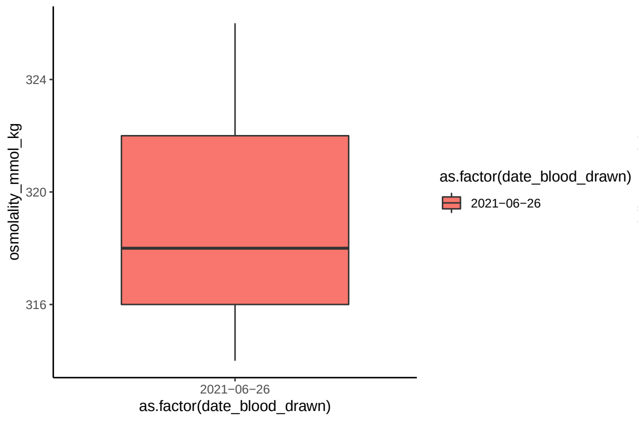
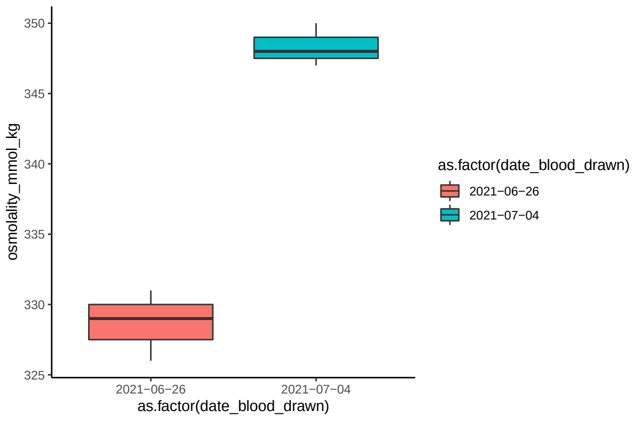
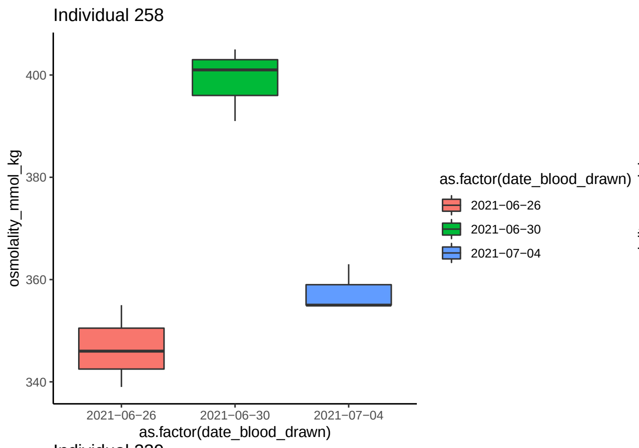
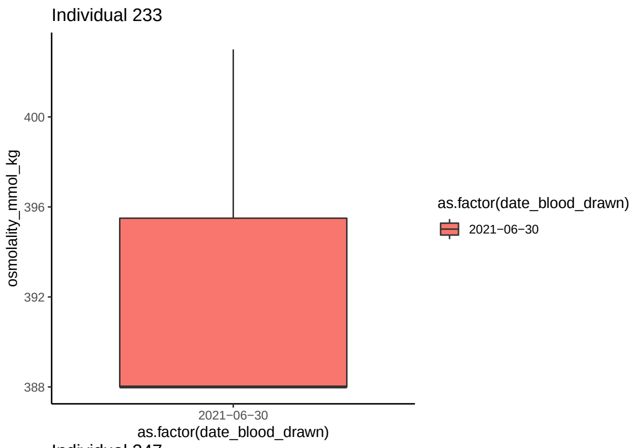
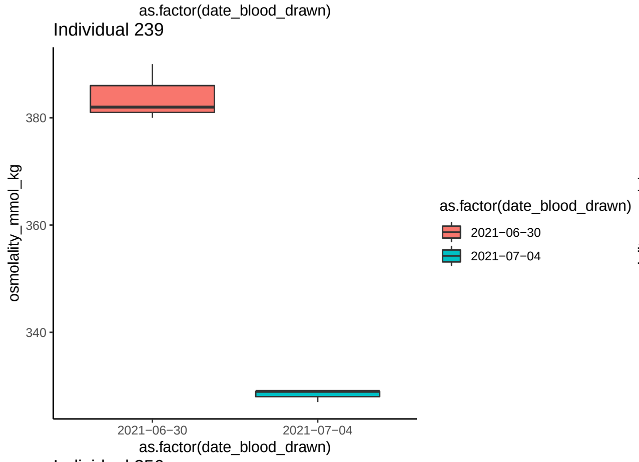


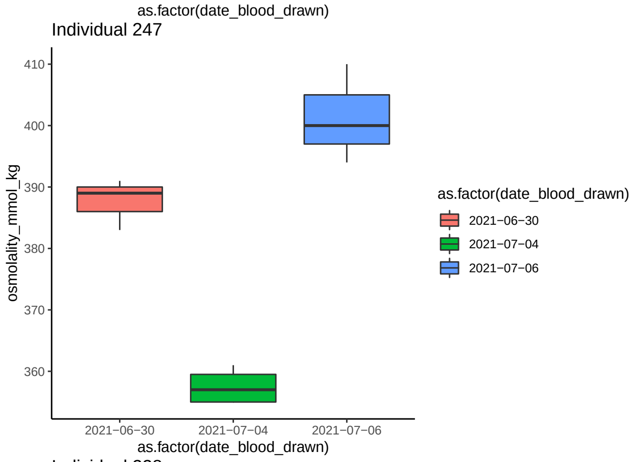
Individual 255

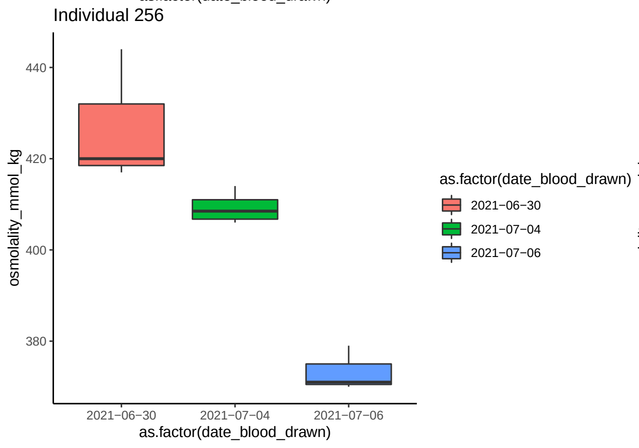


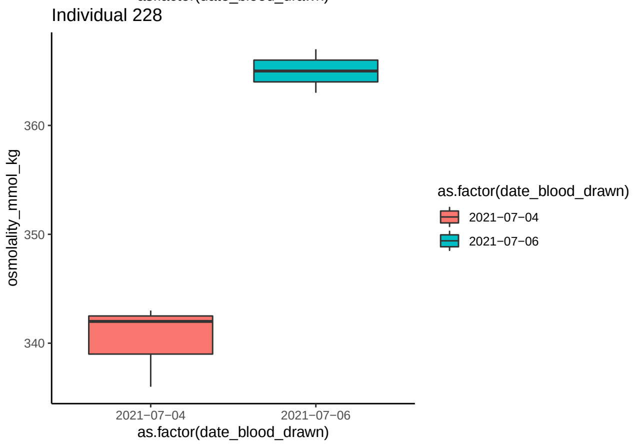

Individual 258
osmolality_mmol_kg
400
380
360
340
2021−06−26
2021−06−30
2021−07−04
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−26
2021−06−30
2021−07−04

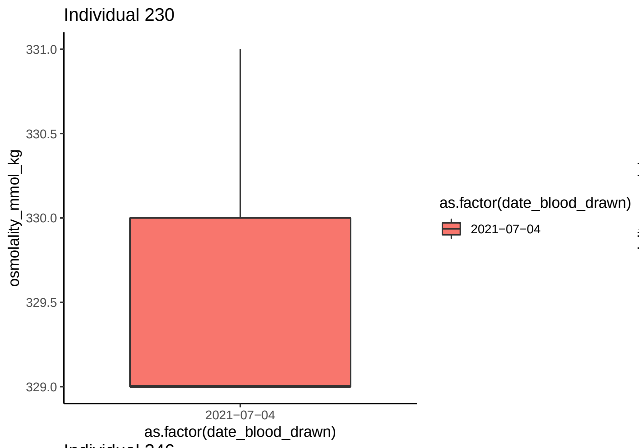

Individual 233
osmolality_mmol_kg
400
396
392
388
2021−06−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−30

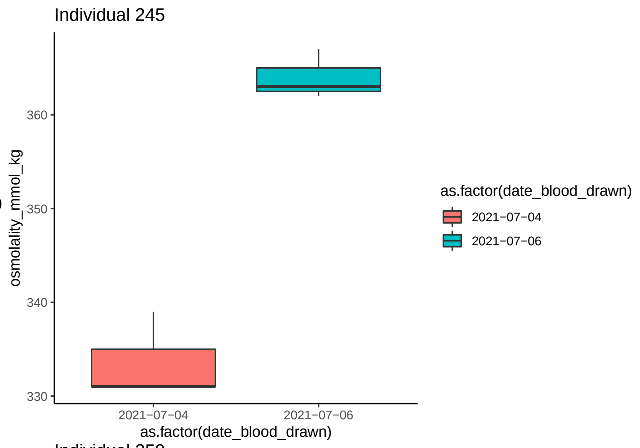

Individual 239
osmolality_mmol_kg
380
360
340
2021−06−30
2021−07−04
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−30
2021−07−04

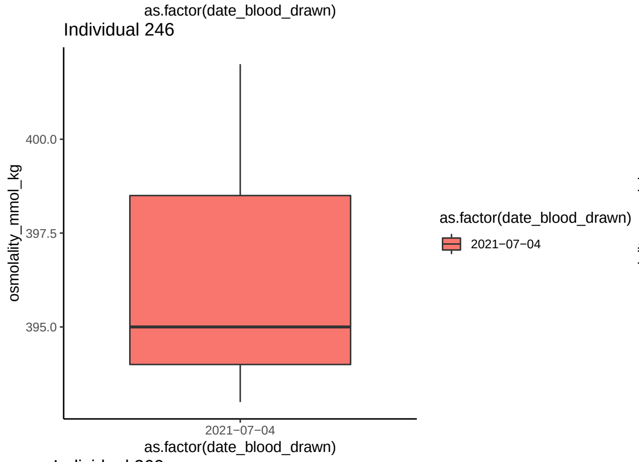

Individual 247
osmolality_mmol_kg
410
400
390
380
370
360
2021−06−30
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−30
2021−07−04
2021−07−06

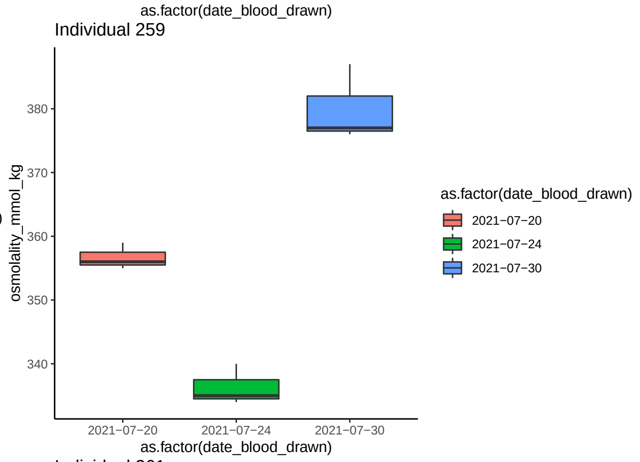

Individual 256
osmolality_mmol_kg
440
420
400
380
2021−06−30
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−06−30
2021−07−04
2021−07−06

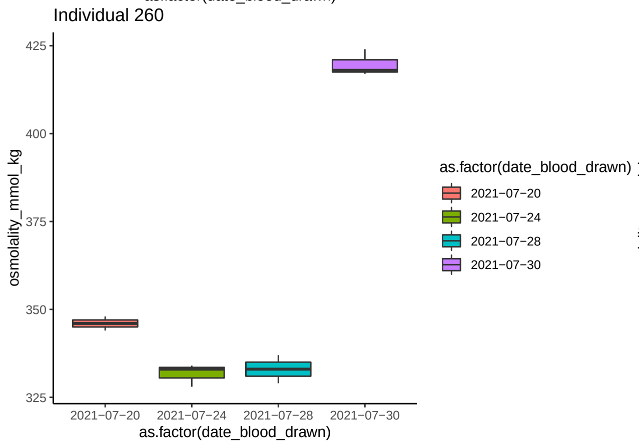

Individual 228
osmolality_mmol_kg
360
350
340
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−04
2021−07−06


