Experimental Data Analysis

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July 2021

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Packages

define not-in function:

```
`%nin%` = Negate(`%in%`)
```

Data

This data was collected in the Spring of 2021 in conjunction with a study carried out in Cal Poly's Herpetology class. Some lizards measured for that primary study were kept to observe physiological changes in response to different climate treatments. See (doi) for full details

Morphometrics & Hydration

Treatment Groups

variables: - individual lizard ID - temp_tmt_C = temperature treatment - humidity_tmt_percent = humidity treatment (high/low, not actually %) - trial_number = which set of lizards that individual was from - conclusion = how that individual's experiment ended (died, canceled, or complete)

```
tmts <- read.csv("./data/exp_tmt_assignment.csv")</pre>
```

Capture Data

variables: - date = date of capture & baseline measurements - individual lizard ID - mass_g = mass in grams - hematocrit_percent = % of blood sample that's red blood cells - osmolality_mmol_kg = concentration of solutes in blood plasma - type = when the measurements were taken along the course of the experiment (all on capture day)

```
##
         date
                        individual_ID
                                                         hematocrit_percent
                                             mass_g
##
   Min.
          :2021-04-19
                        Min. : 31.00
                                         Min.
                                              : 8.20
                                                         Min.
                                                                :16.00
                        1st Qu.: 57.00
                                         1st Qu.:11.10
                                                         1st Qu.:32.75
  1st Qu.:2021-04-26
   Median :2021-04-26
                        Median : 78.00
                                         Median :12.65
                                                         Median :36.00
##
                                               :12.18
## Mean
          :2021-04-29
                        Mean
                              : 77.46
                                         Mean
                                                         Mean
                                                                :36.08
## 3rd Qu.:2021-05-03
                        3rd Qu.: 98.25
                                         3rd Qu.:13.32
                                                         3rd Qu.:39.00
## Max.
           :2021-05-10
                               :122.00
                                                :15.00
                                                         Max.
                                                                :54.00
                        Max.
                                         Max.
## osmolality_mmol_kg
                           type
                      capture:52
          :319.0
## Min.
  1st Qu.:354.2
## Median :373.0
## Mean
          :373.7
## 3rd Qu.:392.2
  Max.
           :423.0
```

extract SVL data separately from capture data:

```
## 1st Qu.: 57.00 1st Qu.:65.00

## Median : 78.00 Median :68.00

## Mean : 77.46 Mean :67.62

## 3rd Qu.: 98.25 3rd Qu.:70.00

## Max. :122.00 Max. :73.00
```

extract capture CEWL cloacal temperature separately:

```
cloacal_temp_C
##
   individual_ID
          : 31.00
##
  Min.
                            :20.00
                     Min.
  1st Qu.: 57.00
                     1st Qu.:22.00
## Median : 78.00
                     Median :24.00
## Mean
          : 77.46
                     Mean
                            :23.68
##
  3rd Qu.: 98.25
                     3rd Qu.:25.00
## Max.
          :122.00
                     Max.
                            :28.00
##
                     NA's
                            :2
```

Experiment Data

variables: - date = date of measurements - individual lizard ID - mass_g = mass in grams - hematocrit_percent = % of blood sample that's red blood cells - osmolality_mmol_kg = concentration of solutes in blood plasma (mean of 1-3 replicates) - type = when the measurements were taken along the course of the experiment (either during experimental treatment or after rehab)

```
##
         date
                         individual_ID
                                                            hematocrit_percent
                                               mass_g
           :2021-04-21
                               : 31.00
                                                 : 6.700
                                                            Min.
                                                                   :12.0
##
  Min.
                         Min.
                                          Min.
   1st Qu.:2021-04-28
                         1st Qu.: 51.25
                                          1st Qu.: 9.875
                                                            1st Qu.:23.0
##
  Median :2021-05-07
                         Median : 87.50
                                          Median :11.250
                                                            Median:28.0
  Mean
           :2021-05-06
                         Mean
                               : 77.85
                                          Mean
                                                  :11.076
                                                            Mean
                                                                   :27.8
   3rd Qu.:2021-05-14
                         3rd Qu.:101.25
                                           3rd Qu.:12.225
                                                            3rd Qu.:33.0
##
   Max.
           :2021-05-20
                         Max.
                                :122.00
                                          Max.
                                                  :14.700
                                                            Max.
                                                                   :43.0
##
##
                                                            NA's
                                                                   :19
##
       type
               osmolality_mmol_kg
##
   exp :98
               Min.
                     :298.0
##
   rehab:34
               1st Qu.:342.0
               Median :355.0
##
```

```
## Mean :360.1
## 3rd Qu.:374.8
## Max. :441.0
## NA's :22
```

Join Dataframes

Now, attach all the dataframes, only use individuals whose treatment was completed, and add a "day" variable for what day of treatment each lizard/observation was on. I also calculate SMI using the equation created in capture_analysis.

```
all_dat <- exp_dat %>%
  # join data
  rbind(capture_hydration) %>%
  # add tmt group info
  left_join(tmts, by = "individual_ID") %>%
  dplyr::select(-notes) %>%
  # add SVL value for each obs of each indiv.
  # for computing BCI and scaled mass indices
  left_join(SVL, by = "individual_ID") %>%
  # only use completed experiment runs
  dplyr::filter(conclusion == "complete") %>%
  group_by(individual_ID) %>%
  # reformat a lot of variables
  mutate(capture_date = min(date),
         day = as.numeric(date - capture_date),
         humidity_tmt_percent = as.factor(humidity_tmt_percent),
         individual_ID = as.factor(individual_ID),
         temp_tmt_C = as.factor(temp_tmt_C),
         trial number = as.factor(trial number),
         conclusion = as.factor(conclusion),
         SMI = mass_g * ((65.02158/SVL_mm) ^ (3.09059/sqrt(0.8944)))
         ) %>%
  # in the first trial we took measurements every 2 days
  # exclude those obs to make consistent across trials
  dplyr::filter(day %nin% c(2,6))
summary(all_dat)
```

```
##
         date
                          individual_ID
                                                          hematocrit_percent
                                             mass_g
##
   Min.
           :2021-04-19
                          37
                                  :
                                     4
                                         Min.
                                                : 6.70
                                                          Min.
                                                                  :12.00
   1st Qu.:2021-05-01
                          39
                                         1st Qu.:10.20
                                                          1st Qu.:24.00
  Median :2021-05-10
                                         Median :11.50
                                                          Median :30.00
##
                          40
                                  :
                                     4
##
   Mean
           :2021-05-07
                          49
                                     4
                                         Mean
                                                 :11.35
                                                          Mean
                                                                  :29.95
                                  :
##
    3rd Qu.:2021-05-14
                          52
                                     4
                                         3rd Qu.:12.70
                                                          3rd Qu.:35.00
           :2021-05-20
                                     4
                                                 :15.00
                                                                  :54.00
##
   {\tt Max.}
                          54
                                  :
                                         Max.
                                                          Max.
##
                           (Other):115
                                                          NA's
                                                                  :12
##
         type
                  osmolality_mmol_kg temp_tmt_C humidity_tmt_percent trial_number
##
                  Min.
                         :298.0
                                      25:139
                                                  dry :68
                                                                        1:23
    exp
           :70
    rehab :34
                  1st Qu.:342.0
                                                  humid:71
                                                                        2:24
##
    capture:35
                  Median :358.5
                                                                        3:44
##
                  Mean
                         :362.4
                                                                        4:48
##
                  3rd Qu.:379.5
##
                  Max.
                         :441.0
                  NA's
##
                         :15
```

```
##
       conclusion
                      SVL mm
                                   capture date
                                                             day
##
   complete:139
                                         :2021-04-19
                                                       Min. : 0.000
                          :59.00
                                  Min.
                  \mathtt{Min}.
                   1st Qu.:66.00
                                                       1st Qu.: 2.000
##
                                  1st Qu.:2021-04-26
##
                  Median :68.00
                                                       Median : 4.000
                                  Median :2021-05-03
##
                   Mean
                          :67.66
                                  Mean
                                        :2021-05-01
                                                        Mean : 5.547
##
                   3rd Qu.:70.00
                                  3rd Qu.:2021-05-10
                                                        3rd Qu.: 9.000
##
                  Max. :73.00
                                  Max.
                                         :2021-05-10
                                                       Max. :11.000
##
##
         SMI
##
   Min. : 7.343
   1st Qu.: 8.976
## Median: 9.977
## Mean
         : 9.958
## 3rd Qu.:10.751
## Max. :13.970
##
unique(all_dat$individual_ID)
## [1] 47 49 39 52 37 40 80 66 54 61 74 73 92 91 95 88 93 96 98
## [20] 89 99 81 97 104 108 122 118 109 113 105 114 101 117 102 103
## 35 Levels: 37 39 40 47 49 52 54 61 66 73 74 80 81 88 89 91 92 93 95 96 ... 122
re-order some factors:
all_dat$humidity_tmt_percent <- factor(all_dat$humidity_tmt_percent,
                                       levels = c("humid", "dry"),
                                       labels = c("Humid", "Dry"))
all_dat$day <- factor(all_dat$day,</pre>
                     levels = c("0", "4", "8", "9", "10", "11"),
                     labels = c("Before Experiment",
                                 "Mid Experiment",
                                 "After Experiment",
                                 "After Experiment",
                                 "After Rehydration",
                                 "After Rehydration"))
summary(all_dat)
                                                       hematocrit_percent
##
        date
                        individual_ID
                                          {\tt mass\_g}
          :2021-04-19
                               : 4
                                      Min. : 6.70
                                                       Min.
                                                             :12.00
                                                       1st Qu.:24.00
  1st Qu.:2021-05-01
                        39
                                :
                                  4
                                      1st Qu.:10.20
## Median :2021-05-10
                        40
                                :
                                  4
                                      Median :11.50
                                                       Median :30.00
## Mean
         :2021-05-07
                        49
                                : 4
                                      Mean :11.35
                                                       Mean
                                                            :29.95
   3rd Qu.:2021-05-14
                                                       3rd Qu.:35.00
                        52
                                : 4
                                      3rd Qu.:12.70
##
  Max. :2021-05-20
                               : 4
                                      Max. :15.00
                                                       Max.
                                                              :54.00
                        54
                         (Other):115
                                                       NA's
##
                                                              :12
##
         type
                 osmolality_mmol_kg temp_tmt_C humidity_tmt_percent trial_number
##
          :70
                Min.
                        :298.0
                                   25:139
                                              Humid:71
                                                                    1:23
   exp
                                                                    2:24
   rehab :34
                 1st Qu.:342.0
##
                                              Dry :68
##
   capture:35
                Median :358.5
                                                                    3:44
                                                                    4:48
##
                Mean :362.4
##
                 3rd Qu.:379.5
##
                Max.
                       :441.0
##
                NA's
                       :15
##
       conclusion
                      SVL_mm
                                   capture_date
                                                                       day
```

```
##
    complete:139
                           :59.00
                                    Min.
                                           :2021-04-19
                                                          Before Experiment:35
                   Min.
                   1st Qu.:66.00
                                    1st Qu.:2021-04-26
##
                                                          Mid Experiment
                                    Median :2021-05-03
##
                   Median :68.00
                                                          After Experiment: 35
                                                          After Rehydration:34
##
                   Mean
                           :67.66
                                           :2021-05-01
                                    Mean
##
                   3rd Qu.:70.00
                                    3rd Qu.:2021-05-10
                           :73.00
                                           :2021-05-10
##
                   Max.
                                    Max.
##
         SMI
##
##
    Min.
           : 7.343
##
    1st Qu.: 8.976
##
   Median: 9.977
          : 9.958
##
   Mean
    3rd Qu.:10.751
##
  Max.
           :13.970
##
##
```

