

Climate Water Loss Experiment - Treatment Hydration Analysis

Savannah Weaver

2021

Contents

Packages	1
Background and Goals	2
Data	2
Load	2
Clean	4
Format	6
Check	8
Experiment Figures	9
SMI	9
Hct	10
Osmolality	12
Multi-Figure	14
CEWL	15
Experiment Models	17
SMI	17
Hematocrit	21
Osmolality	27
CEWL	34
Conclusion	42
Rehydration Figures	43
SMI	43
Hematocrit	44
Osmolality	46
Multi-Figure	48
Rehydration Models	49
SMI	49
Hematocrit	55
Osmolality	61
Conclusion	67

Packages

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

```

library("tidyverse") # workflow and plots
if (!require("lme4")) install.packages("lme4")
library("lme4") # for LMMs
if (!require("lmerTest")) install.packages("lmerTest")
library("lmerTest") # for p-values
if (!require("ggpubr")) install.packages("ggpubr")
library("ggpubr") # for multi-ggplot figs
if (!require("UsingR")) install.packages("UsingR")
library("UsingR") # simple.eda model assumption checker
if (!require("broom")) install.packages("broom")
library("broom") # lmer model export
if (!require("broom.mixed")) install.packages("broom.mixed")
library("broom.mixed") # lmer model export

```

Background and Goals

This data was collected June - August by Master's student Savannah Weaver, advisor Dr. Emily Taylor, and research assistants Tess McIntyre and Taylor Van Rossum. Adult male *Sceloporus occidentalis* were caught across the Cal Poly campus and in Poly Canyon. This R file analyzes the effect of experimental climate treatments on their body condition, osmotic balance, and osmoregulation. Please refer to **doi:** for the published scientific journal article and full details.

Data

Load

Read-in data that was compiled, formatted, and checked for completeness in 'capture_analysis'. See that file for information related to the variables. Some variables need to be re-formatted because they lose their data class in export/import. Attach treatment assignments and format that data. Also compute scaled mass index using the equation derived in 'capture_analysis'.

```

dat <- read.csv("./data/full_exp_data.csv", # main dataset
  na.strings=c("", "NA")) %>%
  # reformat individual_ID to make joining easy
  mutate(individual_ID = as.numeric(individual_ID)) %>%
  # join tmt assignment info
  left_join(read.csv("./data/tmt_assignments.csv"),
    by = "individual_ID") %>%
  # remove irrelevant variables
  dplyr::select(-X, -capture_date,
    -time_captured, -time_processed, -time_c_temp) %>%
  # format date
  mutate(measurement_date = as.Date(measurement_date,
    format = "%Y-%m-%d"),

  # set factor classes
  type = as.factor(type),
  day = as.factor(day),
  individual_ID = as.factor(individual_ID),
  hemolyzed = as.factor(hemolyzed),
  trial_number = as.factor(trial_number),
  tmt = as.factor(paste(temp_tmt, humidity_tmt)),
  temp_tmt = as.factor(temp_tmt),
  humidity_tmt = as.factor(humidity_tmt),

```

```

conclusion = as.factor(conclusion),
# set numeric classes
day_n = as.numeric(day_n),
mass_g = as.numeric(mass_g),
hematocrit_percent = as.numeric(hematocrit_percent),
osmolality_mmol_kg_mean = as.numeric(osmolality_mmol_kg_mean),
CEWL_g_m2h_mean = as.numeric(CEWL_g_m2h_mean),
cloacal_temp_C = as.numeric(cloacal_temp_C),
SVL_mm = as.numeric(SVL_mm),
# compute scaled mass index
SMI = mass_g * ((67.71/SVL_mm) ^ 2.65)) %>%
# only use data from lizards who underwent the full experiment
# this removes 21 obs for the 7 lizards who didn't complete the exp
dplyr::filter(conclusion == "complete")
summary(dat)

```

```

## measurement_date      type      day      individual_ID      mass_g
## Min.   :2021-06-16    exp :804    capture :134    201   : 7    Min.   : 7.00
## 1st Qu.:2021-07-01    rehab:132    post-exp:134    202   : 7    1st Qu.: 9.50
## Median :2021-07-25                    NA's      :668    203   : 7    Median :10.60
## Mean   :2021-07-22                    204   : 7    Mean   :10.64
## 3rd Qu.:2021-08-14                    205   : 7    3rd Qu.:11.70
## Max.   :2021-09-01                    206   : 7    Max.   :17.40
##                                     (Other):894
## hemolyzed  hematocrit_percent  osmolality_mmol_kg_mean  CEWL_g_m2h_mean
## N   :464    Min.   :13.00      Min.   :295.3      Min.   : 7.152
## Y    : 69    1st Qu.:26.00      1st Qu.:336.3      1st Qu.:19.768
## NA's:403    Median :32.00      Median :352.0      Median :24.159
##                                     Mean   :31.99      Mean   :357.9      Mean   :24.971
##                                     3rd Qu.:37.00      3rd Qu.:371.0      3rd Qu.:28.547
##                                     Max.   :52.00      Max.   :576.0      Max.   :79.267
##                                     NA's   :408      NA's   :404      NA's   :668
## cloacal_temp_C      day_n      trial_number  temp_tmt      humidity_tmt
## Min.   :23.00      Min.   : 0.000    1:175      cool:469      dry :468
## 1st Qu.:25.00      1st Qu.: 4.000    2:203      hot :467      humid:468
## Median :26.00      Median : 6.000    3:231
## Mean   :25.92      Mean   : 5.705    4:189
## 3rd Qu.:27.00      3rd Qu.: 8.000    5:138
## Max.   :30.00      Max.   :10.000
## NA's   :668
## SVL_mm      conclusion      notes      shed
## Min.   :60.00    canceled: 0    Length:936    Length:936
## 1st Qu.:66.00    complete:936    Class :character    Class :character
## Median :67.00                    Mode  :character    Mode  :character
## Mean   :67.74
## 3rd Qu.:70.00
## Max.   :77.00
##
## tail_broken      died      tmt      SMI
## Length:936      Length:936      cool dry :231    Min.   : 6.754
## Class :character    Class :character    cool humid:238    1st Qu.: 9.749
## Mode  :character    Mode  :character    hot dry  :237    Median :10.609
##                                     hot humid :230    Mean   :10.619
##                                     3rd Qu.:11.394

```

```
##                                     Max.      :15.132
##
```

Clean

There are a handful of points that appear erroneous.

Individual 239 had post-treatment CEWL >60, which is incredibly unusual for our experiment. He was in the process of shedding when we took this CEWL measurement, so we assume that his process of shedding confounded potential treatment effects, and we should remove that point from our data. We still want to use the other measurements for this individual, so we will just set that CEWL measurement as an NA.

```
which(dat$CEWL_g_m2h_mean > 60)
```

```
## [1] 332
```

```
dat[332, ]
```

```
##      measurement_date type      day individual_ID mass_g hemolyzed
## 332      2021-07-04  exp post-exp           239    11.2         N
##      hematocrit_percent osmolality_mmol_kg_mean CEWL_g_m2h_mean cloacal_temp_C
## 332              28              328.3333          79.2675          23
##      day_n trial_number temp_tmt humidity_tmt SVL_mm conclusion notes shed
## 332      8            2      cool      humid    69    complete
##      tail_broken died      tmt      SMI
## 332              cool humid 10.65364
```

```
dat[332, "CEWL_g_m2h_mean"]
```

```
## [1] 79.2675
```

```
dat[332, "CEWL_g_m2h_mean"] <- NA
dat[332, "CEWL_g_m2h_mean"]
```

```
## [1] NA
```

```
dat %>%
  dplyr::filter(complete.cases(CEWL_g_m2h_mean)) %>%
  summarise(max(CEWL_g_m2h_mean))
```

```
##      max(CEWL_g_m2h_mean)
## 1              56.066
```

10 trial 1 lizards have unreasonably high osmolality measurements on June 24, which we think are due to an osmometer technical error. The values are way too far outside the usual range to be trustworthy, so we will exclude them.

```
which(dat$osmolality_mmol_kg_mean > 500)
```

```
## [1] 136 137 138 139 140 141 142 143 144 145
```

```
dat[136:145, ]
```

```
##      measurement_date type      day individual_ID mass_g hemolyzed
## 136      2021-06-24  exp post-exp           211     9.1         N
## 137      2021-06-24  exp post-exp           213     9.3         N
## 138      2021-06-24  exp post-exp           214    11.7         N
## 139      2021-06-24  exp post-exp           215    11.7         N
## 140      2021-06-24  exp post-exp           216    10.2         Y
## 141      2021-06-24  exp post-exp           217    11.8         Y
```

```

## 142      2021-06-24  exp post-exp          218   10.7        Y
## 143      2021-06-24  exp post-exp          219    9.3        Y
## 144      2021-06-24  exp post-exp          220    9.4        N
## 145      2021-06-24  exp post-exp          221    8.3        N
##      hematocrit_percent  osmolality_mmol_kg_mean  CEWL_g_m2h_mean  cloacal_temp_C
## 136              26              513.6667              21.4220              24
## 137              29              555.3333              20.6700              25
## 138              29              550.3333              35.7120              25
## 139              35              545.0000              25.9860              26
## 140              30              543.5000              21.7125              25
## 141              36              543.5000              23.6580              25
## 142              37              540.5000              20.9020              27
## 143              34              576.0000              42.7980              23
## 144              29              536.6667              54.8280              25
## 145              36              567.3333              28.4860              25
##      day_n  trial_number  temp_tmt  humidity_tmt  SVL_mm  conclusion      notes
## 136      8              1      hot      dry      71      complete
## 137      8              1      cool      dry      67      complete
## 138      8              1      cool      humid     69      complete
## 139      8              1      cool      dry      70      complete
## 140      8              1      cool      dry      68      complete
## 141      8              1      cool      humid     69      complete
## 142      8              1      cool      humid     71      complete
## 143      8              1      hot      dry      70      complete died in rehab
## 144      8              1      hot      humid     73      complete
## 145      8              1      hot      dry      69      complete
##      shed  tail_broken      died      tmt      SMI
## 136      0              0              hot dry  8.024846
## 137      0              0              cool dry  9.563452
## 138      0              0              cool humid 11.129246
## 139      0              0              cool dry 10.712874
## 140      0              0              cool dry 10.085130
## 141      0              0              cool humid 11.224367
## 142      0              0              cool humid  9.435808
## 143      0              0      6/26/21      hot dry  8.515362
## 144      0              0              hot humid  7.701091
## 145      0              0              hot dry  7.895106

dat[136:145, "osmolality_mmol_kg_mean"]

## [1] 513.6667 555.3333 550.3333 545.0000 543.5000 543.5000 540.5000 576.0000
## [9] 536.6667 567.3333

dat[136:145, "osmolality_mmol_kg_mean"] <- c(rep(NA, 10))
dat[136:145, "osmolality_mmol_kg_mean"]

## [1] NA NA NA NA NA NA NA NA NA NA

dat[136:145, ]

##      measurement_date  type      day  individual_ID  mass_g  hemolyzed
## 136      2021-06-24  exp post-exp          211    9.1        N
## 137      2021-06-24  exp post-exp          213    9.3        N
## 138      2021-06-24  exp post-exp          214   11.7        N
## 139      2021-06-24  exp post-exp          215   11.7        N
## 140      2021-06-24  exp post-exp          216   10.2        Y

```

```

## 141      2021-06-24  exp post-exp          217   11.8        Y
## 142      2021-06-24  exp post-exp          218   10.7        Y
## 143      2021-06-24  exp post-exp          219    9.3        Y
## 144      2021-06-24  exp post-exp          220    9.4        N
## 145      2021-06-24  exp post-exp          221    8.3        N
##      hematocrit_percent  osmolality_mmol_kg_mean  CEWL_g_m2h_mean  cloacal_temp_C
## 136              26              NA              21.4220              24
## 137              29              NA              20.6700              25
## 138              29              NA              35.7120              25
## 139              35              NA              25.9860              26
## 140              30              NA              21.7125              25
## 141              36              NA              23.6580              25
## 142              37              NA              20.9020              27
## 143              34              NA              42.7980              23
## 144              29              NA              54.8280              25
## 145              36              NA              28.4860              25
##      day_n  trial_number  temp_tmt  humidity_tmt  SVL_mm  conclusion      notes
## 136      8              1      hot      dry      71      complete
## 137      8              1      cool      dry      67      complete
## 138      8              1      cool      humid     69      complete
## 139      8              1      cool      dry      70      complete
## 140      8              1      cool      dry      68      complete
## 141      8              1      cool      humid     69      complete
## 142      8              1      cool      humid     71      complete
## 143      8              1      hot      dry      70      complete died in rehab
## 144      8              1      hot      humid     73      complete
## 145      8              1      hot      dry      69      complete
##      shed  tail_broken      died      tmt      SMI
## 136      hot dry      8.024846
## 137      cool dry      9.563452
## 138      cool humid 11.129246
## 139      cool dry 10.712874
## 140      cool dry 10.085130
## 141      cool humid 11.224367
## 142      cool humid  9.435808
## 143      6/26/21      hot dry      8.515362
## 144      hot humid  7.701091
## 145      hot dry      7.895106

```

```

dat %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  summarise(max(osmolality_mmol_kg_mean))

```

```

##      max(osmolality_mmol_kg_mean)
## 1                                471.5

```

Format

Rename some factors:

```

dat$humidity_tmt <- factor(dat$humidity_tmt,
  levels = c("humid", "dry"),
  labels = c("Humid", "Dry"))
dat$temp_tmt <- factor(dat$temp_tmt,
  levels = c("hot", "cool"),

```

```

labels = c("Hot", "Cool"))
dat$tmt <- factor(dat$tmt,
levels = c("hot humid", "hot dry",
"cool humid", "cool dry"),
labels = c("Hot Humid", "Hot Dry",
"Cool Humid", "Cool Dry"))
summary(dat)

## measurement_date      type      day      individual_ID      mass_g
## Min. :2021-06-16      exp :804      capture :134      201 : 7      Min. : 7.00
## 1st Qu.:2021-07-01      rehab:132      post-exp:134      202 : 7      1st Qu.: 9.50
## Median :2021-07-25                        NA's :668      203 : 7      Median :10.60
## Mean :2021-07-22                        204 : 7      Mean :10.64
## 3rd Qu.:2021-08-14                        205 : 7      3rd Qu.:11.70
## Max. :2021-09-01                        206 : 7      Max. :17.40
##                                     (Other):894
## hemolyzed hematocrit_percent osmolality_mmol_kg_mean CEWL_g_m2h_mean
## N :464      Min. :13.00      Min. :295.3      Min. : 7.152
## Y : 69      1st Qu.:26.00      1st Qu.:336.1      1st Qu.:19.755
## NA's:403      Median :32.00      Median :351.3      Median :24.152
##              Mean :31.99      Mean :354.3      Mean :24.767
##              3rd Qu.:37.00      3rd Qu.:370.0      3rd Qu.:28.505
##              Max. :52.00      Max. :471.5      Max. :56.066
##              NA's :408      NA's :414      NA's :669
## cloacal_temp_C      day_n      trial_number temp_tmt      humidity_tmt
## Min. :23.00      Min. : 0.000      1:175      Hot :467      Humid:468
## 1st Qu.:25.00      1st Qu.: 4.000      2:203      Cool:469      Dry :468
## Median :26.00      Median : 6.000      3:231
## Mean :25.92      Mean : 5.705      4:189
## 3rd Qu.:27.00      3rd Qu.: 8.000      5:138
## Max. :30.00      Max. :10.000
## NA's :668
## SVL_mm      conclusion      notes      shed
## Min. :60.00      canceled: 0      Length:936      Length:936
## 1st Qu.:66.00      complete:936      Class :character      Class :character
## Median :67.00                        Mode :character      Mode :character
## Mean :67.74
## 3rd Qu.:70.00
## Max. :77.00
##
## tail_broken      died      tmt      SMI
## Length:936      Length:936      Hot Humid :230      Min. : 6.754
## Class :character      Class :character      Hot Dry :237      1st Qu.: 9.749
## Mode :character      Mode :character      Cool Humid:238      Median :10.609
##                                     Cool Dry :231      Mean :10.619
##                                     3rd Qu.:11.394
##                                     Max. :15.132
##

```

Make sub-dataframes without rehab data / with only rehab-related data:

```

dat_no_rehab <- dat %>%
  dplyr::filter(type == "exp")
dat_for_rehab <- dat %>%
  dplyr::filter(day_n %in% c(8, 10))

```

Check

Dates:

```
unique(dat$measurement_date)
```

```
## [1] "2021-06-16" "2021-06-20" "2021-06-21" "2021-06-22" "2021-06-23"
## [6] "2021-06-24" "2021-06-26" "2021-06-30" "2021-07-01" "2021-07-02"
## [11] "2021-07-03" "2021-07-04" "2021-07-06" "2021-07-20" "2021-07-24"
## [16] "2021-07-25" "2021-07-26" "2021-07-27" "2021-07-28" "2021-07-30"
## [21] "2021-08-08" "2021-08-12" "2021-08-13" "2021-08-14" "2021-08-15"
## [26] "2021-08-16" "2021-08-18" "2021-08-22" "2021-08-26" "2021-08-27"
## [31] "2021-08-28" "2021-08-29" "2021-08-30" "2021-09-01"
```

Number of measurements for each lizard:

```
dat_no_rehab %>%
  group_by(individual_ID) %>%
  summarise(n = n()) %>%
  arrange(n)
```

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

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

Every lizard has 6 experimental measurements: pre-tmt, mid-tmt, post-tmt, and mass checks on each of the 3 days between mid and post-tmt.

Did any of the treatment groups inherently start out with large differences in response variables?

```
dat %>%
  dplyr::filter(day == "capture") %>%
  group_by(tmt) %>%
  summarise(mean(mass_g),
            mean(SMI),
            mean(hematocrit_percent),
            mean(osmolality_mmol_kg_mean),
            mean(CEWL_g_m2h_mean))
```

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

```
## # A tibble: 4 x 6
##   tmt      `mean(mass_g)` `mean(SMI)` `mean(hematocrit_pe~` `mean(osmolality_mmol~`
##   <fct>          <dbl>      <dbl>      <dbl>          <dbl>
## 1 pre-tmt      1.25      0.05      0.05      0.05
## 2 mid-tmt      1.25      0.05      0.05      0.05
## 3 post-tmt     1.25      0.05      0.05      0.05
## 4 mass         1.25      0.05      0.05      0.05
```



```
## 1 Hot Hu~          11.6      11.5          37.9          347.
## 2 Hot Dry          12.0      11.8          38.9          347.
## 3 Cool H~         11.6      11.7          39.6          351.
## 4 Cool D~         11.8      11.8          39.3          346.
## # ... with 1 more variable: mean(CEWL_g_m2h_mean) <dbl>
```

There are slight differences, but overall the starting values across groups are more or less the same.

