Cal Poly Herpetology CURE - Capture Data Analyses

Savannah Weaver

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Packages

Background and Goals

This data was collected April - May 2021 during a course-based undergraduate research experience (CURE) in Herpetology, taught by Emily Taylor at Cal Poly, San Luis Obispo. This part of the study was conducted to describe the variation in hydrophysiology in *Sceloporus occidentalis* and to investigate what drives that variation. Please refer to **doi:** for full details.

In this document, we investigate differences in cutaneous evaporative water loss (CEWL) across body regions and dependent on environment, body size, condition, and hydration.

Data

Morphometrics and Blood Data

This data was collected upon capture of each lizard.

Variables in this data frame: - date - collection/capture time for each lizard - individual ID for each lizard - sock ID used to capture each lizard (removed, not relevant to analyses) - SVL = snout-vent length - mass in grams - sex - if female, whether or not gravid (with eggs) - which eye the blood sample was taken from - percent hematocrit = percent of blood that's red blood cells - osmolality = a proxy of hydration, should be inversely related to water content of a lizard (this is the average of 1-3 replicates) - cloacal temperature at the time of CEWL measurement - processing time for each lizard, when all measurements were finished - hemolyzed = whether or not red blood cells burst and contaminated plasma

Before loading in this data, some incorrectly-measured hematocrit and osmolality were omitted: - hematocrit for individuals 1-16, due to observer error - osmolality for individual 19, due to instrumental error

```
# load and format data
morpho_blood_dat <- read.csv("./data/Herpetology_Data.csv", # filename
                             na.strings=c("","NA") # fix empty cells
                             ) %>%
  dplyr::mutate(# put date and time together
                collect_date_time = (paste(date, collect_time)),
                # replace some date-time values that have missing times
                collect_date_time = replace(collect_date_time,
                                            collect_date_time == "4/5/21 NA", NA),
                # correctly format date-time variable
                collect_date_time = as.POSIXct(collect_date_time,
                                               format = \frac{m}{d} \frac{M}{d} ,
                # correctly format date-only variable
                date = as.Date(date, format = "%m/%d/%y"),
                # correctly format collection time variable
                # format extracts just time after posix adds arbitrary date
                collect_time = (as.POSIXct(collect_time, format = "%H:%M")),
                # correctly format processing time variable
                processing_time = (as.POSIXct(processing_time, format = "%H:%M")),
                # set individual_ID variable as a factor, not numeric
                individual_ID = as.factor(individual_ID),
                # set sex variable as a factor, not character
                sex_M_F = as.factor(sex_M_F),
                # set gravidity variable as a factor, not character
                gravid_Y_N = as.factor(gravid_Y_N),
                # set blood sample eye variable as a factor, not character
                blood_sample_eye = as.factor(blood_sample_eye),
                # set hemolyzed variable as a factor, not character
                hemolyzed = as.factor(hemolyzed),
                # compute holding time as capture time - cloacal measurement time:
                hold_time = as.numeric(processing_time - collect_time)
                ) %>%
  # remove two columns not relevant for statistics
  dplyr::select(-sock_ID, -notes)
summary(morpho_blood_dat)
```

```
##
         date
                          collect_time
                                                        individual_ID
##
   Min.
           :2021-04-05
                                 :2021-07-02 10:17:00
                                                               : 1
                         Min.
                                                        1
##
   1st Qu.:2021-04-19
                         1st Qu.:2021-07-02 12:36:00
                                                        2
                                                                  1
                         Median :2021-07-02 12:48:00
## Median :2021-04-26
                                                        3
                                                                  1
## Mean
           :2021-04-27
                                :2021-07-02 12:51:12
                         Mean
                         3rd Qu.:2021-07-02 13:03:00
##
  3rd Qu.:2021-05-10
                                                        5
                                                               : 1
## Max.
           :2021-05-17
                         Max.
                                 :2021-07-02 15:57:00
                                                        6
                                                               :
##
                         NA's
                                 :3
                                                        (Other):142
```

```
{\tt SVL\_mm}
##
                                       sex_M_F gravid_Y_N blood_sample_eye
                         mass_g
##
    Min.
            :42.00
                            : 2.300
                                                    : 22
                                                            both: 2
                     Min.
                                       F: 48
                                                N
##
    1st Qu.:63.00
                     1st Qu.: 9.125
                                       M:100
                                                    : 26
                                                            L
    Median :67.00
                     Median :11.200
                                                                :142
##
                                                NA's:100
                                                            R
##
    Mean
            :64.97
                     Mean
                             :10.586
##
    3rd Qu.:69.00
                     3rd Qu.:12.725
##
    Max.
            :73.00
                     Max.
                             :15.000
##
##
    hematocrit_percent osmolality_mmol_kg cloacal_temp_C
##
            :16.00
                        Min.
                                :293
                                             Min.
                                                    :20.00
##
    1st Qu.:33.00
                        1st Qu.:341
                                             1st Qu.:22.00
                                             Median :23.00
##
    Median :35.00
                        Median:366
##
                                :365
    Mean
            :35.36
                        Mean
                                             Mean
                                                    :23.48
##
    3rd Qu.:38.00
                        3rd Qu.:387
                                             3rd Qu.:25.00
##
    Max.
            :54.00
                        Max.
                                :436
                                             Max.
                                                    :28.00
##
    NA's
            :27
                        NA's
                                :3
                                             NA's
                                                    :7
##
    processing_time
                                    hemolyzed collect_date_time
            :2021-07-02 12:44:00
                                                      :2021-04-05 10:17:00
    Min.
                                               Min.
##
    1st Qu.:2021-07-02 14:09:00
                                         :39
                                               1st Qu.:2021-04-19 12:49:00
                                    Y
##
    Median :2021-07-02 15:17:30
                                    NA's:24
                                               Median :2021-04-26 15:34:00
##
    Mean
            :2021-07-02 15:12:09
                                               Mean
                                                      :2021-04-28 20:28:01
    3rd Qu.:2021-07-02 16:15:15
                                               3rd Qu.:2021-05-10 12:44:00
##
            :2021-07-02 17:38:00
##
   {\tt Max.}
                                                      :2021-05-17 13:01:00
                                               Max.
    NA's
##
            :8
                                               NA's
                                                      :3
##
      hold_time
##
   Min.
           : 21.0
##
    1st Qu.: 95.0
##
    Median :141.5
##
   Mean
            :143.8
##
    3rd Qu.:197.5
##
    Max.
            :268.0
##
    NA's
            :10
unique(morpho_blood_dat$date)
## [1] "2021-04-05" "2021-04-19" "2021-04-26" "2021-05-03" "2021-05-10"
## [6] "2021-05-17"
# export
write.csv(morpho_blood_dat, "exported_data/capture_hydration.csv")
```

I want to test if any IDs are missing, and which ones if so.

```
test <- c(seq(1, 150, by = 1))
lost <- test[test %nin% morpho_blood_dat$individual_ID]
lost</pre>
```

```
## [1] 23 56
```

Individuals 23 and 56 actually both do not exist because those numbers were skipped when assigning IDs, so we have all the individuals measured in the dataframe.

CEWL Data

First, load it all in and merge.

Variables in this dataframe are: - date - time - date_time combined variable - individual_ID for each lizard measured - region = where on the body CEWL was measured - $TEWL_g_m2h = CEWL$ measurement

value in grams/sq-meter/hour - ambient_temp_C = temperature when and where measurement was taken - ambient_RH_percent = relative humidity when and where measurement was taken - abs_humidity = computed from RH using formula on this website: $\frac{https:}{carnotcycle.wordpress.com/2012/08/04/how-to-convert-relative-humidity-to-absolute-humidity/}$

```
# week 1
CEWL_April_05 <- read.csv("./data/capture_CEWL/4-5-21-CEWL.csv", # filename
                          na.strings=c("","NA")) %>% # fix empty cells
  # rename and select the pertinent variables/cols
  # I have to do this for each one
  # so they all have the same number of columns for joining
  dplyr::select(date = Date,
                Time, Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h.., # rename
                ambient_temp_C = AmbT..C., # rename
                ambient_RH_percent = AmbRH....
                )
# week 2
CEWL_April_19 <- read.csv("./data/capture_CEWL/4-19-21-CEWL.csv",</pre>
                          na.strings=c("","NA")) %>%
  dplyr::select(date = Date,
                Time, Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h.., # rename
                ambient_temp_C = AmbT..C., # rename
                ambient_RH_percent = AmbRH....
                )
# week 3
CEWL_April_26 <- read.csv("./data/capture_CEWL/4-26-21-CEWL.csv",
                          na.strings=c("","NA")) %>%
  dplyr::select(date = Date,
                Time, Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h.., # rename
                ambient_temp_C = AmbT..C., # rename
                ambient RH percent = AmbRH....
                )
# week 4
CEWL_May_3 <- read.csv("./data/capture_CEWL/5-3-21-CEWL.csv",</pre>
                          na.strings=c("","NA")) %>%
  dplyr::select(date = Date,
                Time, Status,
                ID = Comments,
                TEWL_g_m2h = TEWL..g..m2h.., # rename
                ambient_temp_C = AmbT..C., # rename
                ambient_RH_percent = AmbRH....
                )
# week 5
CEWL_May_10 <- read.csv("./data/capture_CEWL/5-10-21-CEWL.csv",</pre>
```

```
na.strings=c("","NA")) %>%
     dplyr::select(date = Date,
                                             Time, Status,
                                             ID = Comments,
                                             TEWL_g_m2h = TEWL..g..m2h.., # rename
                                             ambient_temp_C = AmbT..C., # rename
                                             ambient_RH_percent = AmbRH....
# week 6
CEWL_May_17 <- read.csv("./data/capture_CEWL/5-17-21-CEWL.csv",
                                                                         na.strings=c("","NA")) %>%
     dplyr::select(date = Date,
                                             Time, Status,
                                             ID = Comments,
                                             TEWL_g_m2h = TEWL..g..m2h.., # rename
                                             ambient_temp_C = AmbT..C., # rename
                                             ambient_RH_percent = AmbRH....
# merge all CEWL datafiles & reformat
CEWL <- CEWL_April_05 %>% # week 1
     # join with weeks 2-6
     rbind(., CEWL_April_19,
                      CEWL_April_26,
                      CEWL_May_3,
                      CEWL_May_10,
                      CEWL_May_17
                      ) %>%
     # remove any unsuccessful measurements
     dplyr::filter(Status == "Normal") %>%
     # extract individual_ID and region separately from the "ID" variable
     separate(ID, c("individual_ID", "region")) %>%
      # reformat data
     dplyr::mutate(# paste and format date-time variable
                                             CEWL_date_time = as.POSIXct(paste(date, Time),
                                                                                                                             format = \frac{m}{d} \frac{3m}{d} \frac{3m}{m} \frac{3m}{d} \frac{3m}{m} \frac{
                                             # reformat date only
                                             date = as.Date(date, format = "%m/%d/%y"),
                                             # reformat time
                                             # format extracts just time after posix adds arbitrary date
                                             # but then it's a character again...
                                             Time = format(as.POSIXct(Time, format = "%I:%M:%S %p"),
                                                                                     format = "%H:%M:%S"),
                                             # format individual ID as a factor
                                             individual_ID = as.factor(individual_ID),
                                             # set body region as a factor variable after getting only the consistent characters due
                                             region = as.factor(substring(region, 1, 4)),
                                             # convert RH to absolute humidity
                                             abs_humidity_g_m3 = ((6.112 * exp((17.67*ambient_temp_C)/(ambient_temp_C + 243.5)) * am^2
                                             ) %>%
     # remove cols not relevant to stats
     dplyr::select(-Status) %>%
```

```
# remove any rows with missing values
  dplyr::filter(complete.cases(.))
summary(CEWL)
##
         date
                              Time
                                             individual ID region
##
   Min.
           :2021-04-05
                         Length:699
                                                     : 5
                                                            dewl:139
##
    1st Qu.:2021-04-19
                         Class : character
                                             02
                                                        5
                                                            dors:141
##
   Median :2021-04-26
                         Mode :character
                                             03
                                                     :
                                                       5
                                                            head:141
##
   Mean
           :2021-04-28
                                             04
                                                       5
                                                            mite:137
    3rd Qu.:2021-05-10
                                             05
##
                                                        5
                                                            vent:141
##
           :2021-05-17
                                             06
                                                        5
##
                                              (Other):669
##
      {\tt TEWL\_g\_m2h}
                    ambient_temp_C
                                     ambient_RH_percent
##
                            :22.30
          : 3.41
                                            :34.00
    Min.
                    Min.
                                     Min.
##
    1st Qu.:17.09
                    1st Qu.:23.00
                                     1st Qu.:41.30
   Median :22.00
                    Median :23.20
                                     Median :45.20
##
   Mean
           :25.87
                    Mean
                           :23.44
                                     Mean
                                            :43.56
##
    3rd Qu.:32.59
                    3rd Qu.:23.80
                                     3rd Qu.:46.30
##
   Max.
           :96.16
                    Max.
                            :25.30
                                     Max.
                                            :53.10
##
##
  CEWL_date_time
                                   abs_humidity_g_m3
##
   Min.
           :2021-04-05 13:24:15
                                   Min. : 6.989
                                   1st Qu.: 8.611
##
   1st Qu.:2021-04-19 14:07:34
## Median :2021-04-26 17:10:23
                                   Median: 9.483
## Mean
           :2021-04-28 23:39:45
                                   Mean
                                         : 9.188
##
    3rd Qu.:2021-05-10 16:03:10
                                   3rd Qu.: 9.899
           :2021-05-17 17:22:31
##
   Max.
                                          :10.632
                                   Max.
##
```

Write CEWL dataframe as a csv for use in other analyses:

```
write.csv(CEWL, "exported_data/capture_CEWL.csv")
```

Weather Data

This data was obtained from http://www.itrc.org/databases/precip/ (Adcon Server Data) to test the effect of ambient conditions on CEWL. This is different from the ambient conditions already measured with CEWL, which are the temperature and humidity around the measurement device at the time of measurement. We think that the temperature, humidity, wind speed, and solar radiation the lizard was exposed to prior to capture may also affect CEWL.

tbd = daylight savings

The weather data is only every 15 minutes, but I want to match it to any minute measurement, so I need to

interpolate the values for each minute.