Individual 230
osmolality_mmol_kg
331.0
330.5
330.0
329.5
329.0
2021−07−04
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−04


Individual 245
osmolality_mmol_kg
360
350
340
330
2021−07−04
2021−07−06
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−04
2021−07−06


Individual 246
osmolality_mmol_kg
400.0
397.5
395.0
2021−07−04
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−04


Individual 259
osmolality_mmol_kg
380
370
360
350
340
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 260
osmolality_mmol_kg
425
400
375
350
325
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
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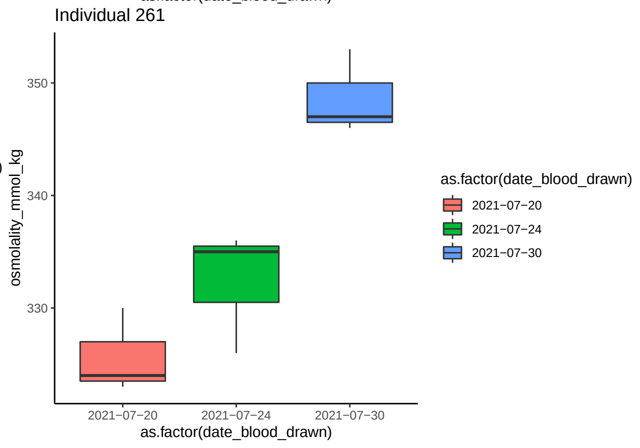
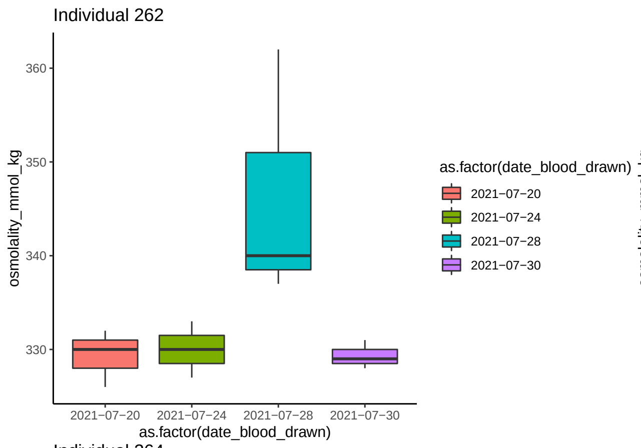
2021−07−28
2021−07−30


Individual 261
osmolality_mmol_kg
350
340
330
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 262
osmolality_mmol_kg
360
350
340
330
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
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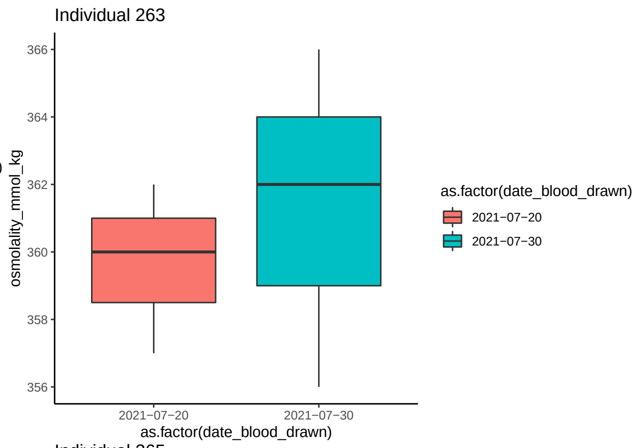
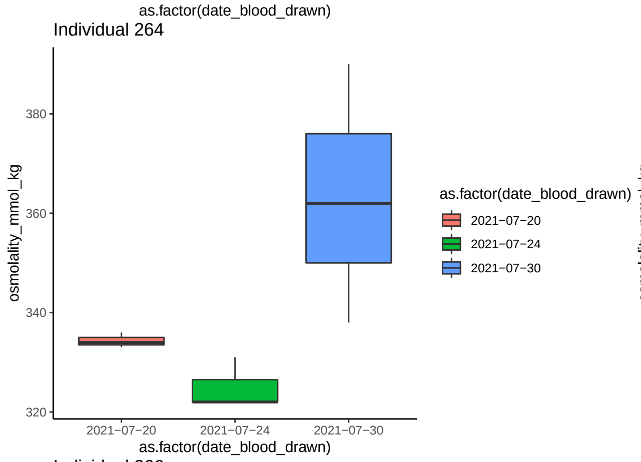
2021−07−30


Individual 263
osmolality_mmol_kg
366
364
362
360
358
356
2021−07−20
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−30


Individual 264
osmolality_mmol_kg
380
360
340
320
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
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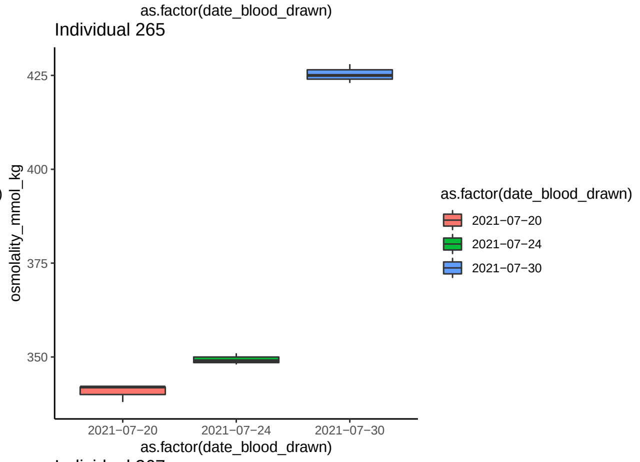
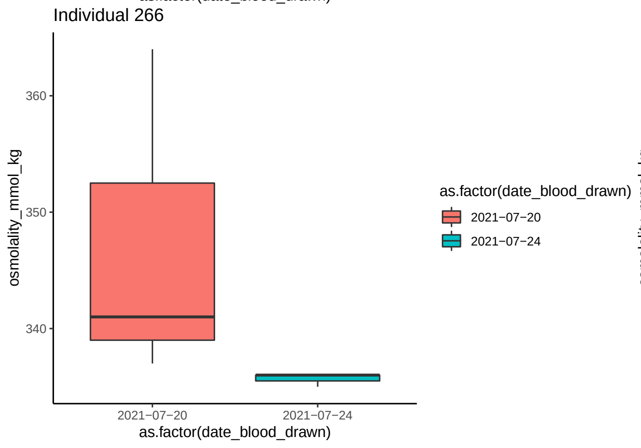
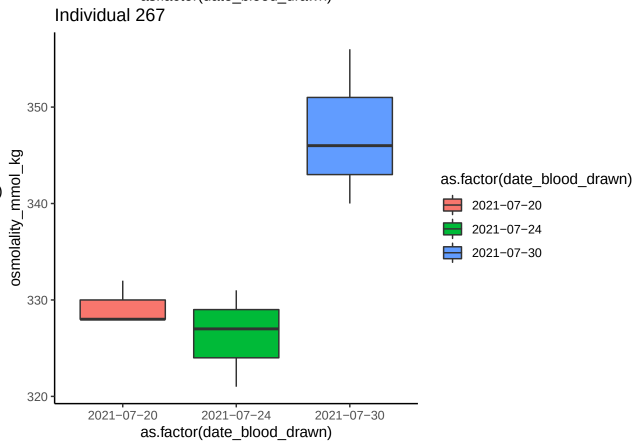
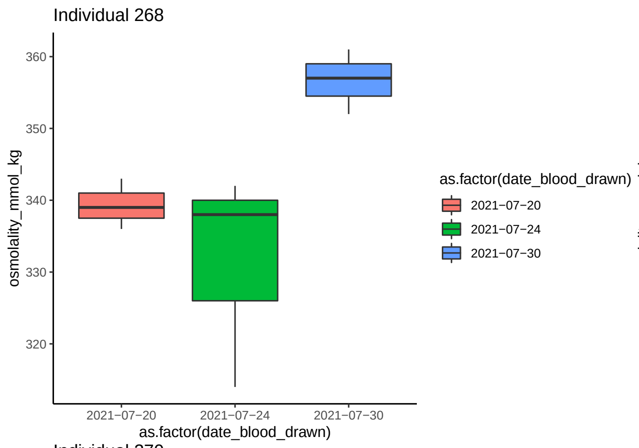
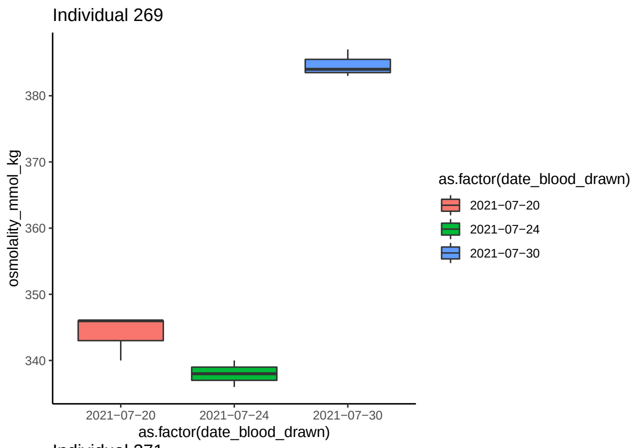
2021−07−30

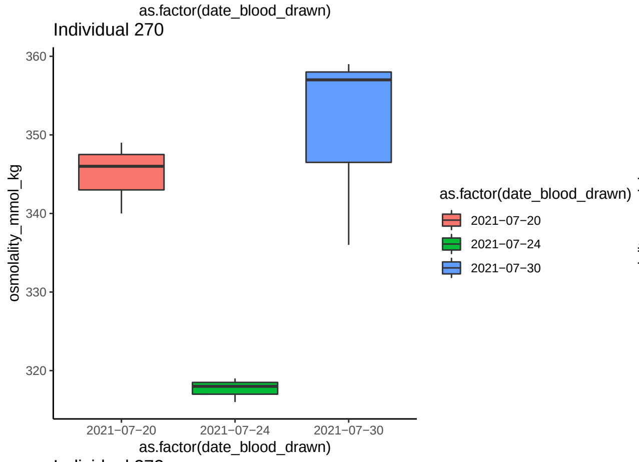

Individual 265
osmolality_mmol_kg
425
400
375
350
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 266
osmolality_mmol_kg
360
350
340
2021−07−20
2021−07−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24


Individual 267
osmolality_mmol_kg
350
340
330
320
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 268
osmolality_mmol_kg
360
350
340
330
320
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 269
osmolality_mmol_kg
380
370
360
350
340
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30