make a sub-dataframe without rehab data to prevent any mix-ups:

```
all_dat_no_rehab <- all_dat %>%
  dplyr::filter(type != "rehab")
```

Checks

Dates:

```
# check that capture dates are valid
unique(all_dat$capture_date)
```

```
## [1] "2021-04-19" "2021-04-26" "2021-05-03" "2021-05-10"
```

Check that each lizard has an accurate number of measurements.

```
all_dat %>%
  group_by(individual_ID, type) %>%
  summarise(n = n()) %>%
  arrange(type)
```

```
## `summarise()` regrouping output by 'individual_ID' (override with `.groups` argument)
## # A tibble: 104 x 3
               individual_ID [35]
## # Groups:
##
      individual_ID type
                               n
##
      <fct>
                     <fct> <int>
##
   1 37
                               2
                     exp
##
   2 39
                               2
                    exp
##
  3 40
                               2
                    exp
## 4 47
                               2
                    exp
## 5 49
                               2
                    exp
##
   6 52
                               2
                    exp
                               2
##
   7 54
                     exp
##
   8 61
                               2
                     exp
## 9 66
                               2
                     exp
## 10 73
                               2
                     exp
## # ... with 94 more rows
```

That all looks good, every lizard has 1 capture measurement, 2 experimental measurements, and 1 rehab measurement.

CEWL

Capture CEWL

variables: - date = date of capture & baseline measurements - individual lizard ID - region = which body area the measurement was taken from - $TEWL_g_m2h$ = evaporative water loss - cloacal_temp_C = taken at measurement; influences CEWL

```
##
        date
                        individual_ID
                                          region
                                                     TEWL_g_m2h
                                                                       day
  Min.
                               : 37.00
##
          :2021-04-19
                        Min.
                                         dewl:32
                                                   Min. : 7.48
                                                                   before:163
   1st Qu.:2021-04-26
                        1st Qu.: 73.00
                                         dors:33
                                                   1st Qu.:20.54
##
## Median :2021-05-03
                        Median : 95.00
                                         head:33
                                                   Median :27.43
          :2021-05-02
                                                         :29.30
## Mean
                        Mean
                              : 87.46
                                         mite:32
                                                   Mean
##
   3rd Qu.:2021-05-10
                        3rd Qu.:104.00
                                         vent:33
                                                   3rd Qu.:36.91
## Max.
          :2021-05-10
                        Max.
                               :122.00
                                                   Max.
                                                          :62.94
##
               cloacal_temp_C
       n_day
##
  Min.
         :0
              Min.
                      :20.00
  1st Qu.:0
               1st Qu.:22.00
##
## Median :0
               Median :24.00
## Mean
         :0
               Mean
                      :23.84
## 3rd Qu.:0
               3rd Qu.:25.00
## Max.
          :0
               Max.
                      :28.00
```

Post-Experiment CEWL

In the future, I could automate this like I did for the HOBO data.

Load in each of the post-rehab datafiles:

```
Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h..
# trial 3
CEWL_t3 <- read.csv("./data/post_exp_CEWL/5-11-21-CEWL.csv",</pre>
                          na.strings=c("","NA")) %>%
  dplyr::select(date = Date,
                Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h..
# trial 4
CEWL_t4 <- read.csv("./data/post_exp_CEWL/5-18-21-CEWL.csv",
                          na.strings=c("","NA")) %>%
  dplyr::select(date = Date,
                Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h..
```

Load in cloacal temperatures:

```
exp_CT <- read.csv("./data/post_exp_CEWL_cloacal_temps.csv") %>%
  mutate(date = as.Date(date, format = "%Y/%m/%d")) %>%
  dplyr::select(-time)
summary(exp_CT)
```

```
individual_ID
                                     cloacal_temp_C
##
       date
## Min.
         :2021-04-28 Min. : 37.00 Min. :19.0
## 1st Qu.:2021-05-04
                     1st Qu.: 69.50
                                    1st Qu.:21.0
## Median: 2021-05-11 Median: 93.00 Median: 23.0
        :2021-05-09 Mean : 85.91
## Mean
                                    Mean :22.4
## 3rd Qu.:2021-05-18 3rd Qu.:103.50
                                    3rd Qu.:23.0
## Max.
        :2021-05-18 Max. :122.00
                                    Max. :26.0
```

Join Dataframes

Merge all post-experiment CEWL, add cloacal temperature, add capture CEWL:

```
# format individual ID
               individual_ID = as.integer(individual_ID),
               # set body region as a factor variable after getting only the consistent characters due
               region = as.factor(substring(region, 1, 4)),
               # add when measurement taken
               day = as.factor("after"),
               n_day = 1 # technically day 8/9, just to help with figures
               ) %>%
 # remove cols not relevant to stats
 dplyr::select(-Status) %>%
  # remove any rows with missing values
  # none actually needed to be removed
 dplyr::filter(complete.cases(.)) %>%
 # add cloacal temperatures
 left_join(exp_CT, by = c("date", "individual_ID")) %>%
 # now matching dataframes, add capture CEWL data
 rbind(cap_CEWL) %>%
 # add tmt assignments
 left_join(tmts, by = "individual_ID") %>%
 mutate(humidity_tmt_percent = as.factor(humidity_tmt_percent),
        individual_ID = as.factor(individual_ID),
        conclusion = as.factor(conclusion),
        trial_number = as.factor(trial_number)
        ) %>%
 # lizards 49 & 80 are missing pre-exp CEWL, so remove them
 dplyr::filter((individual ID %nin% c('49', '80')))
# every lizard should have 10 measurements
summary(CEWL)
##
                        individual_ID region
        date
                                                  TEWL_g_m2h
                                                                    day
                                                                 after :163
## Min.
          :2021-04-19
                               : 10
                                     dewl:65
                        37
                                                Min. : 4.60
## 1st Qu.:2021-05-03
                        39
                                      dors:65
                                                1st Qu.: 20.09
                                                                 before:163
                               : 10
                                                Median : 27.18
## Median :2021-05-10
                       40
                               : 10 head:66
## Mean :2021-05-06
                       47
                               : 10
                                     mite:64
                                               Mean : 30.69
## 3rd Qu.:2021-05-11
                                     vent:66
                                                3rd Qu.: 38.72
                      52
                              : 10
## Max.
          :2021-05-18
                       54
                               : 10
                                                Max.
                                                      :106.38
##
                        (Other):266
##
                 cloacal_temp_C
                                   temp_tmt_C humidity_tmt_percent trial_number
       n_day
## Min.
          :0.0
                Min. :19.00 Min. :25
                                              dry :158
                                                                  1: 50
## 1st Qu.:0.0
                 1st Qu.:21.00
                                1st Qu.:25
                                              humid:168
                                                                  2: 48
## Median :0.5
                 Median :23.00
                                Median:25
                                                                  3:110
                       :23.11
## Mean
         :0.5
                                 Mean
                                       :25
                                                                  4:118
                 Mean
## 3rd Qu.:1.0
                                 3rd Qu.:25
                 3rd Qu.:24.75
## Max.
          :1.0
                       :28.00
                                        :25
                Max.
                               {\tt Max.}
##
##
      conclusion
                     notes
##
   complete:326
                 Length:326
##
                  Class :character
##
                  Mode :character
##
##
##
```

##

Check that data looks correct:

```
CEWL %>%
  group_by(individual_ID, day) %>%
  summarise(n = n()) \%>\%
  arrange(individual_ID, n)
## `summarise()` regrouping output by 'individual_ID' (override with `.groups` argument)
## # A tibble: 66 x 3
## # Groups:
               individual ID [33]
##
      individual_ID day
##
      <fct>
                    <fct>
                            <int>
   1 37
##
                    after
                                5
    2 37
##
                    before
                                5
##
    3 39
                    after
                                5
##
                                5
   4 39
                    before
##
   5 40
                    after
                                5
##
    6 40
                    before
                                5
##
   7 47
                    after
                                5
##
  8 47
                    before
                                5
## 9 52
                                5
                    after
## 10 52
                    before
                                5
## # ... with 56 more rows
```

Everything looks great! (after removing the observations for the two lizards with missing pre-experiment CEWL measurements.)