First, make a separate dataframe with every minute for each of those days.

```
all_times <- data.frame(collect_date_time = c(# April 5
                           seq(from = as.POSIXct("2021-04-05 10:00"),
                                to = as.POSIXct("2021-04-05 16:00"),
                               by="min"),
                           # April 19
                           seq(from = as.POSIXct("2021-04-19 10:00"),
                               to = as.POSIXct("2021-04-19 16:00"),
                               by="min"),
                           # April 26
                           seq(from = as.POSIXct("2021-04-26 10:00"),
                               to = as.POSIXct("2021-04-26 16:00"),
                               by="min"),
                           # May 3
                           seq(from = as.POSIXct("2021-05-03 10:00"),
                               to = as.POSIXct("2021-05-03 16:00"),
                               by="min"),
                           # May 10
                           seq(from = as.POSIXct("2021-05-10 10:00"),
                               to = as.POSIXct("2021-05-10 16:00"),
                               by="min"),
                           # May 17
                           seq(from = as.POSIXct("2021-05-17 10:00"),
                               to = as.POSIXct("2021-05-17 16:00"),
                               by="min")
                           ))
```

Next, merge the weather data into the times dataframe and interpolate the temperature and humidity between measurements.

```
all_times_weather <- all_times %>% # time only dataframe
  # add weather measurements based on matching date-time
 left_join(weather, by = 'collect_date_time') %>%
  # convert temperature units, thanks America
  mutate(temp_C = fahrenheit.to.celsius(Temperature_F, round = 2),
         # interpolate temperatures
         temp_C_interpol = na.approx(temp_C),
         # interpolate humidities
         RH_percent_interpol = na.approx(RH_percent),
         # interpolate Wind Speeds
         Wind_mph_interpol = na.approx(Wind_Speed_mph),
         # interpolate solar radiation
         Solar_rad_Wm2_interpol = na.approx(Pyranometer_W_m),
         # compute absolute humidity
         abs_humidity_g_m3_interpol = ((6.112 * exp((17.67*temp_C_interpol)/(temp_C_interpol + 243.5))
  # keep only the relevant variables
  dplyr::select(collect_date_time,
                temp_C_interpol,
                RH_percent_interpol,
                abs_humidity_g_m3_interpol,
                Wind_mph_interpol,
```

Solar_rad_Wm2_interpol)

summary(all_times_weather)

```
##
   collect_date_time
                                 temp_C_interpol RH_percent_interpol
          :2021-04-05 10:00:00
## Min.
                                      :13.28
                                                Min.
                                                       :38.20
                                 Min.
## 1st Qu.:2021-04-19 13:00:15
                                 1st Qu.:16.54
                                                 1st Qu.:56.77
## Median :2021-04-30 01:00:00
                                 Median :17.78
                                                 Median :67.65
## Mean
         :2021-04-28 21:00:00
                                 Mean :18.78
                                                 Mean
                                                        :65.52
## 3rd Qu.:2021-05-10 12:59:45
                                 3rd Qu.:20.48
                                                 3rd Qu.:72.30
          :2021-05-17 16:00:00
                                                        :92.10
## Max.
                                 Max.
                                        :25.78
                                                 Max.
## abs_humidity_g_m3_interpol Wind_mph_interpol Solar_rad_Wm2_interpol
## Min.
          : 8.497
                              Min.
                                     :0.100
                                                Min. : 356.9
## 1st Qu.: 9.634
                              1st Qu.:4.340
                                                1st Qu.: 743.2
                              Median :4.567
                                                Median: 882.6
## Median :10.616
## Mean
          :10.361
                              Mean
                                    :4.574
                                                Mean : 860.2
## 3rd Qu.:10.912
                              3rd Qu.:5.020
                                                3rd Qu.: 979.5
## Max.
          :11.790
                              Max.
                                     :7.100
                                                Max.
                                                       :1037.5
```

Compute Scaled Mass Index

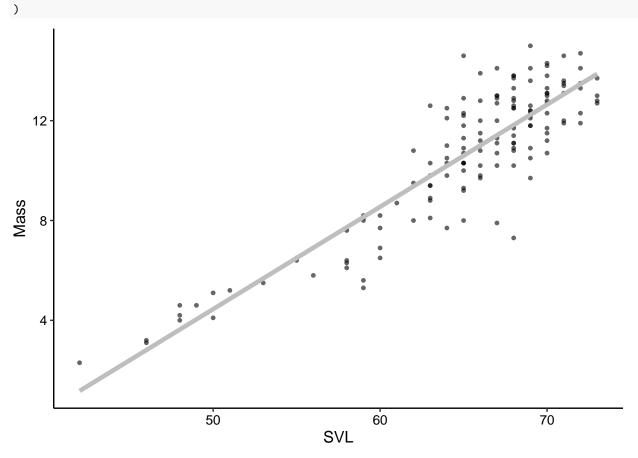
This is also known as the body condition index, or log-log residuals.

I calculate as described by: Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. Oikos, 118(12), 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Step 1: mass $\sim SVL$

plot:

```
morpho_blood_dat %>%
  ggplot(data = .) +
  geom_point(aes(x = SVL_mm,
                 y = mass_g,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = SVL_mm,
                  y = mass_g,
                  ),
              formula = y \sim x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("SVL") +
  ylab("Mass") +
  theme(text = element_text(color = "black",
                             family = "sans",
                             size = 12),
        axis.text = element text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0
```



create a simple linear regression

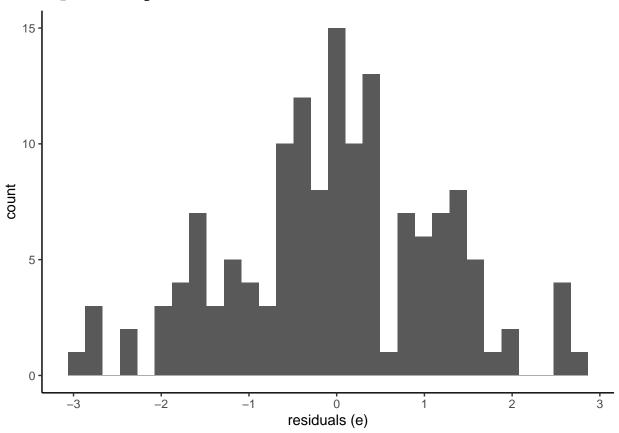
```
mass_SVL_SLR <- lm(data = morpho_blood_dat, mass_g ~ SVL_mm)
summary(mass_SVL_SLR)</pre>
```

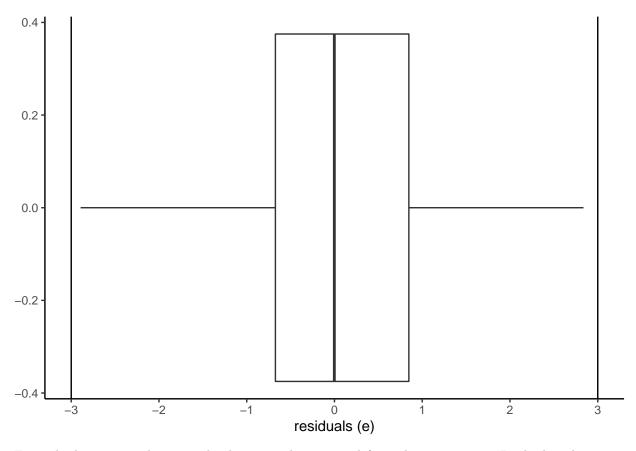
```
##
## Call:
## lm(formula = mass_g ~ SVL_mm, data = morpho_blood_dat)
##
## Residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
  -4.5265 -0.8762 -0.0024 0.6735 4.0031
##
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) -16.04514
                           1.14303 -14.04
                                             <2e-16 ***
## SVL_mm
                0.40988
                           0.01751
                                     23.40
                                             <2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 1.316 on 146 degrees of freedom
## Multiple R-squared: 0.7895, Adjusted R-squared: 0.7881
## F-statistic: 547.7 on 1 and 146 DF, p-value: < 2.2e-16
```

look for outliers by calculating residuals

plot residuals

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.





From the histogram, there are clearly points disconnected from the main curve. In the boxplot, we can distinguish that those outlying points have residuals <-3.5 and >3, so I'll go back and filter those out in the code chunk before making the distribution plots. Now the boxplot has no dots outside the main distribution.

And, check mean residual value:

1 -0.02769117 -0.00174

Not zero, which is not ideal, but pretty close.

Next, check for high leverage points:

high_leverage_dat

```
##
            date
                         collect_time individual_ID SVL_mm mass_g sex_M_F
## 1
      2021-04-05 2021-07-02 10:38:00
                                                   4
                                                          48
                                                                4.2
                                                                           М
## 2
      2021-04-05 2021-07-02 10:17:00
                                                   5
                                                          50
                                                                4.1
                                                                           М
      2021-04-05 2021-07-02 10:47:00
                                                                4.6
## 3
                                                   6
                                                          48
                                                                           М
## 4
      2021-04-05 2021-07-02 10:42:00
                                                   8
                                                          42
                                                                2.3
                                                                           М
## 5
      2021-04-05 2021-07-02 13:27:00
                                                   9
                                                          46
                                                                           F
                                                                3.1
      2021-04-26 2021-07-02 12:38:00
                                                  57
                                                          65
                                                               10.3
                                                                           F
## 7
      2021-04-26 2021-07-02 12:57:00
                                                  64
                                                          65
                                                               10.3
                                                                           М
      2021-04-26 2021-07-02 13:02:00
                                                  67
                                                          64
                                                               10.5
                                                                           Μ
      2021-05-03 2021-07-02 12:51:00
                                                  87
                                                          70
                                                               14.3
                                                                           М
## 10 2021-05-17 2021-07-02 12:19:00
                                                 123
                                                          72
                                                               11.9
                                                                           М
##
      gravid_Y_N blood_sample_eye hematocrit_percent osmolality_mmol_kg
## 1
            <NA>
                                 R
                                                    NA
                                                                        341
                                 R
## 2
            <NA>
                                                                        354
                                                    NA
## 3
            <NA>
                                 L
                                                    NA
                                                                        355
## 4
            <NA>
                                 R
                                                    NA
                                                                        304
## 5
               N
                                 L
                                                    NA
                                                                        NA
                                                                        388
## 6
               N
                                 R
                                                    30
## 7
                                 R
                                                    30
                                                                        378
            <NA>
## 8
            <NA>
                                 R
                                                     32
                                                                        402
## 9
            <NA>
                                 R.
                                                    35
                                                                        394
## 10
            <NA>
                                 R
                                                    52
                                                                        362
##
      cloacal_temp_C
                          processing_time hemolyzed
                                                        collect_date_time hold_time
## 1
                   26 2021-07-02 14:02:00
                                                   Y 2021-04-05 10:38:00
                                                                                 204
                  25 2021-07-02 13:59:00
                                                   Y 2021-04-05 10:17:00
                                                                                 222
## 2
## 3
                  24 2021-07-02 14:06:00
                                                   N 2021-04-05 10:47:00
                                                                                 199
                  23 2021-07-02 14:20:00
                                                   N 2021-04-05 10:42:00
## 4
                                                                                 218
                   23 2021-07-02 14:43:00
                                                <NA> 2021-04-05 13:27:00
                                                                                  76
## 5
## 6
                  26 2021-07-02 13:27:00
                                                   N 2021-04-26 12:38:00
                                                                                  49
## 7
                  22 2021-07-02 15:20:00
                                                   Y 2021-04-26 12:57:00
                                                                                 143
## 8
                  24 2021-07-02 15:41:00
                                                   N 2021-04-26 13:02:00
                                                                                 159
## 9
                  24 2021-07-02 14:32:00
                                                   N 2021-05-03 12:51:00
                                                                                 101
## 10
                  27 2021-07-02 12:47:00
                                                <NA> 2021-05-17 12:19:00
                                                                                  28
##
         y_hat
                       e row
       3.62910 -0.57090
## 1
                           4 0.05776372
## 2
       4.44886 0.34886
                           5 0.04645120
## 3
       3.62910 -0.97090
                           6 0.05776372
                           8 0.10020003
## 4
       1.16982 -1.13018
## 5
       2.80934 -0.29066
                           9 0.07049270
## 6
      10.59706 0.29706
                          54 0.07049270
## 7
      10.59706 0.29706
                          60 0.04132611
## 8
      10.18718 -0.31282
                          63 0.05776372
      12.64646 -1.65354
                          83 0.05193040
## 10 13.46622 1.56622 118 0.04645120
```

The points for individuals 4, 5, 6, 8, 9, 57, 64, 67, 87, and 123 seem to be high-leverage, so we will try removing them.

Check for influential points based on Cook's distance:

```
# add to original dataframe
influential <- mass_SVL_SLR_residuals %>%
 mutate(row = rownames(.)) %>%
 left join(., cooks, by = "row")
# see moderately influential points
cook_mod_inf <- influential %>%
  dplyr::filter(c>0.5)
cook_mod_inf
## [1] date
                           collect_time
                                               individual_ID
                                                                   SVL mm
## [5] mass_g
                           sex M F
                                               gravid_Y_N
                                                                   blood_sample_eye
## [9] hematocrit_percent osmolality_mmol_kg cloacal_temp_C
                                                                   processing_time
## [13] hemolyzed
                           collect_date_time hold_time
                                                                   y_hat
## [17] e
                           row
                                               С
## <0 rows> (or 0-length row.names)
There are no even moderately-infuential points, at least based on Cook's distance, so there's nothing to
potentially remove.
Create new SLR and check mean residual value after removing outliers and high leverage points:
# create new dataframe with filtered data
cleaned_SMI_dat <- mass_SVL_SLR_residuals %>% # already had outliers removed
  mutate(row = rownames(.)) %>%
  # add high leverage point info
 left_join(., high_leverage, by = "row") %>%
  # remove high leverage points
  dplyr::filter(H < h_bar)</pre>
# model
mass_SVL_SLR2 <- lm(data = cleaned_SMI_dat, mass_g ~ SVL_mm)</pre>
summary(mass_SVL_SLR2)
##
## lm(formula = mass_g ~ SVL_mm, data = cleaned_SMI_dat)
##
## Residuals:
        Min
                  1Q
                      Median
                                     30
                                             Max
## -2.72134 -0.88611 0.00146 0.70819 2.87193
##
## Coefficients:
                Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) -17.32109
                            1.28410 -13.49
                                               <2e-16 ***
                                              <2e-16 ***
## SVL mm
                 0.42953
                            0.01953
                                     21.99
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 1.209 on 133 degrees of freedom
## Multiple R-squared: 0.7843, Adjusted R-squared: 0.7827
## F-statistic: 483.6 on 1 and 133 DF, p-value: < 2.2e-16
# compute residuals
mass_SVL_SLR2_residuals <- cleaned_SMI_dat %>%
```

mutate(y_hat = predict(mass_SVL_SLR2),

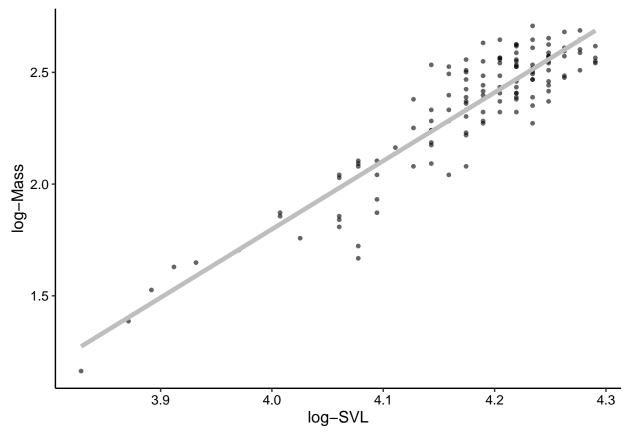
```
## mean_res med
## 1 4.654038e-17 0.001458016
```

The residuals are much much smaller, so I believe it is the right choice to remove the high leverage points. The "cleaned_SMI_dat" will be used to calculate the equation for SMI.