Individual 270
osmolality_mmol_kg
360
350
340
330
320
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
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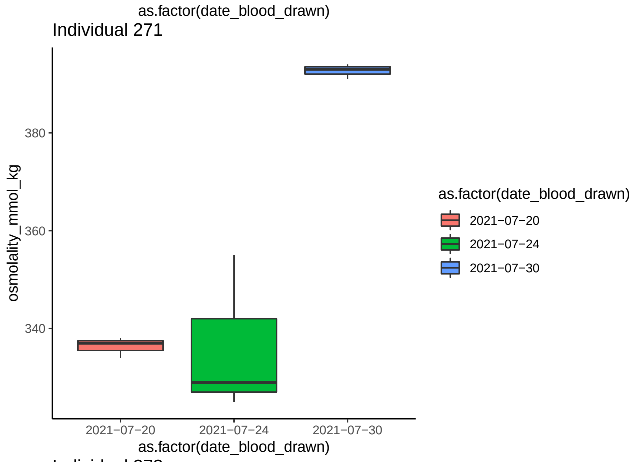
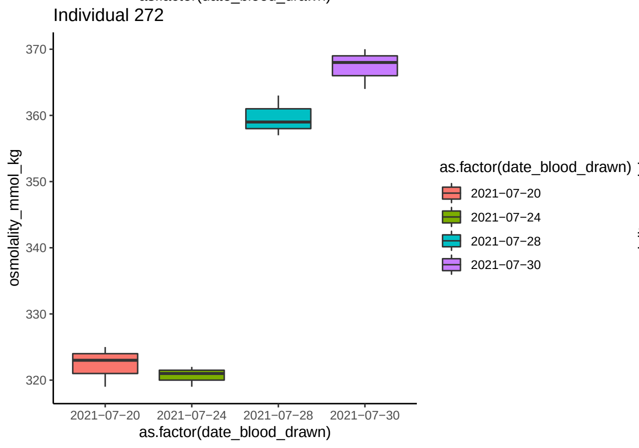
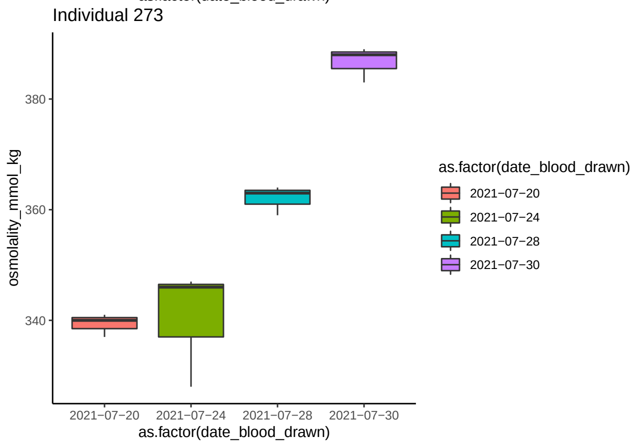
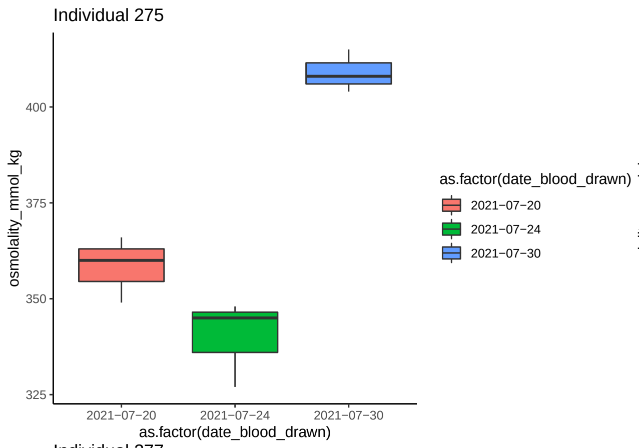
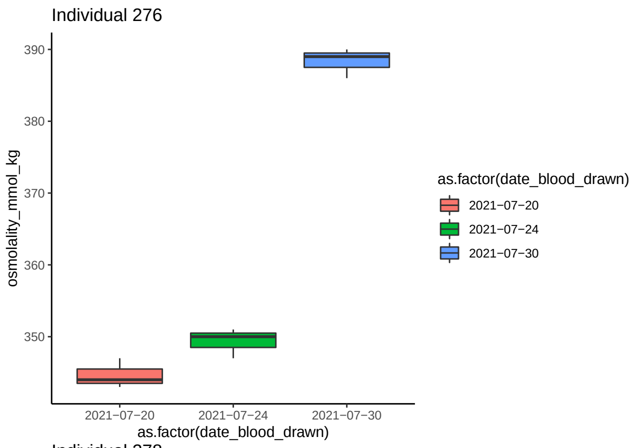
2021−07−30

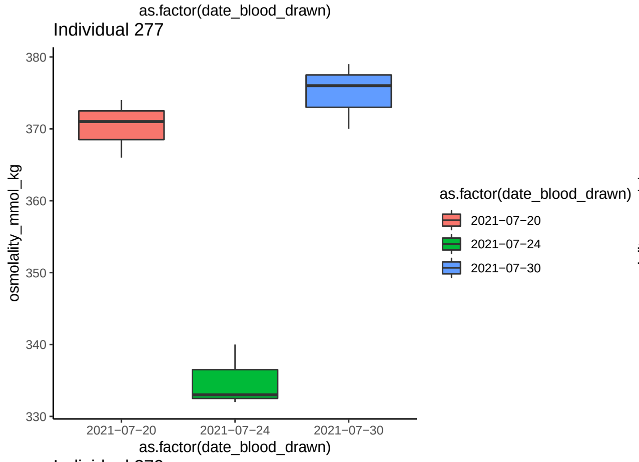

Individual 271
osmolality_mmol_kg
380
360
340
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

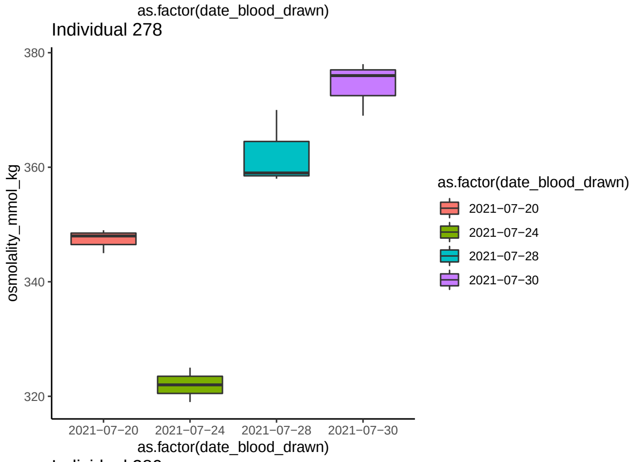

Individual 272
osmolality_mmol_kg
370
360
350
340
330
320
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30

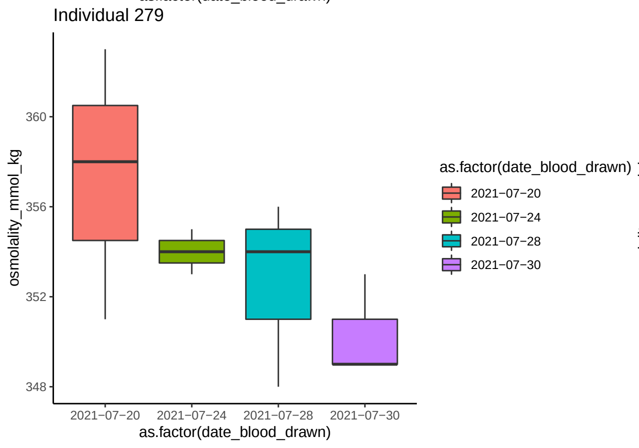

Individual 273
osmolality_mmol_kg
380
360
340
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30

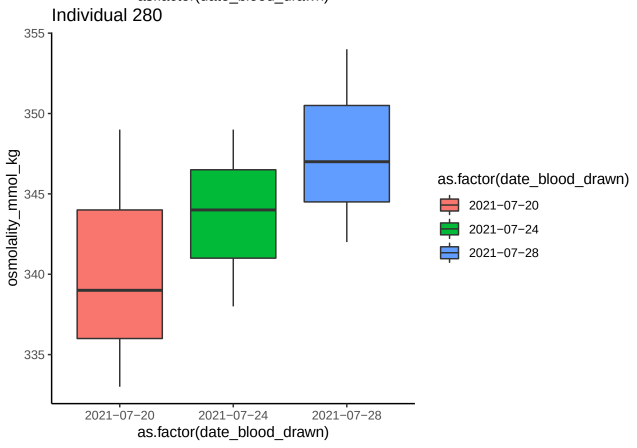

Individual 275
osmolality_mmol_kg
325
350
375
400
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

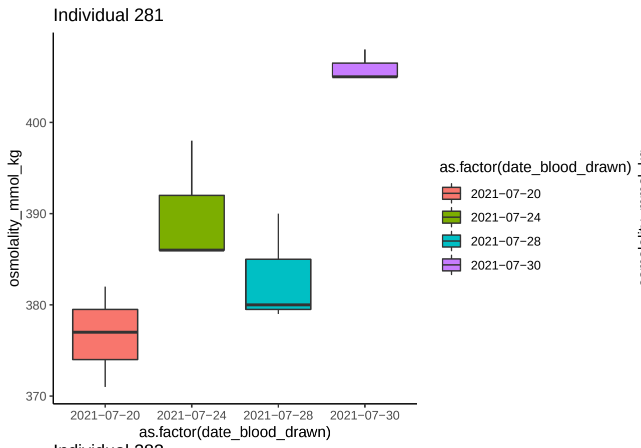

Individual 276
osmolality_mmol_kg
350
360
370
380
390
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

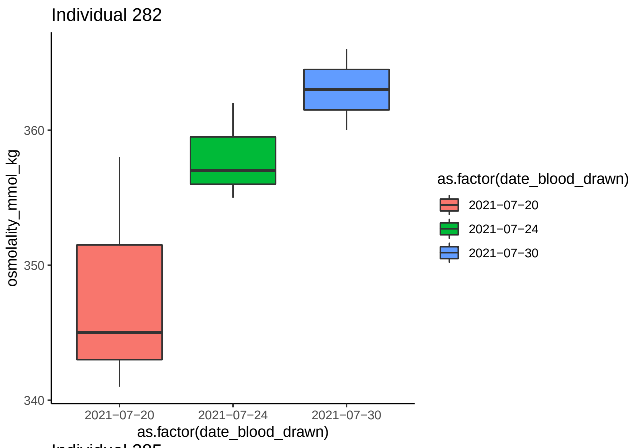

Individual 277
osmolality_mmol_kg
330
340
350
360
370
380
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

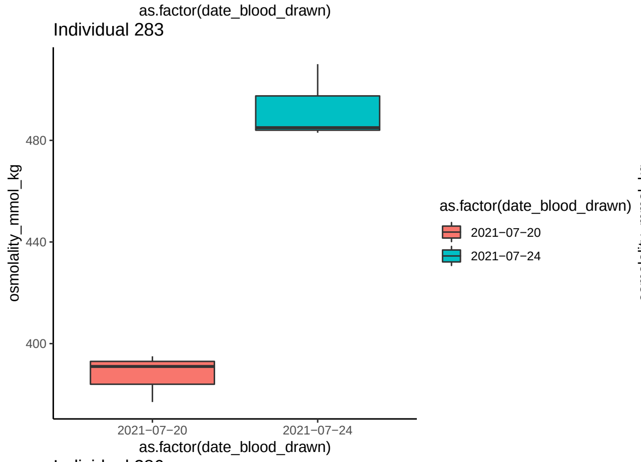

Individual 278
osmolality_mmol_kg
320
340
360
380
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30

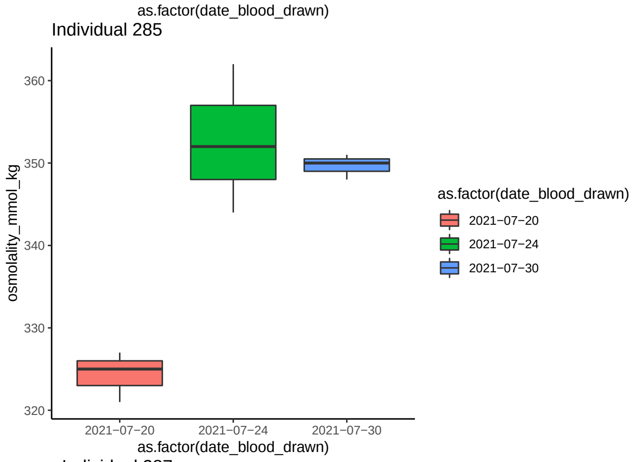

Individual 279
osmolality_mmol_kg
348
352
356
360
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30

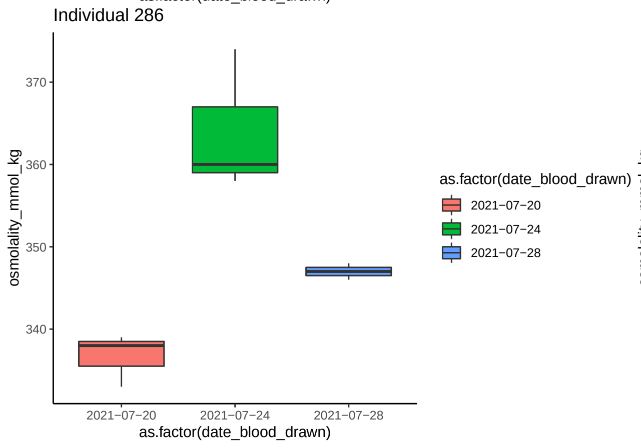

Individual 280
osmolality_mmol_kg
335
340
345
350
355
2021−07−20
2021−07−24
2021−07−28
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28