Experiment Figures

SMI

```
# calculate means to overlay
mean_SMI <- dat %>%
  dplyr::filter(day == "post-exp") %>%
  dplyr::filter(complete.cases(SMI)) %>%
  group_by(tmt) %>%
  summarise(day = 8,
            mean_SMI = mean(SMI))

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

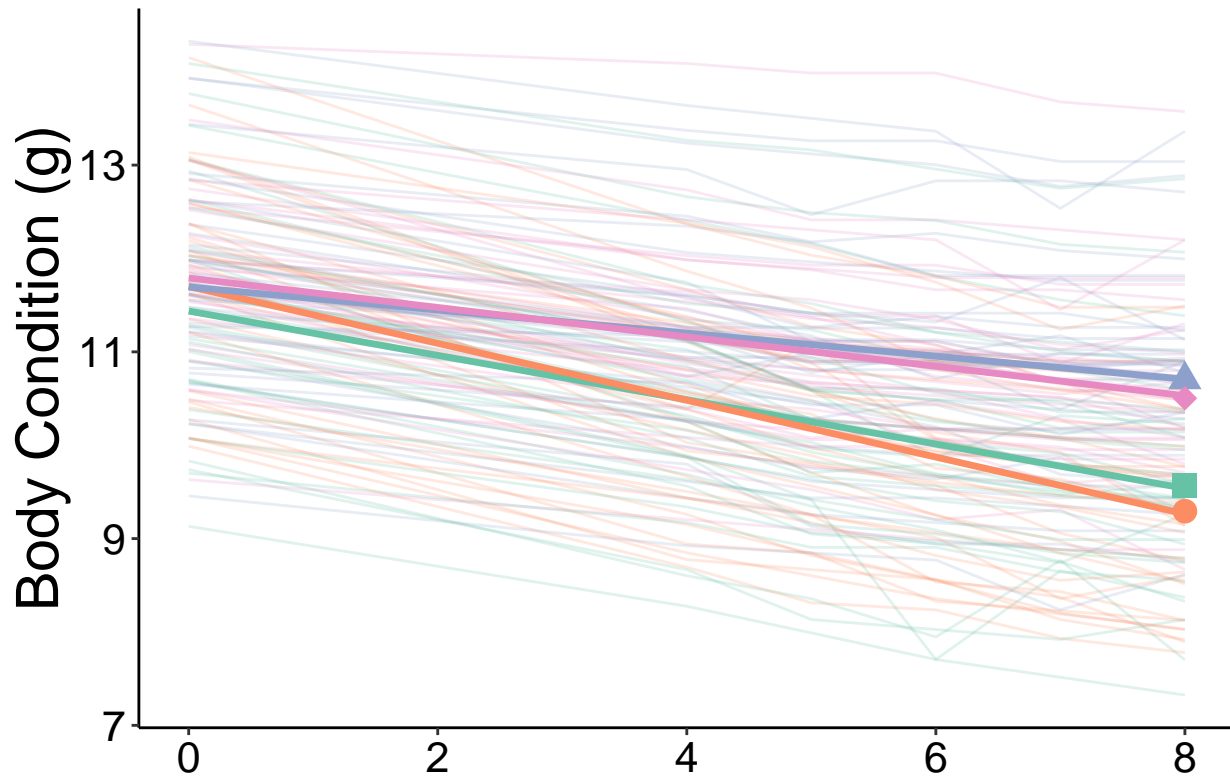
# plot!!
dat_no_rehab %>%
  ggplot(aes(x = day_n,
            y = SMI,
            color = tmt,
            )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
            method = "lm",
            se = F,
            size = 1.2,
            alpha = 1) +
  # this point is more for the legend than for showing means
  geom_point(data = mean_SMI,
            aes(x = day,
              y = mean_SMI - 0.1, # slight adjustment to center on line
              color = tmt,
              shape = tmt),
            size = 4,
            alpha = 1) +
  theme_classic() +
  scale_shape_manual(values = c(15:18), name = "") +
  scale_x_continuous(breaks = c(0, 2, 4, 6, 8)) +
  scale_color_brewer(palette = "Set2", name = "") +
  xlab("") +
  ylab("Body Condition (g)") +
  guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 22),
        axis.text = element_text(color = "black",
                                  family = "sans",
```

```

        size = 16),
    legend.text = element_text(color = "black",
                                family = "sans",
                                size = 22),

    legend.text.align = 0,
    legend.position = "none"
) -> tmt_effects_SMI
tmt_effects_SMI

```



```

# export figure
ggsave(filename = "tmt_effects_SMI.jpeg",
        plot = tmt_effects_SMI,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)

```

Hct

```

# calculate means to overlay
mean_hct <- dat %>%
  dplyr::filter(day == "post-exp") %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  group_by(tmt) %>%
  summarise(day = 8,
            mean_hct = mean(hematocrit_percent))

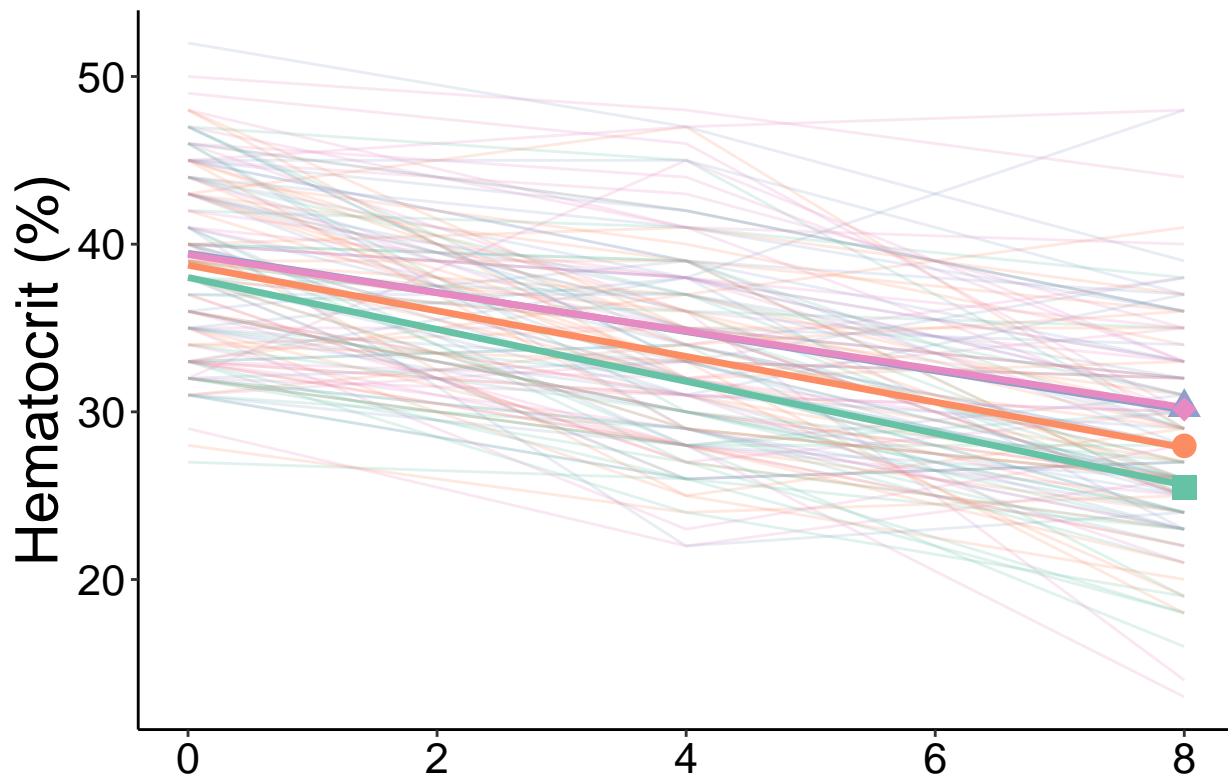
```

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

```

# plot
dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  ggplot(aes(x = day_n,
             y = hematocrit_percent,
             color = tmt,
             )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.2,
              alpha = 1 ) +
  # this point is more for the legend than for showing means
  geom_point(data = mean_hct,
            aes(x = day,
               y = mean_hct,
               color = tmt,
               shape = tmt),
            size = 4,
            alpha = 1) +
  theme_classic() +
  scale_shape_manual(values = c(15:18), name = "") +
  scale_x_continuous(breaks = c(0, 2, 4, 6, 8)) +
  scale_color_brewer(palette = "Set2", name = "") +
  xlab("") +
  ylab("Hematocrit (%)") +
  guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
  theme(text = element_text(color = "black",
                             family = "sans",
                             size = 22),
        axis.text = element_text(color = "black",
                                   family = "sans",
                                   size = 16),
        legend.text = element_text(color = "black",
                                     family = "sans",
                                     size = 22),
        legend.text.align = 0,
        legend.position = "none"
  ) -> tmt_effects_hct
tmt_effects_hct

```



```
# export figure
ggsave(filename = "tmt_effects_hct.jpeg",
        plot = tmt_effects_hct,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Osmolality

```
# calculate means to overlay
mean_osml <- dat %>%
  dplyr::filter(day == "post-exp") %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  group_by(tmt) %>%
  summarise(day = 8,
            mean_osml = mean(osmolality_mmol_kg_mean))

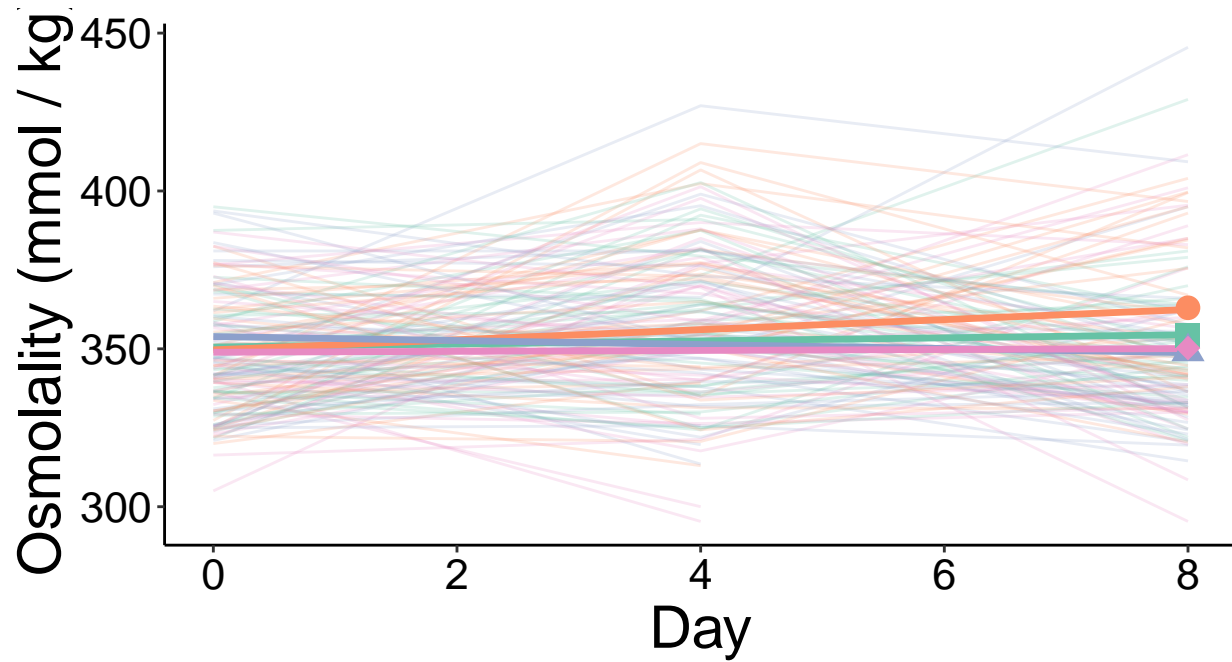
## `summarise()` ungrouping output (override with `.groups` argument)

# plot
dat_no_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  ggplot(aes(x = day_n,
            y = osmolality_mmol_kg_mean,
            color = tmt,
            )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
```

```

        method = "lm",
        se = F,
        size = 1.2,
        alpha = 1 ) +
# this point is more for the legend than for showing means
geom_point(data = mean_osml,
  aes(x = day,
      y = mean_osml + 3, # slight adjust to center on line
      color = tmt,
      shape = tmt),
  size = 4,
  alpha = 1) +
theme_classic() +
scale_shape_manual(values = c(15:18), name = "") +
scale_x_continuous(breaks = c(0, 2, 4, 6, 8)) +
scale_color_brewer(palette = "Set2", name = "") +
xlab("Day") +
ylab("Osmolality (mmol / kg)") +
guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
theme(text = element_text(color = "black",
                           family = "sans",
                           size = 22),
      axis.text = element_text(color = "black",
                                family = "sans",
                                size = 16),
      legend.text = element_text(color = "black",
                                  family = "sans",
                                  size = 22),
      legend.text.align = 0,
      legend.position = "bottom"
    ) -> tmt_effects_osml
tmt_effects_osml

```

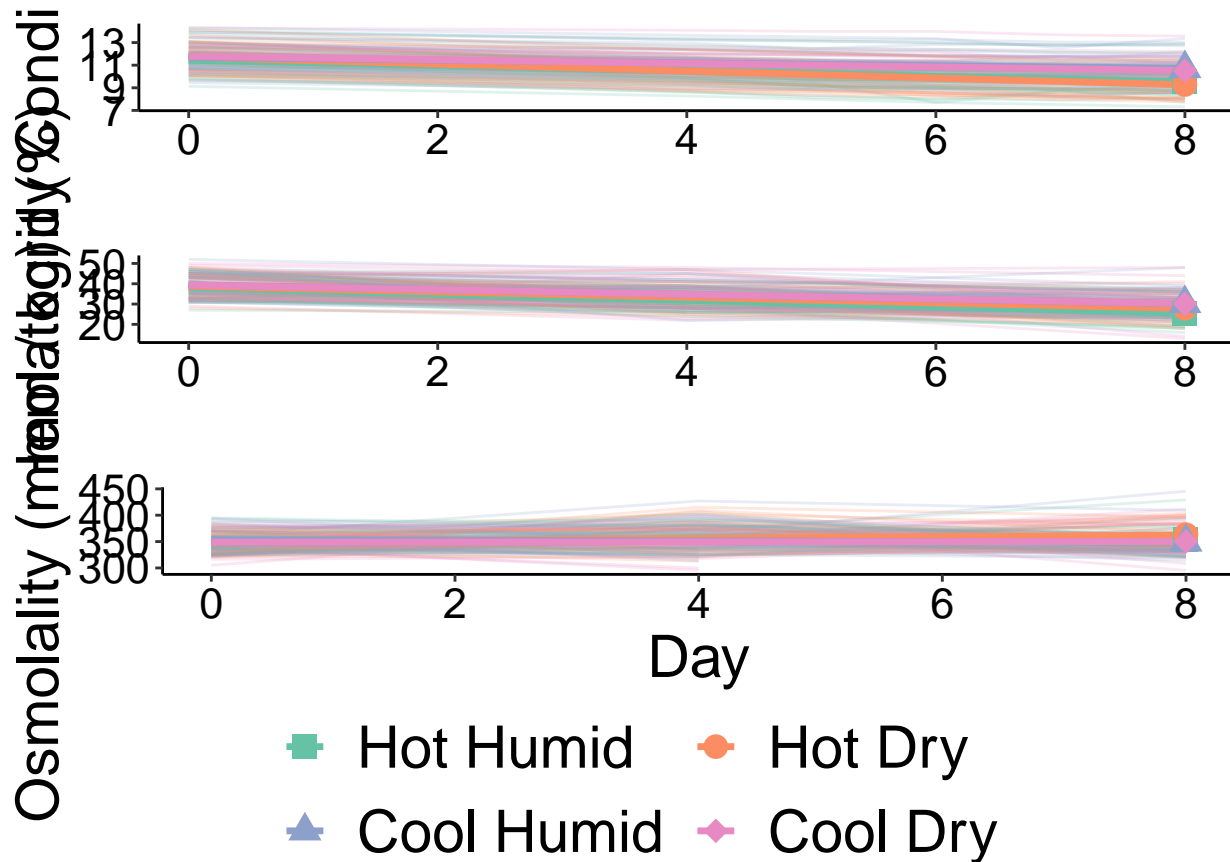


■ Hot Humid ● Hot Dry
▲ Cool Humid ◆ Cool Dry

```
# export figure
ggsave(filename = "tmt_effects_osml.jpeg",
        plot = tmt_effects_osml,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Multi-Figure

```
ggarrange(tmt_effects_SMI,
          tmt_effects_hct,
          tmt_effects_osml,
          ncol = 1, nrow = 3,
          common.legend = TRUE,
          legend = "bottom"
          ) -> tmt_multi_fig
tmt_multi_fig
```



```
# export figure
ggsave(filename = "tmt_multi_fig.jpeg",
  plot = tmt_multi_fig,
  path = "./results_figures",
  device = "jpeg",
  dpi = 1200,
  width = 6, height = 12)
```

CEWL

```
mean_CEWL <- dat %>%
  dplyr::filter(day == "post-exp") %>%
  dplyr::filter(complete.cases(CEWL_g_m2h_mean)) %>%
  group_by(tmt) %>%
  summarise(day = 8,
    mean_CEWL = mean(CEWL_g_m2h_mean))
```

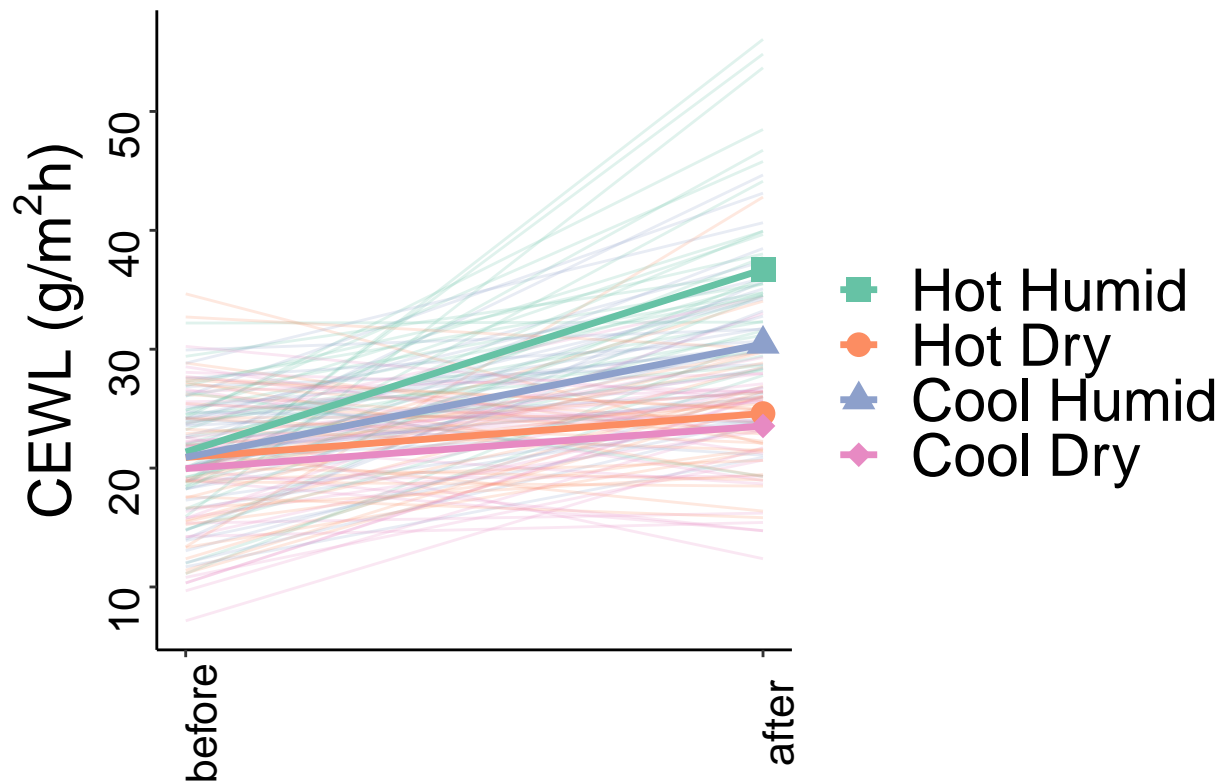
```
## `summarise()` ungrouping output (override with `.groups` argument)
```

```
dat %>%
  dplyr::filter(complete.cases(CEWL_g_m2h_mean)) %>%
  ggplot(aes(x = day_n,
    y = CEWL_g_m2h_mean,
    color = tmt,
  )) +
  geom_line(aes(group = individual_ID),
    alpha = 0.2) +
```

```

stat_smooth(formula = y ~ x,
             method = "lm",
             se = F,
             size = 1.2,
             alpha = 1) +
geom_point(data = mean_CEWL,
           aes(x = day,
               y = mean_CEWL,
               color = tmt,
               shape = tmt),
           size = 4,
           alpha = 1) +
theme_classic() +
scale_shape_manual(values = c(15:18),
                   name = "") +
scale_color_brewer(palette = "Set2",
                   name = "") +
scale_x_continuous(breaks = c(0, 8),
                   labels = c("0" = "before", "8" = "after")
                   ) +
xlab("") +
ylab(bquote('CEWL (g/*m2*h)')) +
theme(text = element_text(color = "black",
                           family = "sans",
                           size = 22),
      axis.text = element_text(color = "black",
                                family = "sans",
                                size = 16,
                                angle = 90),
      legend.text = element_text(color = "black",
                                  family = "sans",
                                  size = 22),
      legend.text.align = 0,
      legend.position = "right"
) -> tmt_effects_CEWL
tmt_effects_CEWL

```

```
# export figure
ggsave(filename = "tmt_effects_CEWL.jpeg",
        plot = tmt_effects_CEWL,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Experiment Models

SMI

```
SMI_mod1 <- lme4::lmer(data = dat_no_rehab,
                      SMI ~
                        day_n*humidity_tmt*temp_tmt +
                        (1|trial_number/individual_ID))
summary(SMI_mod1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## SMI ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## Data: dat_no_rehab
##
## REML criterion at convergence: 540.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -5.5913 -0.4608 -0.0531  0.3972  4.2277
```