Step 2: make log-log relationship

plot and calculate SLR for filtered data

```
cleaned SMI dat %>%
  ggplot(data = .) +
  geom_point(aes(x = log(SVL_mm),
                 y = log(mass_g),
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = log(SVL_mm),
                  y = log(mass_g),
                  ),
              formula = y ~ x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("log-SVL") +
 ylab("log-Mass") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 10),
       legend.text.align = 0
```



```
SLR
```

```
log_mass_SVL_SLR <- lm(data = cleaned_SMI_dat,</pre>
                       log(mass_g) ~ log(SVL_mm))
summary(log_mass_SVL_SLR)
##
## Call:
## lm(formula = log(mass_g) ~ log(SVL_mm), data = cleaned_SMI_dat)
##
## Residuals:
##
        Min
                  1Q
                       Median
                                    ЗQ
   -0.36738 -0.07518 0.00334 0.06423 0.29781
##
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) -10.4465
                            0.4858
                                   -21.50
                                              <2e-16 ***
                                      26.34
                                              <2e-16 ***
## log(SVL_mm)
                 3.0611
                            0.1162
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.1175 on 133 degrees of freedom
## Multiple R-squared: 0.8391, Adjusted R-squared: 0.8379
## F-statistic: 693.6 on 1 and 133 DF, p-value: < 2.2e-16
compute standardized major axis using this regression equation:
```

r <- sqrt(0.8391) # Pearson's correlection coefficient (sqrt of R-squared)

b_OLS <- 3.0611 # regression slope

```
b_SMA <- b_OLS/r
also get a value for L0:
L0 <- mean(cleaned_SMI_dat$SVL_mm)</pre>
```

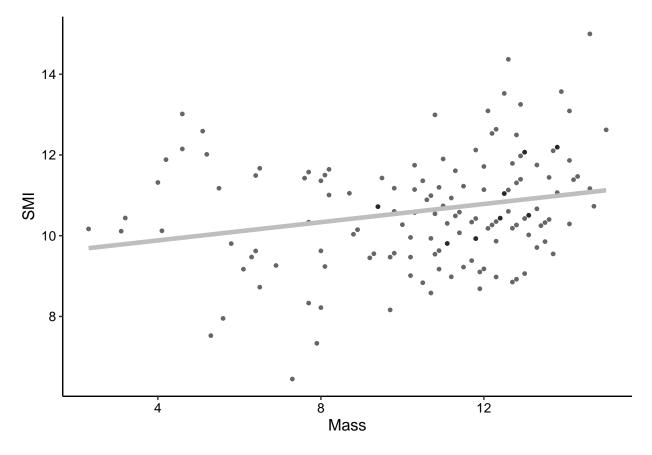
Step 3: calculate scaled mass index

(And join weather data.)

Check

Look at the difference between regular mass and SMI:

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = mass_g,
                 y = SMI,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = mass_g,
                  y = SMI,
                  ),
              formula = y \sim x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Mass") +
  ylab("SMI") +
  theme(text = element_text(color = "black",
                            family = "sans",
                             size = 12),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0
```



Join Data

There are several CEWL measurements for each of the other measures, so I'm going to join two ways. Each way allows slightly different analyses.

First, with CEWL as the primary dataframe. This means each of the other variables will be duplicated for each lizards CEWL measurements.

```
##
         date
                               Time
                                                individual_ID
                                                                region
                                                               dewl:139
##
    {\tt Min.}
            :2021-04-05
                           Length:699
                                                01
                                                        :
                                                           5
##
    1st Qu.:2021-04-19
                           Class :character
                                                02
                                                           5
                                                               dors:141
    Median :2021-04-26
                                                03
##
                           Mode
                                                           5
                                                               head:141
                                :character
                                                04
##
    Mean
            :2021-04-28
                                                           5
                                                               mite:137
    3rd Qu.:2021-05-10
                                                05
                                                           5
                                                               vent:141
##
##
    Max.
            :2021-05-17
                                                06
##
                                                (Other):669
##
                     ambient_temp_C
                                       ambient_RH_percent
      TEWL_g_m2h
                             :22.30
                                               :34.00
##
    Min.
           : 3.41
                     Min.
                                       Min.
                     1st Qu.:23.00
    1st Qu.:17.09
##
                                       1st Qu.:41.30
##
    Median :22.00
                     Median :23.20
                                       Median :45.20
            :25.87
##
    Mean
                     Mean
                             :23.44
                                       Mean
                                               :43.56
                                       3rd Qu.:46.30
##
    3rd Qu.:32.59
                     3rd Qu.:23.80
```

```
Max.
          :96.16
                  Max.
                          :25.30
                                   Max. :53.10
##
##
   CEWL date time
                                 abs_humidity_g_m3 collect_time
   Min. :2021-04-05 13:24:15
                                 Min. : 6.989
                                                   Min. :2021-07-02 11:29:00
   1st Qu.:2021-04-19 14:07:34
                                 1st Qu.: 8.611
                                                   1st Qu.:2021-07-02 12:37:00
##
   Median :2021-04-26 17:10:23
                                 Median : 9.483
                                                   Median :2021-07-02 12:48:00
   Mean :2021-04-28 23:39:45
                                 Mean : 9.188
                                                   Mean :2021-07-02 12:55:48
   3rd Qu.:2021-05-10 16:03:10
                                 3rd Qu.: 9.899
                                                   3rd Qu.:2021-07-02 13:02:15
##
##
         :2021-05-17 17:22:31
                                 Max. :10.632
                                                   Max.
                                                          :2021-07-02 15:44:00
##
                                                   NA's
                                                          :59
##
       SVL\_mm
                                   sex_M_F
                                              gravid_Y_N blood_sample_eye
                       mass_g
##
          :46.00
                   Min. : 3.20
                                   F
                                      :216
                                                  : 91
                                                         both: 10
   Min.
                                              N
   1st Qu.:64.00
                   1st Qu.: 9.70
                                   М
                                       :439
                                              Y
                                                  :125
                                                         L : 0
##
   Median :67.00
                                              NA's:483
                                                         R :645
                   Median :11.40
                                   NA's: 44
##
   Mean
         :65.81
                   Mean :10.88
                                                         NA's: 44
##
   3rd Qu.:69.00
                   3rd Qu.:12.80
##
   Max.
          :73.00
                   Max.
                          :15.00
##
   NA's
          :44
                   NA's
                          :44
   hematocrit_percent osmolality_mmol_kg cloacal_temp_C
##
         :16.0
                      Min. :293.0
                                         Min.
                                               :20.0
##
   1st Qu.:33.0
                      1st Qu.:347.0
                                         1st Qu.:22.0
   Median:36.0
                      Median :368.0
                                         Median:23.0
##
   Mean :35.4
                             :366.8
                                         Mean :23.4
                      Mean
   3rd Qu.:38.0
                      3rd Qu.:387.0
                                         3rd Qu.:25.0
##
##
  Max.
         :54.0
                      Max.
                             :436.0
                                         Max.
                                                :28.0
   NA's
          :119
                      NA's
                                         NA's
                                               :49
##
   processing_time
                                 hemolyzed collect_date_time
         :2021-07-02 12:44:00
   Min.
                                 N
                                    :368
                                            Min.
                                                   :2021-04-05 13:25:00
   1st Qu.:2021-07-02 14:14:00
                                 Y
                                     :179
                                            1st Qu.:2021-04-19 13:09:30
  Median :2021-07-02 15:25:00
                                 NA's:152
                                            Median :2021-05-03 12:40:00
##
   Mean
         :2021-07-02 15:16:48
                                            Mean
                                                   :2021-05-01 01:04:48
   3rd Qu.:2021-07-02 16:18:00
                                            3rd Qu.:2021-05-10 12:52:00
##
   Max.
          :2021-07-02 17:38:00
                                            Max.
                                                   :2021-05-17 13:01:00
##
   NA's
          :59
                                            NA's
                                                   :59
##
     hold time
                        SMI
                                    temp_C_interpol RH_percent_interpol
##
         : 21.0
                                         :15.67
                                                    Min.
   Min.
                  \mathtt{Min}.
                         : 6.450
                                    Min.
                                                           :44.29
   1st Qu.: 91.0
                   1st Qu.: 9.624
                                    1st Qu.:16.66
                                                    1st Qu.:57.51
##
   Median :132.0
                   Median :10.505
                                    Median :18.68
                                                    Median :68.10
##
   Mean :140.5
                   Mean :10.573
                                    Mean :18.77
                                                    Mean
                                                           :66.53
##
   3rd Qu.:189.0
                   3rd Qu.:11.444
                                    3rd Qu.:19.96
                                                    3rd Qu.:72.54
                                           :23.61
          :268.0
                   Max.
                          :14.999
                                    {\tt Max.}
                                                    Max.
                                                           :81.10
##
   NA's
          :69
                   NA's
                          :44
                                    NA's
                                           :59
                                                    NA's
                                                           :59
   abs_humidity_g_m3_interpol Wind_mph_interpol Solar_rad_Wm2_interpol
##
                                                      : 587.0
  Min.
         : 8.912
                              Min.
                                     :3.773
                                                Min.
   1st Qu.: 9.948
                              1st Qu.:4.577
                                                1st Qu.: 741.9
## Median :10.743
                              Median :5.000
                                                Median : 951.3
   Mean :10.568
                              Mean
                                    :4.945
                                                Mean : 892.3
##
   3rd Qu.:10.908
                              3rd Qu.:5.233
                                                3rd Qu.:1032.9
##
   Max.
          :11.520
                              Max.
                                     :6.200
                                                Max.
                                                       :1037.5
##
   NA's
           :59
                              NA's
                                     :59
                                                NA's
                                                       :59
unique(CEWL_data_full$individual_ID)
                                    08 09 10
##
     [1] 01 02 03 04
                        05
                            06 07
                                               11 12
                                                        13
                                                            14
                                                               15
                                                                   16 17
                                                                            18
  [19] 19 20 21 26 27
                            30 29
                                    24 31 32 34 33
                                                        35
                                                           36 37 38 39
```

```
43
                     44
                          45
                              46
                                  47
                                      52
                                          53
                                               48
                                                   50
                                                       51
                                                           54
                                                               55
                                                                        58
##
    [55] 61
             62
                 63
                     64
                          65
                              66
                                  67
                                      68
                                          69
                                              70
                                                   71
                                                       72
                                                           73
                                                               74
                                                                    75
                                                                        76
                                                                            77
                                                                                78
    [73] 79
                     83
                          84
                              85
                                  86
                                      87
                                           88
                                                   90
                                                       91
                                                           92
             99
    [91] 98
                 100 101 102 103 104 105 106 107 108 109 110 111 112 115 116 119
## [109] 113 114 117 118 122 120 123 124 125 126 127 128 129 130 131 132 133 134
## [127] 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
## 157 Levels: 01 02 03 04 05 06 07 08 09 10 100 101 102 103 104 105 106 ... 121
```

Export Data

I want to save the data as a csv for loading into other analyses.

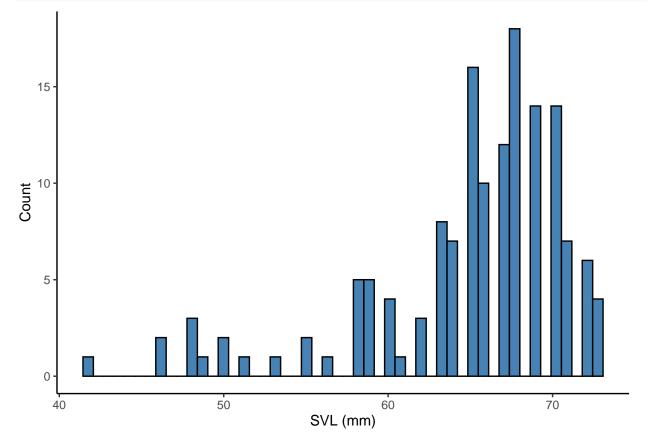
```
write.csv(CEWL_data_full, "exported_data/capture_data_long.csv")
```

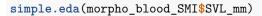
Check Data Distributions

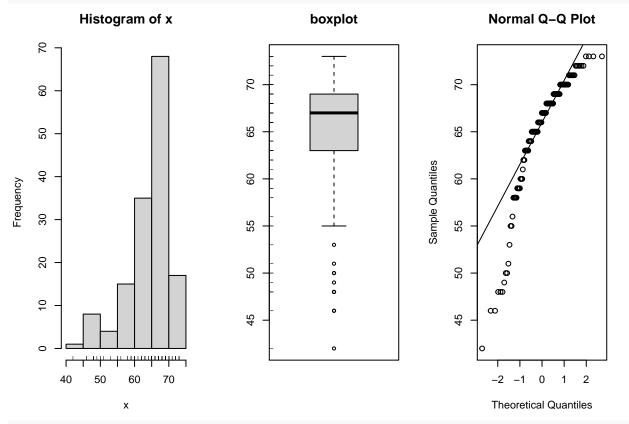
Histograms & Q-Q Plots

SVL