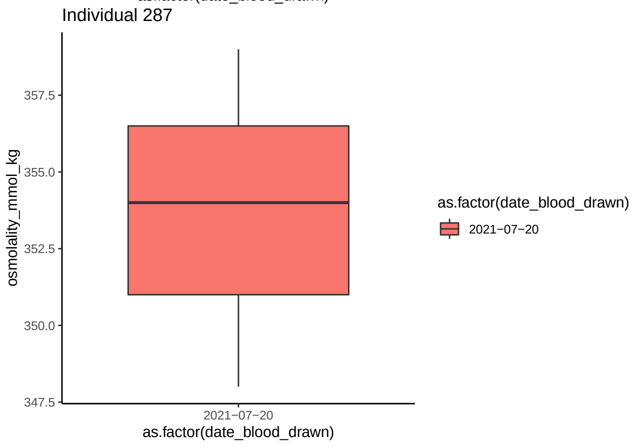

Individual 281
osmolality_mmol_kg
400
390
380
370
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30

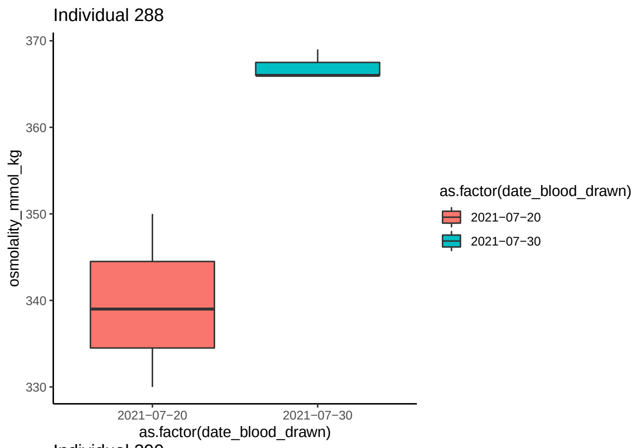

Individual 282
osmolality_mmol_kg
360
350
340
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

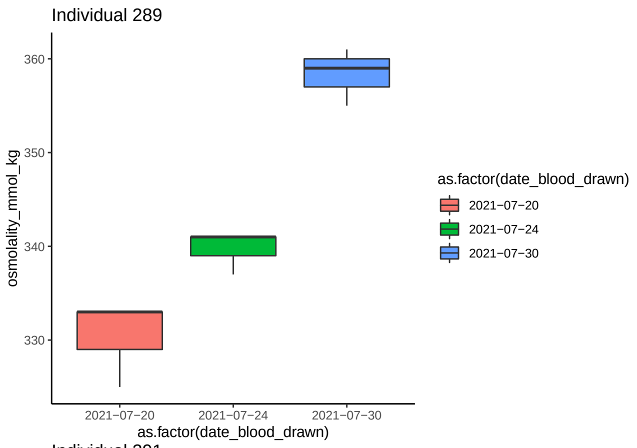

Individual 283
osmolality_mmol_kg
480
440
400
2021−07−20
2021−07−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24

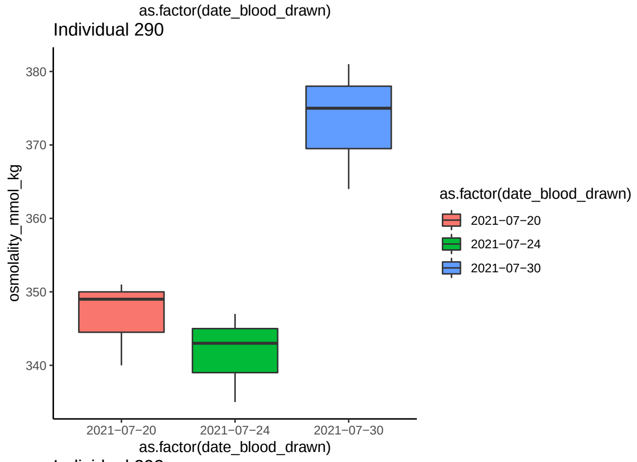

Individual 285
osmolality_mmol_kg
360
350
340
330
320
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

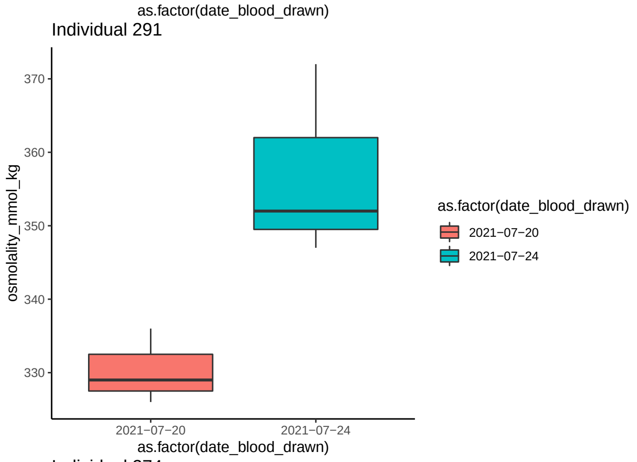

Individual 286
osmolality_mmol_kg
370
360
350
340
2021−07−20
2021−07−24
2021−07−28
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28

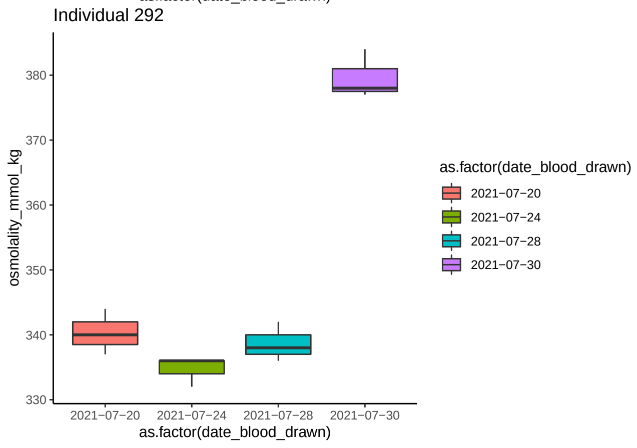

Individual 287
osmolality_mmol_kg
357.5
355.0
352.5
350.0
347.5
2021−07−20
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20

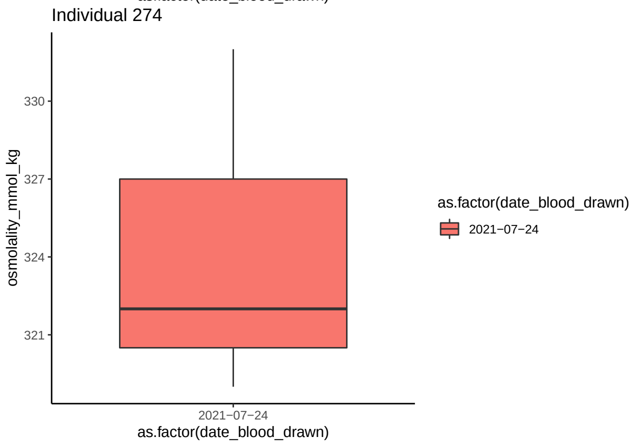

Individual 288
osmolality_mmol_kg
370
360
350
340
330
2021−07−20
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−30

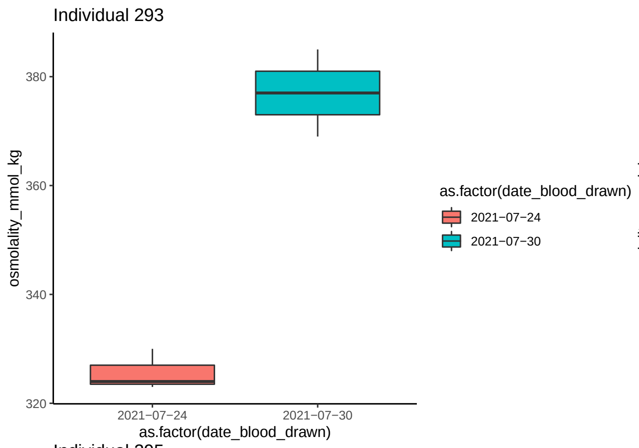

Individual 289
osmolality_mmol_kg
360
350
340
330
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

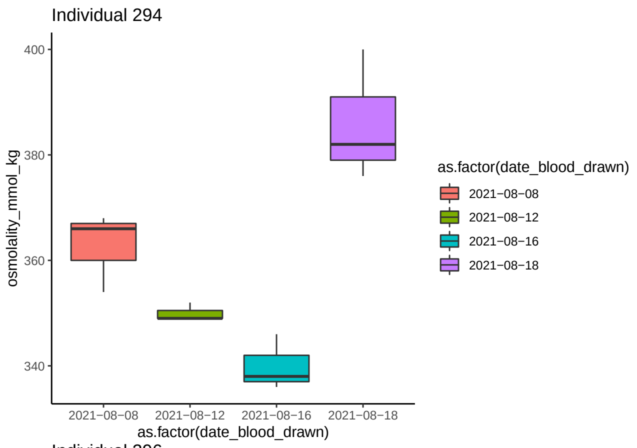

Individual 290
osmolality_mmol_kg
380
370
360
350
340
2021−07−20
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−30

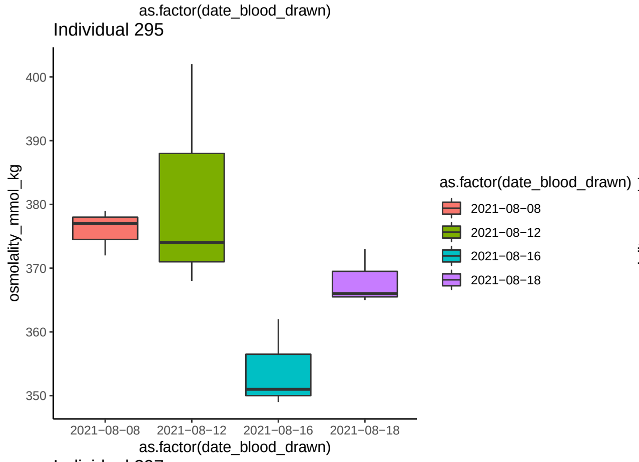

Individual 291
osmolality_mmol_kg
370
360
350
340
330
2021−07−20
2021−07−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24

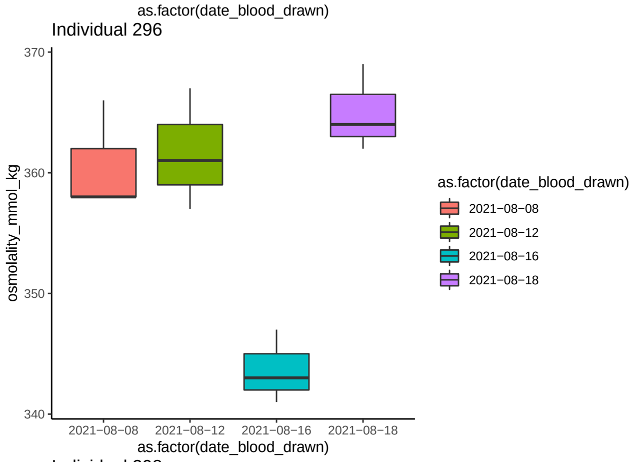

Individual 292
osmolality_mmol_kg
380
370
360
350
340
330
2021−07−20
2021−07−24
2021−07−28
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−20
2021−07−24
2021−07−28
2021−07−30


Individual 274
osmolality_mmol_kg
330
327
324
321
2021−07−24
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−24


Individual 293
osmolality_mmol_kg
320
340
360
380
2021−07−24
2021−07−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−07−24
2021−07−30


Individual 294
osmolality_mmol_kg
340
360
380
400
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 295
osmolality_mmol_kg
350
360
370
380
390
400
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 296
osmolality_mmol_kg
340
350
360
370
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
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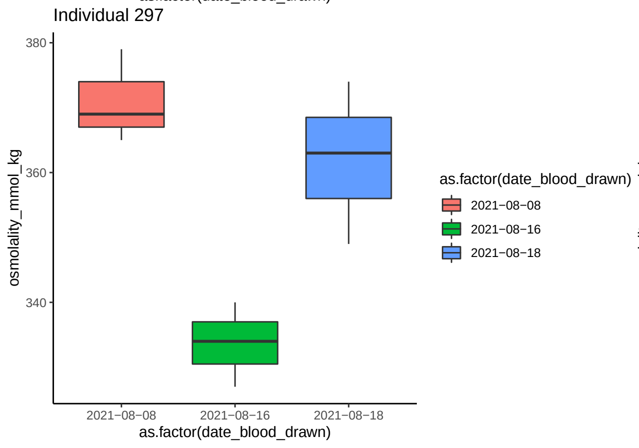
2021−08−16
2021−08−18