```
##
## Random effects:
## Groups Name Variance Std.Dev.
## individual_ID:trial_number (Intercept) 0.9353 0.9671
## trial_number (Intercept) 0.2389 0.4888
## Residual 0.0495 0.2225
## Number of obs: 804, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
## Estimate Std. Error t value
## (Intercept) 11.408711 0.278547 40.958
## day_n -0.237429 0.006124 -38.772
## humidity_tmtDry 0.323569 0.241733 1.339
## temp_tmtCool 0.277697 0.241277 1.151
## day_n:humidity_tmtDry -0.067156 0.008596 -7.812
## day_n:temp_tmtCool 0.114000 0.008596 13.261
## humidity_tmtDry:temp_tmtCool -0.165061 0.341264 -0.484
## day_n:humidity_tmtDry:temp_tmtCool 0.032929 0.012157 2.709
##
## Correlation of Fixed Effects:
## (Intr) day_n hmdt_D tmp_tC dy_:_D dy_:_C h_D:_C
## day_n -0.110
## hmdty_tmtDr -0.441 0.127
## temp_tmtCol -0.440 0.127 0.507
## dy_n:hmdt_D 0.078 -0.712 -0.178 -0.090
## dy_n:tmp_tC 0.078 -0.712 -0.090 -0.178 0.507
## hmdty_tD:_C 0.311 -0.090 -0.706 -0.707 0.126 0.126
## dy_n:h_D:_C -0.055 0.504 0.126 0.126 -0.707 -0.707 -0.178
```

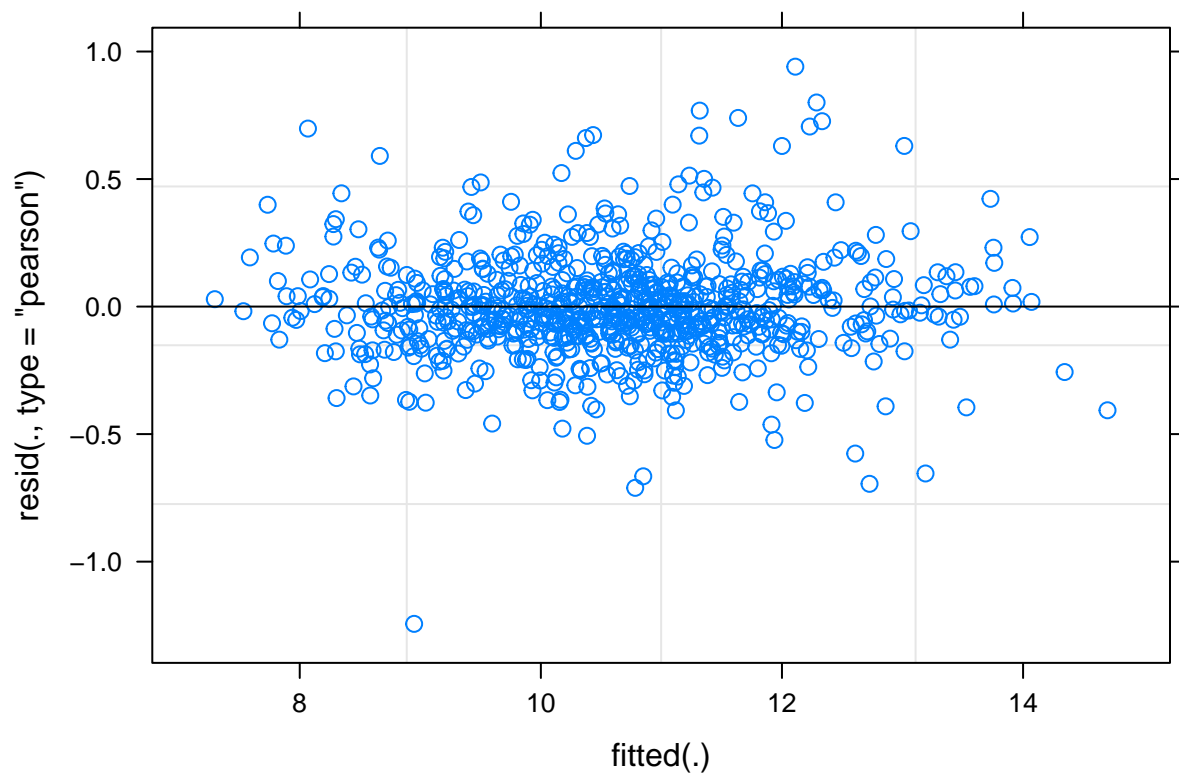
```
drop1(SMI_mod1)
```

```
## Single term deletions
##
## Model:
## SMI ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## npar AIC
## <none> 524.14
## day_n:humidity_tmt:temp_tmt 1 529.48
```

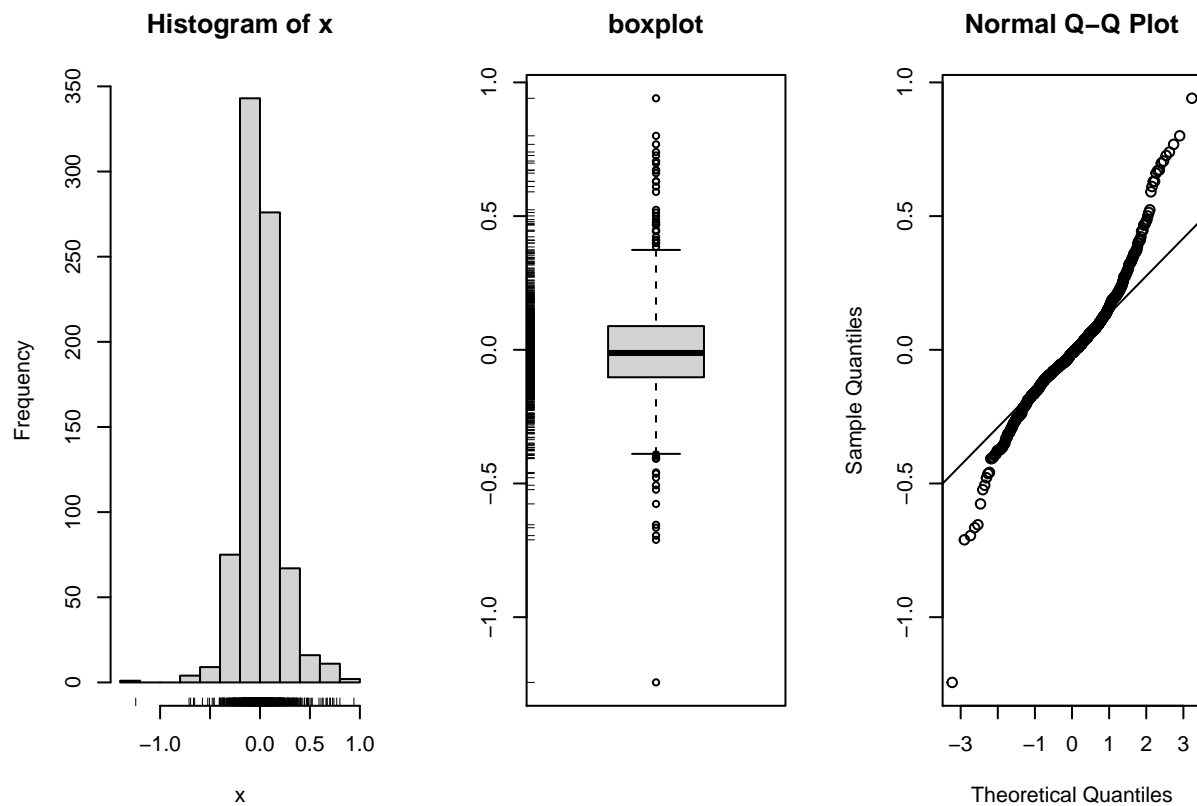
The full model is the best model.

Check linear regression assumptions/conditions:

```
plot(SMI_mod1)
```



```
simple.eda(residuals(SMI_mod1))
```



```

shapiro.test(residuals(SMI_mod1))

##
##  Shapiro-Wilk normality test
##
## data:  residuals(SMI_mod1)
## W = 0.94357, p-value < 2.2e-16

Save best model stats:
SMI_mod_best <- lmerTest::lmer(data = dat_no_rehab,
                             SMI ~
                             day_n*humidity_tmt*temp_tmt +
                             (1|trial_number/individual_ID))
summary(SMI_mod_best)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## SMI ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## Data: dat_no_rehab
##
## REML criterion at convergence: 540.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -5.5913 -0.4608 -0.0531  0.3972  4.2277
##
## Random effects:
## Groups              Name                Variance Std.Dev.
## individual_ID:trial_number (Intercept) 0.9353   0.9671
## trial_number            (Intercept) 0.2389   0.4888
## Residual                  0.0495   0.2225
## Number of obs: 804, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error      df t value
## (Intercept)    11.408711   0.278547   7.742481  40.958
## day_n          -0.237429   0.006124  666.000080 -38.772
## humidity_tmtDry    0.323569   0.241733  134.422291   1.339
## temp_tmtCool       0.277697   0.241277  134.296200   1.151
## day_n:humidity_tmtDry -0.067156   0.008596  666.000082  -7.812
## day_n:temp_tmtCool    0.114000   0.008596  666.000082  13.261
## humidity_tmtDry:temp_tmtCool -0.165061   0.341264  134.303428  -0.484
## day_n:humidity_tmtDry:temp_tmtCool  0.032929   0.012157  666.000083   2.709
##
##              Pr(>|t|)
## (Intercept) 2.48e-10 ***
## day_n       < 2e-16 ***
## humidity_tmtDry 0.18298
## temp_tmtCool  0.25180
## day_n:humidity_tmtDry 2.20e-14 ***
## day_n:temp_tmtCool < 2e-16 ***
## humidity_tmtDry:temp_tmtCool 0.62940
## day_n:humidity_tmtDry:temp_tmtCool 0.00693 **
## ---

```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) day_n  hmdt_D tmp_tC dy_:_D dy_:_C h_D:_C
## day_n          -0.110
## hmdty_tmtDr    -0.441  0.127
## temp_tmtCol   -0.440  0.127  0.507
## dy_n:hmdt_D    0.078 -0.712 -0.178 -0.090
## dy_n:tmp_tC    0.078 -0.712 -0.090 -0.178  0.507
## hmdty_tD:_C    0.311 -0.090 -0.706 -0.707  0.126  0.126
## dy_n:h_D:_C   -0.055  0.504  0.126  0.126 -0.707 -0.707 -0.178

write.csv(broom.mixed::tidy(SMI_mod_best),
          "./results_statistics/exp_effects_SMI.csv")
```

Hematocrit

```
hct_mod1 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
             hematocrit_percent ~
               day_n*humidity_tmt*temp_tmt +
               (1|trial_number/individual_ID))

summary(hct_mod1)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 2379
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.2942 -0.5445 -0.0585  0.5502  2.7781
##
## Random effects:
## Groups              Name                Variance Std.Dev.
## individual_ID:trial_number (Intercept) 12.407   3.522
## trial_number            (Intercept)   6.958   2.638
## Residual                                14.953   3.867
## Number of obs: 398, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    38.2097    1.4673  26.040
## day_n          -1.5536    0.1216 -12.773
## humidity_tmtDry  0.3449    1.2218   0.282
## temp_tmtCool    1.3092    1.2199   1.073
## day_n:humidity_tmtDry  0.1965    0.1697   1.158
## day_n:temp_tmtCool  0.3844    0.1689   2.276
## humidity_tmtDry:temp_tmtCool -0.7963    1.7247  -0.462
## day_n:humidity_tmtDry:temp_tmtCool -0.1676    0.2381  -0.704
##
```

```
## Correlation of Fixed Effects:
##          (Intr) day_n  hmdt_D tmp_tC dy_:_D dy_:_C h_D:_C
## day_n      -0.321
## hmdty_tmtDr -0.423  0.385
## temp_tmtCol -0.423  0.386  0.508
## dy_n:hmdt_D  0.230 -0.717 -0.543 -0.276
## dy_n:tmp_tC  0.231 -0.720 -0.277 -0.544  0.516
## hmdty_tD:_C  0.299 -0.273 -0.706 -0.707  0.385  0.385
## dy_n:h_D:_C -0.164  0.511  0.387  0.386 -0.713 -0.709 -0.546
```

```
drop1(hct_mod1)
```

```
## Single term deletions
```

```
##
```

```
## Model:
```

```
## hematocrit_percent ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
```

```
##          npar      AIC
```

```
## <none>                2398.3
```

```
## day_n:humidity_tmt:temp_tmt    1 2396.8
```

Based on t-values and AIC improvements, we should remove the temp*humidity interaction as well as humidity as a standalone variable:

```
hct_mod2 <- dat_no_rehab %>%
```

```
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
```

```
  lme4::lmer(data = .,
```

```
              hematocrit_percent ~
```

```
              day_n:humidity_tmt + day_n + temp_tmt +
```

```
              (1|trial_number/individual_ID))
```

```
summary(hct_mod2)
```

```
## Linear mixed model fit by REML ['lmerMod']
```

```
## Formula: hematocrit_percent ~ day_n:humidity_tmt + day_n + temp_tmt +
```

```
##      (1 | trial_number/individual_ID)
```

```
##      Data: .
```

```
##
```

```
## REML criterion at convergence: 2387.5
```

```
##
```

```
## Scaled residuals:
```

```
##      Min       1Q   Median       3Q      Max
```

```
## -3.1815 -0.5571 -0.0560  0.5392  2.9775
```

```
##
```

```
## Random effects:
```

```
##      Groups              Name              Variance Std.Dev.
```

```
## individual_ID:trial_number (Intercept) 12.189   3.491
```

```
## trial_number              (Intercept)   6.991   2.644
```

```
## Residual                      15.225   3.902
```

```
## Number of obs: 398, groups: individual_ID:trial_number, 134; trial_number, 5
```

```
##
```

```
## Fixed effects:
```

```
##              Estimate Std. Error t value
```

```
## (Intercept)    37.78979    1.31037  28.839
```

```
## day_n          -1.35225    0.07853 -17.220
```

```
## temp_tmtCool    2.09520    0.72014   2.909
```

```
## day_n:humidity_tmtDry 0.10158    0.10039   1.012
```

```
##
```

```
## Correlation of Fixed Effects:
##           (Intr) day_n  tmp_tC
## day_n      -0.135
## temp_tmtCol -0.275 -0.015
## dy_n:hmdt_D -0.004 -0.644  0.014

anova(hct_mod1, hct_mod2)

## refitting model(s) with ML (instead of REML)

## Data: .
## Models:
## hct_mod2: hematocrit_percent ~ day_n:humidity_tmt + day_n + temp_tmt +
## hct_mod2:      (1 | trial_number/individual_ID)
## hct_mod1: hematocrit_percent ~ day_n * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
##           npar      AIC      BIC logLik deviance Chisq Df Pr(>Chisq)
## hct_mod2      7 2398.2 2426.1 -1192.1  2384.2
## hct_mod1     11 2398.3 2442.2 -1188.2  2376.3 7.8844  4   0.09591 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
drop1(hct_mod2)
```

```
## Single term deletions
##
## Model:
## hematocrit_percent ~ day_n:humidity_tmt + day_n + temp_tmt +
##      (1 | trial_number/individual_ID)
##           npar      AIC
## <none>           2398.2
## temp_tmt           1 2404.5
## day_n:humidity_tmt   1 2397.3
```

We should drop the day* humidity interaction.

```
hct_mod3 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
             hematocrit_percent ~
               day_n + temp_tmt +
               (1|trial_number/individual_ID))
summary(hct_mod3)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 2385.7
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.11072 -0.56258 -0.04207  0.57441  2.90790
##
## Random effects:
## Groups              Name             Variance Std.Dev.
## individual_ID:trial_number (Intercept) 12.165   3.488
```

```
## trial_number          (Intercept)  7.102   2.665
## Residual              15.232   3.903
## Number of obs: 398, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)  37.79474    1.31872  28.660
## day_n        -1.30105    0.06007 -21.660
## temp_tmtCool  2.08472    0.71963   2.897
##
## Correlation of Fixed Effects:
##           (Intr) day_n
## day_n      -0.178
## temp_tmtCol -0.273 -0.008
anova(hct_mod2, hct_mod3)

## refitting model(s) with ML (instead of REML)

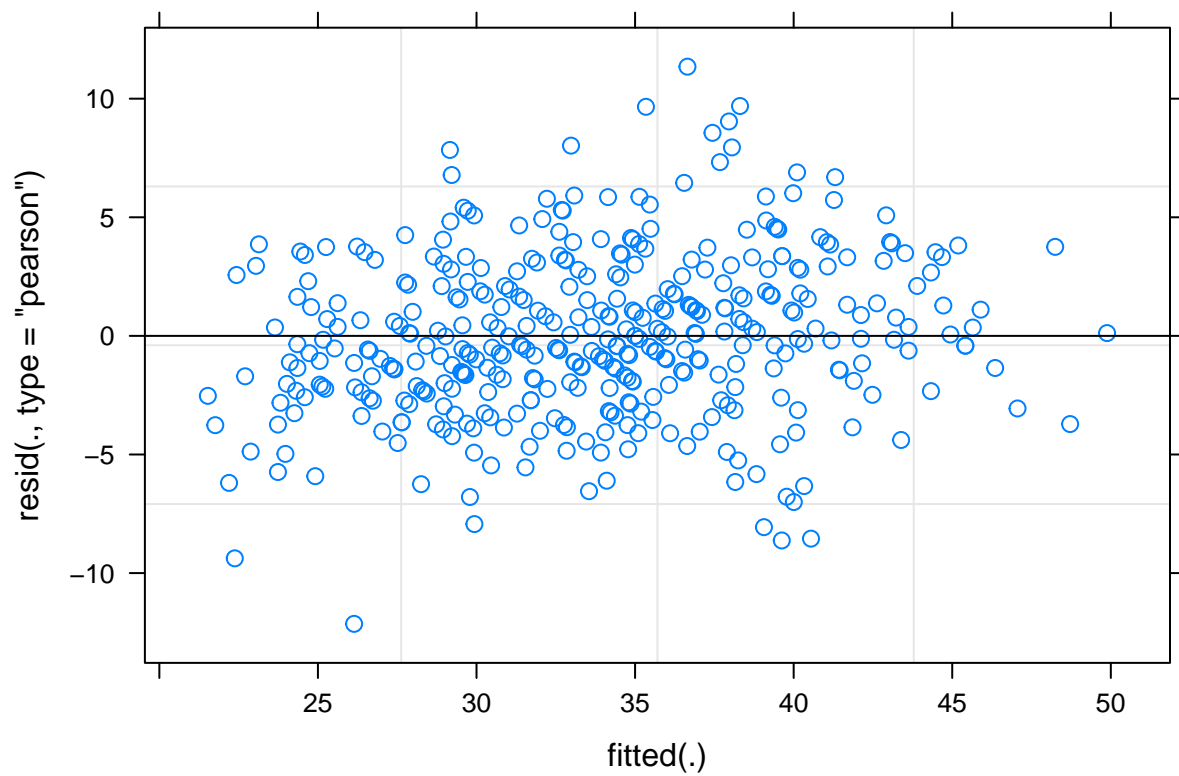
## Data: .
## Models:
## hct_mod3: hematocrit_percent ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
## hct_mod2: hematocrit_percent ~ day_n:humidity_tmt + day_n + temp_tmt +
## hct_mod2:      (1 | trial_number/individual_ID)
##           npar      AIC      BIC logLik deviance Chisq Df Pr(>Chisq)
## hct_mod3      6 2397.3 2421.2 -1192.6  2385.3
## hct_mod2      7 2398.2 2426.1 -1192.1  2384.2 1.0432  1    0.3071
drop1(hct_mod3)

## Single term deletions
##
## Model:
## hematocrit_percent ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
##           npar      AIC
## <none>        2397.3
## day_n         1 2665.5
## temp_tmt      1 2403.5
```

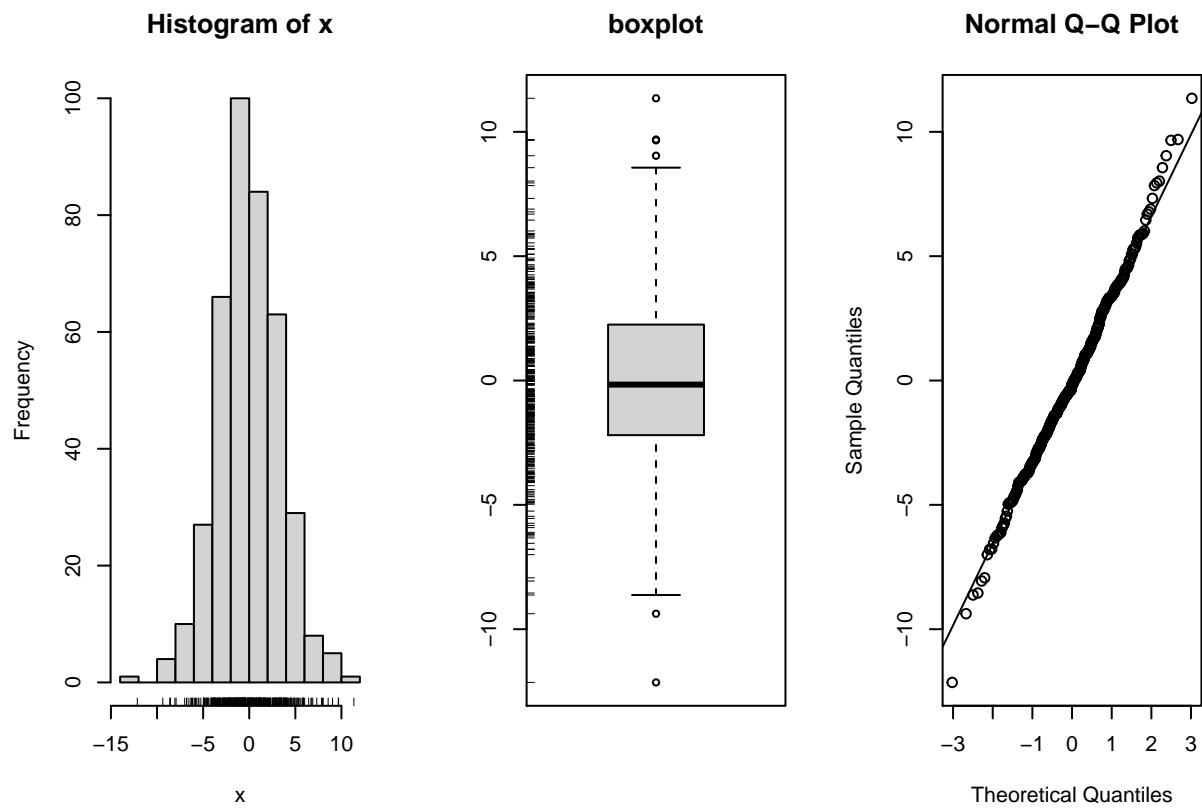
We do not lose predictive power, and AIC suggests not dropping any more variables. This is the best model.