```
morpho_blood_SMI %>%
  ggplot(., aes(x = SVL_mm)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("SVL (mm)") +
  ylab("Count")
```





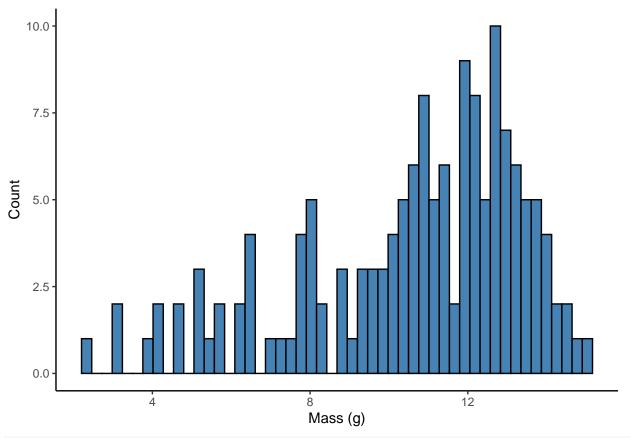


```
# Normality test if p > .05, data is normal. Data is not normal.
shapiro.test(morpho_blood_SMI$SVL_mm)
```

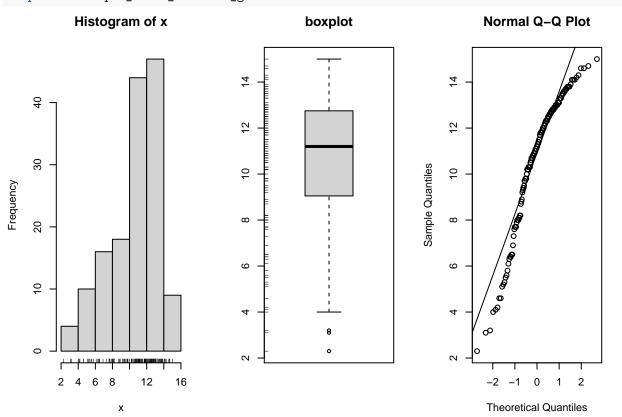
```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$SVL_mm
## W = 0.85469, p-value = 8.85e-11
```

Mass

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



simple.eda(morpho_blood_SMI\$mass_g)

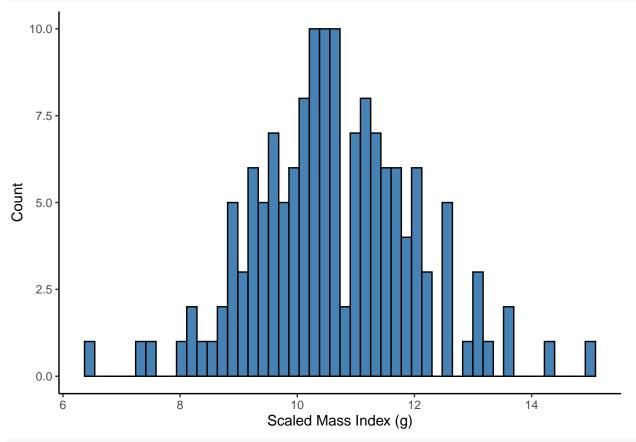


```
# Normality test if p > .05, data is normal. Data is not normal.
shapiro.test(morpho_blood_SMI$mass_g)
```

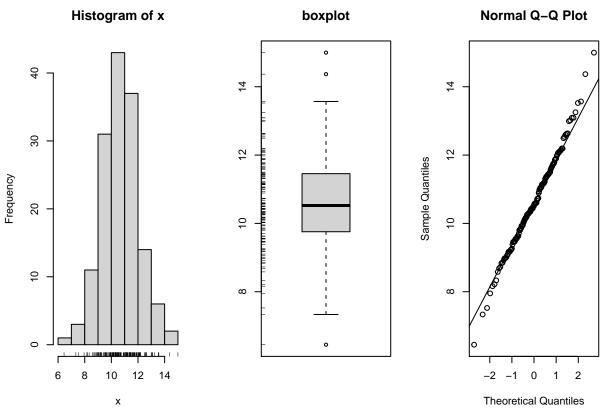
```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$mass_g
## W = 0.92517, p-value = 5.397e-07
```

SMI

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



simple.eda(morpho_blood_SMI\$SMI)



```
# Normality test if p > .05, data is normal. Data is not normal.
shapiro.test(morpho_blood_SMI$SMI)
```

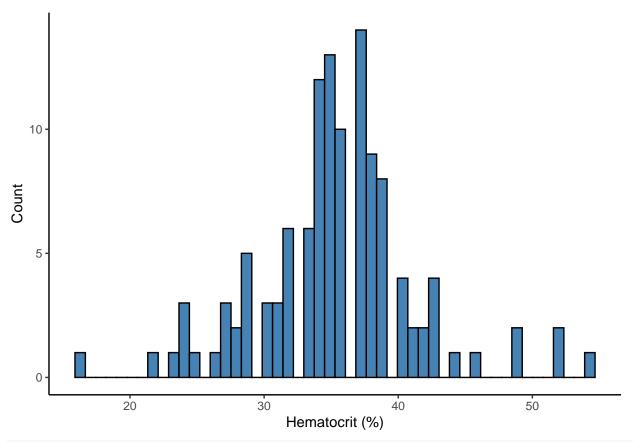
```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$SMI
## W = 0.9937, p-value = 0.7667
```

SMI is one of the few variables actually normally distributed!

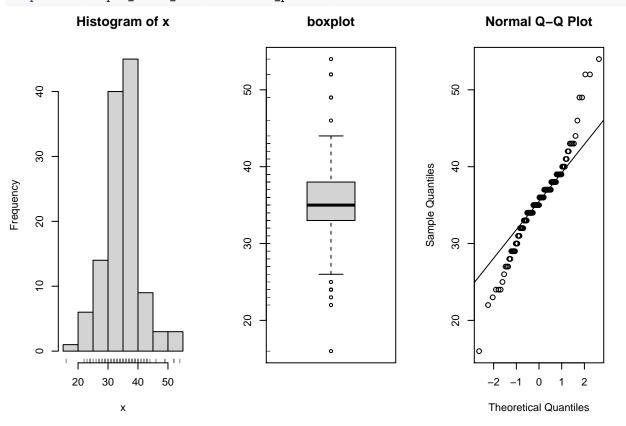
Hematocrit

looks pretty normally distributed around ~35%, but not statistically normal

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



simple.eda(morpho_blood_SMI\$hematocrit_percent)



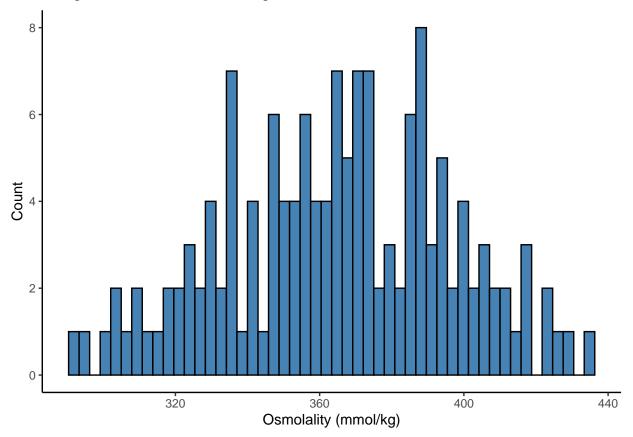
```
# Normality test if p > .05, data is normal. Data is not normal
shapiro.test(morpho_blood_SMI$hematocrit_percent)
```

```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$hematocrit_percent
## W = 0.95933, p-value = 0.001056
```

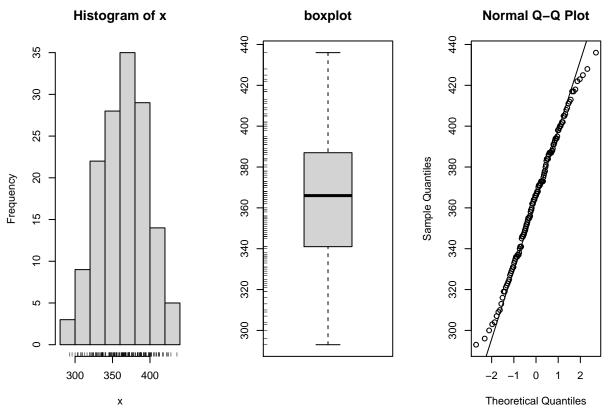
Osmolality

pretty normally distributed around $\sim 370!$:D

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



simple.eda(morpho_blood_SMI\$osmolality_mmol_kg)



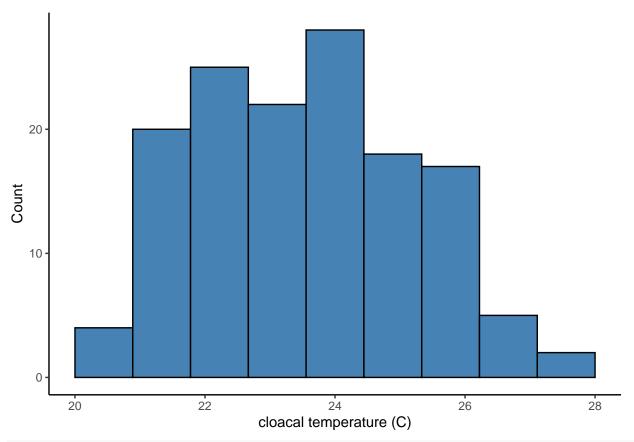
```
# Normality test if p > .05, data is normal. Data is normal
shapiro.test(morpho_blood_SMI$osmolality_mmol_kg)
```

```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$osmolality_mmol_kg
## W = 0.99198, p-value = 0.5876
```

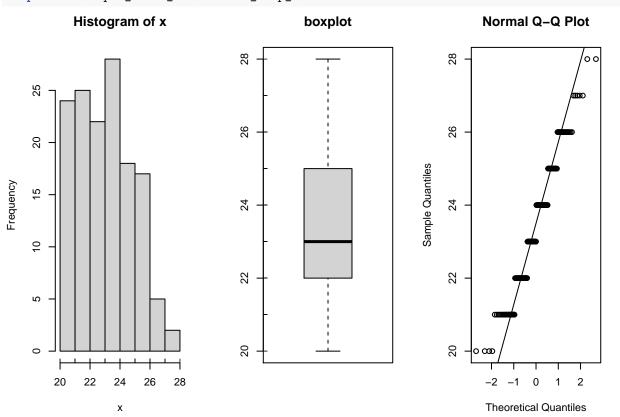
Cloacal Temperature

seems normally distributed, but not normal

```
morpho_blood_SMI %>%
    ggplot(., aes(x = cloacal_temp_C)) +
    geom_histogram(color = "black", fill="steelblue", bins=10) +
    theme_classic() +
    xlab("cloacal temperature (C)") +
    ylab("Count")
```



simple.eda(morpho_blood_SMI\$cloacal_temp_C)

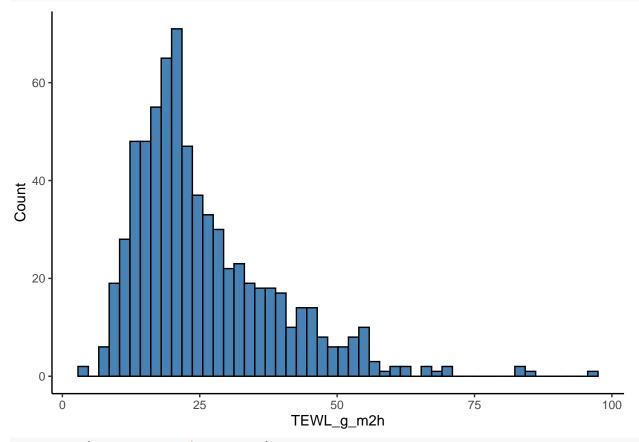


```
# Normality test if p > .05, data is normal. Data is not normal
shapiro.test(morpho_blood_SMI$cloacal_temp_C)
```

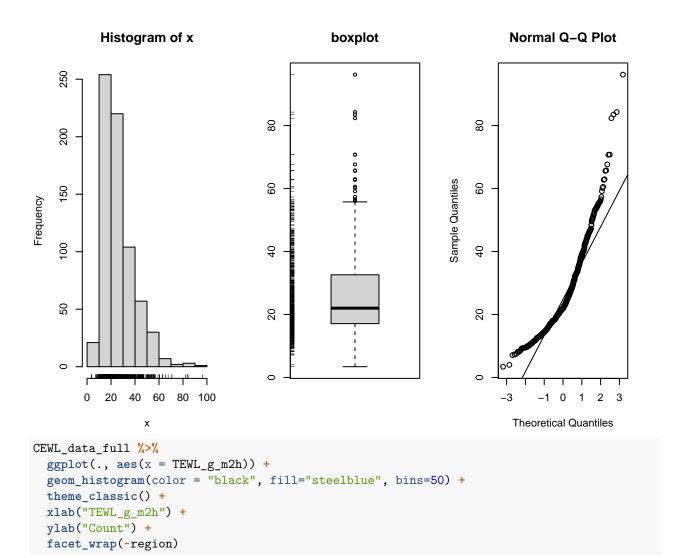
```
##
## Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$cloacal_temp_C
## W = 0.95661, p-value = 0.0002007
```

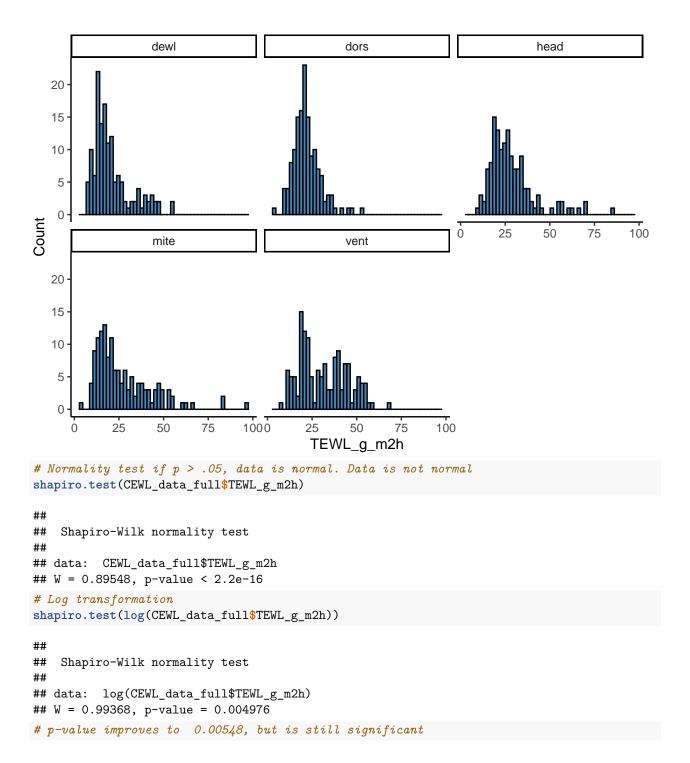
\mathbf{CEWL}