Individual 297
osmolality_mmol_kg
340
360
380
2021−08−08
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
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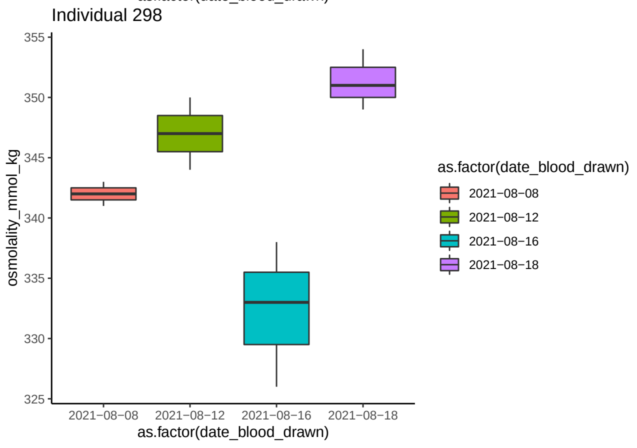
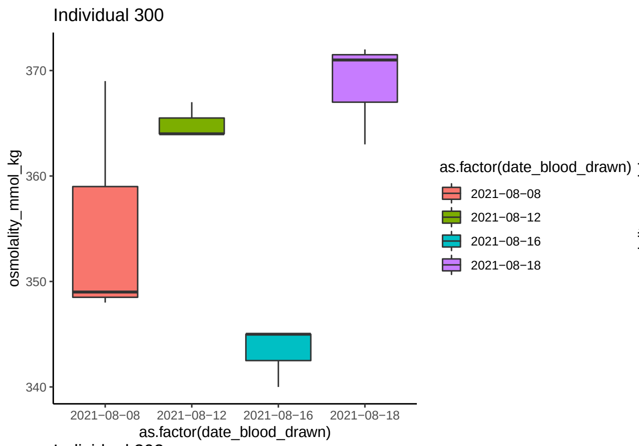
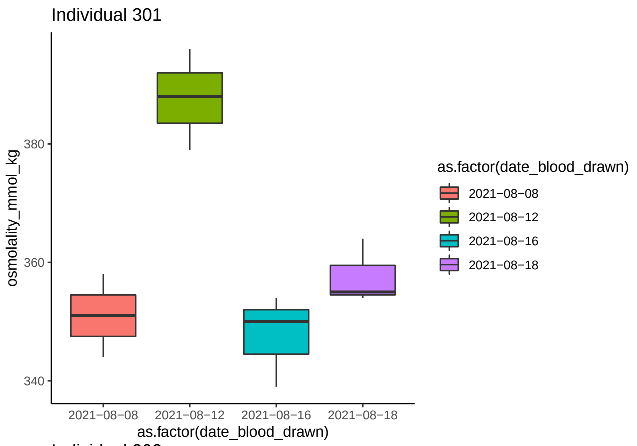
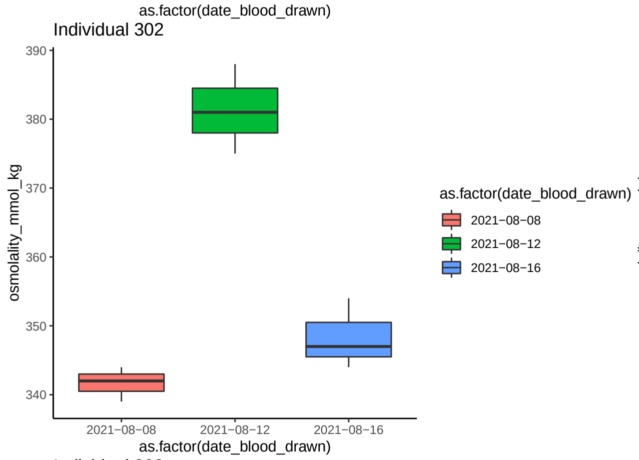
2021−08−16
2021−08−18


Individual 298
osmolality_mmol_kg
325
330
335
340
345
350
355
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 300
osmolality_mmol_kg
370
360
350
340
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 301
osmolality_mmol_kg
380
360
340
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 302
osmolality_mmol_kg
390
380
370
360
350
340
2021−08−08
2021−08−12
2021−08−16
as.factor(date_blood_drawn)
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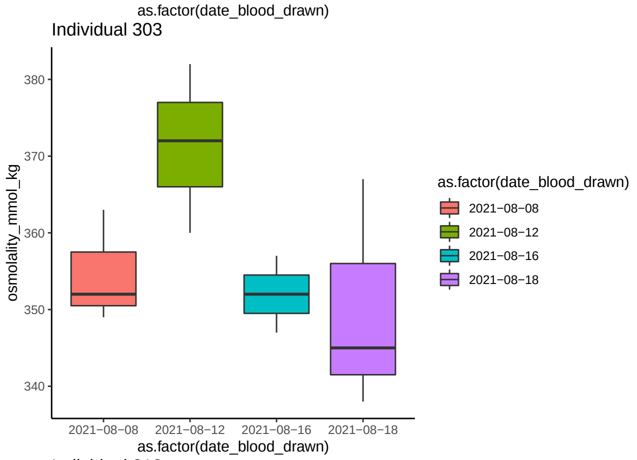
2021−08−12
2021−08−16


Individual 303
osmolality_mmol_kg
380
370
360
350
340
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
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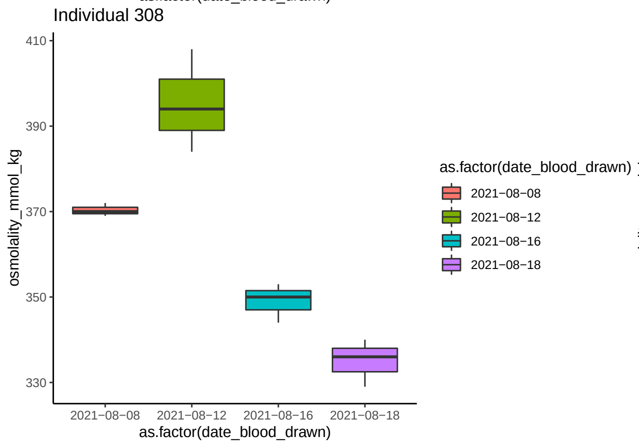
2021−08−16
2021−08−18


Individual 308
osmolality_mmol_kg
410
390
370
350
330
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
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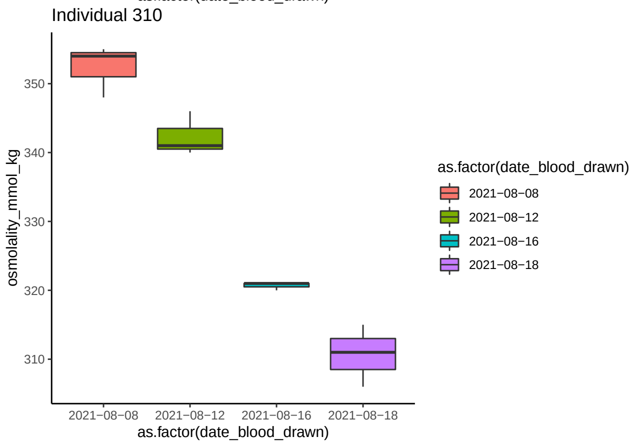
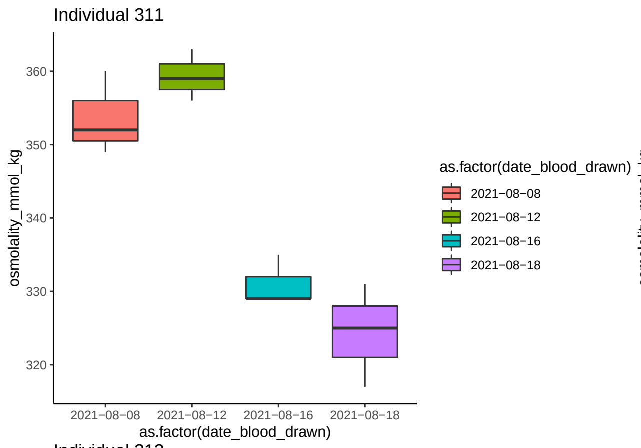
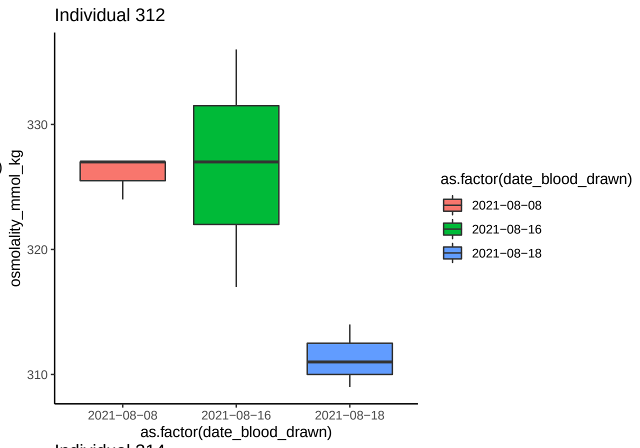

Individual 310
osmolality_mmol_kg
350
340
330
320
310
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 311
osmolality_mmol_kg
360
350
340
330
320
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18


Individual 312
osmolality_mmol_kg
330
320
310
2021−08−08
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
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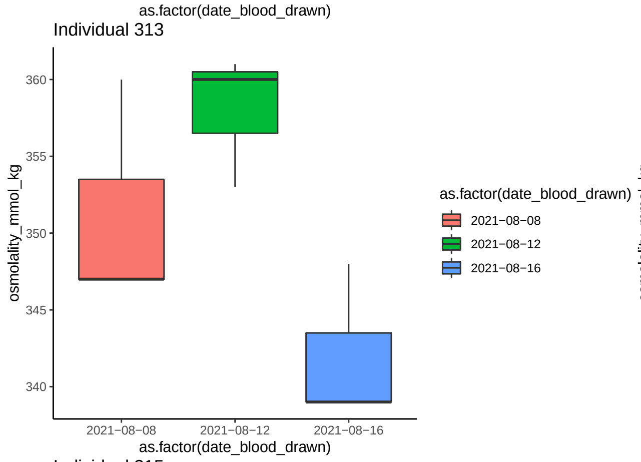
2021−08−16
2021−08−18


Individual 313
osmolality_mmol_kg
360
355
350
345
340
2021−08−08
2021−08−12
2021−08−16
as.factor(date_blood_drawn)
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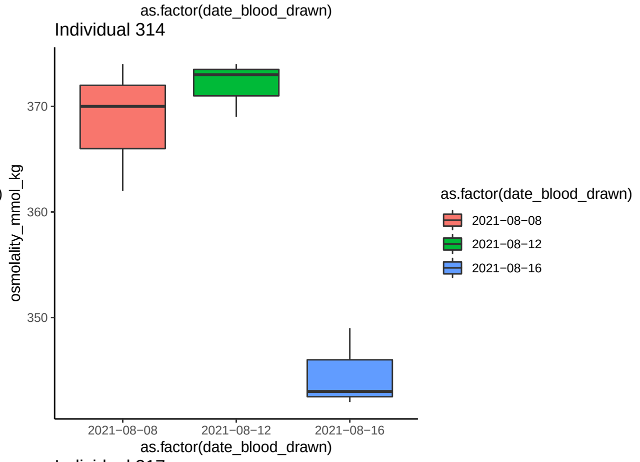
2021−08−12
2021−08−16