Before/after aren't perfectly even because sometimes we were unable to get the AquaFlux to equilibrate and take a measurement.

Finally, make a small edit so the regions are spelled out completely. This requires reordering factor levels:

```
##
                          individual_ID
         date
                                               region
                                                           TEWL_g_m2h
   Min.
           :2021-04-19
                                 : 10
                                        Dorsum
                                                   :65
                                                         Min.
                                                              : 4.60
   1st Qu.:2021-05-03
                                                   :66
                                                         1st Qu.: 20.09
##
                          39
                                 : 10
                                        Ventrum
   Median :2021-05-10
                          40
                                 : 10
                                        Head
                                                   :66
                                                         Median: 27.18
##
                                 : 10
  Mean
           :2021-05-06
                          47
                                                                : 30.69
                                        Dewlap
                                                   :65
                                                         Mean
    3rd Qu.:2021-05-11
                          52
                                 : 10
                                        Mite Patch:64
                                                         3rd Qu.: 38.72
           :2021-05-18
                                 : 10
                                                                :106.38
##
  Max.
                          54
                                                         Max.
##
                          (Other):266
##
                                cloacal_temp_C
        day
                     n_day
                                                   temp_tmt_C humidity_tmt_percent
  Before:163
                         :0.0
                                Min.
                                       :19.00
                                                        :25
                                                              Humid:168
                 Min.
                                                Min.
    After:163
                 1st Qu.:0.0
                                1st Qu.:21.00
                                                 1st Qu.:25
                                                              Dry :158
```

```
Median: 0.5 Median: 23.00 Median: 25
##
               Mean :0.5 Mean :23.11 Mean :25
##
               3rd Qu.:1.0 3rd Qu.:24.75 3rd Qu.:25
##
##
               Max.
                     :1.0 Max. :28.00 Max. :25
##
##
  trial_number
                 conclusion
                               notes
  1: 50
               complete:326 Length:326
## 2: 48
                            Class :character
## 3:110
                            Mode :character
## 4:118
##
##
##
```

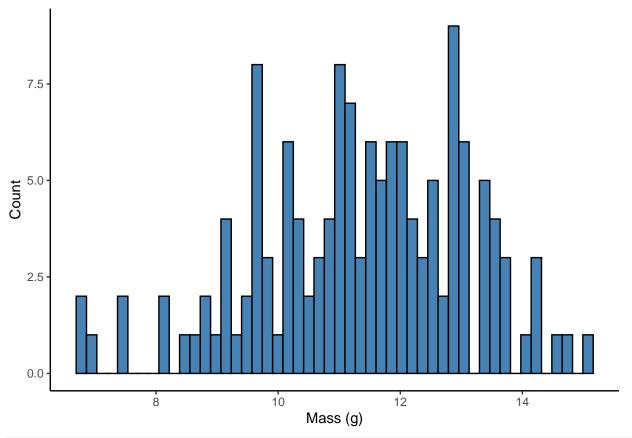
Export Data Frames for Power Analyses

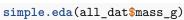
```
#write.csv(all_dat, "exported_data/exp_effects_hydration.csv")
#write.csv(CEWL, "exported_data/exp_effects_CEWL.csv")
```

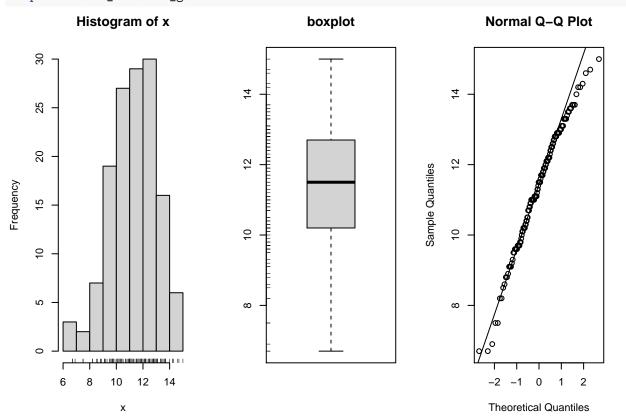
Data Distributions

Mass

```
all_dat %>%
  ggplot(., aes(x = mass_g)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("Mass (g)") +
  ylab("Count")
```







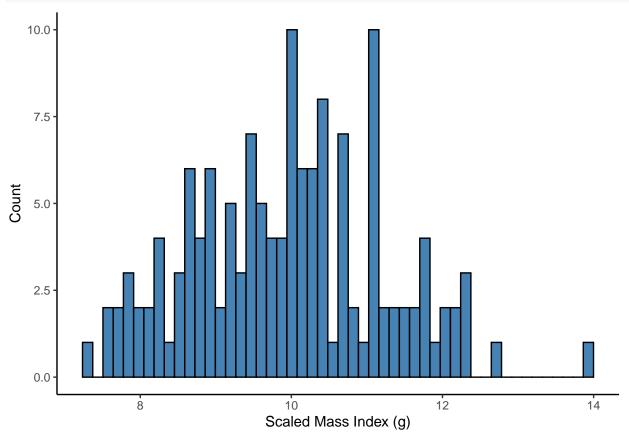
shapiro.test(all_dat\$mass_g)

```
##
## Shapiro-Wilk normality test
##
## data: all_dat$mass_g
## W = 0.98437, p-value = 0.1144
```

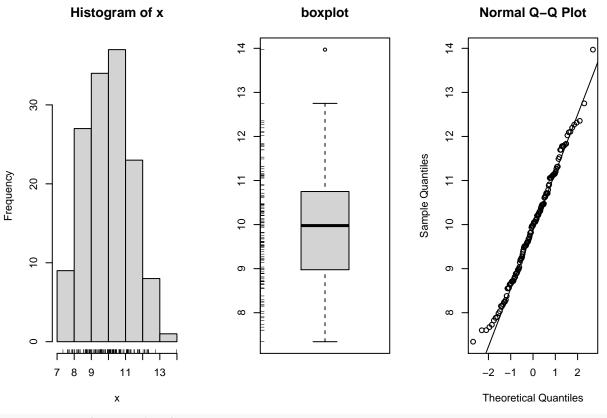
Mass distribution not normal, skewed to the left.

Scaled Mass Index

```
all_dat %>%
  ggplot(., aes(x = SMI)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("Scaled Mass Index (g)") +
  ylab("Count")
```



simple.eda(all_dat\$SMI)



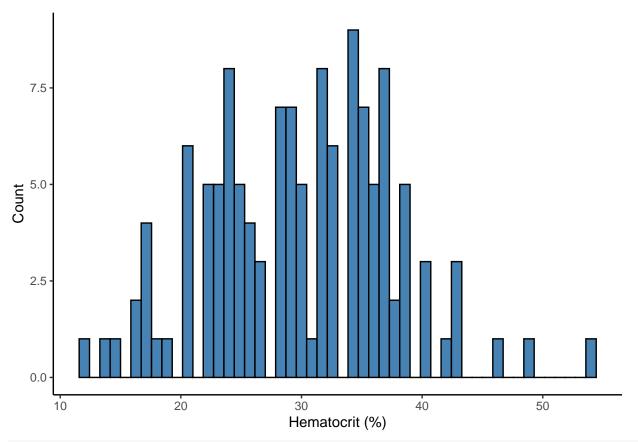
```
shapiro.test(all_dat$SMI)
```

```
##
## Shapiro-Wilk normality test
##
## data: all_dat$SMI
## W = 0.98975, p-value = 0.4014
```

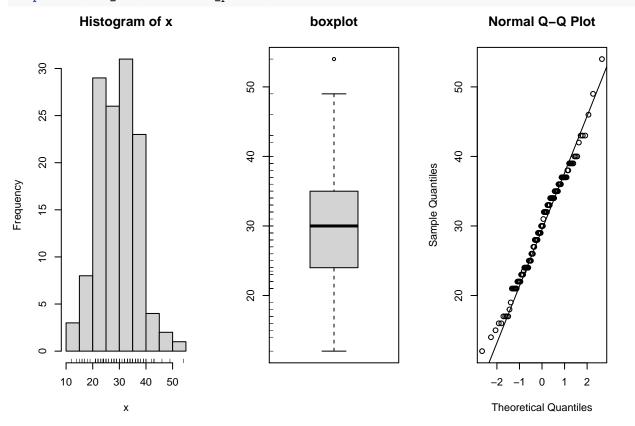
Hematocrit

```
all_dat %>%
  ggplot(., aes(x = hematocrit_percent)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("Hematocrit (%)") +
  ylab("Count")
```

Warning: Removed 12 rows containing non-finite values (stat_bin).



simple.eda(all_dat\$hematocrit_percent)



shapiro.test(all_dat\$hematocrit_percent)

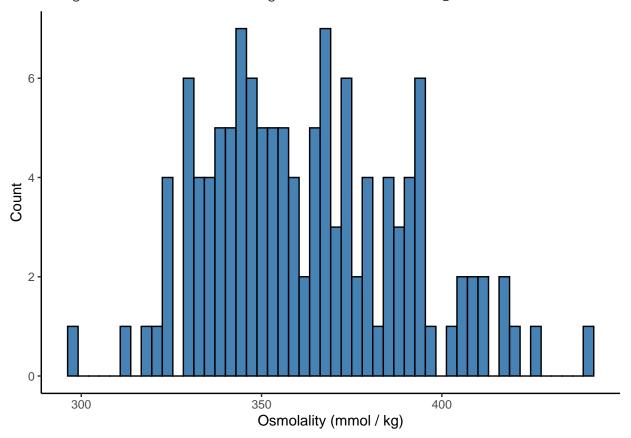
```
##
## Shapiro-Wilk normality test
##
## data: all_dat$hematocrit_percent
## W = 0.98872, p-value = 0.385
```

Visually, looks slightly skewed to the right, but statistically, the distribution of hematocrit is normal.