```
CEWL_data_full %>%
  ggplot(., aes(x = TEWL_g_m2h)) +
  geom_histogram(color = "black", fill="steelblue", bins=50) +
  theme_classic() +
  xlab("TEWL_g_m2h") +
  ylab("Count")
```



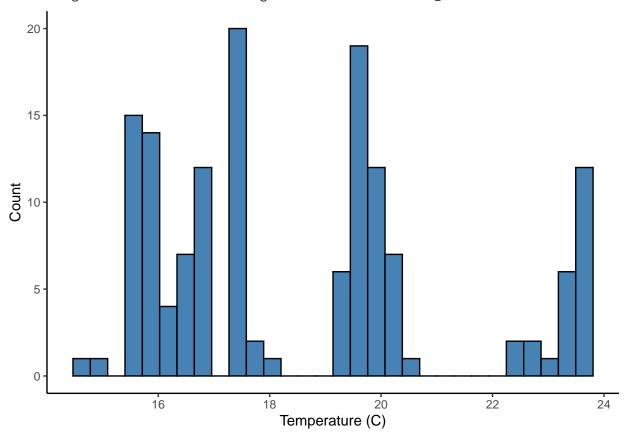
simple.eda(CEWL_data_full\$TEWL_g_m2h)



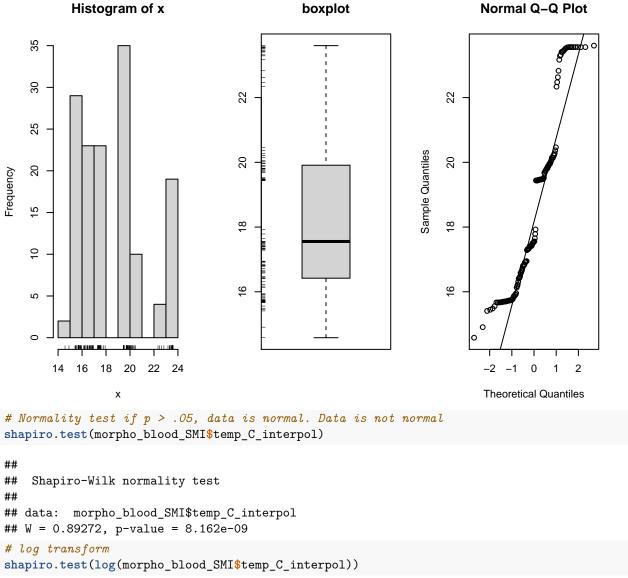


Capture Temperature

```
morpho_blood_SMI %>%
  ggplot(., aes(x = temp_C_interpol)) +
  geom_histogram(color = "black", fill="steelblue", bins=30) +
  theme_classic() +
  xlab("Temperature (C)") +
  ylab("Count")
```



simple.eda(morpho_blood_SMI\$temp_C_interpol)



```
shapiro.test(log(morpho_blood_SMI$temp_C_interp

##

## Shapiro-Wilk normality test

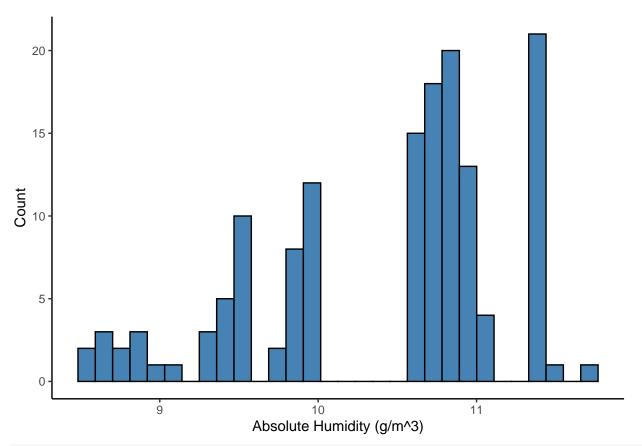
##

## data: log(morpho_blood_SMI$temp_C_interpol)
```

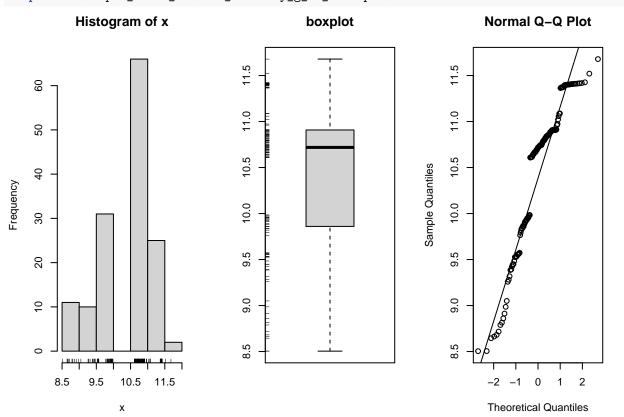
Capture Absolute Humidity

W = 0.91155, p-value = 9.439e-08

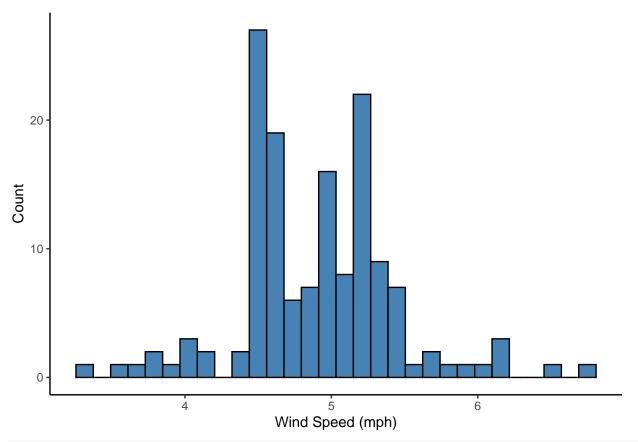
```
morpho_blood_SMI %>%
  ggplot(., aes(x = abs_humidity_g_m3_interpol)) +
  geom_histogram(color = "black", fill="steelblue", bins=30) +
  theme_classic() +
  xlab("Absolute Humidity (g/m^3)") +
  ylab("Count")
```



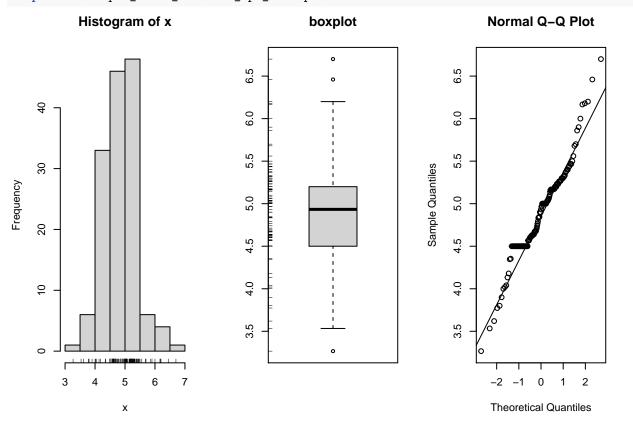
simple.eda(morpho_blood_SMI\$abs_humidity_g_m3_interpol)



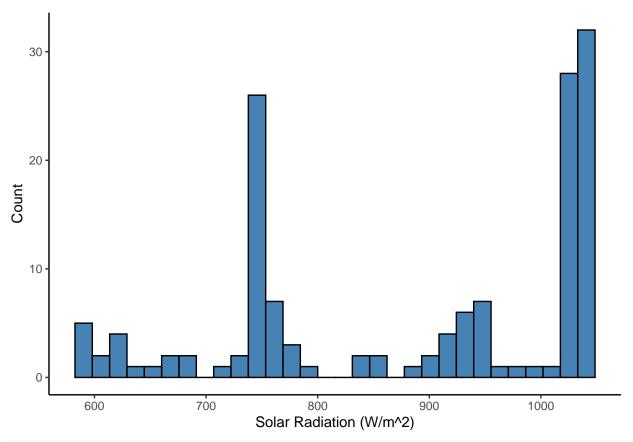
```
# Normality test if p > .05, data is normal. Data is not normal
shapiro.test(morpho_blood_SMI$abs_humidity_g_m3_interpol)
##
##
   Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$abs_humidity_g_m3_interpol
## W = 0.90867, p-value = 6.365e-08
# Doesn't fix non normality
shapiro.test(log(morpho_blood_SMI$abs_humidity_g_m3_interpol))
##
   Shapiro-Wilk normality test
##
##
## data: log(morpho_blood_SMI$abs_humidity_g_m3_interpol)
## W = 0.89617, p-value = 1.252e-08
Wind Speed at Capture
morpho_blood_SMI %>%
  ggplot(., aes(x = Wind_mph_interpol)) +
  geom_histogram(color = "black", fill="steelblue", bins=30) +
  theme_classic() +
  xlab("Wind Speed (mph)") +
  ylab("Count")
```



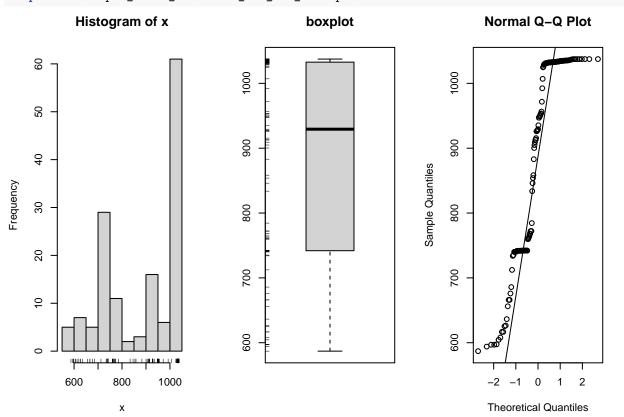
simple.eda(morpho_blood_SMI\$Wind_mph_interpol)



```
# Normality test if p > .05, data is normal. Data is not normal
shapiro.test(morpho_blood_SMI$Wind_mph_interpol)
##
##
   Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$Wind_mph_interpol
## W = 0.96149, p-value = 0.0004364
# In transformation doesn't fix non normality
shapiro.test(log(morpho_blood_SMI$Wind_mph_interpol))
##
   Shapiro-Wilk normality test
##
##
## data: log(morpho_blood_SMI$Wind_mph_interpol)
## W = 0.95827, p-value = 0.0002241
Solar Radiation at Capture
morpho_blood_SMI %>%
  ggplot(., aes(x = Solar_rad_Wm2_interpol)) +
  geom_histogram(color = "black", fill="steelblue", bins=30) +
  theme_classic() +
  xlab("Solar Radiation (W/m^2)") +
  ylab("Count")
```



simple.eda(morpho_blood_SMI\$Solar_rad_Wm2_interpol)



```
# Normality test if p > .05, data is normal. Data is not normal
shapiro.test(morpho_blood_SMI$Solar_rad_Wm2_interpol)
##
##
   Shapiro-Wilk normality test
##
## data: morpho_blood_SMI$Solar_rad_Wm2_interpol
## W = 0.82946, p-value = 1.072e-11
# Doesn't fix non normality
shapiro.test(log(morpho_blood_SMI$Solar_rad_Wm2_interpol))
##
##
   Shapiro-Wilk normality test
##
## data: log(morpho_blood_SMI$Solar_rad_Wm2_interpol)
## W = 0.829, p-value = 1.028e-11
```

Conclusion

Osmolality and SMI were the only normally distributed variables.

The following variables each had non-normal distributions: - SVL (skewed left) - mass (skewed left) - hct (looks like a bell curve, but not very pretty) - cloacal temp (skewed right) - CEWL (skewed right, overall and for each region individually) - capture temp (multimodal) - capture humidity (multimodal and skewed left) - wind speed (multimodal but sorta bell curve) - solar radiation (multimodal)

All of the non-normal variables are likely to need transformation if they're included in the final model.

Basic Figs & LMs

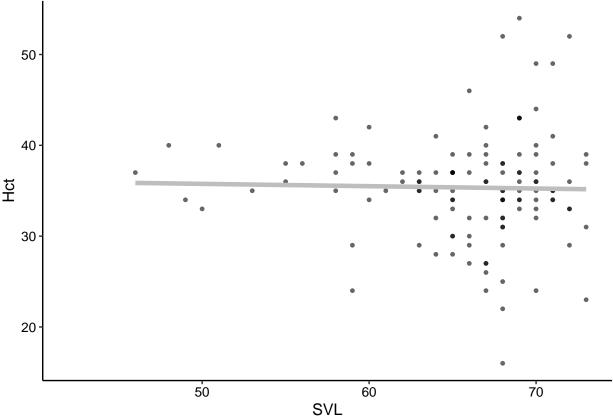
What affects osmolality & hematocrit?

 $Hct \sim SVL$

```
# plot
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = SVL_mm,
                 y = hematocrit_percent,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = SVL_mm,
                  y = hematocrit_percent,
                  ),
              formula = y \sim x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("SVL") +
  ylab("Hct") +
```

Warning: Removed 27 rows containing non-finite values (stat_smooth).

Warning: Removed 27 rows containing missing values (geom_point).