Check linear regression assumptions/conditions:

```
plot(hct_mod3)
```

```
simple.eda(residuals(hct_mod3))
```



```

shapiro.test(residuals(hct_mod3))

##
## Shapiro-Wilk normality test
##
## data: residuals(hct_mod3)
## W = 0.99603, p-value = 0.4209

Save best model stats:

hct_mod_best <- dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lmerTest::lmer(data = .,
                 hematocrit_percent ~
                   day_n + temp_tmt +
                   (1|trial_number/individual_ID))
summary(hct_mod_best)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## hematocrit_percent ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 2385.7
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.11072 -0.56258 -0.04207  0.57441  2.90790
##
## Random effects:
## Groups              Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 12.165   3.488
## trial_number              (Intercept)  7.102   2.665
## Residual                  15.232   3.903
## Number of obs: 398, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)  37.79474    1.31872  4.95996  28.660 1.06e-06 ***
## day_n        -1.30105    0.06007 264.55052 -21.660 < 2e-16 ***
## temp_tmtCool  2.08472    0.71963 128.00945   2.897 0.00443 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) day_n
## day_n        -0.178
## temp_tmtCol -0.273 -0.008

write.csv(broom::tidy(hct_mod_best),
          "./results_statistics/exp_effects_hct.csv")

```

Osmolality

```
osml_mod1 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               day_n*humidity_tmt*temp_tmt +
               (1|trial_number/individual_ID))

summary(osml_mod1)

## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ day_n * humidity_tmt * temp_tmt + (1 |
##      trial_number/individual_ID)
##      Data: .
##
## REML criterion at convergence: 3553.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.4680 -0.7182 -0.1420  0.6284  4.0713
##
## Random effects:
##      Groups                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 12.23    3.497
## trial_number              (Intercept) 11.60    3.406
## Residual                    537.81   23.191
## Number of obs: 391, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)    350.7348    4.0472  86.661
## day_n           0.4808    0.7193   0.668
## humidity_tmtDry -0.5965    5.2582  -0.113
## temp_tmtCool    3.3416    5.2548   0.636
## day_n:humidity_tmtDry 1.1063    1.0178   1.087
## day_n:temp_tmtCool -1.1269    1.0178  -1.107
## humidity_tmtDry:temp_tmtCool -4.1327    7.4296  -0.556
## day_n:humidity_tmtDry:temp_tmtCool -0.3301    1.4454  -0.228
##
## Correlation of Fixed Effects:
##      (Intr) day_n  hmdt_D tmp_tC dy_:_D dy_:_C h_D:_C
## day_n      -0.703
## hmdty_tmtDr -0.660  0.541
## temp_tmtCol -0.660  0.542  0.508
## dy_n:hmdt_D 0.497 -0.707 -0.758 -0.383
## dy_n:tmp_tC 0.498 -0.706 -0.383 -0.759  0.500
## hmdty_tD:_C 0.466 -0.383 -0.707 -0.707  0.537  0.537
## dy_n:h_D:_C -0.350  0.497  0.534  0.534 -0.704 -0.704 -0.758

drop1(osml_mod1)

## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ day_n * humidity_tmt * temp_tmt + (1 |
```

```

##      trial_number/individual_ID)
##                                npar      AIC
## <none>                        3595.2
## day_n:humidity_tmt:temp_tmt    1 3593.2

Drop temperature from the 3-way interaction:

osml_mod2 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               day_n * humidity_tmt + temp_tmt +
               (1|trial_number/individual_ID))
summary(osml_mod2)

## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ day_n * humidity_tmt + temp_tmt + (1 |
##      trial_number/individual_ID)
##      Data: .
##
## REML criterion at convergence: 3566.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.6465 -0.7128 -0.1399  0.6169  4.0249
##
## Random effects:
##      Groups                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 10.68    3.268
## trial_number                (Intercept) 11.54    3.396
## Residual                    541.38   23.268
## Number of obs: 391, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    354.3311     3.2867 107.808
## day_n          -0.0858     0.5107  -0.168
## humidity_tmtDry -2.7421     3.7252  -0.736
## temp_tmtCool   -3.7456     2.4219  -1.547
## day_n:humidity_tmtDry  0.9581     0.7250   1.321
##
## Correlation of Fixed Effects:
##              (Intr) day_n  hmdt_D tmp_tC
## day_n        -0.611
## hmdty_tmtDr  -0.572  0.536
## temp_tmtCol -0.378  0.008  0.014
## dy_n:hmdt_D  0.431 -0.704 -0.758 -0.006

anova(osml_mod1, osml_mod2)

## refitting model(s) with ML (instead of REML)
## Data: .
## Models:
## osml_mod2: osmolality_mmol_kg_mean ~ day_n * humidity_tmt + temp_tmt + (1 |
## osml_mod2:      trial_number/individual_ID)

```

```
## osml_mod1: osmolality_mmol_kg_mean ~ day_n * humidity_tmt * temp_tmt + (1 |
## osml_mod1:      trial_number/individual_ID)
##      npar      AIC      BIC logLik deviance Chisq Df Pr(>Chisq)
## osml_mod2      8 3593.7 3625.4 -1788.8   3577.7
## osml_mod1     11 3595.2 3638.8 -1786.6   3573.2 4.5097  3      0.2114
```

```
drop1(osml_mod2)
```

```
## Single term deletions
```

```
##
```

```
## Model:
```

```
## osmolality_mmol_kg_mean ~ day_n * humidity_tmt + temp_tmt + (1 |
```

```
##      trial_number/individual_ID)
```

```
##      npar      AIC
```

```
## <none>          3593.7
```

```
## temp_tmt        1 3594.1
```

```
## day_n:humidity_tmt 1 3593.4
```

Drop the day* humidity interaction:

```
osml_mod3 <- dat_no_rehab %>%
```

```
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
```

```
  lme4::lmer(data = .,
```

```
              osmolality_mmol_kg_mean ~
```

```
              day_n + humidity_tmt + temp_tmt +
```

```
              (1|trial_number/individual_ID))
```

```
summary(osml_mod3)
```

```
## Linear mixed model fit by REML ['lmerMod']
```

```
## Formula: osmolality_mmol_kg_mean ~ day_n + humidity_tmt + temp_tmt + (1 |
```

```
##      trial_number/individual_ID)
```

```
## Data: .
```

```
##
```

```
## REML criterion at convergence: 3569.4
```

```
##
```

```
## Scaled residuals:
```

```
##      Min      1Q  Median      3Q      Max
```

```
## -2.5592 -0.7250 -0.1629  0.6100  3.9372
```

```
##
```

```
## Random effects:
```

```
## Groups              Name      Variance Std.Dev.
```

```
## individual_ID:trial_number (Intercept) 10.94   3.307
```

```
## trial_number              (Intercept) 11.51   3.393
```

```
## Residual                  542.22  23.286
```

```
## Number of obs: 391, groups: individual_ID:trial_number, 134; trial_number, 5
```

```
##
```

```
## Fixed effects:
```

```
##      Estimate Std. Error t value
```

```
## (Intercept)  352.4603    2.9679 118.756
```

```
## day_n        0.3889     0.3633   1.070
```

```
## humidity_tmtDry 0.9905     2.4316   0.407
```

```
## temp_tmtCool  -3.7272     2.4252  -1.537
```

```
##
```

```
## Correlation of Fixed Effects:
```

```
##      (Intr) day_n  hmdt_D
```

```
## day_n      -0.480
```

```
## hmdty_tmtDr -0.417 0.004
## temp_tmtCol -0.417 0.006 0.014
anova(osml_mod2, osml_mod3)

## refitting model(s) with ML (instead of REML)

## Data: .
## Models:
## osml_mod3: osmolality_mmol_kg_mean ~ day_n + humidity_tmt + temp_tmt + (1 |
## osml_mod3: trial_number/individual_ID)
## osml_mod2: osmolality_mmol_kg_mean ~ day_n * humidity_tmt + temp_tmt + (1 |
## osml_mod2: trial_number/individual_ID)
##          npar    AIC    BIC logLik deviance Chisq Df Pr(>Chisq)
## osml_mod3     7 3593.4 3621.2 -1789.7   3579.4
## osml_mod2     8 3593.7 3625.4 -1788.8   3577.7 1.758 1    0.1849

drop1(osml_mod3)

## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ day_n + humidity_tmt + temp_tmt + (1 |
## trial_number/individual_ID)
##          npar    AIC
## <none>          3593.4
## day_n           1 3592.6
## humidity_tmt     1 3591.6
## temp_tmt         1 3593.8

Drop humidity:
osml_mod4 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               day_n + temp_tmt +
               (1|trial_number/individual_ID))
summary(osml_mod4)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## osmolality_mmol_kg_mean ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 3573.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.5390 -0.7138 -0.1561  0.6040  3.9262
##
## Random effects:
##      Groups              Name              Variance Std.Dev.
## individual_ID:trial_number (Intercept)    9.812    3.132
## trial_number              (Intercept)   11.287    3.360
## Residual                                542.155   23.284
## Number of obs: 391, groups: individual_ID:trial_number, 134; trial_number, 5
```

```

##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept) 352.9616      2.6860 131.410
## day_n        0.3890      0.3632   1.071
## temp_tmtCool -3.7404      2.4178  -1.547
##
## Correlation of Fixed Effects:
##           (Intr) day_n
## day_n      -0.528
## temp_tmtCol -0.453  0.006
anova(osml_mod3, osml_mod4)

## refitting model(s) with ML (instead of REML)

## Data: .
## Models:
## osml_mod4: osmolality_mmol_kg_mean ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
## osml_mod3: osmolality_mmol_kg_mean ~ day_n + humidity_tmt + temp_tmt + (1 |
## osml_mod3: trial_number/individual_ID)
##           npar      AIC      BIC logLik deviance Chisq Df Pr(>Chisq)
## osml_mod4      6 3591.6 3615.4 -1789.8   3579.6
## osml_mod3      7 3593.4 3621.2 -1789.7   3579.4 0.157  1    0.6919
drop1(osml_mod4)

## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ day_n + temp_tmt + (1 | trial_number/individual_ID)
##           npar      AIC
## <none>         3591.6
## day_n          1 3590.7
## temp_tmt       1 3592.0

We do not lose predictive power. Drop day:

osml_mod5 <- dat_no_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               temp_tmt +
               (1|trial_number/individual_ID))
summary(osml_mod5)

## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 3574.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.4733 -0.6993 -0.1804  0.6000  3.9889
##
## Random effects:

```

```

## Groups               Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 11.65   3.413
## trial_number           (Intercept) 11.31   3.363
## Residual                540.62  23.251
## Number of obs: 391, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  354.476    2.287 155.016
## temp_tmtCool  -3.757    2.426  -1.549
##
## Correlation of Fixed Effects:
##             (Intr)
## temp_tmtCol -0.530
anova(osml_mod3, osml_mod5)

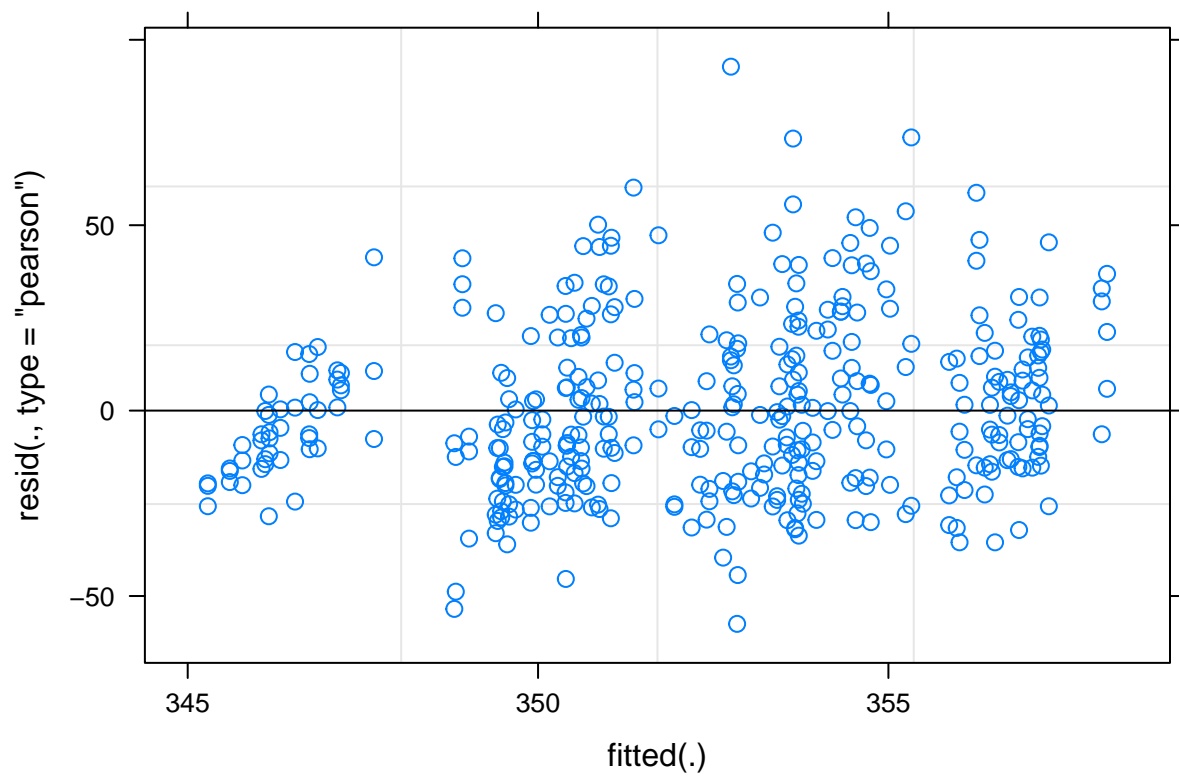
## refitting model(s) with ML (instead of REML)

## Data: .
## Models:
## osml_mod5: osmolality_mmol_kg_mean ~ temp_tmt + (1 | trial_number/individual_ID)
## osml_mod3: osmolality_mmol_kg_mean ~ day_n + humidity_tmt + temp_tmt + (1 |
## osml_mod3: trial_number/individual_ID)
##           npar    AIC    BIC logLik deviance Chisq Df Pr(>Chisq)
## osml_mod5    5 3590.7 3610.6 -1790.4  3580.7
## osml_mod3    7 3593.4 3621.2 -1789.7  3579.4 1.3131  2    0.5186
drop1(osml_mod5)

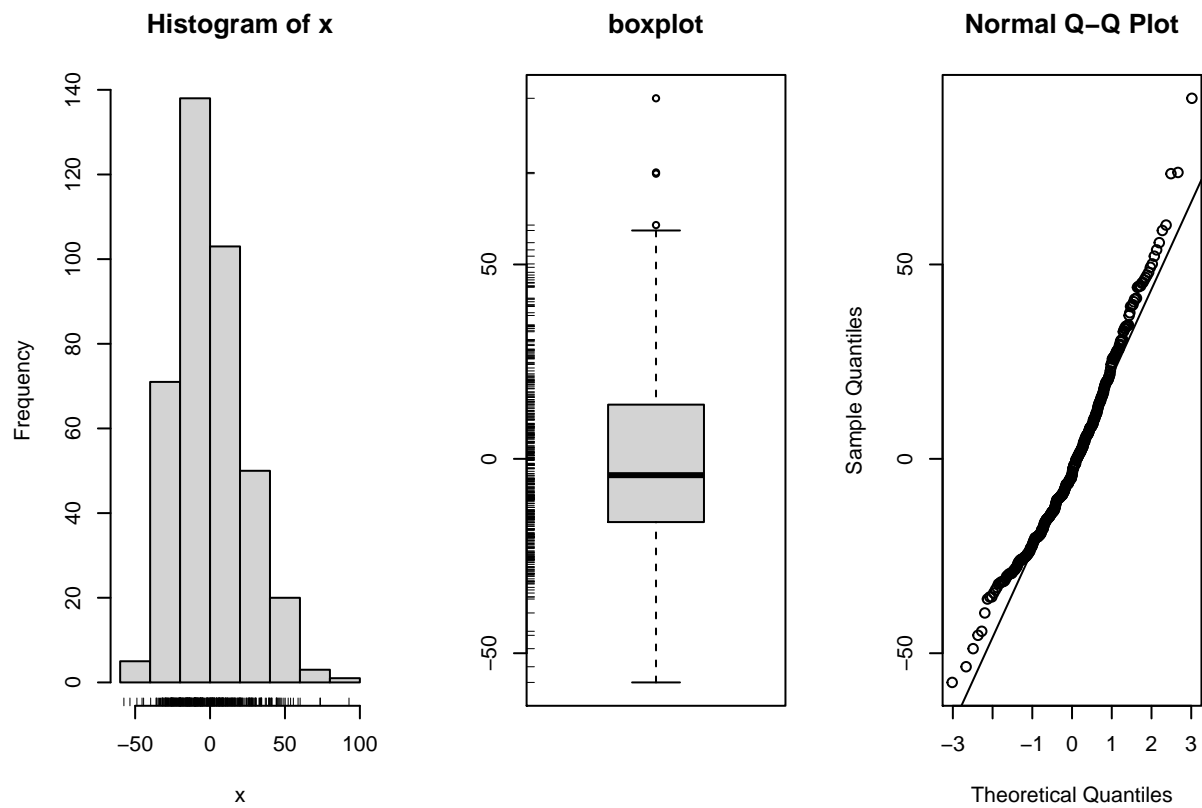
## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ temp_tmt + (1 | trial_number/individual_ID)
##           npar    AIC
## <none>        3590.7
## temp_tmt     1 3591.1

This is the best model, with only temperature as a predictor.
Check linear regression assumptions/conditions:
plot(osml_mod5)

```

```
simple.eda(residuals(osml_mod5))
```



```
shapiro.test(residuals(osml_mod5))
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: residuals(osml_mod5)  
## W = 0.97201, p-value = 7.746e-07
```