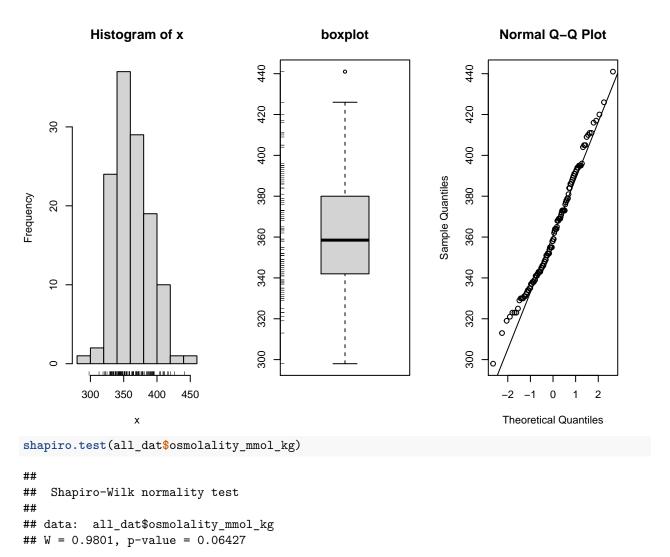
Osmolality

```
all_dat %>%
  ggplot(., aes(x = osmolality_mmol_kg)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("Osmolality (mmol / kg)") +
  ylab("Count")
```

Warning: Removed 15 rows containing non-finite values (stat_bin).



simple.eda(all_dat\$osmolality_mmol_kg)



Visually, looks slightly skewed to the right, but statistically, the distribution of osmolality is normal.

Figures

Means to Overlay

```
all_dat_mean_SMI <- all_dat %>%
  group_by(humidity_tmt_percent, day) %>%
  summarise(SMI_mean = mean(SMI))

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

all_dat_mean_hct <- all_dat %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  group_by(humidity_tmt_percent, day) %>%
  summarise(hct_mean = mean(hematocrit_percent))

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

all_dat_mean_osml <- all_dat %>%
  dplyr::filter(complete.cases(osmolality_mmol_kg)) %>%
```

```
group_by(humidity_tmt_percent, day) %>%
summarise(osml_mean = mean(osmolality_mmol_kg))
```

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

SMI ~ Time

```
ggplot() +
  geom_point(data = all_dat,
             aes(x = day,
                 y = SMI,
                 color = humidity tmt percent
                 ),
             size = 1,
             alpha = 0.6) +
  geom_line(data = all_dat,
            aes(x = day,
                v = SMI,
                group = individual_ID,
                color = humidity_tmt_percent),
            alpha = 0.2) +
  geom_line(data = all_dat_mean_SMI,
            aes(x = day,
                y = SMI_mean,
                group = humidity_tmt_percent,
                color = humidity_tmt_percent),
            size = 1.6,
            alpha = 1) +
  theme classic() +
  scale_color_brewer(palette = "Set2",
                     name = "") +
  xlab("") +
  ylab("Body Condition (g)") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 22),
       axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 16),
        axis.text.x = element_blank(),
        legend.text = element_text(color = "black",
                                 family = "sans",
                                 size = 24),
        legend.text.align = 0,
        legend.position = "none",
        plot.margin = unit(c(0.2,0,0,0.4), "cm")
) -> tmt_effects_SMI
tmt_effects_SMI
```

```
# export figure
#ggsave(filename = "tmt_effects_SMI.jpeg",
# plot = tmt_effects_SMI,
# path = "./final_figures",
# device = "jpeg",
# device = "jpeg",
# difference = "tope =
```

$Hct \sim Time$

```
ggplot() +
  geom_point(data = all_dat,
             aes(x = day,
                 y = hematocrit_percent,
                 color = humidity_tmt_percent
                 ),
             size = 1,
             alpha = 0.6) +
  geom_line(data = all_dat,
            aes(x = day,
                y = hematocrit_percent,
                group = individual_ID,
                color = humidity_tmt_percent),
            alpha = 0.2) +
  geom_line(data = all_dat_mean_hct,
            aes(x = day,
                y = hct_mean,
                group = humidity_tmt_percent,
```

```
color = humidity_tmt_percent),
            size = 1.6,
            alpha = 1) +
  theme_classic() +
  scale_color_brewer(palette = "Set2",
                     name = "") +
  scale_x_discrete(labels = c("Before\nExperiment",
                              "Mid\nExperiment",
                              "After\nExperiment",
                              "After\nRehydration")) +
 xlab("") +
 ylab("Hematocrit (%)") +
 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 = 24),
        legend.text.align = 0,
        legend.position = "none",
        plot.margin = unit(c(0.2,0,0,0.4), "cm")
        ) -> tmt_effects_hct
tmt_effects_hct
```

- ## Warning: Removed 12 rows containing missing values (geom_point).
- ## Warning: Removed 12 row(s) containing missing values (geom_path).

```
50-
(%) 40-
10

Before Mid After After Experiment Experiment Rehydration
```

```
# export figure
#ggsave(filename = "tmt_effects_hct.jpeg",

#          plot = tmt_effects_hct,

#          path = "./final_figures",

#          device = "jpeg",

#          dpi = 1200,

#          width = 5, height = 4)
```

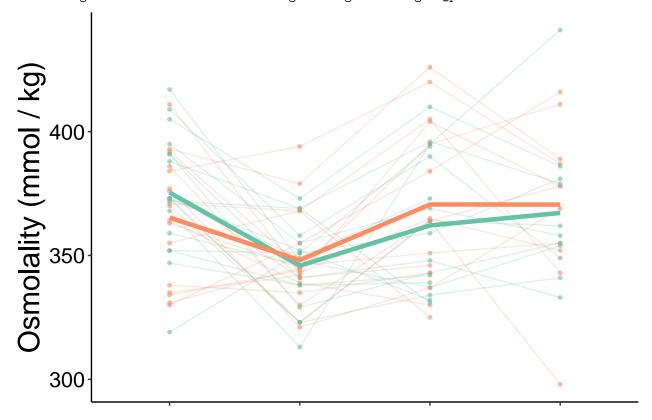
$Osml \sim Time$

```
ggplot() +
  geom_point(data = all_dat,
             aes(x = day,
                 y = osmolality_mmol_kg,
                 color = humidity_tmt_percent
                 ),
             size = 1,
             alpha = 0.6) +
  geom_line(data = all_dat,
            aes(x = day,
                y = osmolality_mmol_kg,
                group = individual_ID,
                color = humidity_tmt_percent),
            alpha = 0.2) +
  geom_line(data = all_dat_mean_osml,
            aes(x = day,
                y = osml_mean,
                group = humidity_tmt_percent,
```

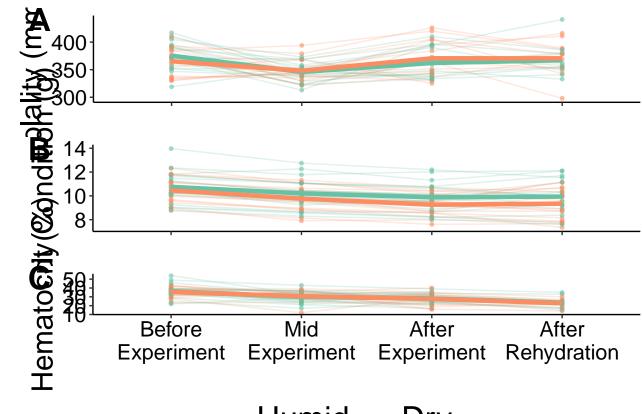
```
color = humidity_tmt_percent),
            size = 1.6,
            alpha = 1) +
  theme_classic() +
  scale_color_brewer(palette = "Set2",
                     name = "") +
  xlab("") +
  ylab("Osmolality (mmol / kg)") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 22),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 16),
        axis.text.x = element_blank(),
        legend.text = element_text(color = "black",
                                 family = "sans",
                                  size = 24),
        legend.text.align = 0,
        legend.position = "none",
        plot.margin = unit(c(0.2,0,0,0.1), "cm")
        ) -> tmt_effects_osml
tmt_effects_osml
```

Warning: Removed 15 rows containing missing values (geom_point).

Warning: Removed 15 row(s) containing missing values (geom_path).