Individual 314
osmolality_mmol_kg
370
360
350
2021−08−08
2021−08−12
2021−08−16
as.factor(date_blood_drawn)
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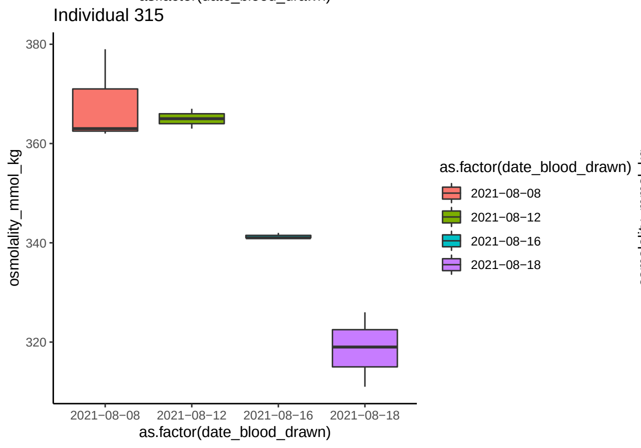
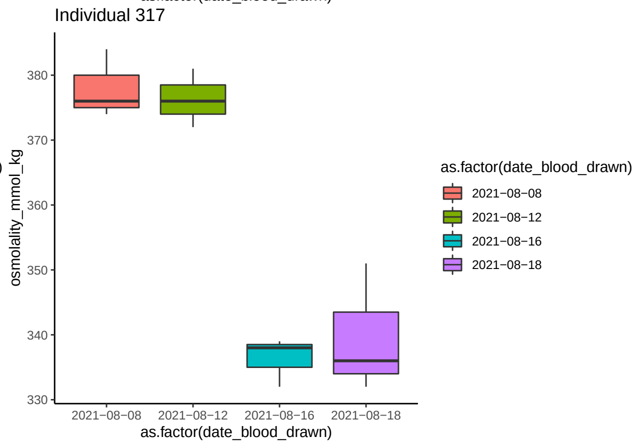
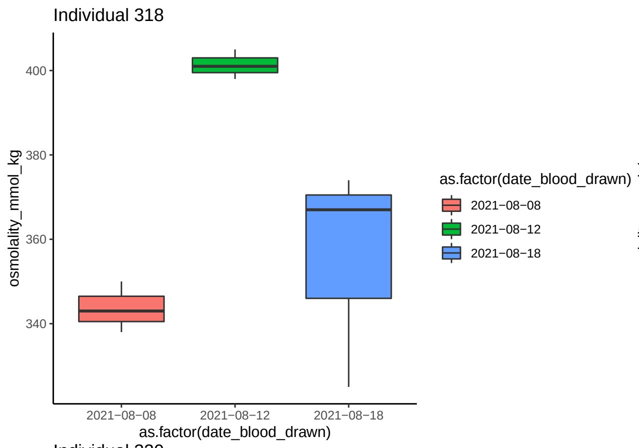
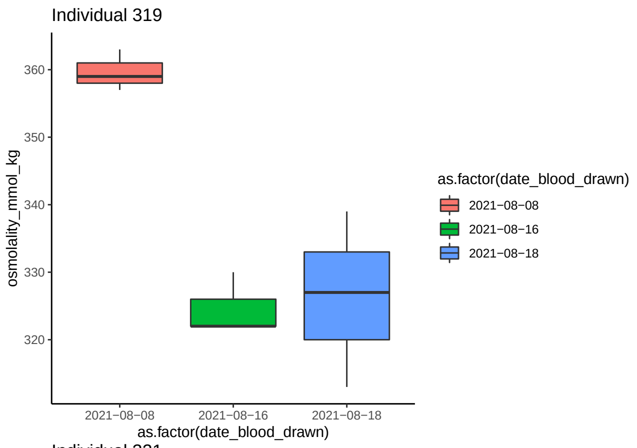
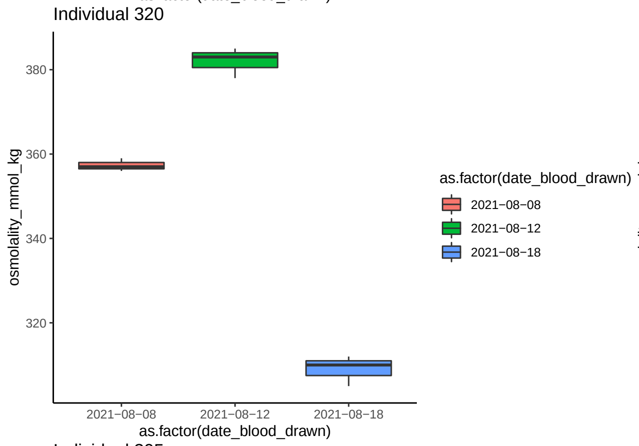
2021−08−12
2021−08−16

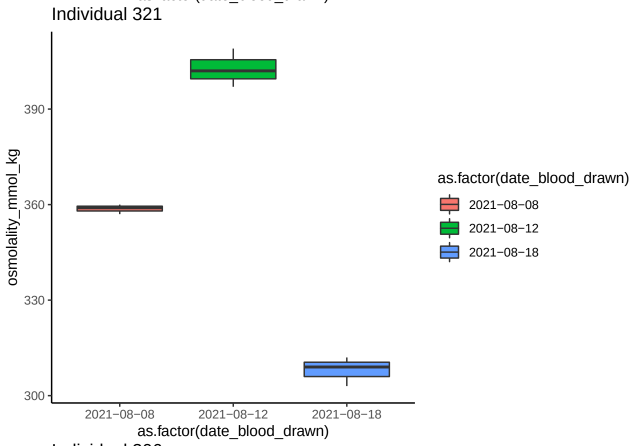

Individual 315
osmolality_mmol_kg
380
360
340
320
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18

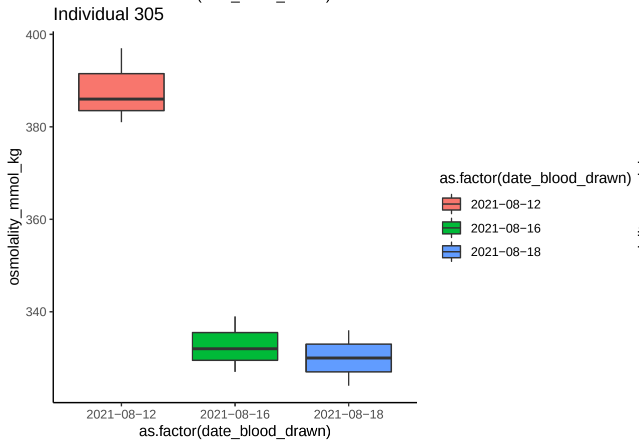

Individual 317
osmolality_mmol_kg
380
370
360
350
340
330
2021−08−08
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−16
2021−08−18

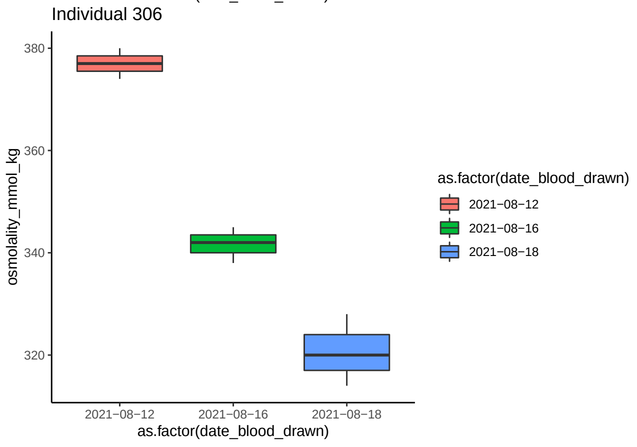

Individual 318
osmolality_mmol_kg
400
380
360
340
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−18
as.factor(date_blood_drawn)

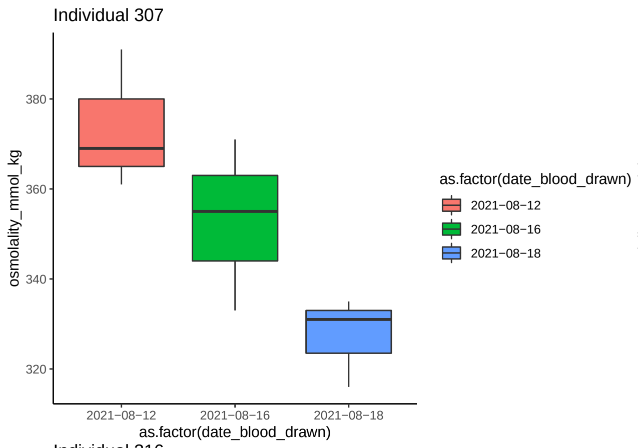

Individual 319
osmolality_mmol_kg
360
350
340
330
320
as.factor(date_blood_drawn)
2021−08−08
2021−08−16
2021−08−18
as.factor(date_blood_drawn)

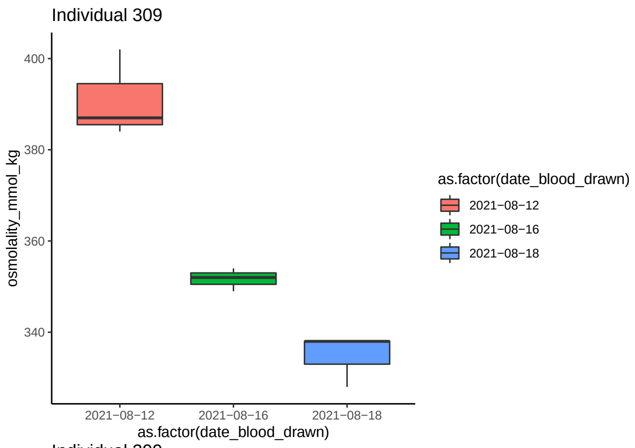

Individual 320
osmolality_mmol_kg
380
360
340
320
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−18
as.factor(date_blood_drawn)

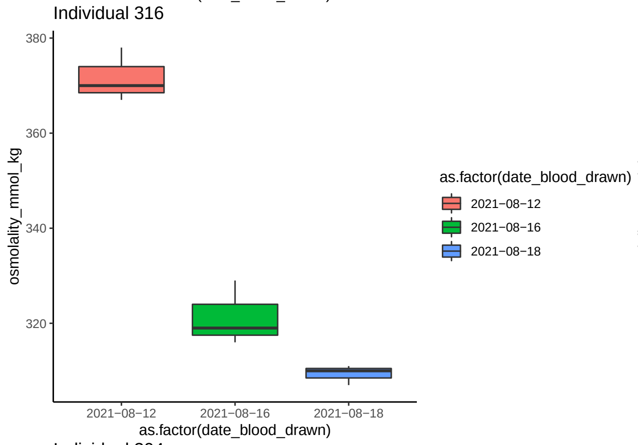

Individual 321
osmolality_mmol_kg
390
360
330
300
as.factor(date_blood_drawn)
2021−08−08
2021−08−12
2021−08−18
as.factor(date_blood_drawn)

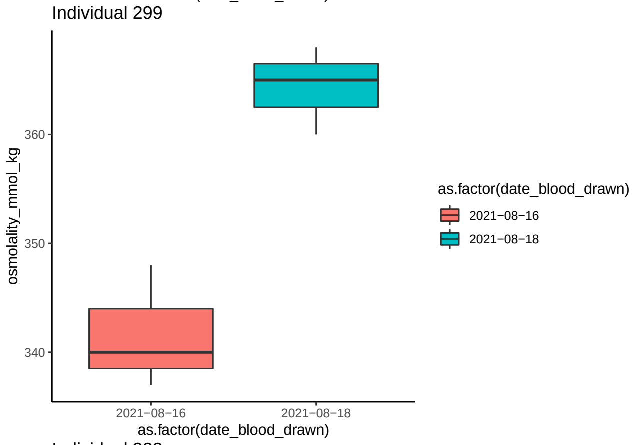

Individual 305
osmolality_mmol_kg
400
380
360
340
as.factor(date_blood_drawn)
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)

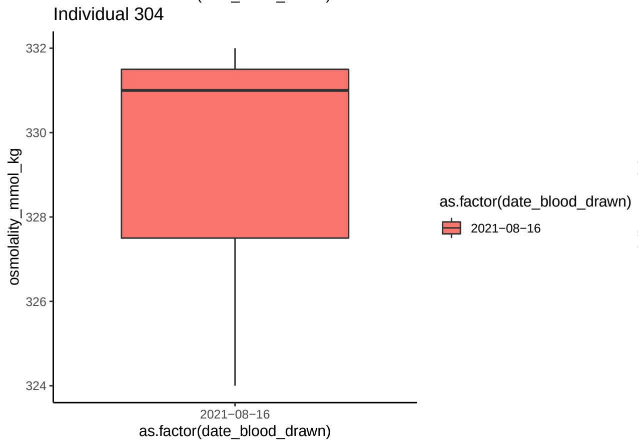

Individual 306
osmolality_mmol_kg
380
360
340
320
as.factor(date_blood_drawn)
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)