```
##
## lm(formula = hematocrit_percent ~ SVL_mm, data = morpho_blood_SMI)
##
## Residuals:
##
        Min
                  1Q
                       Median
                                            Max
                                    3Q
  -19.2957 -2.7565 -0.2189
                                2.7043 18.7299
##
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept) 37.03634 6.51586 5.684 9.54e-08 ***
## SVL_mm -0.02560 0.09888 -0.259 0.796
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 5.953 on 119 degrees of freedom
## (27 observations deleted due to missingness)
## Multiple R-squared: 0.0005628, Adjusted R-squared: -0.007836
## F-statistic: 0.06702 on 1 and 119 DF, p-value: 0.7962
```

Osml ~ SVL

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = SVL_mm,
                 y = osmolality_mmol_kg,
                 ),
             size = 1.
             alpha = 0.6) +
  stat_smooth(aes(x = SVL_mm,
                  y = osmolality_mmol_kg,
                  ),
              formula = y ~ x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme classic() +
  xlab("SVL") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black",
                             family = "sans",
                             size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 10),
        legend.text.align = 0,
)
```

- ## Warning: Removed 3 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 3 rows containing missing values (geom_point).

```
440 -
   400
Osmolality
   360
   320
                             50
                                                     60
                                                                             70
                                              SVL
lm_osml_SVL <- lm(osmolality_mmol_kg ~ SVL_mm,</pre>
           data = morpho_blood_SMI)
summary(lm_osml_SVL)
##
## lm(formula = osmolality_mmol_kg ~ SVL_mm, data = morpho_blood_SMI)
##
## Residuals:
##
       Min
                1Q Median
##
   -72.716 -20.584
                     1.268 22.088 72.055
##
## Coefficients:
               Estimate Std. Error t value Pr(>|t|)
                                    12.507
## (Intercept) 352.1404
                           28.1557
                                              <2e-16 ***
## SVL_mm
                 0.1967
                            0.4305
                                      0.457
                                               0.648
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 31.27 on 143 degrees of freedom
     (3 observations deleted due to missingness)
## Multiple R-squared: 0.001458, Adjusted R-squared: -0.005525
```

F-statistic: 0.2088 on 1 and 143 DF, p-value: 0.6484

$Hct \sim SMI$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = SMI,
                 y = hematocrit_percent,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = SMI,
                  y = hematocrit_percent,
                  ),
              formula = y ~ x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Scaled Mass Index") +
  ylab("Hematocrit") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```

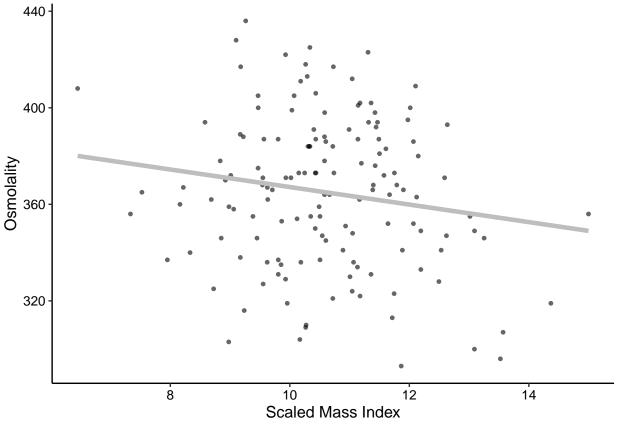
- ## Warning: Removed 27 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 27 rows containing missing values (geom_point).

```
50
  40
Hematocrit
  30
  20
                      8
                                        10
                                                          12
                                                                           14
                                     Scaled Mass Index
lm_hct_mass <- lm(hematocrit_percent ~ SMI,</pre>
           data = morpho_blood_SMI)
summary(lm_hct_mass)
##
## Call:
## lm(formula = hematocrit_percent ~ SMI, data = morpho_blood_SMI)
## Residuals:
##
       Min
                1Q Median
                                ЗQ
   -19.990 -2.680 -0.120
##
                             2.927
                                    17.845
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) 31.3051
                            4.3249
                                     7.238 4.84e-11 ***
## SMI
                 0.3842
                            0.4071
                                     0.944
                                              0.347
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 5.933 on 119 degrees of freedom
     (27 observations deleted due to missingness)
## Multiple R-squared: 0.007431,
                                    Adjusted R-squared: -0.0009099
## F-statistic: 0.8909 on 1 and 119 DF, p-value: 0.3471
```

$Osml \sim SMI$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = SMI,
                 y = osmolality_mmol_kg,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = SMI,
                 y = osmolality_mmol_kg),
              formula = y ~ x,
              method = "lm",
              se = F,
              color = "gray",
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Scaled Mass Index") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```

- ## Warning: Removed 3 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 3 rows containing missing values (geom_point).



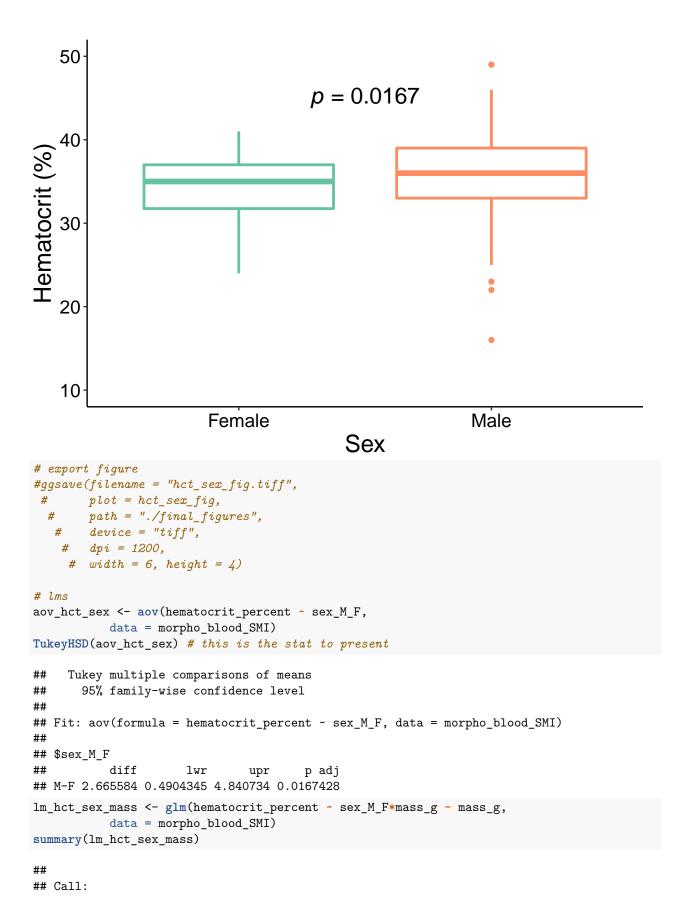
```
##
## Call:
## lm(formula = osmolality_mmol_kg ~ SMI, data = morpho_blood_SMI)
## Residuals:
##
       Min
                1Q Median
                               ЗQ
                     1.245
   -67.879 -22.935
                           21.677
                                   66.154
##
##
## Coefficients:
##
              Estimate Std. Error t value Pr(>|t|)
   (Intercept) 403.490
                            20.121
                                  20.053
                                             <2e-16 ***
## SMI
                 -3.632
                            1.881 -1.931
                                            0.0555 .
##
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 30.89 on 143 degrees of freedom
     (3 observations deleted due to missingness)
## Multiple R-squared: 0.02541,
                                   Adjusted R-squared: 0.0186
## F-statistic: 3.729 on 1 and 143 DF, p-value: 0.05545
```

$\mathbf{Hct} \sim \mathbf{Sex}$

males have significantly higher hematocrit %

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = sex_M_F,
                 y = hematocrit_percent,
                 color = sex_M_F
                 ),
              size = 1,
              alpha = 1) +
 theme_classic() +
  xlab("Sex") +
 ylab("Hematocrit (%)") +
  annotate("text", x = 1.5, y = 45,
          parse = TRUE,
          size = 6) +
 ylim(10, 50) +
  scale_x_discrete(labels = c("F" = "Female",
                            "M" = "Male")) +
  scale_color_brewer(palette = "Set2") +
  theme(text = element_text(color = "black",
                          family = "sans",
                          size = 18),
       axis.text = element_text(color = "black",
                               family = "sans",
                               size = 14),
       legend.text.align = 0,
       legend.position = "none"
) -> hct_sex_fig
hct_sex_fig
```

Warning: Removed 30 rows containing non-finite values (stat_boxplot).



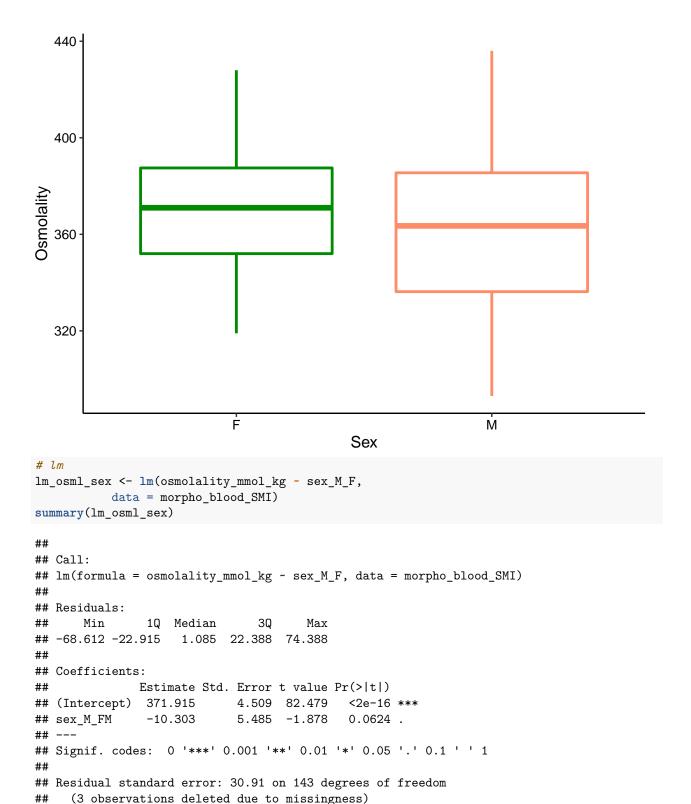
```
## glm(formula = hematocrit_percent ~ sex_M_F * mass_g - mass_g,
##
       data = morpho_blood_SMI)
##
## Deviance Residuals:
       Min
                   1Q
                        Median
                                       3Q
                                                Max
## -20.6306
            -2.6187
                        0.6422
                                            17.2271
                                   3.1481
## Coefficients:
##
                  Estimate Std. Error t value Pr(>|t|)
                                       9.646
## (Intercept)
                   35.9163
                                3.7236
                                                 <2e-16 ***
## sex_M_FM
                   -0.9224
                                4.7553 -0.194
                                                  0.847
## sex_M_FF:mass_g -0.2244
                                0.3596 -0.624
                                                  0.534
## sex_M_FM:mass_g 0.1186
                                0.2568
                                       0.462
                                                  0.645
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Dispersion parameter for gaussian family taken to be 34.18947)
##
      Null deviance: 4219.7 on 120 degrees of freedom
##
## Residual deviance: 4000.2 on 117 degrees of freedom
     (27 observations deleted due to missingness)
## AIC: 776.68
##
## Number of Fisher Scoring iterations: 2
```

Hematocrit is significantly predicted by sex, but the interaction between sex and mass is ~nonexistent.

$Osml \sim Sex$

```
morpho blood SMI %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = sex_M_F,
                   y = osmolality_mmol_kg,
                   color = sex_M_F
                   ),
               size = 1.
               alpha = 0.6) +
  scale_colour_manual(name = "Sex",
                      values = c("green4", "salmon1") ) +
  theme_classic() +
  xlab("Sex") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
```

Warning: Removed 3 rows containing non-finite values (stat_boxplot).



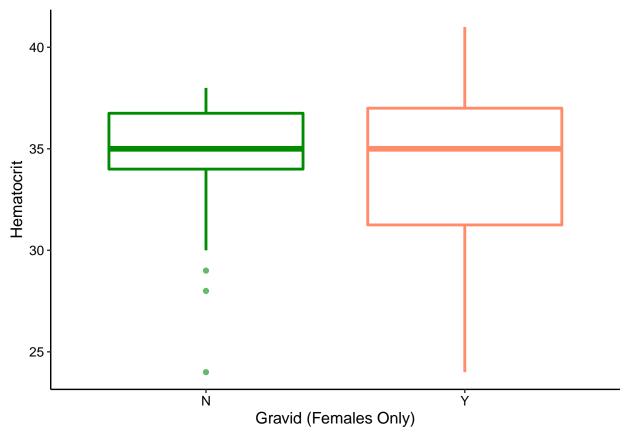
Multiple R-squared: 0.02408, Adjusted R-squared: 0.01725

F-statistic: 3.528 on 1 and 143 DF, p-value: 0.06237

Hct ~ Gravidity

```
morpho_blood_SMI %>%
  dplyr::filter(sex_M_F == 'F') %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = gravid_Y_N,
                   y = hematocrit_percent,
                   color = gravid_Y_N
                   ),
               size = 1,
               alpha = 0.6) +
  scale_colour_manual(name = "Gravid",
                      values = c("green4", "salmon1") ) +
  theme_classic() +
  xlab("Gravid (Females Only)") +
  ylab("Hematocrit") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
```

Warning: Removed 4 rows containing non-finite values (stat_boxplot).

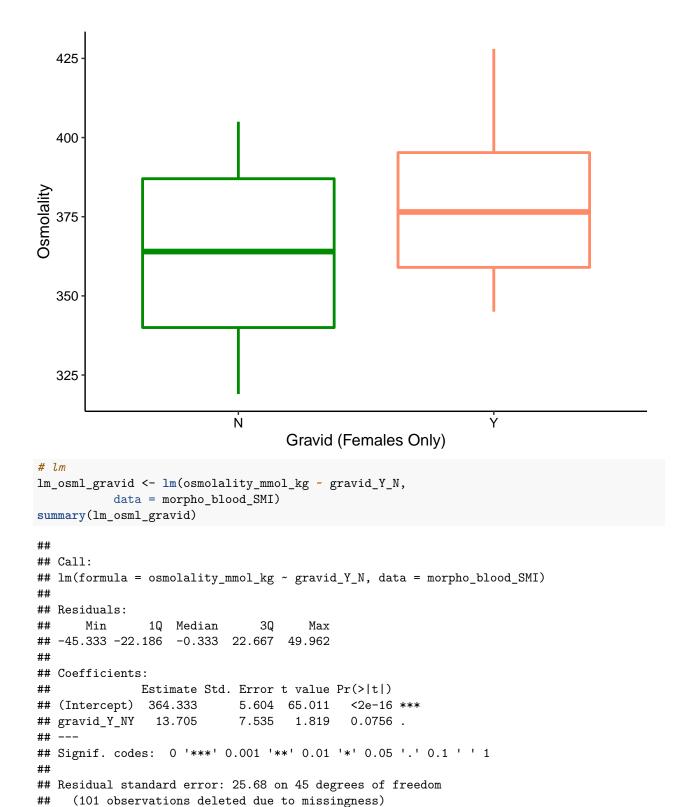