Multi-Figure

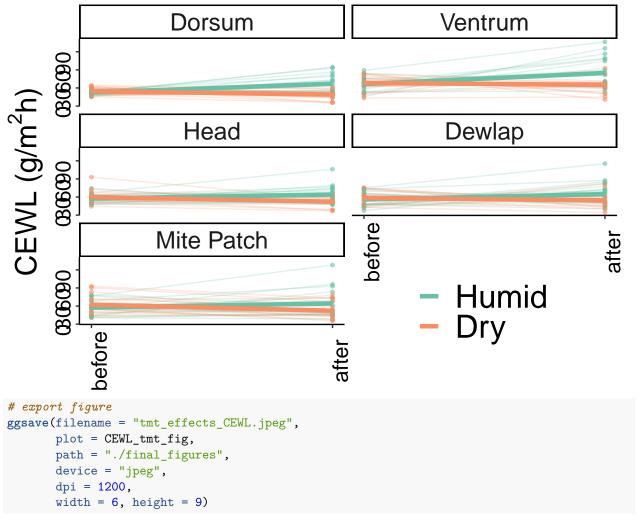


Humid – Dry

CEWL

```
CEWL %>%
  ggplot(data = .) +
  geom_point(aes(x = n_day,
                 y = TEWL_g_m2h,
                 color = humidity_tmt_percent
                 ),
             size = 1,
             alpha = 0.6) +
  geom_line(aes(x = n_day,
                y = TEWL_g_m2h,
                group = individual_ID,
                color = humidity_tmt_percent),
            alpha = 0.2) +
  stat_smooth(aes(x = n_day,
                  y = TEWL_g_m2h,
                  color = humidity_tmt_percent
```

```
formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  scale_color_brewer(palette = "Set2",
                     name = ""
                     #name = "Humidity\nTreatment"
                     ) +
  facet_wrap(~region, ncol = 2) +
  scale_x_continuous(breaks = c(0, 1),
                     labels = c("0" = "before", "1" = "after")
                     ) +
  xlab("") +
  ylab(bquote('CEWL (g/'*m^2*'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 = 24),
        legend.text.align = 0,
        legend.position = c(0.75,0.12),
        \#legend.justification = c(1, 1)
) -> CEWL_tmt_fig
CEWL_tmt_fig
```



I saved the legend separately to make the figure layout better.

Models

SMI

Check whether means started out different:

```
SMI_diff_lm <- all_dat_no_rehab %>%
    dplyr::filter(day == "Before Experiment") %>%
    lm(data = ., SMI ~ humidity_tmt_percent)
summary(SMI_diff_lm)

##
## Call:
## lm(formula = SMI ~ humidity_tmt_percent, data = .)
##
## Residuals:
## Min    1Q Median    3Q Max
## -1.9405 -0.7429 -0.0401    0.7385    3.2183
##
## Coefficients:
```

```
##
                          Estimate Std. Error t value Pr(>|t|)
                           10.7517
                                     0.2811
                                                38.25
                                                        <2e-16 ***
## (Intercept)
## humidity_tmt_percentDry -0.2904
                                       0.4033 - 0.72
                                                         0.476
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.192 on 33 degrees of freedom
## Multiple R-squared: 0.01547,
                                 Adjusted R-squared:
## F-statistic: 0.5187 on 1 and 33 DF, p-value: 0.4765
NOT significantly different, which is good.
Check whether means ended differently:
SMI_diff_lm_end <- all_dat_no_rehab %>%
 dplyr::filter(day == "After Experiment") %>%
 lm(data = ., SMI ~ humidity_tmt_percent)
summary(SMI_diff_lm_end)
##
## Call:
## lm(formula = SMI ~ humidity_tmt_percent, data = .)
##
## Residuals:
##
       Min
                 1Q Median
                                   3Q
                                           Max
## -1.85144 -0.69873 -0.07453 0.80895 2.31088
## Coefficients:
##
                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                            9.8852
                                       0.2585 38.246
                                                        <2e-16 ***
## humidity_tmt_percentDry -0.6287
                                       0.3709 -1.695
                                                        0.0994 .
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 1.097 on 33 degrees of freedom
## Multiple R-squared: 0.08012,
                                   Adjusted R-squared:
## F-statistic: 2.874 on 1 and 33 DF, p-value: 0.09943
Build Model
SMI_mod <- lme4::lmer(data = all_dat_no_rehab,</pre>
              SMI ~ day*humidity_tmt_percent +
               (1|trial_number))
## boundary (singular) fit: see ?isSingular
summary(SMI_mod)
## Linear mixed model fit by REML ['lmerMod']
## Formula: SMI ~ day * humidity_tmt_percent + (1 | trial_number)
     Data: all_dat_no_rehab
## REML criterion at convergence: 325.9
## Scaled residuals:
##
       Min
                                   3Q
                1Q
                     Median
                                           Max
```

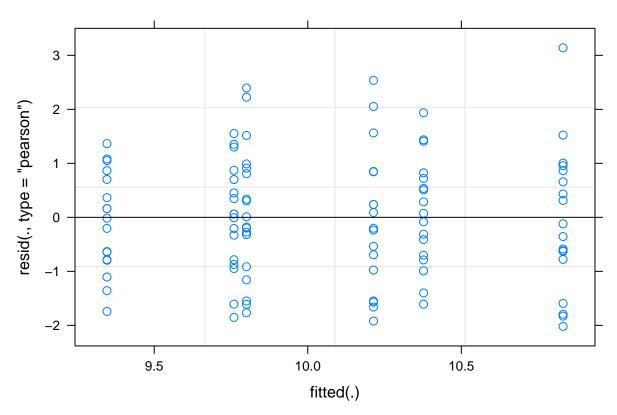
```
## -1.68654 -0.68344 -0.01058 0.71830 2.79715
##
## Random effects:
                             Variance Std.Dev.
## Groups Name
## trial_number (Intercept) 2.368e-20 1.539e-10
                             1.324e+00 1.151e+00
## Residual
## Number of obs: 105, groups: trial_number, 4
##
## Fixed effects:
##
                                               Estimate Std. Error t value
## (Intercept)
                                                10.7517
                                                           0.2712 39.646
                                                            0.3835 -1.415
## dayMid Experiment
                                                -0.5428
## dayAfter Experiment
                                                -0.8665
                                                            0.3835 -2.259
## humidity_tmt_percentDry
                                                -0.2904
                                                            0.3891 - 0.746
## dayMid Experiment:humidity_tmt_percentDry
                                                -0.1534
                                                            0.5503 -0.279
## dayAfter Experiment:humidity_tmt_percentDry -0.3383
                                                            0.5503 -0.615
##
## Correlation of Fixed Effects:
##
               (Intr) dyMdEx dyAftE hmd__D dME:__
## dyMdExprmnt -0.707
## dyAftrExprm -0.707 0.500
## hmdty tmt D -0.697 0.493 0.493
## dyMExpr:__D 0.493 -0.697 -0.348 -0.707
## dyAExpr:__D 0.493 -0.348 -0.697 -0.707 0.500
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see ?isSingular
drop1(SMI mod)
## boundary (singular) fit: see ?isSingular
## Single term deletions
##
## Model:
## SMI ~ day * humidity_tmt_percent + (1 | trial_number)
##
                            npar
                                   AIC
## <none>
                                 337.25
                               2 333.66
## day:humidity_tmt_percent
# drop interaction term
SMI_mod2 <- lme4::lmer(data = all_dat_no_rehab,</pre>
               SMI ~ day + humidity_tmt_percent +
               (1 trial number))
## boundary (singular) fit: see ?isSingular
summary(SMI_mod2)
## Linear mixed model fit by REML ['lmerMod']
## Formula: SMI ~ day + humidity_tmt_percent + (1 | trial_number)
     Data: all_dat_no_rehab
##
## REML criterion at convergence: 327.3
## Scaled residuals:
                 10
                     Median
                                    3Q
## -1.76998 -0.68801 -0.00593 0.73878 2.75012
```

```
##
## Random effects:
  Groups
                Name
                             Variance Std.Dev.
  trial_number (Intercept) 0.000
                                      0.000
## Residual
                             1.303
                                      1.141
## Number of obs: 105, groups: trial_number, 4
## Fixed effects:
##
                           Estimate Std. Error t value
## (Intercept)
                                       0.2212 48.964
                           10.8313
## dayMid Experiment
                           -0.6173
                                        0.2728 -2.263
## dayAfter Experiment
                            -1.0308
                                        0.2728 -3.778
## humidity_tmt_percentDry -0.4543
                                        0.2229 -2.039
##
## Correlation of Fixed Effects:
               (Intr) dyMdEx dyAftE
##
## dyMdExprmnt -0.617
## dyAftrExprm -0.617
## hmdty_tmt_D -0.489 0.000 0.000
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see ?isSingular
drop1(SMI_mod2)
## boundary (singular) fit: see ?isSingular
## boundary (singular) fit: see ?isSingular
## Single term deletions
##
## Model:
## SMI ~ day + humidity_tmt_percent + (1 | trial_number)
##
                        npar
                                AIC
## <none>
                             333.66
## day
                           2 343.71
## humidity_tmt_percent
                           1 335.89
```

Check Conditions

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

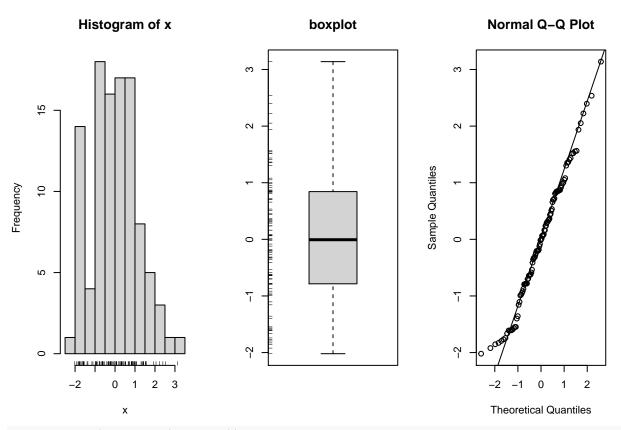
```
plot(SMI_mod2)
```



L & E both look good.