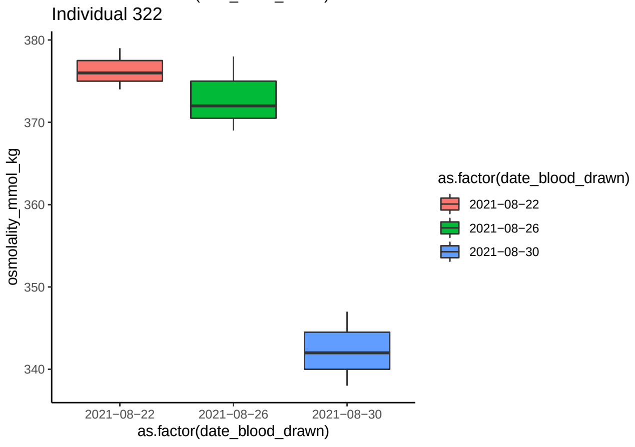

Individual 307
osmolality_mmol_kg
380
360
340
320
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−12
2021−08−16
2021−08−18

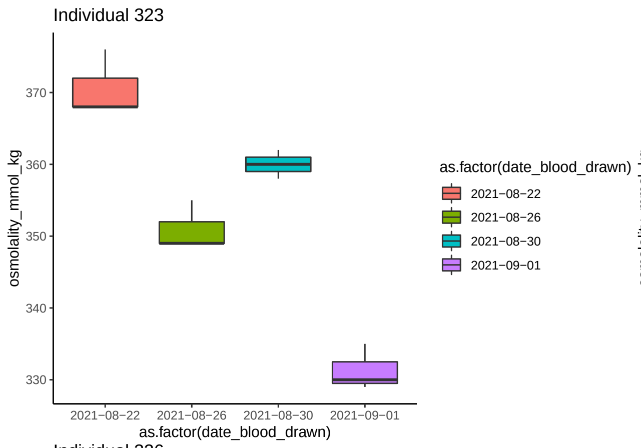

Individual 309
osmolality_mmol_kg
400
380
360
340
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−12
2021−08−16
2021−08−18

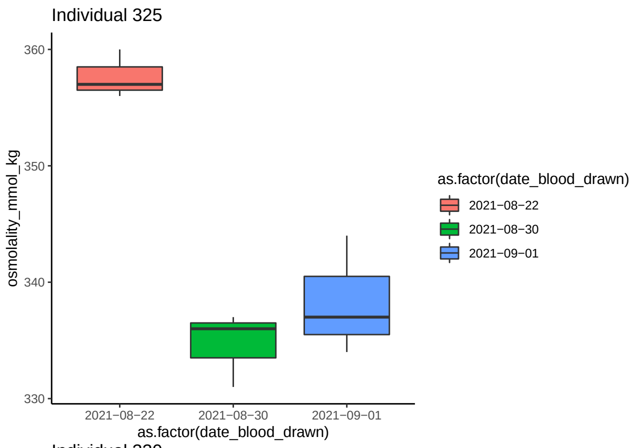

Individual 316
osmolality_mmol_kg
380
360
340
320
2021−08−12
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−12
2021−08−16
2021−08−18

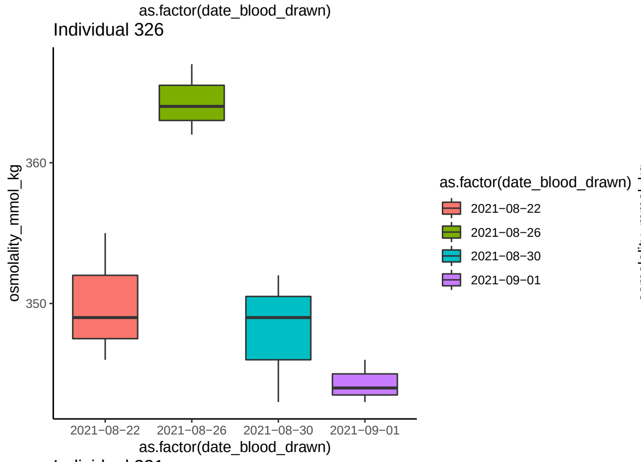

Individual 299
osmolality_mmol_kg
360
350
340
2021−08−16
2021−08−18
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−16
2021−08−18

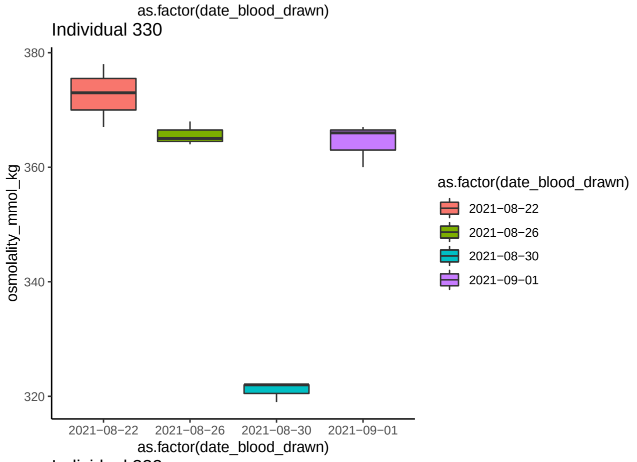

Individual 304
osmolality_mmol_kg
332
330
328
326
324
2021−08−16
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−16

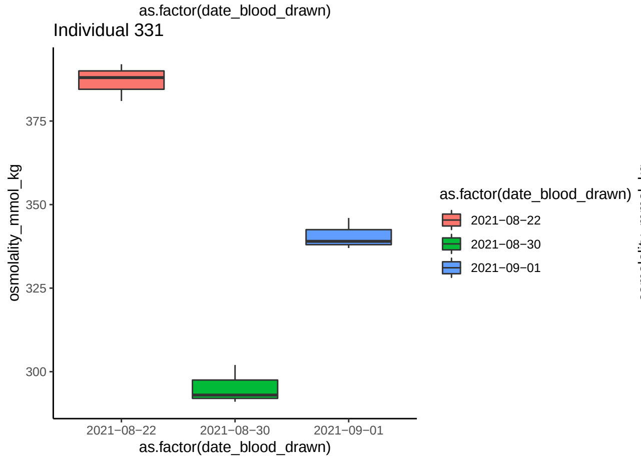

Individual 322
osmolality_mmol_kg
380
370
360
350
340
2021−08−22
2021−08−26
2021−08−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30

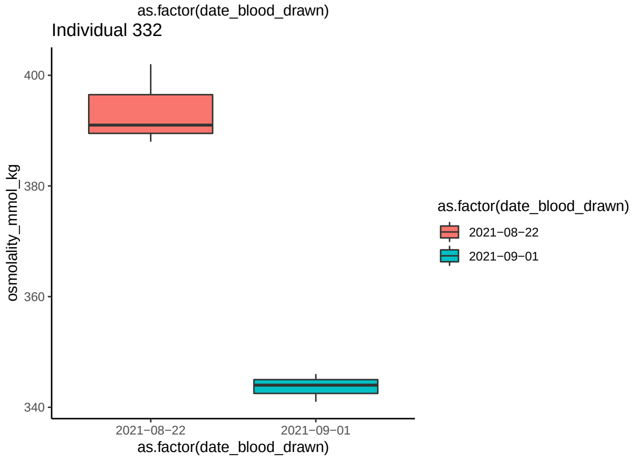

Individual 323
osmolality_mmol_kg
330
340
350
360
370
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

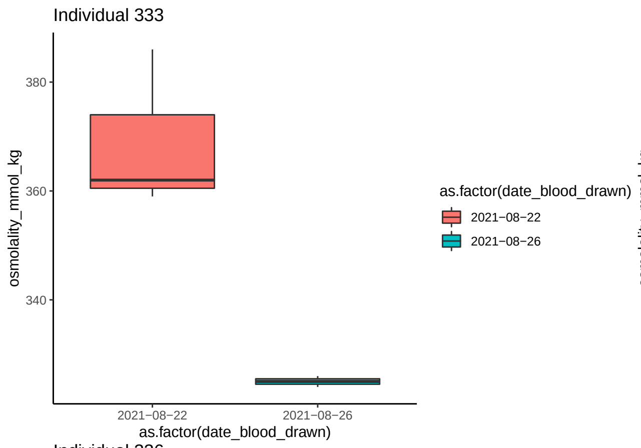

Individual 325
osmolality_mmol_kg
330
340
350
360
2021−08−22
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−30
2021−09−01

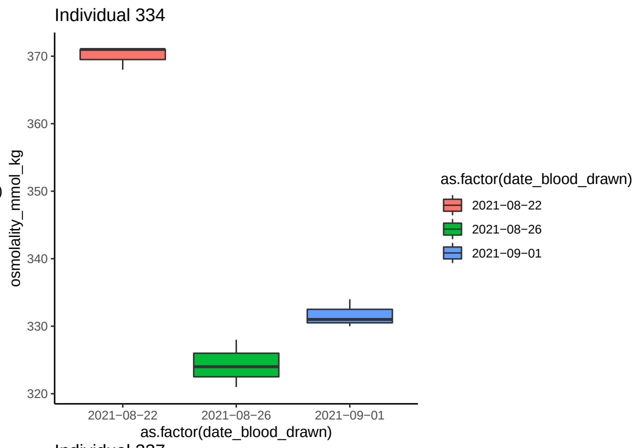

Individual 326
osmolality_mmol_kg
350
360
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

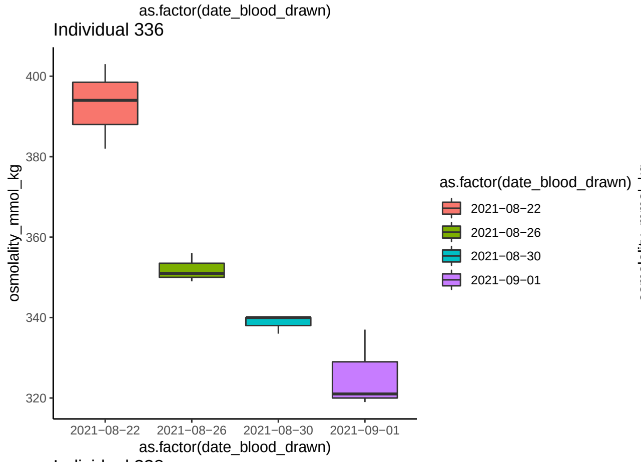

Individual 330
osmolality_mmol_kg
320
340
360
380
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

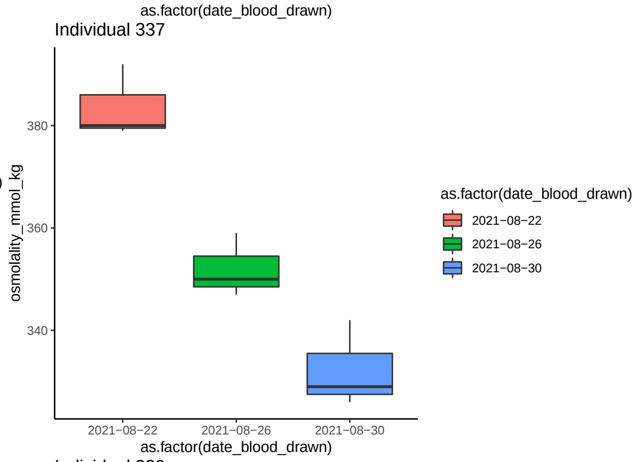

Individual 331
osmolality_mmol_kg
300
325
350
375
2021−08−22
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−30
2021−09−01

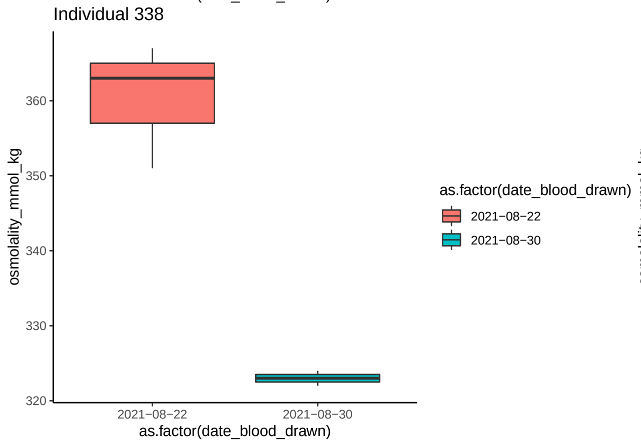

Individual 332
osmolality_mmol_kg
340
360
380
400
2021−08−22
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−09−01