```
##
## lm(formula = hematocrit_percent ~ gravid_Y_N, data = morpho_blood_SMI)
## Residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -10.111 -1.596
                   1.271
                            3.080
                                    7.654
##
## Coefficients:
              Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                34.111
                            1.017 33.546
                                            <2e-16 ***
## gravid_Y_NY
                -0.765
                            1.323 -0.578
                                             0.566
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 4.314 on 42 degrees of freedom
     (104 observations deleted due to missingness)
## Multiple R-squared: 0.0079, Adjusted R-squared: -0.01572
## F-statistic: 0.3344 on 1 and 42 DF, p-value: 0.5662
```

Osml ~ Gravidity

```
morpho_blood_SMI %>%
  dplyr::filter(sex_M_F == 'F') %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = gravid_Y_N,
                   y = osmolality_mmol_kg,
                   color = gravid Y N
                   ),
               size = 1,
               alpha = 0.6) +
  scale_colour_manual(name = "Gravid",
                      values = c("green4", "salmon1") ) +
  theme_classic() +
  xlab("Gravid (Females Only)") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
)
```

Warning: Removed 1 rows containing non-finite values (stat_boxplot).



Adjusted R-squared: 0.04778

Multiple R-squared: 0.06848,

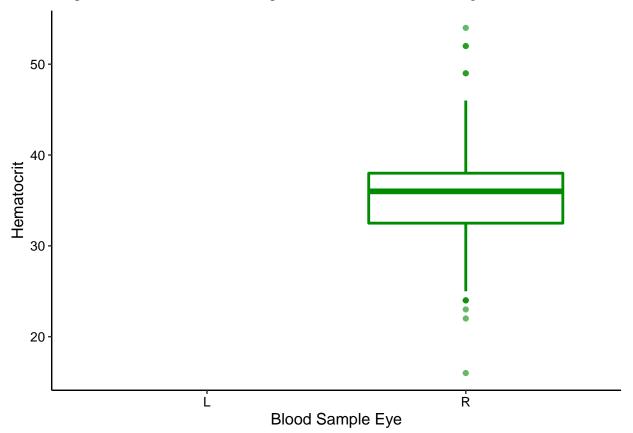
F-statistic: 3.308 on 1 and 45 DF, p-value: 0.07559

Hct ~ Sample Eye

Actually, any blood samples not taken from the right eye ended up being excluded or didn't have hematocrit values, so we can't test this difference.

```
morpho_blood_SMI %>%
  dplyr::filter(blood_sample_eye %in% c("R", "L")) %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = blood_sample_eye,
                   y = hematocrit_percent,
                   color = blood_sample_eye
                   ),
               size = 1,
               alpha = 0.6) +
  scale_colour_manual(name = "Blood Sample Eye",
                      values = c("green4", "salmon1") ) +
  theme_classic() +
  xlab("Blood Sample Eye") +
  ylab("Hematocrit") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
```

Warning: Removed 27 rows containing non-finite values (stat_boxplot).



$Osml \sim Sample \ Eye$

```
morpho_blood_SMI %>%
  dplyr::filter(blood_sample_eye %in% c("R", "L")) %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = blood_sample_eye,
                   y = osmolality_mmol_kg,
                   color = blood_sample_eye
                   ),
               size = 1,
               alpha = 0.6) +
  scale_colour_manual(name = "Blood Sample Eye",
                      values = c("green4", "salmon1") ) +
  theme_classic() +
  xlab("Blood Sample Eye") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
## Warning: Removed 3 rows containing non-finite values (stat_boxplot).
  440 -
  400
Osmolality
  360
  320
                                                                   Ŕ
                                       Blood Sample Eye
# lm
lm_osml_eye <- lm(osmolality_mmol_kg ~ blood_sample_eye,</pre>
           data = morpho_blood_SMI)
summary(lm_osml_eye)
```

```
##
## Call:
## lm(formula = osmolality_mmol_kg ~ blood_sample_eye, data = morpho_blood_SMI)
##
## Residuals:
##
      Min
               1Q Median
                                3Q
                                       Max
## -71.986 -19.986
                     1.014 22.014 71.014
##
## Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                       400.50
                                   21.85
                                         18.330
                                                   <2e-16 ***
                       -60.83
                                   28.21 -2.157
                                                   0.0327 *
## blood_sample_eyeL
## blood_sample_eyeR
                       -35.51
                                   22.01 -1.614
                                                   0.1088
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 30.9 on 142 degrees of freedom
     (3 observations deleted due to missingness)
## Multiple R-squared: 0.03175,
                                   Adjusted R-squared:
## F-statistic: 2.328 on 2 and 142 DF, p-value: 0.1012
summary(morpho_blood_SMI$blood_sample_eye)
```

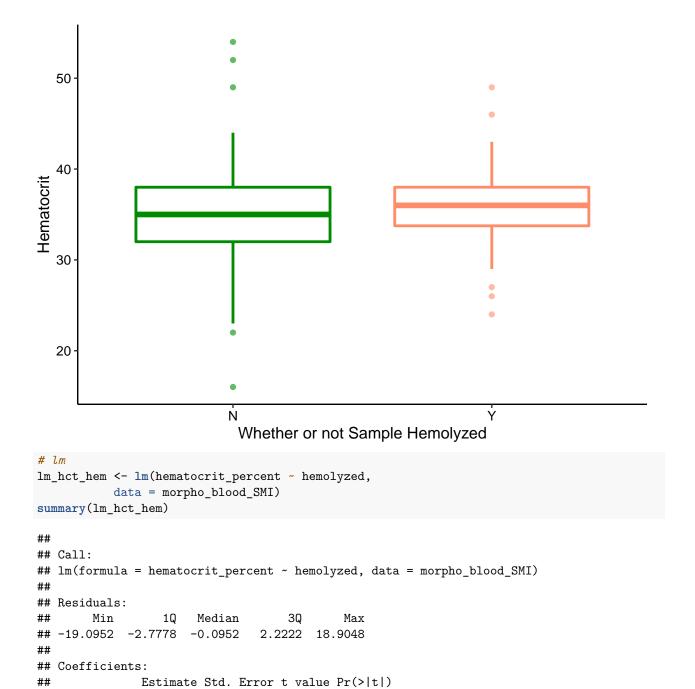
both L R ## 2 4 142

There IS a significant difference, but upon further inspection, the number of blood draws taken from the right postorbital sinus vs left or both is so uneven, this would be unhelpful in the actual model.

Hct ~ Hemolyzed/Not

```
morpho blood SMI %>%
  dplyr::filter(hemolyzed %in% c("Y", "N")) %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = hemolyzed,
                   y = hematocrit_percent,
                   color = hemolyzed
                   ),
               size = 1.
               alpha = 0.6) +
  scale_colour_manual(name = "Blood Sample Eye",
                      values = c("green4", "salmon1", "green4", "salmon1") ) +
  theme_classic() +
  xlab("Whether or not Sample Hemolyzed") +
  ylab("Hematocrit") +
  theme(text = element text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
)
```

Warning: Removed 25 rows containing non-finite values (stat_boxplot).



<2e-16 ***

Adjusted R-squared: -0.007293

0.591

0.7636 45.958

0.539

1.2664

Residual standard error: 6.061 on 97 degrees of freedom
(49 observations deleted due to missingness)

F-statistic: 0.2905 on 1 and 97 DF, p-value: 0.5911

Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1

(Intercept) 35.0952

0.6825

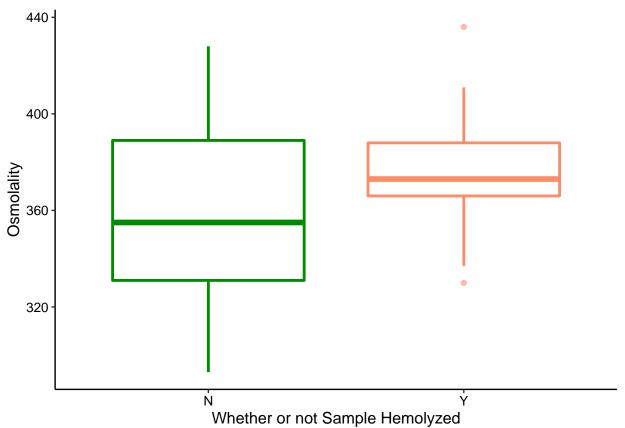
Multiple R-squared: 0.002986,

hemolyzedY

$Osml \sim Hemolyzed/Not$

```
morpho_blood_SMI %>%
  dplyr::filter(hemolyzed %in% c("Y", "N")) %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = hemolyzed,
                   y = osmolality_mmol_kg,
                   color = hemolyzed
                   ),
               size = 1,
               alpha = 0.6) +
  scale_colour_manual(name = "Blood Sample Eye",
                      values = c("green4", "salmon1", "green4", "salmon1") ) +
  theme_classic() +
  xlab("Whether or not Sample Hemolyzed") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
```

Warning: Removed 1 rows containing non-finite values (stat_boxplot).



```
# lm
lm_osml_hem <- lm(osmolality_mmol_kg ~ hemolyzed,</pre>
```

summary(lm_osml_hem)

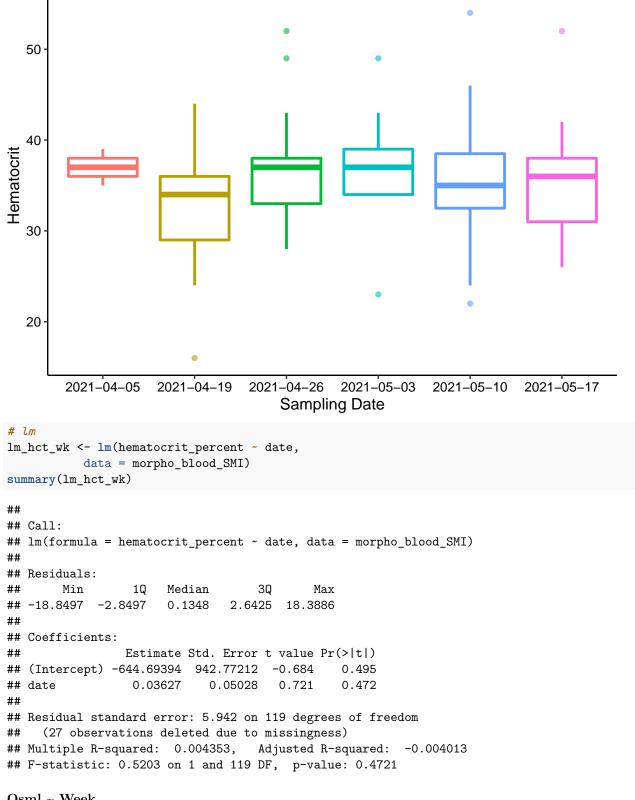
data = morpho_blood_SMI)

```
##
## Call:
## lm(formula = osmolality_mmol_kg ~ hemolyzed, data = morpho_blood_SMI)
##
## Residuals:
##
      Min
               1Q Median
                               3Q
                                      Max
## -66.690 -23.858 -2.026 24.310
##
## Coefficients:
              Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) 359.690
                            3.523 102.109
                                            <2e-16 ***
                15.335
                            6.256
                                    2.451
                                            0.0157 *
## hemolyzedY
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 32.29 on 121 degrees of freedom
     (25 observations deleted due to missingness)
## Multiple R-squared: 0.04731,
                                   Adjusted R-squared:
## F-statistic: 6.009 on 1 and 121 DF, p-value: 0.01566
```

Osmolality in hemolyzed samples versus not are significantly different! This is an unfortunate trait of the dataset, and not something we are specifically interested in testing, so we will include it as a random factor in the LMM.

Hct ~ Week

Warning: Removed 27 rows containing non-finite values (stat_boxplot).

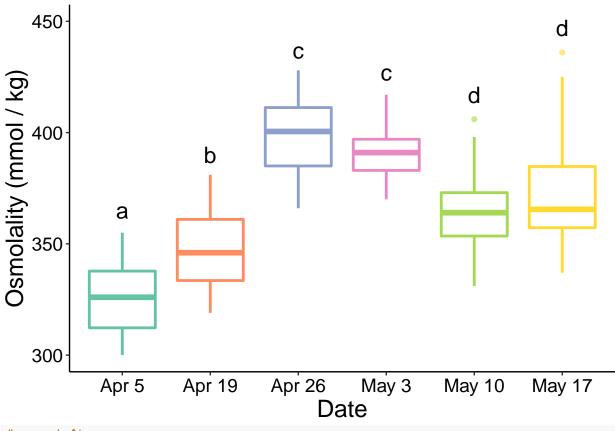


$Osml \sim Week$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
```

```
geom_boxplot(aes(x = as.factor(date),
                   y = osmolality_mmol_kg,
                   color = as.factor(date)
                   ),
               size = 1,
               alpha = 0.6) +
  theme_classic() +
  xlab("Date") +
  ylab("Osmolality (mmol / kg)") +
  annotate("text", x = 1, y = 365, label = "a", size = 6) +
  annotate("text", x = 2, y = 390, label = "b", size = 6) +
  annotate("text", x = 3, y = 437, label = "c", size = 6) +
  annotate("text", x = 4, y = 427, label = "c", size = 6) +
  annotate("text", x = 5, y = 417, label = "d", size = 6) +
  annotate("text", x = 6, y = 447, label = "d", size = 6) +
  scale_x_discrete(labels = c("2021-04-05" = "Apr 5",
                              "2021-04-19" = "Apr 19",
                              "2021-04-26" = "Apr 26",
                              "2021-05-03" = "May 3",
                              "2021-05-10" = "May 10",
                              "2021-05-17" = "May 17")) +
  scale_color_brewer(palette = "Set2") +
 ylim(300, 450) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 14),
        legend.text.align = 0,
       legend.position = "none"
) -> osml_date_fig
osml_date_fig
```