Is the distribution of residuals **normal**? use Shapiro-Wilk normality test: H0: data is NOT significantly different from normal distribution HA: data IS significantly different from normal distribution

simple.eda(residuals(SMI_mod2))



shapiro.test(residuals(SMI_mod2))

```
##
## Shapiro-Wilk normality test
##
## data: residuals(SMI_mod2)
## W = 0.98165, p-value = 0.1551
```

Normality is fine.

Export Best

SMI is best predicted by day and treatment, but not including their interaction.

Hematocrit

Build Model

```
hct_mod <- all_dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
```

```
hematocrit_percent ~ day * humidity_tmt_percent +
               (1 trial_number))
summary(hct mod)
## Linear mixed model fit by REML ['lmerMod']
## Formula: hematocrit_percent ~ day * humidity_tmt_percent + (1 | trial_number)
##
     Data: .
##
## REML criterion at convergence: 665.8
##
## Scaled residuals:
      Min
               1Q Median
                                3Q
                                       Max
## -3.1677 -0.5501 0.0365 0.5956 2.6381
##
## Random effects:
## Groups
                             Variance Std.Dev.
                Name
## trial_number (Intercept) 7.185
                                      2.681
## Residual
                             38.959
                                      6.242
## Number of obs: 105, groups: trial_number, 4
## Fixed effects:
                                               Estimate Std. Error t value
##
## (Intercept)
                                                36.0220 1.9995 18.016
## dayMid Experiment
                                                -6.6111
                                                            2.0806 -3.178
## dayAfter Experiment
                                                -8.2222
                                                            2.0806 -3.952
## humidity_tmt_percentDry
                                                -0.8797
                                                            2.1115 -0.417
## dayMid Experiment:humidity_tmt_percentDry
                                                 1.7288
                                                            2.9853
                                                                   0.579
## dayAfter Experiment:humidity_tmt_percentDry
                                                 0.3399
                                                            2.9853
                                                                   0.114
## Correlation of Fixed Effects:
##
               (Intr) dyMdEx dyAftE hmd__D dME:__
## dyMdExprmnt -0.520
## dyAftrExprm -0.520 0.500
## hmdty_tmt_D -0.514 0.493 0.493
## dyMExpr:__D 0.363 -0.697 -0.348 -0.707
## dyAExpr:__D 0.363 -0.348 -0.697 -0.707 0.500
drop1(hct_mod)
## Single term deletions
##
## hematocrit_percent ~ day * humidity_tmt_percent + (1 | trial_number)
                                    AIC
                            npar
## <none>
                                 699.17
## day:humidity_tmt_percent
                               2 695.56
# drop day*humidity interaction
hct_mod2 <- all_dat_no_rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
              hematocrit_percent ~ day + humidity_tmt_percent +
               (1|trial_number))
summary(hct mod2)
```

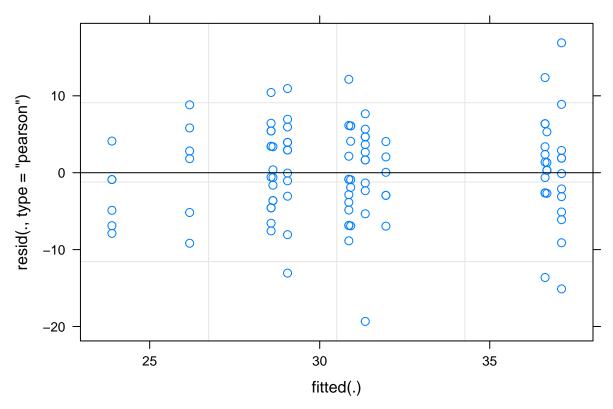
```
## Linear mixed model fit by REML ['lmerMod']
## Formula: hematocrit_percent ~ day + humidity_tmt_percent + (1 | trial_number)
      Data: .
##
## REML criterion at convergence: 674
## Scaled residuals:
##
       Min 1Q
                     Median
                                   3Q
## -3.10869 -0.60033 0.02219 0.62265 2.71370
##
## Random effects:
## Groups
                            Variance Std.Dev.
                Name
## trial_number (Intercept) 7.224
                                    2.688
## Residual
                                      6.190
                            38.312
## Number of obs: 105, groups: trial_number, 4
##
## Fixed effects:
##
                         Estimate Std. Error t value
## (Intercept)
                           35.685
                                       1.812 19.698
                                        1.480 -3.901
## dayMid Experiment
                            -5.771
## dayAfter Experiment
                            -8.057
                                       1.480 -5.445
## humidity_tmt_percentDry -0.190
                                       1.210 -0.157
##
## Correlation of Fixed Effects:
##
               (Intr) dyMdEx dyAftE
## dyMdExprmnt -0.408
## dyAftrExprm -0.408 0.500
## hmdty_tmt_D -0.326 0.000 0.000
drop1(hct_mod2)
## Single term deletions
##
## Model:
## hematocrit_percent ~ day + humidity_tmt_percent + (1 | trial_number)
                        npar
                                AIC
                             695.56
## <none>
## day
                           2 719.67
## humidity_tmt_percent
                          1 693.59
# drop humidity tmt
hct mod3 <- all dat no rehab %>%
  dplyr::filter(complete.cases(hematocrit_percent)) %>%
  lme4::lmer(data = .,
              hematocrit_percent ~ day +
               (1 trial_number))
summary(hct_mod3)
## Linear mixed model fit by REML ['lmerMod']
## Formula: hematocrit_percent ~ day + (1 | trial_number)
##
      Data: .
## REML criterion at convergence: 676.2
##
## Scaled residuals:
```

```
##
                  1Q
                       Median
## -3.14009 -0.58850 0.00871 0.64072 2.74219
##
## Random effects:
##
    Groups
                 Name
                             Variance Std.Dev.
   trial_number (Intercept) 7.253
                                       2.693
##
   Residual
                             37.932
                                       6.159
## Number of obs: 105, groups: trial_number, 4
##
## Fixed effects:
##
                       Estimate Std. Error t value
                                      1.712 20.794
## (Intercept)
                         35.592
## dayMid Experiment
                         -5.771
                                      1.472 -3.920
## dayAfter Experiment
                         -8.057
                                      1.472 -5.473
##
## Correlation of Fixed Effects:
##
               (Intr) dyMdEx
## dyMdExprmnt -0.430
## dyAftrExprm -0.430
                       0.500
drop1(hct_mod3)
## Single term deletions
##
## Model:
## hematocrit_percent ~ day + (1 | trial_number)
          npar
##
                  AIC
## <none>
               693.59
             2 717.69
## day
```

Check Conditions

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

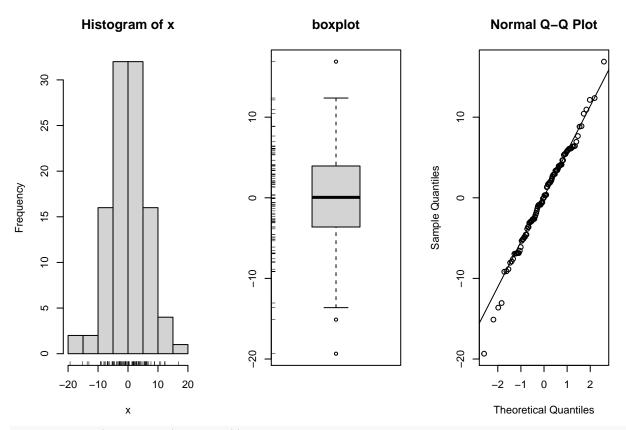
```
plot(hct_mod3)
```



L & E both seem fine. Slight fanning to the right, but otherwise no big pattern, so L is definitely okay. E could use help.

Is the distribution of residuals **normal**? use Shapiro-Wilk normality test: H0: data is NOT significantly different from normal distribution HA: data IS significantly different from normal distribution

simple.eda(residuals(hct_mod3))



shapiro.test(residuals(hct_mod3))

```
##
## Shapiro-Wilk normality test
##
## data: residuals(hct_mod3)
## W = 0.98869, p-value = 0.5248
residuals are normally distributed!
```

Export Best

Osmolality

Build Model

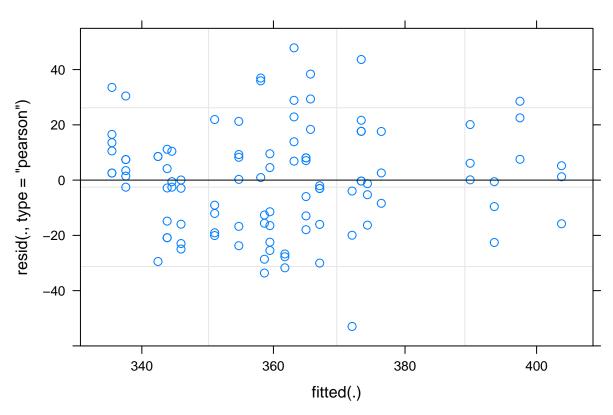
```
(1|trial_number))
summary(osml_mod)
## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg ~ day * humidity_tmt_percent + (1 | trial_number)
##
      Data: .
##
## REML criterion at convergence: 872.1
## Scaled residuals:
##
       Min
                  10
                       Median
                                    30
                                             Max
## -2.66849 -0.79364 0.00361 0.51469
##
## Random effects:
## Groups
                             Variance Std.Dev.
                 Name
## trial_number (Intercept) 313.5
                                      17.71
## Residual
                             393.8
                                       19.84
## Number of obs: 102, groups: trial_number, 4
##
## Fixed effects:
                                               Estimate Std. Error t value
##
## (Intercept)
                                                 378.509
                                                             10.035 37.719
## dayMid Experiment
                                                 -29.500
                                                              6.615 -4.460
## dayAfter Experiment
                                                 -13.885
                                                              6.713 -2.068
## humidity_tmt_percentDry
                                                 -10.215
                                                              6.713 - 1.522
## dayMid Experiment:humidity_tmt_percentDry
                                                  12.324
                                                              9.491
                                                                      1.298
## dayAfter Experiment:humidity_tmt_percentDry
                                                  17.783
                                                              9.721
                                                                      1.829
##
## Correlation of Fixed Effects:
##
               (Intr) dyMdEx dyAftE hmd__D dME:__
## dyMdExprmnt -0.330
## dyAftrExprm -0.326
                      0.493
## hmdty_tmt_D -0.326 0.493 0.485
## dyMExpr:__D 0.230 -0.697 -0.343 -0.707
## dyAExpr:__D 0.224 -0.340 -0.690 -0.690 0.488
drop1(osml_mod)
## Single term deletions
##
## Model:
## osmolality_mmol_kg ~ day * humidity_tmt_percent + (1 | trial_number)
##
                            npar
                                    AIC
                                 920.78
## <none>
## day:humidity_tmt_percent
                               2 920.47
```

This is the best model.