Save best model stats:

```
osml_mod_best <- dat_no_rehab %>%  
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%  
  lmerTest::lmer(data = .,  
                 osmolality_mmol_kg_mean ~  
                 temp_tmt +  
                 (1|trial_number/individual_ID))  
summary(osml_mod_best)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: osmolality_mmol_kg_mean ~ temp_tmt + (1 | trial_number/individual_ID)  
## Data: .  
##  
## REML criterion at convergence: 3574.1  
##  
## Scaled residuals:  
##      Min       1Q   Median       3Q      Max   
## -2.4733 -0.6993 -0.1804  0.6000  3.9889   
##  
## Random effects:  
## Groups                Name      Variance Std.Dev.  
## individual_ID:trial_number (Intercept) 11.65   3.413  
## trial_number              (Intercept) 11.31   3.363  
## Residual                  540.62  23.251  
## Number of obs: 391, groups: individual_ID:trial_number, 134; trial_number, 5  
##  
## Fixed effects:  
##              Estimate Std. Error    df t value Pr(>|t|)      
## (Intercept)  354.476    2.287   8.167 155.016 1.85e-15 ***  
## temp_tmtCool  -3.757    2.426 123.484  -1.549   0.124      
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
##  
## Correlation of Fixed Effects:  
##              (Intr)  
## temp_tmtCol -0.530
```

```
write.csv(broom::tidy(osml_mod_best),  
          "./results_statistics/exp_effects_osml.csv")
```

CEWL

```
CEWL_mod1a <- lme4::lmer(data = dat_no_rehab,  
                        CEWL_g_m2h_mean ~  
                        day_n*humidity_tmt*temp_tmt +
```

```

                                cloacal_temp_C +
                                (1|trial_number/individual_ID))

## boundary (singular) fit: see ?isSingular

summary(CEWL_mod1a)

## Linear mixed model fit by REML ['lmerMod']
## Formula: CEWL_g_m2h_mean ~ day_n * humidity_tmt * temp_tmt + cloacal_temp_C +
##      (1 | trial_number/individual_ID)
##      Data: dat_no_rehab
##
## REML criterion at convergence: 1658.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.7135 -0.6132 -0.0884  0.5389  3.8868
##
## Random effects:
##      Groups                Name         Variance Std.Dev.
## individual_ID:trial_number (Intercept) 5.446e-12 2.334e-06
## trial_number                (Intercept) 7.798e+00 2.792e+00
## Residual                    2.860e+01 5.348e+00
## Number of obs: 267, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)      56.9778     9.6182   5.924
## day_n              1.6681     0.1775   9.397
## humidity_tmtDry    -0.4167     1.3085  -0.318
## temp_tmtCool       -0.2284     1.3076  -0.175
## cloacal_temp_C     -1.3485     0.3585  -3.762
## day_n:humidity_tmtDry -1.3446     0.2329  -5.773
## day_n:temp_tmtCool  -0.7156     0.2320  -3.085
## humidity_tmtDry:temp_tmtCool -0.7086     1.8500  -0.383
## day_n:humidity_tmtDry:temp_tmtCool  0.7486     0.3274   2.286
##
## Correlation of Fixed Effects:
##              (Intr) day_n  hmdt_D tmp_tC clc__C dy_:_D dy_:_C h_D:_C
## day_n          -0.433
## hmdty_tmtDr   -0.080  0.471
## temp_tmtCol  -0.042  0.456  0.507
## clocl_tmp_C  -0.987  0.375  0.011 -0.028
## dy_n:hmdt_D   0.174 -0.703 -0.702 -0.352 -0.127
## dy_n:tmp_tC   0.071 -0.666 -0.357 -0.703 -0.023  0.504
## hmdty_tD:_C   0.010 -0.315 -0.705 -0.707  0.039  0.490  0.496
## dy_n:h_D:_C  -0.013  0.458  0.498  0.499 -0.022 -0.697 -0.708 -0.706
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see ?isSingular

drop1(CEWL_mod1a)

## boundary (singular) fit: see ?isSingular
## boundary (singular) fit: see ?isSingular

## Single term deletions

```

```
##
## Model:
## CEWL_g_m2h_mean ~ day_n * humidity_tmt * temp_tmt + cloacal_temp_C +
##   (1 | trial_number/individual_ID)
##               npar    AIC
## <none>                1681.5
## cloacal_temp_C        1 1693.3
## day_n:humidity_tmt:temp_tmt 1 1684.8
```

Actually, re-run without individual ID as a random effect, since it accounts for none of the variation in CEWL:

```
CEWL_mod1b <- lme4::lmer(data = dat_no_rehab,
                        CEWL_g_m2h_mean ~
                          day_n*humidity_tmt*temp_tmt +
                          cloacal_temp_C +
                          (1|trial_number))
summary(CEWL_mod1b)

## Linear mixed model fit by REML ['lmerMod']
## Formula: CEWL_g_m2h_mean ~ day_n * humidity_tmt * temp_tmt + cloacal_temp_C +
##   (1 | trial_number)
##   Data: dat_no_rehab
##
## REML criterion at convergence: 1658.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.7135 -0.6132 -0.0884  0.5389  3.8868
##
## Random effects:
##   Groups       Name             Variance Std.Dev.
##   trial_number (Intercept)  7.798     2.792
##   Residual                28.602     5.348
## Number of obs: 267, groups: trial_number, 5
##
## Fixed effects:
##               Estimate Std. Error t value
## (Intercept)      56.9778    9.6182   5.924
## day_n             1.6681    0.1775   9.397
## humidity_tmtDry   -0.4167    1.3085  -0.318
## temp_tmtCool      -0.2284    1.3076  -0.175
## cloacal_temp_C    -1.3485    0.3585  -3.762
## day_n:humidity_tmtDry -1.3446    0.2329  -5.773
## day_n:temp_tmtCool -0.7156    0.2320  -3.085
## humidity_tmtDry:temp_tmtCool -0.7086    1.8500  -0.383
## day_n:humidity_tmtDry:temp_tmtCool 0.7486    0.3274   2.286
##
## Correlation of Fixed Effects:
##              (Intr) day_n  hmdt_D tmp_tC clc__C dy_:_D dy_:_C h_D:_C
## day_n        -0.433
## hmdty_tmtDr  -0.080  0.471
## temp_tmtCol  -0.042  0.456  0.507
## clocl_tmp_C  -0.987  0.375  0.011 -0.028
## dy_n:hmdt_D  0.174 -0.703 -0.702 -0.352 -0.127
```

```
## dy_n:tmt_tC  0.071 -0.666 -0.357 -0.703 -0.023  0.504
## hmdty_tD:_C  0.010 -0.315 -0.705 -0.707  0.039  0.490  0.496
## dy_n:h_D:_C -0.013  0.458  0.498  0.499 -0.022 -0.697 -0.708 -0.706
```

```
drop1(CEWL_mod1b)
```

```
## Single term deletions
##
## Model:
## CEWL_g_m2h_mean ~ day_n * humidity_tmt * temp_tmt + cloacal_temp_C +
## (1 | trial_number)
##
##               npar      AIC
## <none>                1679.5
## cloacal_temp_C         1 1691.3
## day_n:humidity_tmt:temp_tmt  1 1682.8
```

Based on AIC, this is the best model. Based on t-values, let's try dropping the individual effects of temperature and humidity since they have the lowest t-values.

```
CEWL_mod2 <- lme4::lmer(data = dat_no_rehab,
                        CEWL_g_m2h_mean ~
                        day_n:humidity_tmt:temp_tmt +
                        humidity_tmt:temp_tmt +
                        day_n:temp_tmt +
                        day_n:humidity_tmt +
                        day_n +
                        cloacal_temp_C +
                        (1|trial_number))
```

fixed-effect model matrix is rank deficient so dropping 1 column / coefficient

```
summary(CEWL_mod2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + humidity_tmt:temp_tmt +
## day_n:temp_tmt + day_n:humidity_tmt + day_n + cloacal_temp_C +
## (1 | trial_number)
## Data: dat_no_rehab
##
## REML criterion at convergence: 1658.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.7135 -0.6132 -0.0884  0.5389  3.8868
##
## Random effects:
##  Groups      Name      Variance Std.Dev.
## trial_number (Intercept)  7.798   2.792
## Residual                28.602   5.348
## Number of obs: 267, groups: trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)    55.6241    9.5674   5.814
## day_n           1.6681    0.1775   9.397
## cloacal_temp_C  -1.3485    0.3585  -3.762
```

```

## humidity_tmtHumid:temp_tmtHot      1.3537      1.3201      1.025
## humidity_tmtDry:temp_tmtHot         0.9370      1.3079      0.716
## humidity_tmtHumid:temp_tmtCool      1.1254      1.3117      0.858
## day_n:temp_tmtCool                  -0.7156      0.2320     -3.085
## day_n:humidity_tmtDry               -1.3446      0.2329     -5.773
## day_n:humidity_tmtDry:temp_tmtCool   0.7486      0.3274      2.286
##
## Correlation of Fixed Effects:
##          (Intr) day_n  clc__C h_H:_H h_D:_H h_H:_C dy_:_C dy_:_D
## day_n      -0.370
## clocl_tmp_C -0.987  0.375
## hmdty_tH:_H -0.030 -0.477 -0.039
## hmdty_tD:_H -0.042 -0.010 -0.028  0.504
## hmdty_tH:_C -0.003 -0.025 -0.067  0.506  0.508
## dy_n:tmp_tC  0.023 -0.666 -0.023  0.355  0.001 -0.344
## dy_n:hmdt_D  0.126 -0.703 -0.127  0.357 -0.342  0.009  0.504
## dy_n:h_D:_C -0.013  0.458 -0.022  0.001  0.499  0.498 -0.708 -0.697
## fit warnings:
## fixed-effect model matrix is rank deficient so dropping 1 column / coefficient
anova(CEWL_mod1b, CEWL_mod2)

## refitting model(s) with ML (instead of REML)

## Data: dat_no_rehab
## Models:
## CEWL_mod1b: CEWL_g_m2h_mean ~ day_n * humidity_tmt * temp_tmt + cloacal_temp_C +
## CEWL_mod1b:      (1 | trial_number)
## CEWL_mod2: CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + humidity_tmt:temp_tmt +
## CEWL_mod2:      day_n:temp_tmt + day_n:humidity_tmt + day_n + cloacal_temp_C +
## CEWL_mod2:      (1 | trial_number)
##          npar    AIC    BIC   logLik deviance Chisq Df Pr(>Chisq)
## CEWL_mod1b   11 1679.5 1719 -828.76   1657.5
## CEWL_mod2    11 1679.5 1719 -828.76   1657.5      0  0
drop1(CEWL_mod2)

## fixed-effect model matrix is rank deficient so dropping 1 column / coefficient
## fixed-effect model matrix is rank deficient so dropping 1 column / coefficient

## Single term deletions
##
## Model:
## CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + humidity_tmt:temp_tmt +
##      day_n:temp_tmt + day_n:humidity_tmt + day_n + cloacal_temp_C +
##      (1 | trial_number)
##          npar    AIC
## <none>          1679.5
## cloacal_temp_C      1 1691.3
## day_n:humidity_tmt:temp_tmt  1 1682.8

It seems like we lost no predictive power. We can try dropping the interaction between temperature and
humidity:
CEWL_mod3 <- lme4::lmer(data = dat_no_rehab,
                      CEWL_g_m2h_mean ~
                      day_n:humidity_tmt:temp_tmt +

```

```

        day_n:temp_tmt +
        day_n:humidity_tmt +
        day_n +
        cloacal_temp_C +
        (1|trial_number))
summary(CEWL_mod3)

## Linear mixed model fit by REML ['lmerMod']
## Formula: CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + day_n:temp_tmt +
##      day_n:humidity_tmt + day_n + cloacal_temp_C + (1 | trial_number)
##      Data: dat_no_rehab
##
## REML criterion at convergence: 1666
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.7253 -0.6161 -0.0815  0.5676  3.9063
##
## Random effects:
##      Groups          Name          Variance Std.Dev.
## trial_number (Intercept)  7.839      2.800
## Residual                28.402      5.329
## Number of obs: 267, groups: trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)      55.9226     9.5233   5.872
## day_n              1.7338     0.1451  11.952
## cloacal_temp_C    -1.3274     0.3564  -3.724
## day_n:temp_tmtCool -0.7447     0.1643  -4.532
## day_n:humidity_tmtDry -1.3979     0.1653  -8.456
## day_n:humidity_tmtDry:temp_tmtCool  0.6603     0.2312   2.856
##
## Correlation of Fixed Effects:
##              (Intr) day_n  clc__C dy_:_C dy_:_D
## day_n          -0.465
## clocl_tmp_C    -0.990  0.450
## dy_n:tmp_tC    0.059 -0.591 -0.060
## dy_n:hmdt_D    0.165 -0.638 -0.167  0.506
## dy_n:h_D:_C    -0.008  0.405  0.008 -0.709 -0.695
anova(CEWL_mod2, CEWL_mod3)

## refitting model(s) with ML (instead of REML)
## Data: dat_no_rehab
## Models:
## CEWL_mod3: CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + day_n:temp_tmt +
## CEWL_mod3:      day_n:humidity_tmt + day_n + cloacal_temp_C + (1 | trial_number)
## CEWL_mod2: CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + humidity_tmt:temp_tmt +
## CEWL_mod2:      day_n:temp_tmt + day_n:humidity_tmt + day_n + cloacal_temp_C +
## CEWL_mod2:      (1 | trial_number)
##              npar      AIC      BIC logLik deviance Chisq Df Pr(>Chisq)
## CEWL_mod3       8 1674.8 1703.5 -829.38  1658.8
## CEWL_mod2      11 1679.5 1719.0 -828.76  1657.5 1.2559  3      0.7396

```

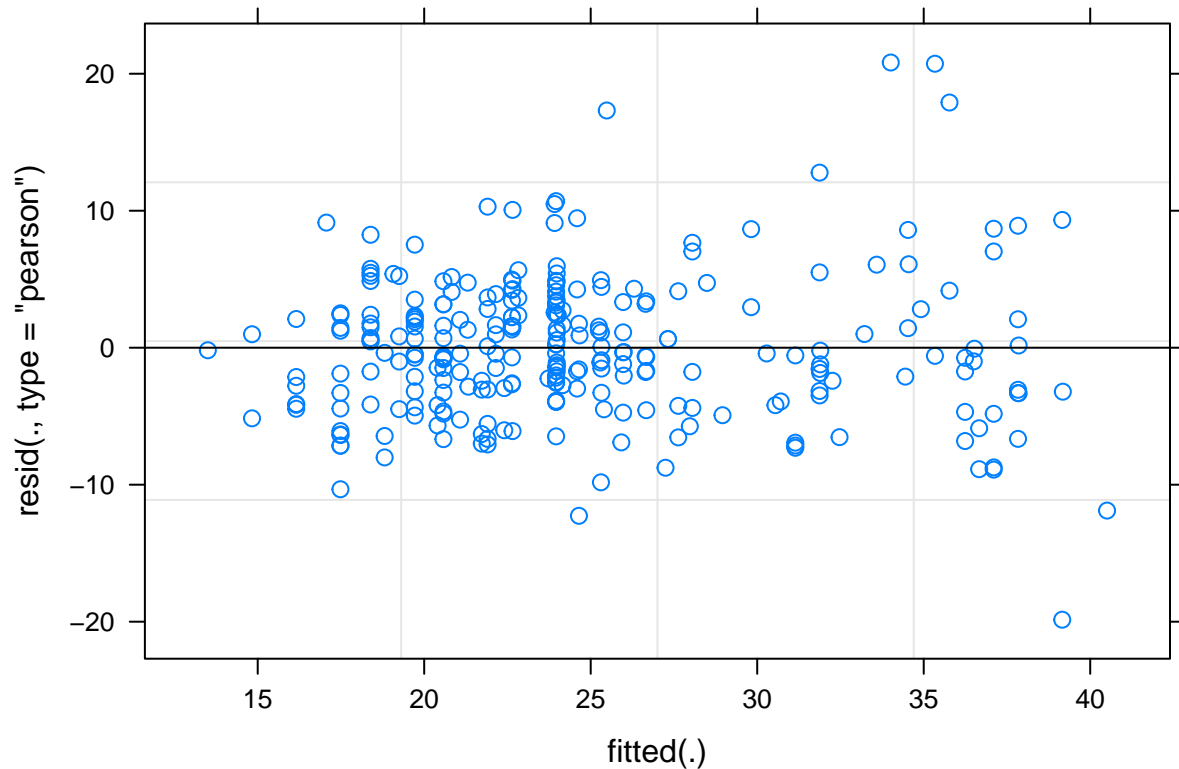
```
drop1(CEWL_mod3)
```

```
## Single term deletions
##
## Model:
## CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + day_n:temp_tmt +
##   day_n:humidity_tmt + day_n + cloacal_temp_C + (1 | trial_number)
##               npar      AIC
## <none>                1674.8
## cloacal_temp_C         1 1686.1
## day_n:humidity_tmt:temp_tmt 1 1681.0
```

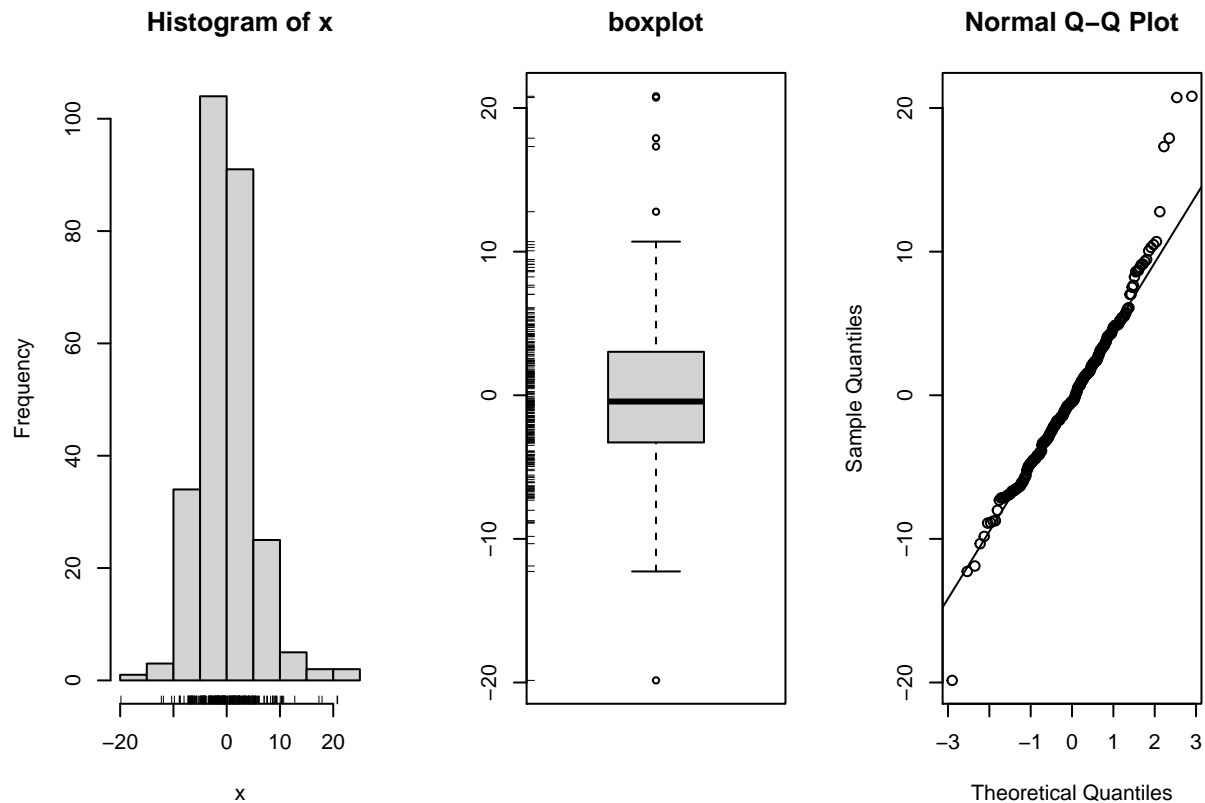
This model has a lower AIC than the previous model, all the remaining t-values are large, and we lose no predictive power. This is the best model.