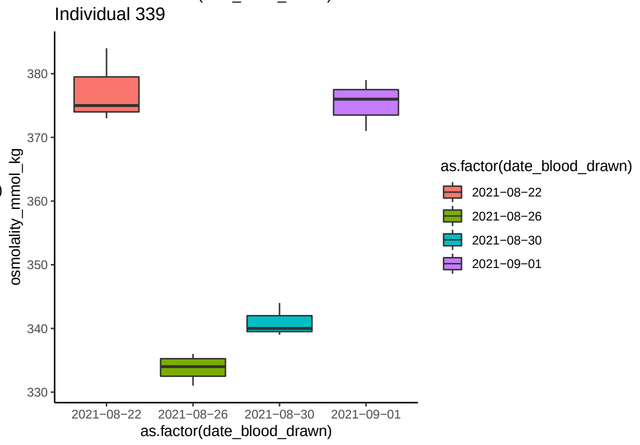

Individual 333
osmolality_mmol_kg
380
360
340
2021−08−22
2021−08−26
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26

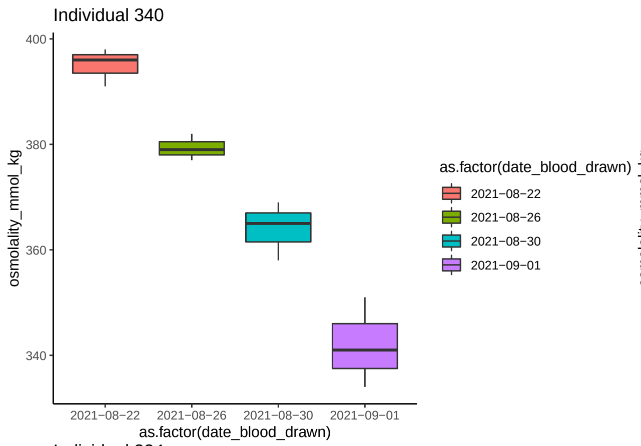

Individual 334
osmolality_mmol_kg
370
360
350
340
330
320
2021−08−22
2021−08−26
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−09−01

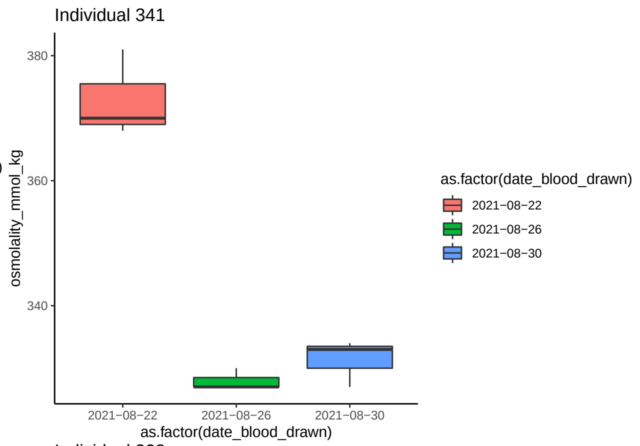

Individual 336
osmolality_mmol_kg
400
380
360
340
320
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

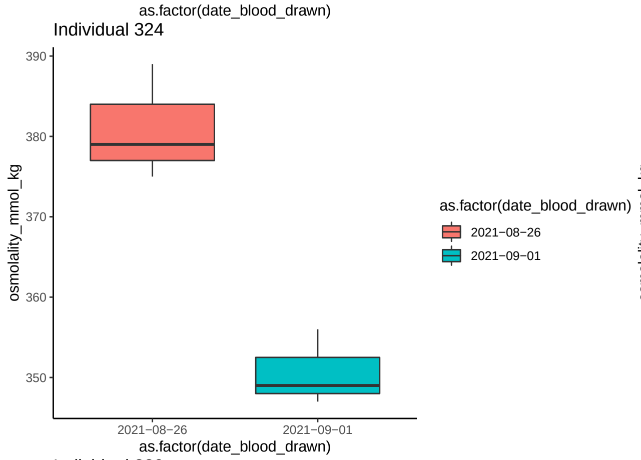

Individual 337
osmolality_mmol_kg
380
360
340
2021−08−22
2021−08−26
2021−08−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30

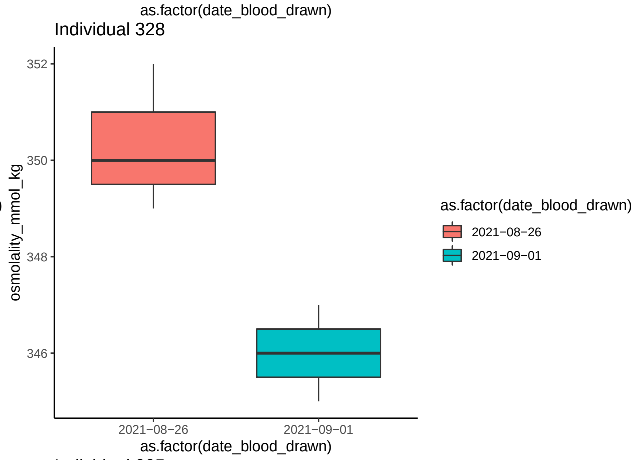

Individual 338
osmolality_mmol_kg
360
350
340
330
320
2021−08−22
2021−08−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−30

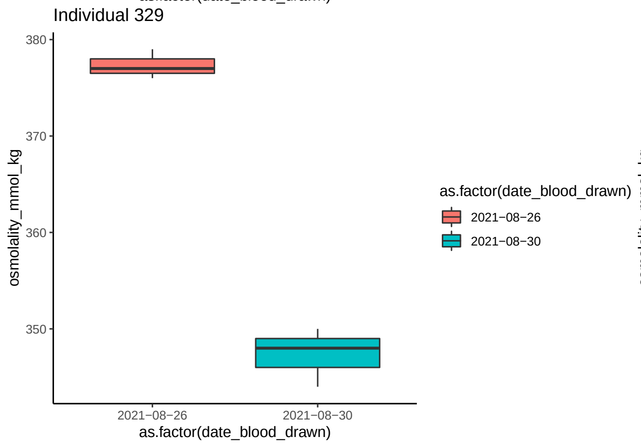

Individual 339
osmolality_mmol_kg
380
370
360
350
340
330
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

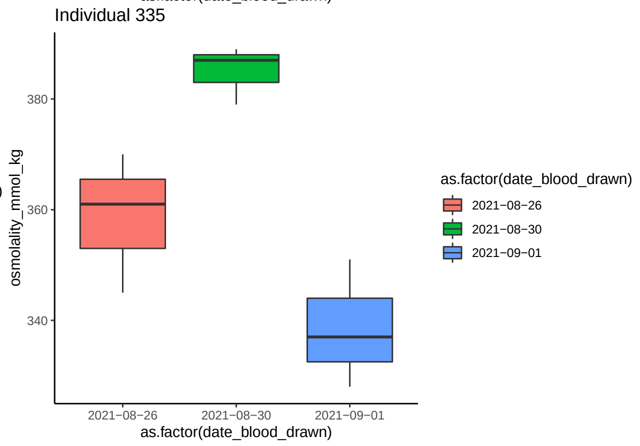

Individual 340
400
380
360
340
osmolality_mmol_kg
2021−08−22
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30
2021−09−01

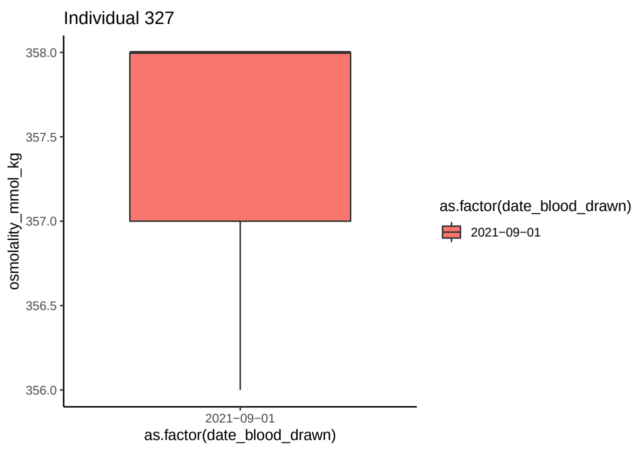

Individual 341
380
360
340
osmolality_mmol_kg
2021−08−22
2021−08−26
2021−08−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−22
2021−08−26
2021−08−30


Individual 324
390
380
370
360
350
osmolality_mmol_kg
2021−08−26
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−26
2021−09−01


Individual 328
352
350
348
346
osmolality_mmol_kg
2021−08−26
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−26
2021−09−01


Individual 329
380
370
360
350
osmolality_mmol_kg
2021−08−26
2021−08−30
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−26
2021−08−30


Individual 335
380
360
340
osmolality_mmol_kg
2021−08−26
2021−08−30
2021−09−01
as.factor(date_blood_drawn)
as.factor(date_blood_drawn)
2021−08−26
2021−08−30
2021−09−01



Based on boxplots, there are no outliers.

Remove Outlier

EVen though there are no outliers based on boxplot IQRs, we still want to find and omit the point severely increasing CV for one individual on one date, so find that one.

```
CVs %>% dplyr::filter(CV > 10)
```

```
## # A tibble: 1 x 8
## # Groups:   individual_ID [1]
##   individual_ID date_blood_drawn  mean    SD    CV   min   max osml_range
##   <fct>         <date>           <dbl> <dbl> <dbl> <dbl> <dbl>      <dbl>
## 1 225           2021-06-26        325.  69.8  21.5   247   381        134
```

Determine which replicate of that group is an outlier and pulls the CV above our acceptable range (10-15%).

```
enuf_reps %>%
  dplyr::filter(individual_ID == 225 & date_blood_drawn == "2021-06-26")
```

```
## # A tibble: 3 x 8
## # Groups:   individual_ID, date_blood_drawn [1]
##   date_blood_drawn date_osmom_run time_osmom_run      individual_ID replicate_no
##   <date>           <date>         <dttm>              <fct>         <fct>
## 1 2021-06-26       2021-06-26     2022-10-03 09:42:00 225           1
## 2 2021-06-26       2021-06-26     2022-10-03 09:42:00 225           2
## 3 2021-06-26       2021-06-26     2022-10-03 09:42:00 225           3
## # ... with 3 more variables: osmolality_mmol_kg <dbl>, notes <lgl>, count <int>
```

We should remove replicate 2 for individual 225 on June 26, which is >100 mmol/kg away from the closest values, and the other two values are within 35 mmol/kg.

```
enuf_reps_trimmed <- enuf_reps %>%
  # remove the one outlier
  dplyr::filter(!(individual_ID == 225 &
                date_blood_drawn == "2021-06-26" &
                replicate_no == 2))

# check
enuf_reps_trimmed %>%
  dplyr::filter(individual_ID == 225 &
                date_blood_drawn == "2021-06-26")
```

```
## # A tibble: 2 x 8
## # Groups:   individual_ID, date_blood_drawn [1]
##   date_blood_drawn date_osmom_run time_osmom_run      individual_ID replicate_no
##   <date>           <date>         <dttm>              <fct>         <fct>
## 1 2021-06-26       2021-06-26     2022-10-03 09:42:00 225           1
## 2 2021-06-26       2021-06-26     2022-10-03 09:42:00 225           3
## # ... with 3 more variables: osmolality_mmol_kg <dbl>, notes <lgl>, count <int>
```

Average Remaining Replicates

Now that the single outlier has been removed from the technical replicates when there were enough replicates to identify them, I will rejoin the data for lizards with 1-2 (not_reps) and 3-4 (enuf_reps) replicates, then average the technical replicates for each lizard on each of their measurement dates.

```
osml_means <- not_reps %>%
  rbind(enuf_reps_trimmed) %>%
  group_by(date_blood_drawn, individual_ID) %>%
  summarise(osmolality_mmol_kg_mean = mean(osmolality_mmol_kg))
```

```
## `summarise()` regrouping output by 'date_blood_drawn' (override with `.groups` argument)
```

Other Cleaning

10 lizards in trial 1 have unreasonably high osmolality measurements on June 24, which we think are due to an osmometer technical error, as they were all from the same time period. The values are way too far outside the usual range to be trustworthy, and these measurements were taken just before the osmometer had to be recalibrated, so we will exclude them.

```
osml_means_clean <- osml_means %>%
  dplyr::filter(!(osmolality_mmol_kg_mean > 500))
```

Export

```
write_rds(osml_means_clean, "./data/osml_means_clean.RDS")
```

Reporting

We only removed one measurement that was an outlier within its technical replicate group.

We omitted 10 mean measurements (after taking tech rep mean) for lizards in trial 1 which had unreasonably high osmolality measurements on June 24 that can be confidently attributed to intrumental error.