Check Conditions

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

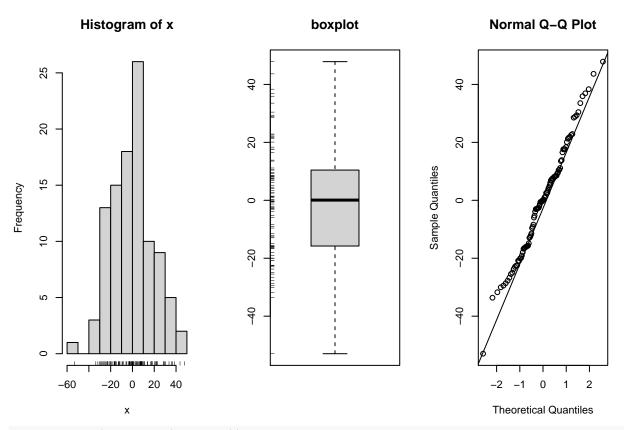
```
plot(osml_mod)
```



L & E both seem fine.

Is the distribution of residuals **normal**? use Shapiro-Wilk normality test: H0: data is NOT significantly different from normal distribution HA: data IS significantly different from normal distribution

simple.eda(residuals(osml_mod))



shapiro.test(residuals(osml_mod))

```
##
## Shapiro-Wilk normality test
##
## data: residuals(osml_mod)
## W = 0.98957, p-value = 0.616
Normality is fine.
```

Export Best

The model seems good as-is.

CEWL

Build Model

```
cloacal_temp_C +
               (1|trial_number/individual_ID))
summary(CEWL mod)
## Linear mixed model fit by REML ['lmerMod']
## Formula: TEWL_g_m2h ~ day * humidity_tmt_percent * region + cloacal_temp_C +
##
       (1 | trial number/individual ID)
##
      Data: .
##
## REML criterion at convergence: 2441.8
## Scaled residuals:
      Min
               10 Median
                               3Q
                                      Max
## -2.4361 -0.5707 -0.0822 0.4555 4.1852
##
## Random effects:
## Groups
                              Name
                                          Variance Std.Dev.
## individual_ID:trial_number (Intercept) 38.212
                                                    6.182
## trial number
                               (Intercept)
                                            8.141
                                                    2.853
## Residual
                                          123.274 11.103
## Number of obs: 326, groups: individual_ID:trial_number, 33; trial_number, 4
##
## Fixed effects:
##
                                                     Estimate Std. Error t value
## (Intercept)
                                                    -69.91895
                                                               11.98454 -5.834
## dayAfter
                                                     20.55665
                                                                 3.96057
                                                                           5.190
                                                                           0.021
## humidity_tmt_percentDry
                                                      0.09390
                                                                 4.44521
## regionVentrum
                                                     14.06471
                                                                 3.80826
                                                                           3.693
                                                                 3.80826
## regionHead
                                                      5.20176
                                                                           1.366
                                                                 3.87231
## regionDewlap
                                                      2.76945
                                                                           0.715
## regionMite Patch
                                                      5.02118
                                                                 3.80826
                                                                           1.318
## cloacal_temp_C
                                                                 0.48902 7.982
                                                      3.90353
                                                                 5.52280 -4.014
## dayAfter:humidity_tmt_percentDry
                                                    -22.17103
## dayAfter:regionVentrum
                                                      4.82016
                                                                 5.43126
                                                                          0.887
## dayAfter:regionHead
                                                     -7.17102
                                                                 5.43126 -1.320
## dayAfter:regionDewlap
                                                     -2.93470
                                                                 5.46212 -0.537
## dayAfter:regionMite Patch
                                                                 5.47797 -1.104
                                                     -6.04716
## humidity_tmt_percentDry:regionVentrum
                                                     -0.34596
                                                                 5.46920 -0.063
## humidity tmt percentDry:regionHead
                                                     -0.03395
                                                                 5.46920 -0.006
## humidity_tmt_percentDry:regionDewlap
                                                                 5.51399
                                                      0.90930
                                                                          0.165
## humidity tmt percentDry:regionMite Patch
                                                      2.90477
                                                                 5.52066
                                                                           0.526
## dayAfter:humidity_tmt_percentDry:regionVentrum
                                                                 7.76641 -0.306
                                                     -2.37516
## dayAfter:humidity_tmt_percentDry:regionHead
                                                      5.28070
                                                                 7.76641
                                                                          0.680
## dayAfter:humidity_tmt_percentDry:regionDewlap
                                                      5.66684
                                                                 7.82338
                                                                           0.724
## dayAfter:humidity_tmt_percentDry:regionMite Patch
                                                      1.47809
                                                                 7.83657
                                                                           0.189
## Correlation matrix not shown by default, as p = 21 > 12.
## Use print(x, correlation=TRUE) or
       vcov(x)
                     if you need it
drop1(CEWL_mod)
## Single term deletions
```

##

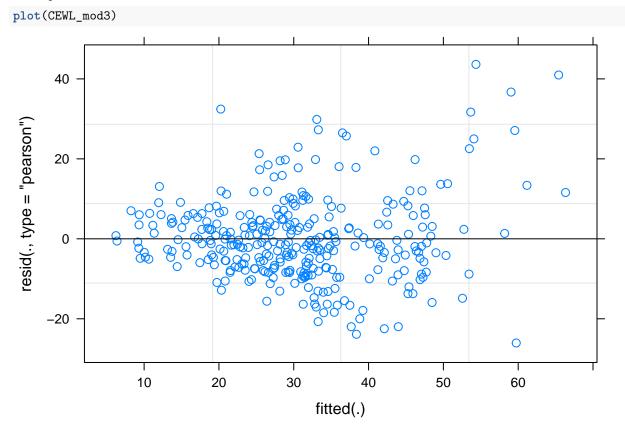
```
## Model:
## TEWL_g_m2h ~ day * humidity_tmt_percent * region + cloacal_temp_C +
       (1 | trial number/individual ID)
##
                                           AIC
## <none>
                                        2570.1
## cloacal temp C
                                      1 2628.2
## day:humidity_tmt_percent:region
                                      4 2563.8
Drop triple interaction.
CEWL mod2 <- CEWL %>%
  lme4::lmer(data = .,
              TEWL g m2h ~
              humidity_tmt_percent * (day + region) +
               cloacal_temp_C +
               (1|trial_number/individual_ID))
summary(CEWL_mod2)
## Linear mixed model fit by REML ['lmerMod']
## Formula: TEWL_g_m2h ~ humidity_tmt_percent * (day + region) + cloacal_temp_C +
##
       (1 | trial_number/individual_ID)
##
      Data: .
##
## REML criterion at convergence: 2490.3
## Scaled residuals:
              1Q Median
      Min
                                3Q
## -2.4790 -0.6180 -0.0854 0.4480 4.0055
## Random effects:
                                           Variance Std.Dev.
## Groups
                               Name
## individual_ID:trial_number (Intercept)
                                            38.017
                                                     6.166
## trial_number
                               (Intercept)
                                             8.677
                                                     2.946
## Residual
                                           123.628 11.119
## Number of obs: 326, groups: individual_ID:trial_number, 33; trial_number, 4
##
## Fixed effects:
##
                                            Estimate Std. Error t value
## (Intercept)
                                            -68.6125
                                                      11.9022 -5.765
## humidity_tmt_percentDry
                                             -0.8482
                                                         3.7242 -0.228
## dayAfter
                                             18.2857
                                                         1.9294
                                                                 9.478
## regionVentrum
                                             16.5143
                                                         2.7189
                                                                  6.074
## regionHead
                                              1.6557
                                                         2.7189 0.609
## regionDewlap
                                                         2.7199 0.486
                                              1.3212
## regionMite Patch
                                              2.0988
                                                         2.7414 0.766
## cloacal_temp_C
                                              3.8933
                                                         0.4894
                                                                  7.956
## humidity_tmt_percentDry:dayAfter
                                            -20.1573
                                                         2.4933 -8.084
## humidity_tmt_percentDry:regionVentrum
                                             -1.5730
                                                         3.8883 -0.405
                                                                 0.660
## humidity_tmt_percentDry:regionHead
                                              2.5669
                                                         3.8883
                                                                 0.940
## humidity_tmt_percentDry:regionDewlap
                                              3.6718
                                                         3.9063
## humidity_tmt_percentDry:regionMite Patch
                                                                  0.884
                                              3.4656
                                                         3.9211
##
## Correlation matrix not shown by default, as p = 13 > 12.
## Use print(x, correlation=TRUE) or
      vcov(x)
                     if you need it
```

```
drop1(CEWL_mod2)
## Single term deletions
##
## Model:
## TEWL_g_m2h ~ humidity_tmt_percent * (day + region) + cloacal_temp_C +
##
       (1 | trial_number/individual_ID)
##
                               npar
                                       AIC
                                    2563.5
## <none>
## cloacal temp C
                                  1 2619.8
## humidity_tmt_percent:day
                                  1 2622.6
## humidity_tmt_percent:region
                                  4 2558.4
We can drop the humidity:region interaction.
CEWL_mod3 <- CEWL %>%
  dplyr::filter(complete.cases(.)) %>%
  lme4::lmer(data = .,
               TEWL_g_m2h ~
               day*humidity_tmt_percent + region + cloacal_temp_C +
               (1|trial_number/individual_ID))
summary(CEWL_mod3)
## Linear mixed model fit by REML ['lmerMod']
## Formula: TEWL_g_m2h ~ day * humidity_tmt_percent + region + cloacal_temp_C +
       (1 | trial_number/individual_ID)
##
##
      Data: .
##
## REML criterion at convergence: 2510.2
## Scaled residuals:
##
       Min
                1Q Median
                                3Q
                                       Max
## -2.3478 -0.6058 -0.1117 0.4446 3.9319
##
## Random effects:
## Groups
                               Name
                                           Variance Std.Dev.
## individual_ID:trial_number (Intercept) 38.023
                                                     6.166
## trial_number
                               (Intercept)
                                             8.872
                                                     2.979
## Residual
                                           123.111 11.096
## Number of obs: 326, groups: individual_ID:trial_number, 33; trial_number, 4
##
## Fixed effects:
##
                                    Estimate Std. Error t value
## (Intercept)
                                    -69.3758
                                              11.8197 -5.869
## dayAfter
                                     18.2632
                                                 1.9251
                                                         9.487
## humidity_tmt_percentDry
                                      0.7605
                                                 2.7927
                                                          0.272
## regionVentrum
                                     15.7651
                                                 1.9396
                                                          8.128
## regionHead
                                      2.9138
                                                 1.9396
                                                          1.502
## regionDewlap
                                      3.0977
                                                 1.9483
                                                          1.590
## regionMite Patch
                                      3.7912
                                                 1.9565
                                                          1.938
## cloacal_temp_C
                                      3.8920
                                                 0.4886
                                                          7.966
## dayAfter:humidity_tmt_percentDry -20.1375
                                                 2.4881 -8.094
## Correlation of Fixed Effects:
##
               (Intr) dyAftr hmd_D rgnVnt regnHd rgnDwl rgnMtP clc_C
```

```
## dayAfter
               -0.507
## hmdty_tmt_D 0.005
                      0.217
## regionVntrm -0.086 -0.004
                              0.000
## regionHead -0.086 -0.004
                              0.000
                                     0.504
## regionDewlp -0.098 -0.010 -0.006
                                     0.502
                                            0.502
## reginMtPtch -0.108 0.013 0.002
                                     0.500
                                            0.500
                                                   0.498
## clocl tmp C -0.972 0.456 -0.122
                                     0.004
                                            0.004
                                                   0.017 0.026
## dyAftr:h_D 0.191 -0.680 -0.418
                                     0.004
                                            0.004
                                                   0.017 -0.010 -0.146
drop1(CEWL_mod3)
## Single term deletions
##
## Model:
  TEWL_g_m2h ~ day * humidity_tmt_percent + region + cloacal_temp_C +
##
       (1 | trial_number/individual_ID)
##
## <none>
                                 2558.4
## region
                               4 2622.8
## cloacal_temp_C
                               1 2614.1
## day:humidity_tmt_percent
                               1 2616.8
```