Check linear regression assumptions/conditions:

```
plot(CEWL_mod3)
```



```
simple.eda(residuals(CEWL_mod3))
```

```
shapiro.test(residuals(CEWL_mod3))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(CEWL_mod3)
## W = 0.96724, p-value = 8.744e-06
```

Save best model stats:

```
CEWL_mod_best <- lmerTest::lmer(data = dat_no_rehab,
                                CEWL_g_m2h_mean ~
                                  day_n:humidity_tmt:temp_tmt +
                                  day_n:temp_tmt +
                                  day_n:humidity_tmt +
                                  day_n +
                                  cloacal_temp_C +
                                  (1|trial_number))
summary(CEWL_mod_best)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: CEWL_g_m2h_mean ~ day_n:humidity_tmt:temp_tmt + day_n:temp_tmt +
##          day_n:humidity_tmt + day_n + cloacal_temp_C + (1 | trial_number)
## Data: dat_no_rehab
##
## REML criterion at convergence: 1666
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
```

```

## -3.7253 -0.6161 -0.0815  0.5676  3.9063
##
## Random effects:
## Groups          Name          Variance Std.Dev.
## trial_number (Intercept)  7.839   2.800
## Residual                28.402   5.329
## Number of obs: 267, groups: trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error    df t value
## (Intercept)    55.9226    9.5233 257.7583   5.872
## day_n           1.7338    0.1451 258.1416  11.952
## cloacal_temp_C -1.3274    0.3564 260.6308  -3.724
## day_n:temp_tmtCool -0.7447    0.1643 256.9944  -4.532
## day_n:humidity_tmtDry -1.3979    0.1653 257.2239  -8.456
## day_n:humidity_tmtDry:temp_tmtCool  0.6603    0.2312 257.0031   2.856
##
##              Pr(>|t|)
## (Intercept)  1.32e-08 ***
## day_n        < 2e-16 ***
## cloacal_temp_C  0.00024 ***
## day_n:temp_tmtCool  8.98e-06 ***
## day_n:humidity_tmtDry  2.09e-15 ***
## day_n:humidity_tmtDry:temp_tmtCool  0.00464 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) day_n  clc__C dy_:_C dy_:_D
## day_n          -0.465
## clocl_tmp_C    -0.990  0.450
## dy_n:tmp_tC     0.059 -0.591 -0.060
## dy_n:hmdt_D     0.165 -0.638 -0.167  0.506
## dy_n:h_D:_C    -0.008  0.405  0.008 -0.709 -0.695

write.csv(broom::tidy(CEWL_mod3),
          "./results_statistics/exp_effects_CEWL.csv")

```

Conclusion

The change in body condition due to experimental treatment was best described by the full model including time in acclimation treatment, acclimation temperature, acclimation humidity, and both pairwise and three-way interactions. Hematocrit was best-predicted by a reduced model including time in acclimation treatment and acclimation temperature. Plasma osmolality was best-predicted by a reduced model including only acclimation temperature. In model selection for hematocrit and plasma osmolality, each dropped variable only improved the model by $<2\text{-}\Delta\text{AIC}$, so each stepwise model predicted the variability in the response variable equally well. However, the variables dropped also had very low t-values, evidence for their lack of influence thus exclusion from the model. The best model to predict the changes in CEWL included time in acclimation treatment, its interactions with acclimation temperature and acclimation humidity, and their three-way interaction. Nested effects of trial number and individual lizard ID were included as random effects in the LMMs predicting body condition, hematocrit, and plasma osmolality. Individual ID did not account for any variation in CEWL, so only trial number was included as a random effect in that model.

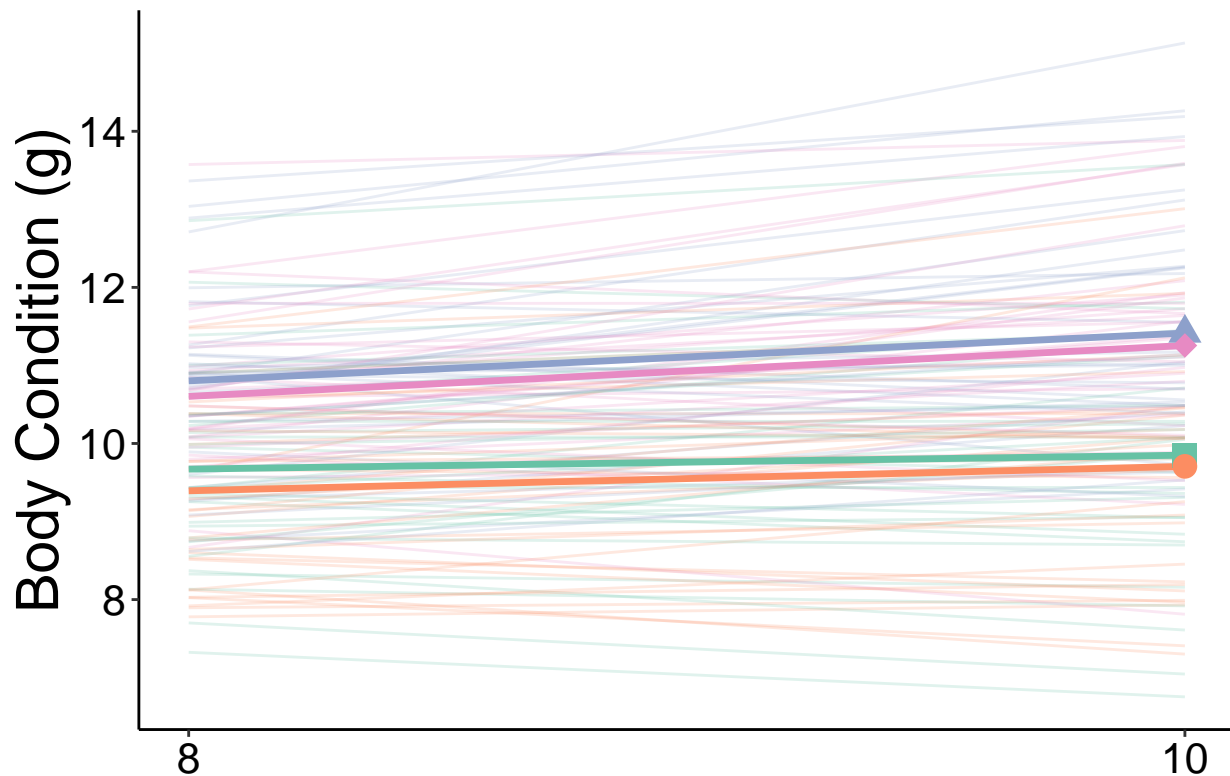
Rehydration Figures

SMI

```
# calculate means to overlay
mean_SMI_rehab <- dat %>%
  dplyr::filter(type == "rehab") %>%
  dplyr::filter(complete.cases(SMI)) %>%
  group_by(tmt) %>%
  summarise(day = 10,
            mean_SMI = mean(SMI))

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

# plot!!
dat_for_rehab %>%
  ggplot(aes(x = day_n,
            y = SMI,
            color = tmt,
            )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.2,
              alpha = 1) +
  # this point is more for the legend than for showing means
  geom_point(data = mean_SMI_rehab,
            aes(x = day,
              y = mean_SMI,
              color = tmt,
              shape = tmt),
            size = 4,
            alpha = 1) +
  theme_classic() +
  scale_shape_manual(values = c(15:18), name = "") +
  scale_x_continuous(breaks = c(8, 10)) +
  scale_color_brewer(palette = "Set2", name = "") +
  xlab("") +
  ylab("Body Condition (g)") +
  guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 22),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 16),
        legend.text = element_text(color = "black",
                                    family = "sans",
                                    size = 22),
        legend.text.align = 0,
        legend.position = "none"
  ) -> rehab_effects_SMI
rehab_effects_SMI
```



```
# export figure
ggsave(filename = "rehab_effects_SMI.jpeg",
        plot = rehab_effects_SMI,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Hematocrit

```
# calculate means to overlay
mean_hct_rehab <- dat %>%
  dplyr::filter(type == "rehab") %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  group_by(tmt) %>%
  summarise(day = 10,
            mean_hct = mean(hematocrit_percent))

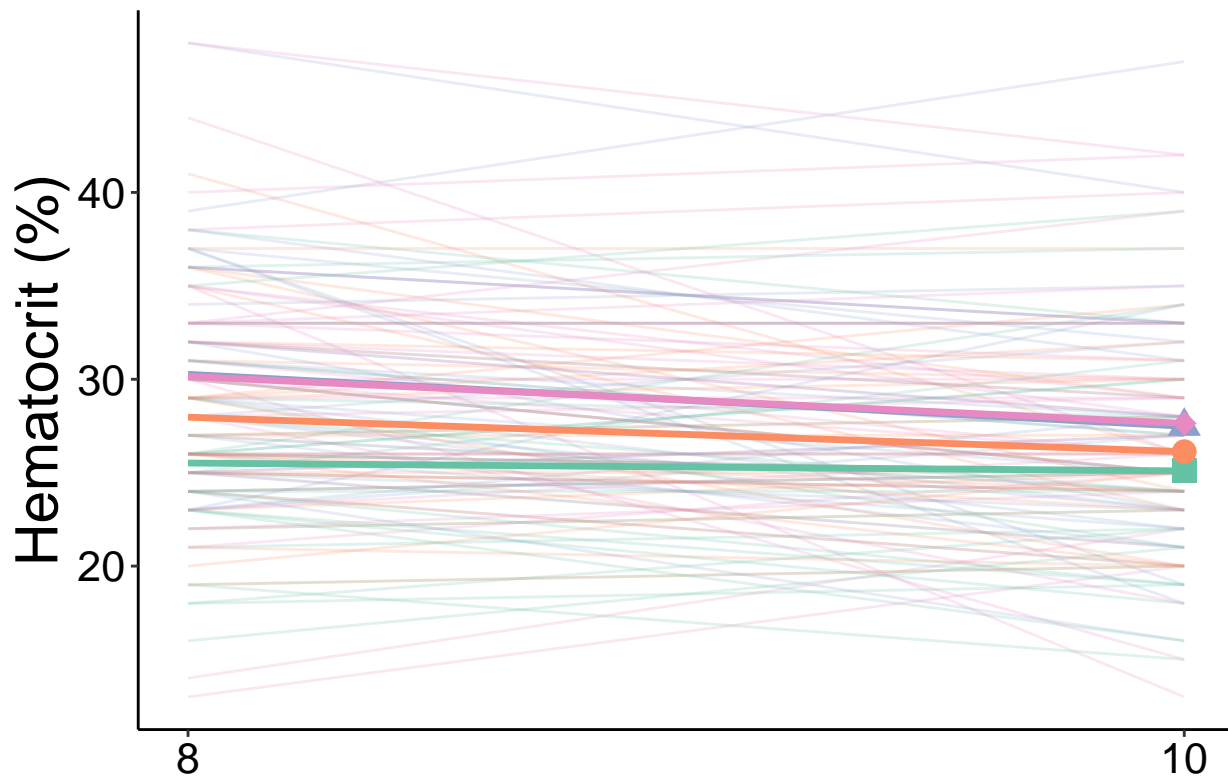
## `summarise()` ungrouping output (override with `.groups` argument)

# plot
dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  ggplot(aes(x = day_n,
             y = hematocrit_percent,
             color = tmt,
             )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
```

```

        method = "lm",
        se = F,
        size = 1.2,
        alpha = 1 ) +
# this point is more for the legend than for showing means
geom_point(data = mean_hct_rehab,
          aes(x = day,
              y = mean_hct,
              color = tmt,
              shape = tmt),
          size = 4,
          alpha = 1) +
theme_classic() +
scale_shape_manual(values = c(15:18), name = "") +
scale_x_continuous(breaks = c(8, 10)) +
scale_color_brewer(palette = "Set2", name = "") +
xlab("") +
ylab("Hematocrit (%)") +
guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
theme(text = element_text(color = "black",
                           family = "sans",
                           size = 22),
      axis.text = element_text(color = "black",
                                family = "sans",
                                size = 16),
      legend.text = element_text(color = "black",
                                  family = "sans",
                                  size = 22),
      legend.text.align = 0,
      legend.position = "none"
    ) -> rehab_effects_hct
rehab_effects_hct

```



```
# export figure
ggsave(filename = "rehab_effects_hct.jpeg",
        plot = rehab_effects_hct,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Osmolality

```
# calculate means to overlay
mean_osml_rehab <- dat %>%
  dplyr::filter(type == "rehab") %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  group_by(tmt) %>%
  summarise(day = 10,
            mean_osml = mean(osmolality_mmol_kg_mean))

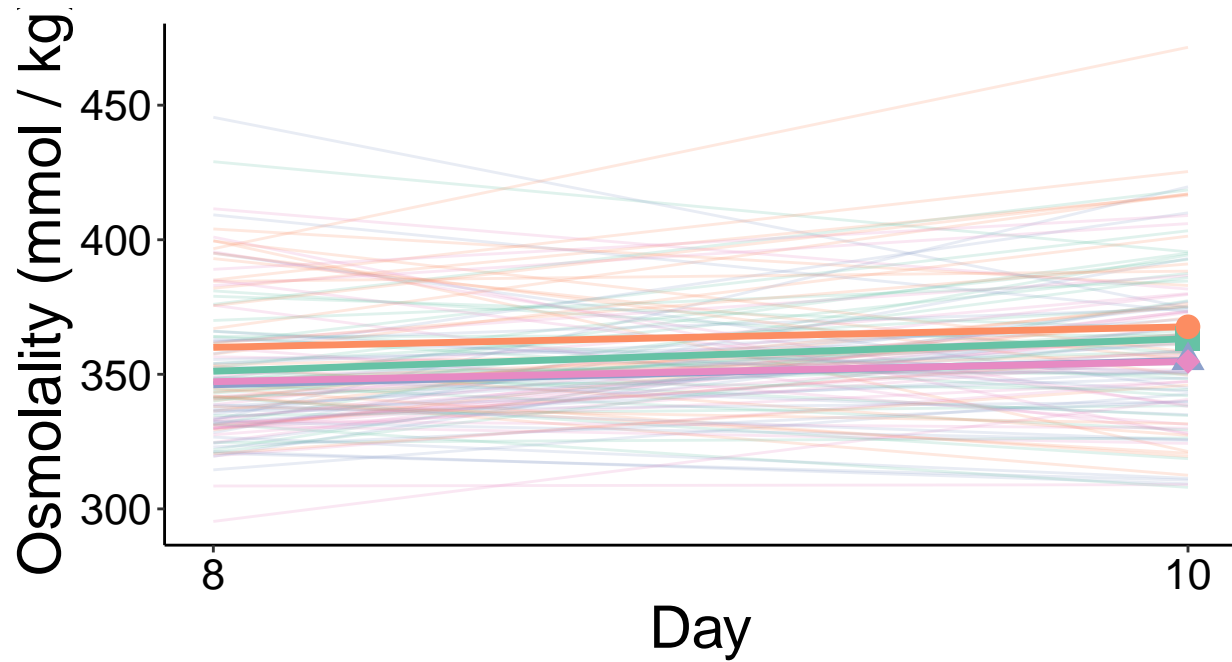
## `summarise()` ungrouping output (override with `.groups` argument)

# plot
dat_for_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  ggplot(aes(x = day_n,
             y = osmolality_mmol_kg_mean,
             color = tmt,
             )) +
  geom_line(aes(group = individual_ID),
            alpha = 0.2) +
  stat_smooth(formula = y ~ x,
```

```

        method = "lm",
        se = F,
        size = 1.2,
        alpha = 1 ) +
# this point is more for the legend than for showing means
geom_point(data = mean_osml_rehab,
          aes(x = day,
              y = mean_osml,
              color = tmt,
              shape = tmt),
          size = 4,
          alpha = 1) +
theme_classic() +
scale_shape_manual(values = c(15:18), name = "") +
scale_x_continuous(breaks = c(8, 10)) +
scale_color_brewer(palette = "Set2", name = "") +
xlab("Day") +
ylab("Osmolality (mmol / kg)") +
guides(shape = guide_legend(nrow = 2, byrow = TRUE)) +
theme(text = element_text(color = "black",
                           family = "sans",
                           size = 22),
      axis.text = element_text(color = "black",
                                family = "sans",
                                size = 16),
      legend.text = element_text(color = "black",
                                  family = "sans",
                                  size = 22),
      legend.text.align = 0,
      legend.position = "bottom"
    ) -> rehab_effects_osml
rehab_effects_osml

```

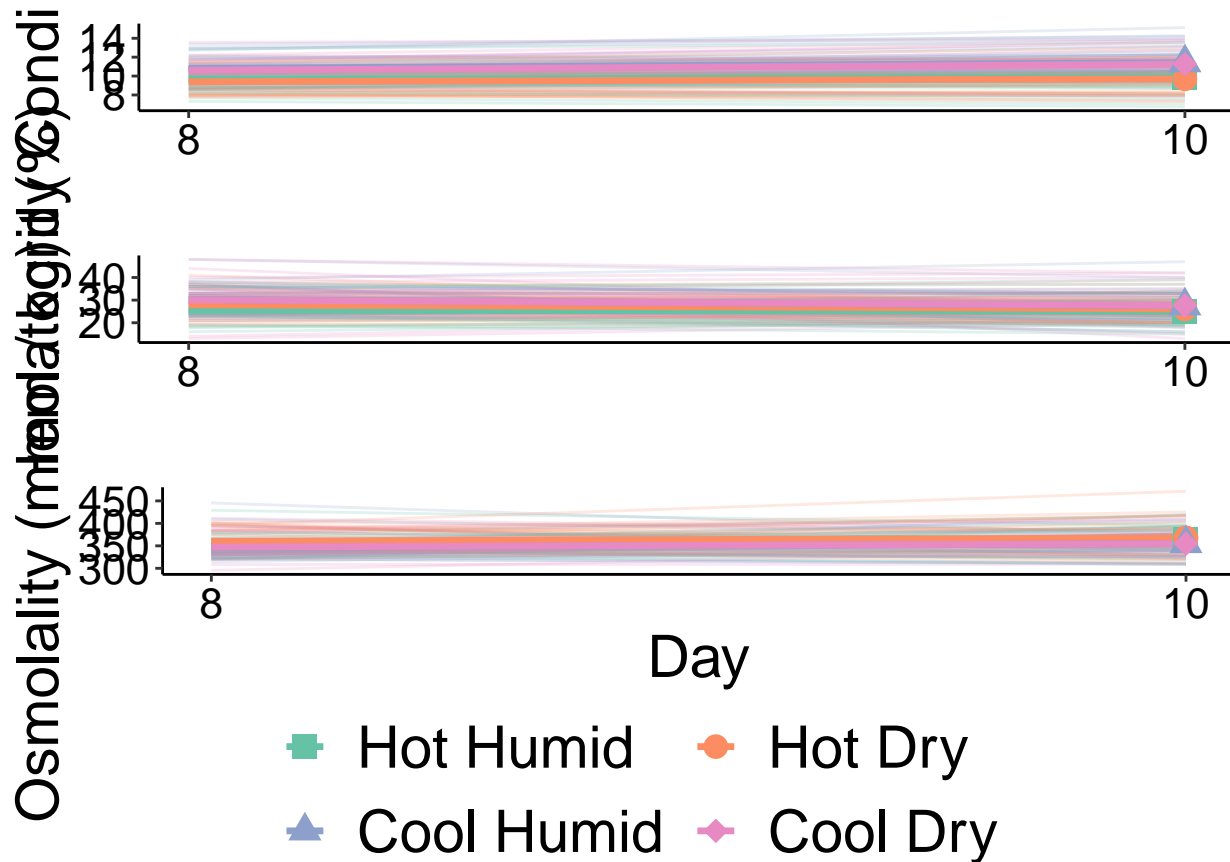


■ Hot Humid ● Hot Dry
▲ Cool Humid ◆ Cool Dry

```
# export figure
ggsave(filename = "rehab_effects_osml.jpeg",
        plot = rehab_effects_osml,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 4)
```

Multi-Figure

```
ggarrange(rehab_effects_SMI,
          rehab_effects_hct,
          rehab_effects_osml,
          ncol = 1, nrow = 3,
          common.legend = TRUE,
          legend = "bottom"
          ) -> rehab_multi_fig
rehab_multi_fig
```

```
# export figure
ggsave(filename = "rehab_multi_fig.jpeg",
        plot = rehab_multi_fig,
        path = "./results_figures",
        device = "jpeg",
        dpi = 1200,
        width = 6, height = 12)
```