Check Conditions

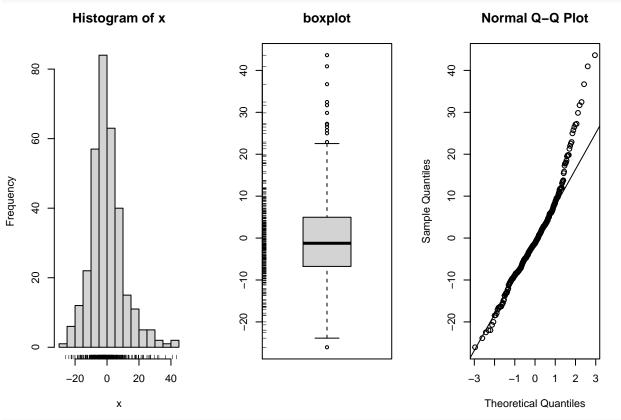
Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). Plotting residuals shows us whether the data meets linearity and equal variance assumptions:



clear fanning shape... L & E are not satisfied.

Is the distribution of residuals **normal**? use Shapiro-Wilk normality test: H0: data is NOT significantly different from normal distribution HA: data IS significantly different from normal distribution





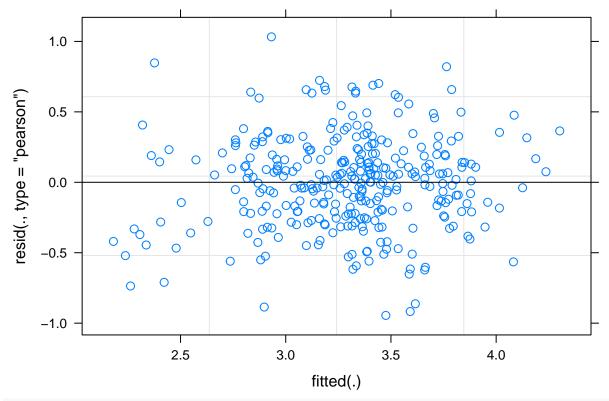
shapiro.test(residuals(CEWL_mod3))

```
##
## Shapiro-Wilk normality test
##
## data: residuals(CEWL_mod3)
## W = 0.95465, p-value = 1.701e-08
```

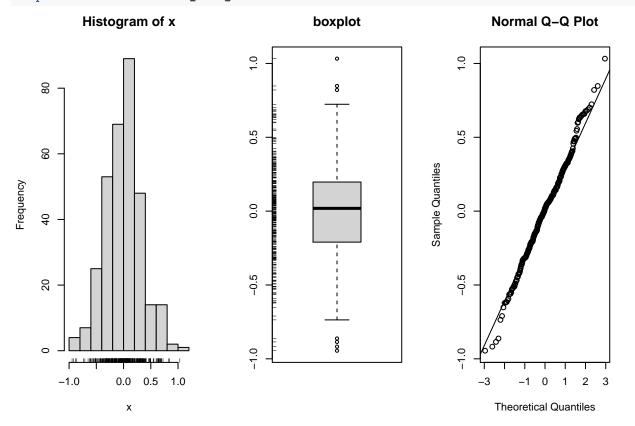
Transform CEWL

distribution not normal

```
##
## REML criterion at convergence: 302.8
## Scaled residuals:
                 1Q
                     Median
                                   3Q
## -2.76069 -0.61262 0.05481 0.57357 3.01606
## Random effects:
                                          Variance Std.Dev.
## Groups
                              Name
## individual_ID:trial_number (Intercept) 0.02787 0.1669
## trial_number
                              (Intercept) 0.03971 0.1993
## Residual
                                          0.11720 0.3423
## Number of obs: 326, groups: individual_ID:trial_number, 33; trial_number, 4
##
## Fixed effects:
##
                                   Estimate Std. Error t value
## (Intercept)
                                               0.37173 -0.436
                                   -0.16197
## dayAfter
                                    0.51526
                                               0.05931
                                                         8.688
## humidity_tmt_percentDry
                                    0.03209
                                              0.08029
                                                         0.400
## regionVentrum
                                    0.48102
                                              0.05984
                                                        8.038
## regionHead
                                    0.12591 0.05984
                                                        2.104
## regionDewlap
                                    0.08143 0.06011 1.355
## regionMite Patch
                                    0.05704 0.06036 0.945
## cloacal temp C
                                    0.13641
                                               0.01496
                                                        9.117
## dayAfter:humidity_tmt_percentDry -0.65376 0.07676 -8.517
## Correlation of Fixed Effects:
              (Intr) dyAftr hmd__D rgnVnt regnHd rgnDwl rgnMtP clc__C
## dayAfter
              -0.491
## hmdty_tmt_D 0.028 0.231
## regionVntrm -0.085 -0.004 0.000
## regionHead -0.085 -0.004 0.000 0.504
## regionDewlp -0.096 -0.010 -0.006 0.502 0.502
## reginMtPtch -0.106 0.013 0.002 0.500 0.500 0.498
## clocl_tmp_C -0.946  0.453 -0.139  0.004  0.004  0.017  0.027
## dyAftr:h_D 0.186 -0.680 -0.448 0.004 0.004 0.017 -0.010 -0.145
drop1(CEWL_mod3_t)
## Single term deletions
##
## Model:
## log(TEWL_g_m2h) ~ day * humidity_tmt_percent + region + cloacal_temp_C +
       (1 | trial_number/individual_ID)
##
                           npar
                                   AIC
## <none>
                                289.48
## region
                              4 355.11
## cloacal_temp_C
                              1 362.73
## day:humidity_tmt_percent
                              1 353.42
plot(CEWL_mod3_t)
```



simple.eda(residuals(CEWL_mod3_t))



shapiro.test(residuals(CEWL_mod3_t))

```
##
## Shapiro-Wilk normality test
##
## data: residuals(CEWL_mod3_t)
## W = 0.99524, p-value = 0.4174
L, E, and N are all satisfied now.:)
```

Export Best

The model is best with all the parameters currently included in model 3.