Rehydration Models

SMI

```
SMI_rmod1 <- lme4::lmer(data = dat_for_rehab,
                      SMI ~
                        type*humidity_tmt*temp_tmt +
                        (1|trial_number/individual_ID))
summary(SMI_rmod1)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## SMI ~ type * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## Data: dat_for_rehab
##
## REML criterion at convergence: 721.4
##
## Scaled residuals:
```

```

##      Min      1Q   Median      3Q      Max
## -2.23035 -0.45572 -0.02395  0.51218  2.24644
##
## Random effects:
##      Groups              Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 1.0357  1.0177
## trial_number              (Intercept) 0.4166  0.6454
## Residual                  0.2895  0.5380
## Number of obs: 266, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)      9.63612    0.35190  27.383
## typerehab         0.18011    0.13430   1.341
## humidity_tmtDry  -0.19855    0.28187  -0.704
## temp_tmtCool      1.15702    0.28136   4.112
## typerehab:humidity_tmtDry  0.16808    0.18847   0.892
## typerehab:temp_tmtCool    0.43241    0.18726   2.309
## humidity_tmtDry:temp_tmtCool 0.08418    0.39796   0.212
## typerehab:humidity_tmtDry:temp_tmtCool -0.12846    0.26476  -0.485
##
## Correlation of Fixed Effects:
##      (Intr) typrhb hmdt_D tmp_tC typ:_D typ:_C h_D:_C
## typerehab  -0.185
## hmdty_tmtDr -0.407  0.232
## temp_tmtCol -0.406  0.232  0.507
## typrhb:hm_D  0.132 -0.712 -0.325 -0.165
## typrhb:tm_C  0.133 -0.717 -0.166 -0.328  0.511
## hmdty_tD:_C  0.287 -0.164 -0.706 -0.707  0.230  0.232
## typrh:_D:_C -0.094  0.507  0.232  0.232 -0.712 -0.707 -0.328
drop1(SMI_rmod1)

## Single term deletions
##
## Model:
## SMI ~ type * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
##              npar      AIC
## <none>              730.66
## type:humidity_tmt:temp_tmt    1 728.90

Remove three-way interaction and day*humidity interaction.
SMI_rmod2 <- lme4::lmer(data = dat_for_rehab,
                      SMI ~
                        type*temp_tmt + humidity_tmt +
                        (1|trial_number/individual_ID))
summary(SMI_rmod2)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## SMI ~ type * temp_tmt + humidity_tmt + (1 | trial_number/individual_ID)
##      Data: dat_for_rehab
##
## REML criterion at convergence: 719.1
##

```

```
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.21974 -0.48494 -0.03309  0.51616  2.33210
##
## Random effects:
##   Groups                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 1.0269   1.0134
## trial_number              (Intercept) 0.4170   0.6457
## Residual                    0.2871   0.5358
## Number of obs: 266, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    9.58896    0.33525  28.602
## typerehab       0.26544    0.09384   2.829
## temp_tmtCool    1.19989    0.19816   6.055
## humidity_tmtDry -0.10557    0.18796  -0.562
## typerehab:temp_tmtCool 0.36659    0.13182   2.781
##
## Correlation of Fixed Effects:
##              (Intr) typrhb tmp_tC hmdt_D
## typerehab    -0.136
## temp_tmtCol  -0.300  0.230
## hmdty_tmtDr  -0.285  0.000  0.016
## typrhb:tm_C   0.097 -0.712 -0.328  0.000
```

```
drop1(SMI_rmod2)
```

```
## Single term deletions
##
## Model:
## SMI ~ type * temp_tmt + humidity_tmt + (1 | trial_number/individual_ID)
##              npar      AIC
## <none>              725.52
## humidity_tmt        1 723.86
## type:temp_tmt        1 731.16
```

Remove temperature and humidity single effects:

```
SMI_rmod3 <- lme4::lmer(data = dat_for_rehab,
                      SMI ~
                        type:temp_tmt + type +
                        (1|trial_number/individual_ID))
summary(SMI_rmod3)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: SMI ~ type:temp_tmt + type + (1 | trial_number/individual_ID)
##   Data: dat_for_rehab
##
## REML criterion at convergence: 717.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.23546 -0.48143 -0.02646  0.52425  2.32206
##
## Random effects:
```

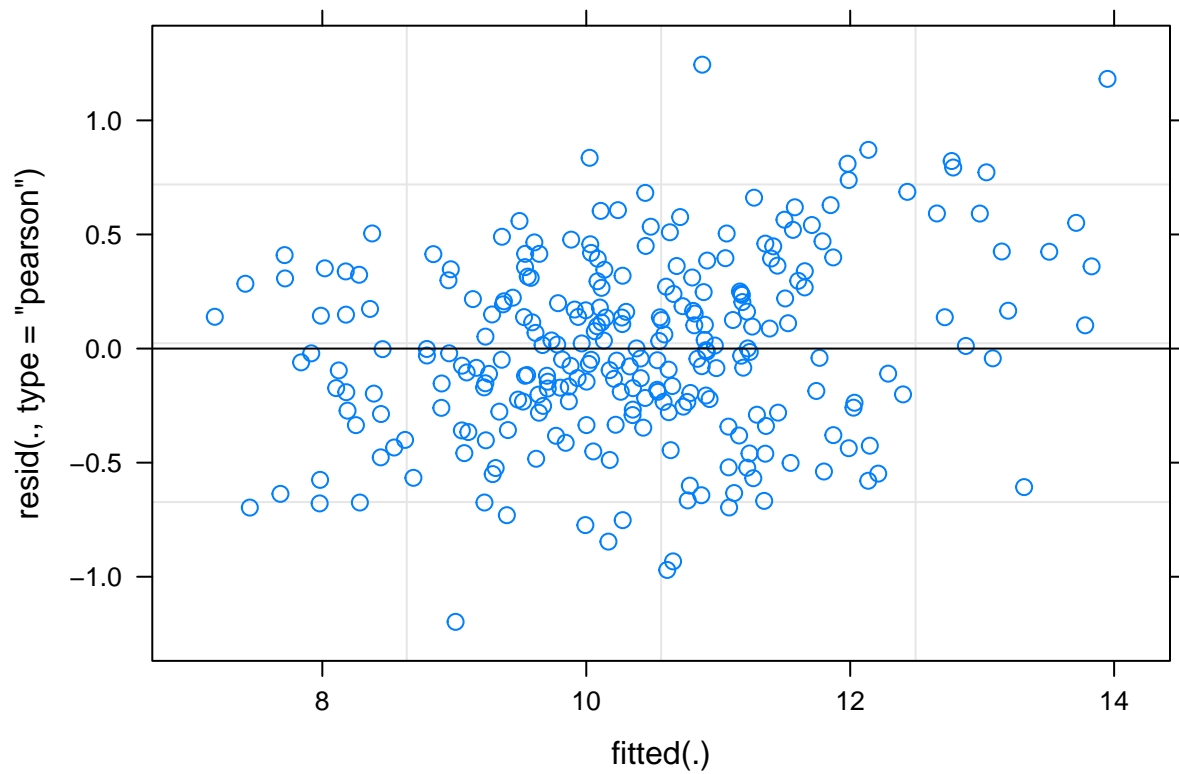
```
## Groups                                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 1.0203   1.0101
## trial_number                    (Intercept) 0.4235   0.6507
## Residual                        0.2871   0.5358
## Number of obs: 266, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##                                Estimate Std. Error t value
## (Intercept)                   9.53530    0.32320  29.503
## typerehab                     0.26545    0.09384   2.829
## typeexp:temp_tmtCool          1.20175    0.19763   6.081
## typerehab:temp_tmtCool        1.56833    0.19823   7.912
##
## Correlation of Fixed Effects:
##              (Intr) typrhb typx:_C
## typerehab    -0.141
## typxp:tmp_C  -0.306  0.231
## typrhb:tm_C  -0.239 -0.243  0.778
drop1(SMI_rmod3)

## Single term deletions
##
## Model:
## SMI ~ type:temp_tmt + type + (1 | trial_number/individual_ID)
##              npar      AIC
## <none>          723.86
## type:temp_tmt    2 773.39
```

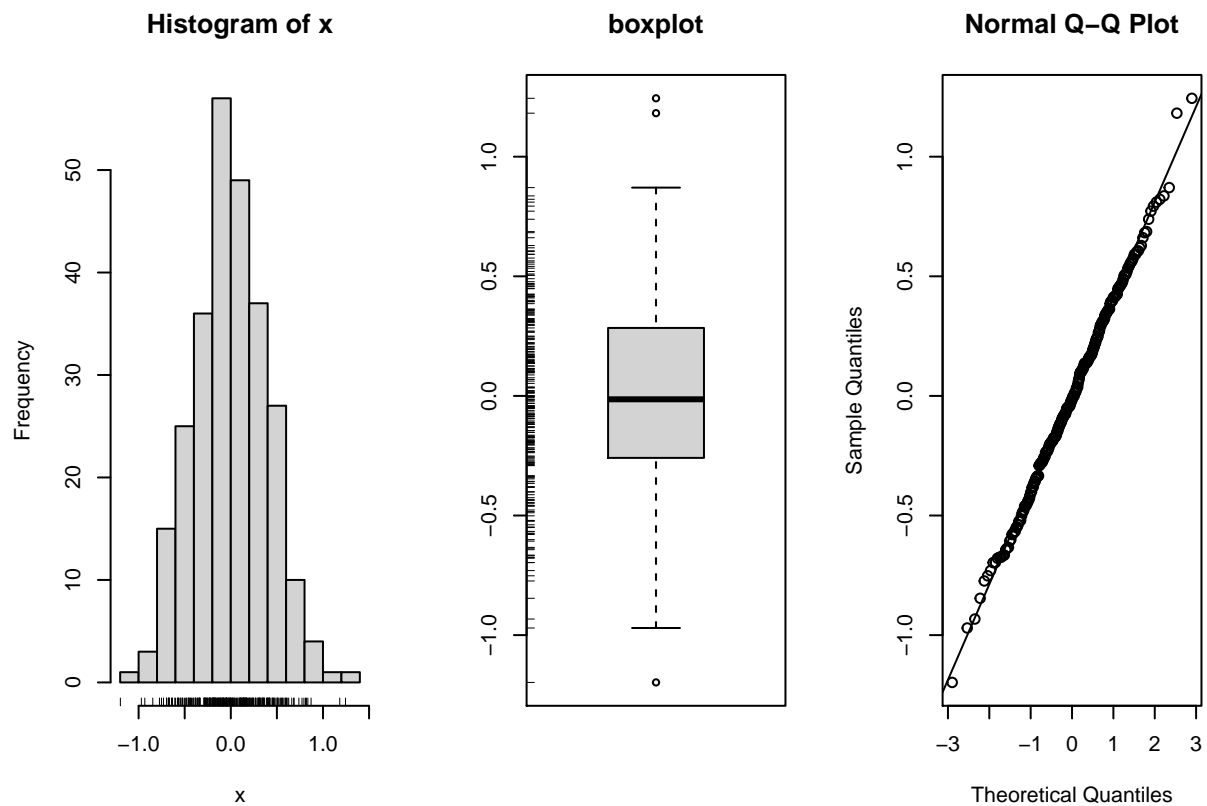
This is the best model.

Check linear regression assumptions/conditions:

```
plot(SMI_rmod3)
```



```
simple.eda(residuals(SMI_rmod3))
```



```

shapiro.test(residuals(SMI_rmod3))

##
##  Shapiro-Wilk normality test
##
## data:  residuals(SMI_rmod3)
## W = 0.9978, p-value = 0.9781

Save best model stats:
SMI_rmod_best <- lmerTest::lmer(data = dat_for_rehab,
                               SMI ~
                                   type:temp_tmt + type +
                                   (1|trial_number/individual_ID))
summary(SMI_rmod_best)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: SMI ~ type:temp_tmt + type + (1 | trial_number/individual_ID)
## Data: dat_for_rehab
##
## REML criterion at convergence: 717.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.23546 -0.48143 -0.02646  0.52425  2.32206
##
## Random effects:
## Groups              Name                Variance Std.Dev.
## individual_ID:trial_number (Intercept) 1.0203   1.0101
## trial_number              (Intercept) 0.4235   0.6507
## Residual                  0.2871   0.5358
## Number of obs: 266, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)    9.53530    0.32320   4.90353  29.503 1.04e-06 ***
## typerehab       0.26545    0.09384  131.02585   2.829 0.00541 **
## typeexp:temp_tmtCool 1.20175    0.19763  158.89957   6.081 8.58e-09 ***
## typerehab:temp_tmtCool 1.56833    0.19823  160.37496   7.912 3.91e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) typrhb typx:_C
## typerehab    -0.141
## typxp:tmp_C  -0.306  0.231
## typrhb:tm_C  -0.239 -0.243  0.778

write.csv(broom.mixed::tidy(SMI_rmod_best),
          "./results_statistics/rehab_effects_SMI.csv")

```

Hematocrit

```
hct_rmod1 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
             hematocrit_percent ~
               type*humidity_tmt*temp_tmt +
               (1|trial_number/individual_ID))
summary(hct_rmod1)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ type * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 1583.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.08683 -0.44747 -0.02418  0.47715  2.42538
##
## Random effects:
## Groups              Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 14.787   3.845
## trial_number              (Intercept)  8.829   2.971
## Residual                  15.344   3.917
## Number of obs: 261, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##                               Estimate Std. Error t value
## (Intercept)                   25.7524     1.6525  15.584
## typerehab                     -0.6506     0.9994  -0.651
## humidity_tmtDry                1.9501     1.3678   1.426
## temp_tmtCool                   4.4725     1.3583   3.293
## typerehab:humidity_tmtDry      -1.3491     1.4016  -0.963
## typerehab:temp_tmtCool         -2.2377     1.3861  -1.614
## humidity_tmtDry:temp_tmtCool  -2.5055     1.9144  -1.309
## typerehab:humidity_tmtDry:temp_tmtCool  1.7222     1.9538   0.881
##
## Correlation of Fixed Effects:
##              (Intr) typrhb hmdt_D tmp_tC typ:_D typ:_C h_D:_C
## typerehab    -0.309
## hmdty_tmtDr  -0.425  0.374
## temp_tmtCol  -0.427  0.376  0.516
## typrhb:hm_D   0.221 -0.713 -0.513 -0.268
## typrhb:tm_C   0.223 -0.721 -0.269 -0.511  0.514
## hmdty_tD:_C   0.302 -0.267 -0.712 -0.709  0.366  0.362
## typrh:_D:_C  -0.159  0.511  0.368  0.363 -0.717 -0.709 -0.508
drop1(hct_rmod1)

## Single term deletions
##
## Model:
## hematocrit_percent ~ type * humidity_tmt * temp_tmt + (1 | trial_number/individual_ID)
```

```

##                                npar    AIC
## <none>                        1620.4
## type:humidity_tmt:temp_tmt    1 1619.2

Remove humidity from the three-way interaction:
hct_rmod2 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
             hematocrit_percent ~
               type*temp_tmt + humidity_tmt +
               (1|trial_number/individual_ID))
summary(hct_rmod2)

## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ type * temp_tmt + humidity_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 1593
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.10684 -0.44086 -0.03292  0.45501  2.43909
##
## Random effects:
## Groups              Name            Variance Std.Dev.
## individual_ID:trial_number (Intercept) 14.846   3.853
## trial_number              (Intercept)  8.846   2.974
## Residual                  15.223   3.902
## Number of obs: 261, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    26.5279    1.5555  17.054
## typerehab       -1.3427    0.6984  -1.922
## temp_tmtCool     3.2044    0.9567   3.349
## humidity_tmtDry   0.4439    0.8282   0.536
## typerehab:temp_tmtCool -1.3630    0.9733  -1.400
##
## Correlation of Fixed Effects:
##              (Intr) typrhb tmp_tC hmdt_D
## typerehab    -0.226
## temp_tmtCol -0.318  0.365
## hmdty_tmtDr -0.273  0.010  0.019
## typrhb:tm_C  0.163 -0.717 -0.506 -0.010
drop1(hct_rmod2)

## Single term deletions
##
## Model:
## hematocrit_percent ~ type * temp_tmt + humidity_tmt + (1 | trial_number/individual_ID)
##              npar    AIC
## <none>          1616.5
## humidity_tmt    1 1614.8

```



```
## type:temp_tmt      1 1616.5
```

Drop humidity from the model completely:

```
hct_rmod3 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
             hematocrit_percent ~
               type * temp_tmt +
               (1|trial_number/individual_ID))
summary(hct_rmod3)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ type * temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 1594.8
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.09360 -0.42924 -0.02434  0.45256  2.42497
##
## Random effects:
## Groups              Name            Variance Std.Dev.
## individual_ID:trial_number (Intercept) 14.715   3.836
## trial_number              (Intercept)  8.972   2.995
## Residual                  15.221   3.901
## Number of obs: 261, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    26.7554    1.5040  17.789
## typerehab       -1.3464    0.6983  -1.928
## temp_tmtCool     3.1946    0.9545   3.347
## typerehab:temp_tmtCool -1.3576    0.9731  -1.395
##
## Correlation of Fixed Effects:
##              (Intr) typrhb tmp_tC
## typerehab    -0.231
## temp_tmtCol -0.323  0.366
## typrhb:tm_C  0.166 -0.717 -0.507
```

```
drop1(hct_rmod3)
```

```
## Single term deletions
##
## Model:
## hematocrit_percent ~ type * temp_tmt + (1 | trial_number/individual_ID)
##              npar      AIC
## <none>          1614.8
## type:temp_tmt    1 1614.8
```

The model is the same whether we drop the interaction or not, so we choose to drop it to simplify the model.

```
hct_rmod4 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
```

```

lme4::lmer(data = .,
            hematocrit_percent ~
              type + temp_tmt +
              (1|trial_number/individual_ID))
summary(hct_rmod4)

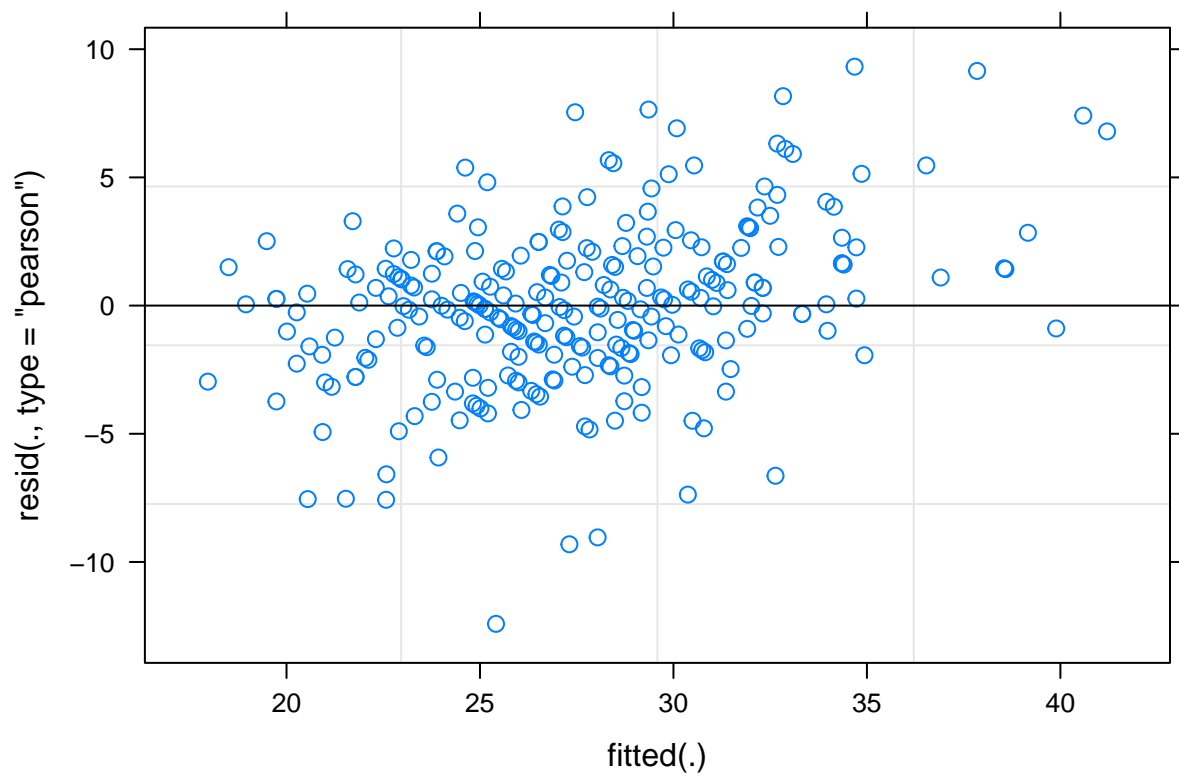
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## hematocrit_percent ~ type + temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 1598.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.1704 -0.4600  0.0080  0.4204  2.3797
##
## Random effects:
##   Groups                Name         Variance Std.Dev.
## individual_ID:trial_number (Intercept) 14.658   3.829
## trial_number              (Intercept)  9.015   3.003
## Residual                  15.333   3.916
## Number of obs: 261, groups:  individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)  27.1037    1.4862  18.237
## typerehab    -2.0452    0.4882  -4.189
## temp_tmtCool  2.5192    0.8225   3.063
##
## Correlation of Fixed Effects:
##              (Intr) typrhb
## typerehab    -0.163
## temp_tmtCol -0.281  0.004
drop1(hct_rmod4)

## Single term deletions
##
## Model:
## hematocrit_percent ~ type + temp_tmt + (1 | trial_number/individual_ID)
##              npar      AIC
## <none>          1614.8
## type           1 1629.3
## temp_tmt       1 1621.9

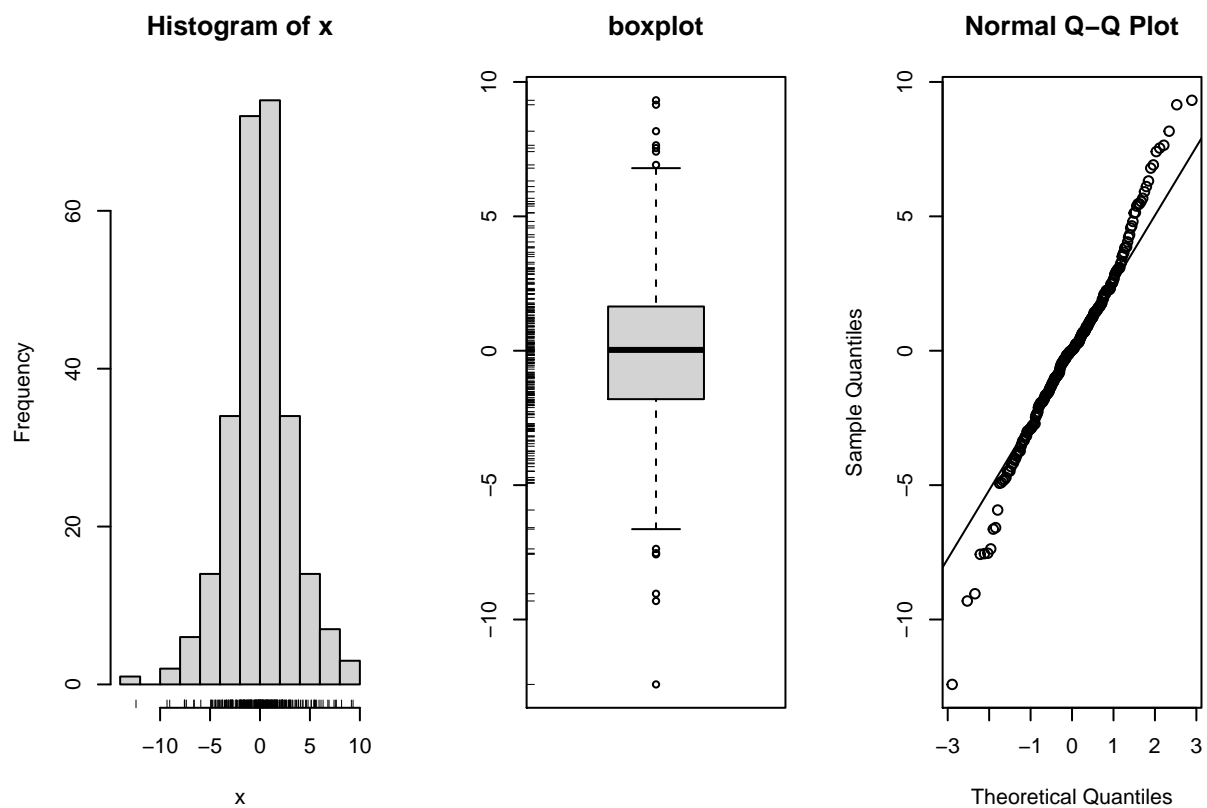
This seems to be the best model.

Check linear regression assumptions/conditions:
plot(hct_rmod4)

```



```
simple.eda(residuals(hct_rmod4))
```



```

shapiro.test(residuals(hct_rmod4))

##
##  Shapiro-Wilk normality test
##
## data:  residuals(hct_rmod4)
## W = 0.98196, p-value = 0.00217

Save best model stats:

hct_rmod_best <- dat_for_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lmerTest::lmer(data = .,
                 hematocrit_percent ~
                   type + temp_tmt +
                   (1|trial_number/individual_ID))
summary(hct_rmod_best)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## hematocrit_percent ~ type + temp_tmt + (1 | trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 1598.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.1704 -0.4600  0.0080  0.4204  2.3797
##
## Random effects:
## Groups              Name            Variance Std.Dev.
## individual_ID:trial_number (Intercept) 14.658   3.829
## trial_number              (Intercept)  9.015   3.003
## Residual                  15.333   3.916
## Number of obs: 261, groups: individual_ID:trial_number, 134; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)  27.1037     1.4862    4.9451  18.237 1.00e-05 ***
## typerehab    -2.0452     0.4882   128.0452  -4.189 5.18e-05 ***
## temp_tmtCool  2.5192     0.8225   126.9259   3.063 0.00268 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) typrhb
## typerehab    -0.163
## temp_tmtCol  -0.281  0.004

write.csv(broom::tidy(hct_rmod_best),
          "./results_statistics/rehab_effects_hct.csv")

```

Osmolality

```
osml_rmod1 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               type*humidity_tmt*temp_tmt +
               (1|trial_number/individual_ID))

summary(osml_rmod1)

## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ type * humidity_tmt * temp_tmt + (1 |
##      trial_number/individual_ID)
##      Data: .
##
## REML criterion at convergence: 2313.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.2333 -0.6220 -0.0734  0.5582  3.7278
##
## Random effects:
##      Groups                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept) 65.36   8.085
## trial_number              (Intercept) 161.32  12.701
## Residual                  523.96   22.890
## Number of obs: 255, groups: individual_ID:trial_number, 133; trial_number, 5
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                       351.416     7.130  49.288
## typerehab                          11.482     5.735   2.002
## humidity_tmtDry                     7.406     6.120   1.210
## temp_tmtCool                       -4.877     6.116  -0.797
## typerehab:humidity_tmtDry           -4.498     8.144  -0.552
## typerehab:temp_tmtCool              -3.084     8.086  -0.381
## humidity_tmtDry:temp_tmtCool        -8.507     8.721  -0.975
## typerehab:humidity_tmtDry:temp_tmtCool 3.284    11.503   0.286
##
## Correlation of Fixed Effects:
##      (Intr) typrhb hmdt_D tmp_tC typ:_D typ:_C h_D:_C
## typerehab    -0.402
## hmdty_tmtDr  -0.422  0.468
## temp_tmtCol  -0.421  0.468  0.492
## typrhb:hm_D   0.282 -0.703 -0.671 -0.330
## typrhb:tm_C   0.284 -0.708 -0.332 -0.678  0.499
## hmdty_tD:_C   0.295 -0.328 -0.700 -0.701  0.471  0.475
## typrh:_D:_C  -0.200  0.498  0.475  0.476 -0.708 -0.703 -0.680

drop1(osml_rmod1)

## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ type * humidity_tmt * temp_tmt + (1 |
```

```
##      trial_number/individual_ID)
##                                npar      AIC
## <none>                        2375.5
## type:humidity_tmt:temp_tmt    1 2373.6
```

Drop the interaction with day:

```
osml_rmod2 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               type + humidity_tmt*temp_tmt +
               (1|trial_number/individual_ID))
summary(osml_rmod2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ type + humidity_tmt * temp_tmt + (1 |
##      trial_number/individual_ID)
##      Data: .
##
## REML criterion at convergence: 2330.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.2800 -0.6464 -0.0801  0.5779  3.6801
##
## Random effects:
##      Groups                Name      Variance Std.Dev.
## individual_ID:trial_number (Intercept)  71.99     8.485
## trial_number              (Intercept) 160.83    12.682
## Residual                  512.08    22.629
## Number of obs: 255, groups:  individual_ID:trial_number, 133; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    352.868     6.679  52.832
## typerehab        8.553     2.853   2.998
## humidity_tmtDry  5.139     4.541   1.132
## temp_tmtCool   -6.429     4.501  -1.429
## humidity_tmtDry:temp_tmtCool -6.881     6.402  -1.075
##
## Correlation of Fixed Effects:
##              (Intr) typrhb hmdt_D tmp_tC
## typerehab    -0.216
## hmdty_tmtDr  -0.337 -0.004
## temp_tmtCol  -0.337 -0.016  0.500
## hmdty_tD:_C   0.237  0.004 -0.707 -0.703
```

```
drop1(osml_rmod2)
```

```
## Single term deletions
```

```
##
```

```
## Model:
```

```
## osmolality_mmol_kg_mean ~ type + humidity_tmt * temp_tmt + (1 |
##      trial_number/individual_ID)
##                                npar      AIC
```

```
## <none>                2369.9
## type                   1 2376.5
## humidity_tmt:temp_tmt  1 2369.1
```

Drop the interaction between temperature and humidity:

```
osml_rmod3 <- dat_for_rehab %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%
  lme4::lmer(data = .,
             osmolality_mmol_kg_mean ~
               type + humidity_tmt + temp_tmt +
               (1|trial_number/individual_ID))
summary(osml_rmod3)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg_mean ~ type + humidity_tmt + temp_tmt + (1 |
##   trial_number/individual_ID)
## Data: .
##
## REML criterion at convergence: 2337.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.2183 -0.6314 -0.0763  0.5420  3.7383
##
## Random effects:
## Groups              Name             Variance Std.Dev.
## individual_ID:trial_number (Intercept)  72.55     8.518
## trial_number              (Intercept) 160.10    12.653
## Residual                  512.00    22.627
## Number of obs: 255, groups:  individual_ID:trial_number, 133; trial_number, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   354.568     6.478  54.730
## typerehab       8.568     2.853   3.003
## humidity_tmtDry  1.689     3.215   0.525
## temp_tmtCool   -9.829     3.204  -3.067
##
## Correlation of Fixed Effects:
##              (Intr) typrhb hmdt_D
## typerehab    -0.223
## hmdty_tmtDr -0.247 -0.002
## temp_tmtCol -0.247 -0.019  0.006
```

```
drop1(osml_rmod3)
```

```
## Single term deletions
##
## Model:
## osmolality_mmol_kg_mean ~ type + humidity_tmt + temp_tmt + (1 |
##   trial_number/individual_ID)
##              npar      AIC
## <none>         2369.1
## type          1 2375.7
## humidity_tmt  1 2367.4
```

```
## temp_tmt          1 2376.3
```

Drop humidity:

```
osml_rmod4 <- dat_for_rehab %>%  
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%  
  lme4::lmer(data = .,  
            osmolality_mmol_kg_mean ~  
              type + temp_tmt +  
              (1|trial_number/individual_ID))  
summary(osml_rmod4)
```

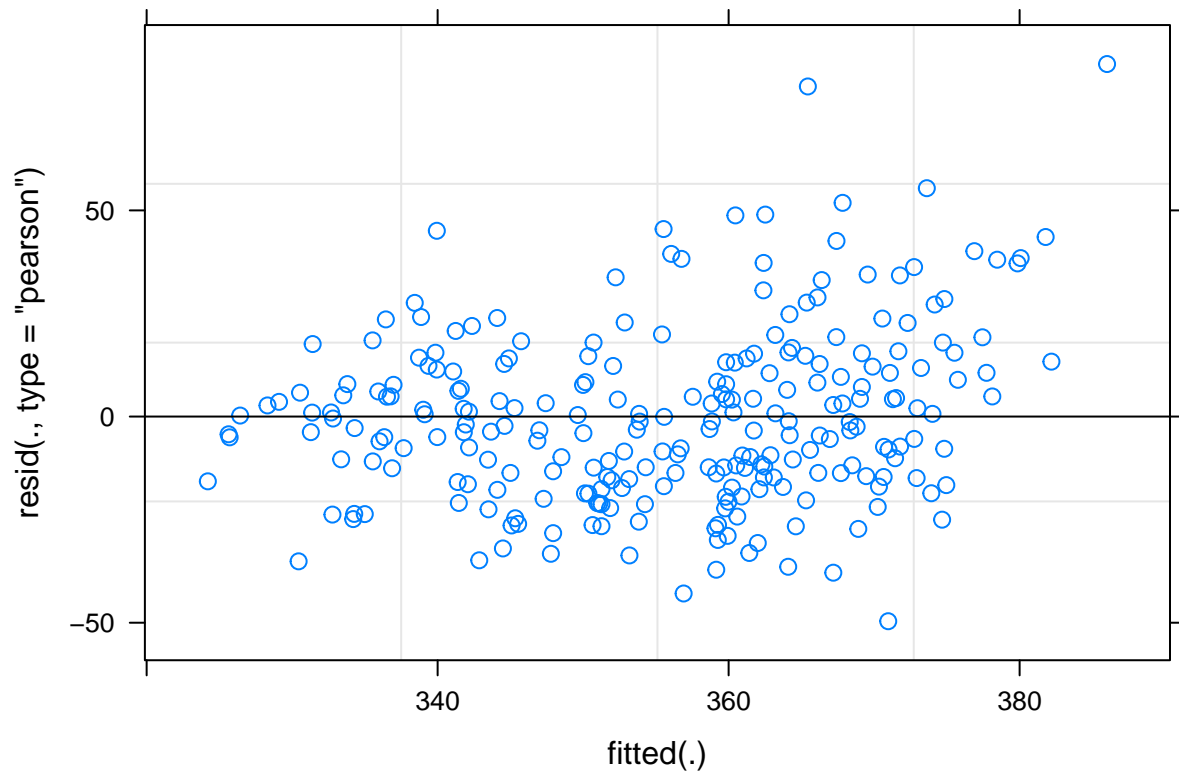
```
## Linear mixed model fit by REML ['lmerMod']  
## Formula:  
## osmolality_mmol_kg_mean ~ type + temp_tmt + (1 | trial_number/individual_ID)  
## Data: .  
##  
## REML criterion at convergence: 2342  
##  
## Scaled residuals:  
##      Min       1Q   Median       3Q      Max   
## -2.1943 -0.6527 -0.0856  0.5430  3.7792   
##  
## Random effects:  
## Groups                Name      Variance Std.Dev.  
## individual_ID:trial_number (Intercept) 70.8      8.414  
## trial_number              (Intercept) 162.1     12.733  
## Residual                  511.7     22.621  
## Number of obs: 255, groups: individual_ID:trial_number, 133; trial_number, 5  
##  
## Fixed effects:  
##              Estimate Std. Error t value  
## (Intercept)  355.413      6.307  56.350  
## typerehab      8.565      2.852   3.003  
## temp_tmtCool -9.838      3.195  -3.079  
##  
## Correlation of Fixed Effects:  
##              (Intr) typrhb  
## typerehab    -0.230  
## temp_tmtCol -0.251 -0.019  
drop1(osml_rmod4)
```

```
## Single term deletions  
##  
## Model:  
## osmolality_mmol_kg_mean ~ type + temp_tmt + (1 | trial_number/individual_ID)  
##      npar    AIC  
## <none>    2367.4  
## type      1 2374.1  
## temp_tmt  1 2374.6
```

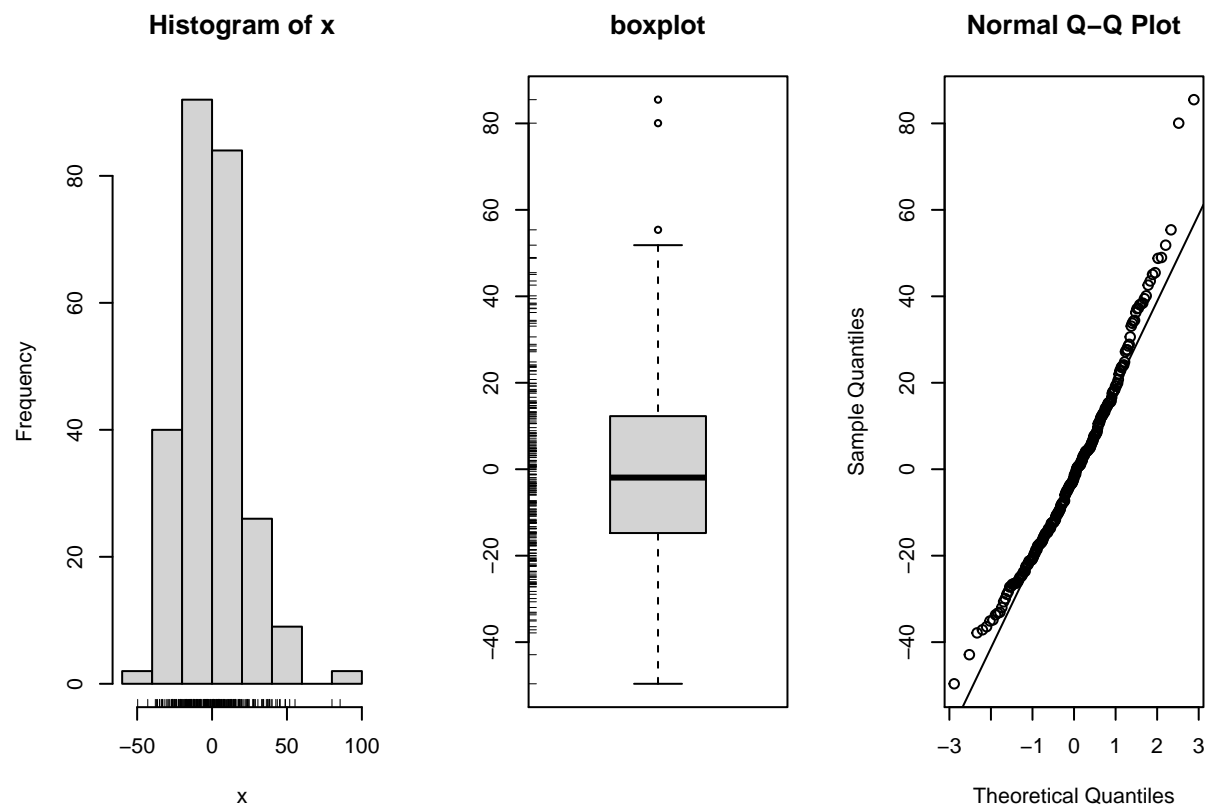
The best model to predict the rehydration effects on plasma osmolality included only additive effects of rehab and acclimation temp tmt prior to rehab.

Check linear regression assumptions/conditions:


```
plot(osml_rmod4)
```



```
simple.eda(residuals(osml_rmod4))
```



```
shapiro.test(residuals(osml_rmod4))
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: residuals(osml_rmod4)  
## W = 0.97061, p-value = 4.119e-05
```

Save best model stats:

```
osml_rmod_best <- dat_for_rehab %>%  
  dplyr::filter(complete.cases(osmolality_mmol_kg_mean)) %>%  
  lmerTest::lmer(data = .,  
                 osmolality_mmol_kg_mean ~  
                 type + temp_tmt +  
                 (1|trial_number/individual_ID))  
summary(osml_rmod_best)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula:  
## osmolality_mmol_kg_mean ~ type + temp_tmt + (1 | trial_number/individual_ID)  
## Data: .  
##  
## REML criterion at convergence: 2342  
##  
## Scaled residuals:  
##      Min       1Q   Median       3Q      Max   
## -2.1943 -0.6527 -0.0856  0.5430  3.7792   
##  
## Random effects:  
## Groups              Name      Variance Std.Dev.  
## individual_ID:trial_number (Intercept)  70.8      8.414  
## trial_number              (Intercept) 162.1     12.733  
## Residual                    511.7     22.621  
## Number of obs: 255, groups: individual_ID:trial_number, 133; trial_number, 5  
##  
## Fixed effects:  
##              Estimate Std. Error    df t value Pr(>|t|)      
## (Intercept)  355.413      6.307   5.147  56.350  2.2e-08 ***  
## typerehab      8.565      2.852 126.589   3.003  0.00322 **  
## temp_tmtCool  -9.838      3.195 122.874  -3.079  0.00256 **  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
##  
## Correlation of Fixed Effects:  
##              (Intr) typrhb  
## typerehab    -0.230  
## temp_tmtCol -0.251 -0.019
```

```
write.csv(broom::tidy(osml_rmod_best),  
          "./results_statistics/rehab_effects_osml.csv")
```

Conclusion

During the 2-day rehydration opportunity provided to lizards following experimental climate treatment, body condition and plasma osmolality significantly increased and hematocrit significantly decreased. The change in body condition during rehydration was also effected by the interaction between rehydration and acclimation temperature prior to rehydration, with cool-acclimated lizards having overall higher body condition and showing a larger increase in body condition during rehydration. Cool-acclimated lizards also maintained higher hematocrit and lower plasma osmolality during rehydration compared to other treatment groups, although changes in hct and osml during rehydration were not different due to acclimation. Nested effects of trial number and individual lizard ID were included as random effects in all three LMMs.

This is true for pretty much all the model selection I did: In model selection, each dropped variable only improved the model by $<2\text{-}\Delta\text{AIC}$, so each stepwise model predicted the variability in the response variable equally well. However, the variables dropped also had very low t-values, evidence for their lack of influence thus exclusion from the model.