Warning: Removed 5 rows containing non-finite values (stat_boxplot).



```
## Call:
## glm(formula = osmolality_mmol_kg ~ date, data = morpho_blood_SMI)
##
## Deviance Residuals:
##
       Min
                 1Q
                      Median
                                   3Q
                                           Max
## -48.236 -21.636
                      -2.635
                               20.364
                                        65.564
##
## Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) -1.999e+04 2.954e+03 -6.766 3.15e-10 ***
                                       6.889 1.65e-10 ***
## date
                1.086e+00 1.576e-01
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## (Dispersion parameter for gaussian family taken to be 735.2172)
##
## Null deviance: 140031 on 144 degrees of freedom
## Residual deviance: 105136 on 143 degrees of freedom
## (3 observations deleted due to missingness)
## AIC: 1372.5
##
## Number of Fisher Scoring iterations: 2
```

Osmolality was VERY different by week, and that was not easily attributable to climate factors (see below). We also experienced technical difficulties with the osmometer between some weeks, so some of the variation may be attributable to technical error. Thus date/week will be a good random factor to include in the LMM.

distinguish pairwise differences using an ANOVA:

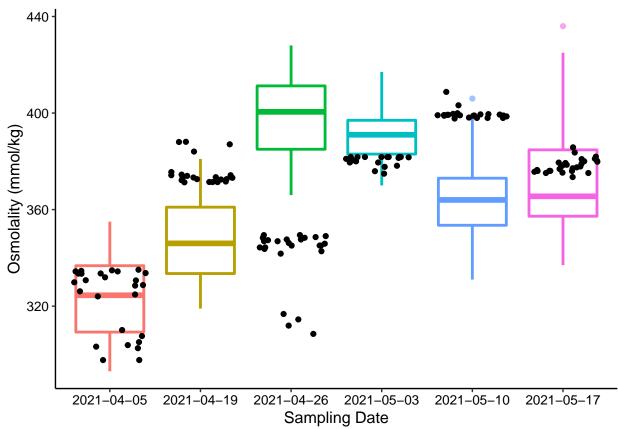
```
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = osmolality_mmol_kg ~ as.factor(date), data = morpho_blood_SMI)
##
## $`as.factor(date)`
##
                               diff
                                           lwr
                                                      upr
                                                              p adj
## 2021-04-19-2021-04-05
                          22.891304
                                      7.979417
                                                37.803191 0.0002634
## 2021-04-26-2021-04-05 75.076923 60.628732 89.525114 0.0000000
## 2021-05-03-2021-04-05
                         66.657895
                                    50.935165 82.380624 0.0000000
## 2021-05-10-2021-04-05
                         41.543478
                                     26.631591
                                               56.455365 0.0000000
## 2021-05-17-2021-04-05
                          46.785714
                                     32.597872
                                                60.973557 0.0000000
## 2021-04-26-2021-04-19
                          52.185619
                                     37.273732
                                                67.097506 0.0000000
## 2021-05-03-2021-04-19
                          43.766590
                                    27.616718
                                                59.916462 0.0000000
## 2021-05-10-2021-04-19
                         18.652174
                                      3.290581
                                                34.013766 0.0078361
## 2021-05-17-2021-04-19
                          23.894410
                                      9.234635
                                                38.554185 0.0000857
## 2021-05-03-2021-04-26 -8.419028 -24.141758
                                                 7.303701 0.6342961
## 2021-05-10-2021-04-26 -33.533445 -48.445332 -18.621558 0.0000000
## 2021-05-17-2021-04-26 -28.291209 -42.479051 -14.103367 0.0000008
## 2021-05-10-2021-05-03 -25.114416 -41.264289
                                                -8.964544 0.0002088
## 2021-05-17-2021-05-03 -19.872180 -35.356006
                                               -4.388355 0.0039942
## 2021-05-17-2021-05-10
                           5.242236 -9.417539
                                               19.902011 0.9059063
```

Osml, Humidity, Week

```
theme_classic() +
   xlab("Sampling Date") +
   ylab("Osmolality (mmol/kg)") +
   theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
)
```

Warning: Removed 3 rows containing non-finite values (stat_boxplot).

Warning: Removed 3 rows containing missing values (geom_point).



Osml ~ Abs. Humidity

```
color = "royalblue",
              size = 1.6,
              alpha = 1) +
 theme_classic() +
 xlab(bquote('Absolute Humidity at Capture (g / '*m^2*')')) +
 ylab("Osmolality (mmol / kg)") +
 xlim(8, 12) +
 ylim(300, 450) +
  annotate("text", x = 8.5, y = 440,
           label = "paste(italic(R) ^2, \" = 0.038\")",
           parse = TRUE,
           size = 6) +
  annotate("text", x = 8.5, y = 428,
           label = "paste(italic(p), \  \  = 0.011\  \  )",
           parse = TRUE,
           size = 6) +
 theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
       axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 14),
        legend.text.align = 0,
) -> osml_abhum_fig
osml_abhum_fig
```

- ## Warning: Removed 7 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 7 rows containing missing values (geom_point).

```
## Call:
## lm(formula = osmolality_mmol_kg ~ abs_humidity_g_m3_interpol,
##
       data = morpho_blood_SMI)
##
## Residuals:
              1Q Median
   -64.06 -21.24 -1.45 21.64
                                66.35
##
##
## Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                               279.229
                                            33.666
                                                     8.294 7.88e-14 ***
## abs_humidity_g_m3_interpol
                                 8.286
                                             3.216
                                                     2.576
                                                              0.011 *
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

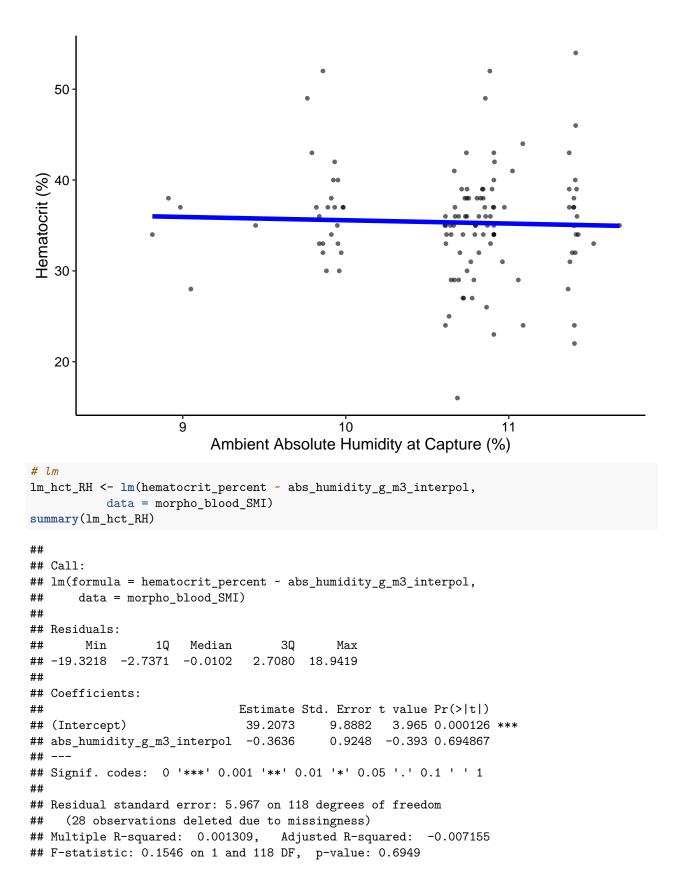
##

```
##
## Residual standard error: 30.11 on 141 degrees of freedom
## (5 observations deleted due to missingness)
## Multiple R-squared: 0.04496, Adjusted R-squared: 0.03818
## F-statistic: 6.637 on 1 and 141 DF, p-value: 0.01101
positive correlation...
```

Hct ~ Humidity

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = abs_humidity_g_m3_interpol,
                 y = hematocrit_percent,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = abs_humidity_g_m3_interpol,
                  y = hematocrit_percent),
              formula = y ~ x,
              method = "lm",
              se = F,
              color = "blue",
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Ambient Absolute Humidity at Capture (%)") +
  ylab("Hematocrit (%)") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
)
```

- ## Warning: Removed 28 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 28 rows containing missing values (geom_point).



$Hct \sim Temperature$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = temp_C_interpol,
                 y = hematocrit_percent,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = temp_C_interpol,
                 y = hematocrit_percent),
              formula = y ~ x,
              method = "lm",
              se = F,
              color = "maroon",
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Ambient Temperature at Capture (C)") +
  ylab("Hematocrit (%)") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```

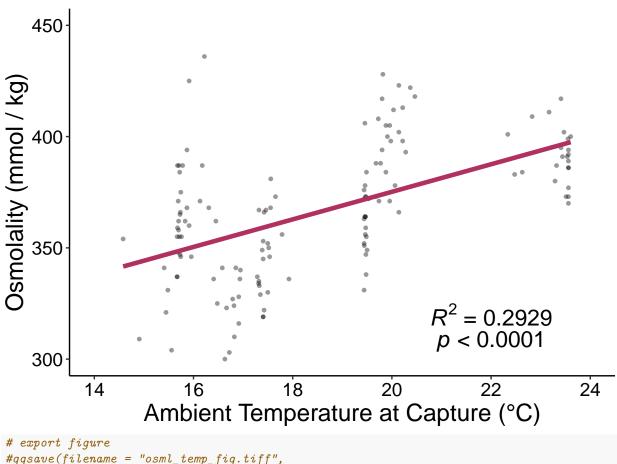
- ## Warning: Removed 28 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 28 rows containing missing values (geom_point).

```
50
Hematocrit (%)
   40
   20
                     16
                                      18
                                                       20
                                                                        22
                                                                                         24
                             Ambient Temperature at Capture (C)
lm_hct_temp <- lm(hematocrit_percent ~ temp_C_interpol,</pre>
           data = morpho_blood_SMI)
summary(lm_hct_temp)
##
## Call:
## lm(formula = hematocrit_percent ~ temp_C_interpol, data = morpho_blood_SMI)
## Residuals:
##
        Min
                       Median
                                     3Q
                  1Q
                                             Max
   -18.6541 -3.0678
                       0.3085
                                         18.5137
##
                                 2.3757
## Coefficients:
##
                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                    27.9447
                                3.9993
                                          6.987 1.78e-10 ***
                                          1.862
## temp_C_interpol
                     0.3872
                                0.2079
                                                  0.0651 .
##
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 5.885 on 118 degrees of freedom
     (28 observations deleted due to missingness)
## Multiple R-squared: 0.02855,
                                     Adjusted R-squared: 0.02032
## F-statistic: 3.468 on 1 and 118 DF, p-value: 0.06505
```

$Osml \sim Temperature$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = temp_C_interpol,
                 y = osmolality_mmol_kg,
                 ),
             size = 1,
             alpha = 0.4) +
  stat_smooth(aes(x = temp_C_interpol,
                 y = osmolality_mmol_kg),
              formula = y ~ x,
              method = "lm",
              se = F,
              color = "maroon",
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Ambient Temperature at Capture (°C)") +
  ylab("Osmolality (mmol / kg)") +
  annotate("text", x = 22, y = 320,
           label = "paste(italic(R) ^2, \" = 0.2929\")",
           parse = TRUE,
           size = 6) +
  annotate("text", x = 22, y = 308,
           label = "paste(italic(p), \" < 0.0001\")",</pre>
           parse = TRUE,
           size = 6) +
  xlim(14, 24) +
  ylim(300,450) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 14),
        legend.text.align = 0,
) -> osml_temp_fig
osml_temp_fig
```

- ## Warning: Removed 7 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 7 rows containing missing values (geom_point).



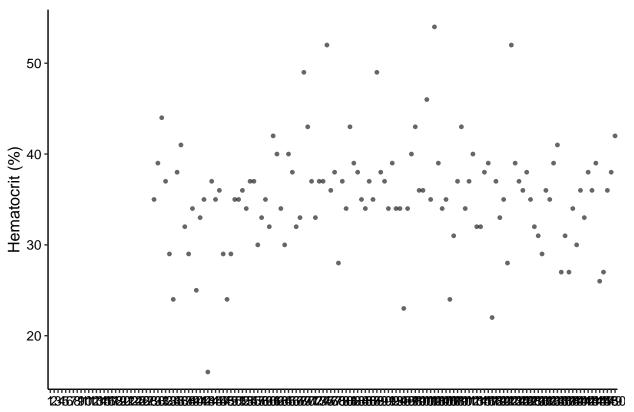
```
##
## lm(formula = osmolality_mmol_kg ~ temp_C_interpol, data = morpho_blood_SMI)
##
## Residuals:
               1Q Median
                               3Q
                                      Max
  -59.723 -16.089
                  -1.996 15.667 85.643
##
## Coefficients:
##
                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                  246.0362
                              15.6239
                                      15.747
                                              < 2e-16 ***
## temp_C_interpol
                    6.4316
                               0.8315
                                       7.735 1.8e-12 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 25.82 on 141 degrees of freedom
## (5 observations deleted due to missingness)
## Multiple R-squared: 0.2979, Adjusted R-squared: 0.2929
## F-statistic: 59.82 on 1 and 141 DF, p-value: 1.796e-12
```

Hct ~ Individual

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = individual_ID,
                 y = hematocrit percent,
                 ),
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = individual_ID,
                  y = hematocrit_percent,
                  ),
              formula = y \sim x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Individual Lizard") +
  ylab("Hematocrit (%)") +
  theme(text = element_text(color = "black",
                             family = "sans",
                             size = 12),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0,
```

- ## Warning: Removed 27 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 27 rows containing missing values (geom_point).



Individual Lizard

$Osml \sim Individual$

```
morpho_blood_SMI %>%
  ggplot(data = .) +
  geom_point(aes(x = individual_ID,
                 y = osmolality_mmol_kg,
             size = 1,
             alpha = 0.6) +
  stat_smooth(aes(x = individual_ID,
                  y = osmolality_mmol_kg,
                  ),
              formula = y ~ x,
              method = "lm",
              color = "gray",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Individual Lizard") +
  ylab("Osmolality") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
```

```
size = 10),
      legend.text.align = 0,
## Warning: Removed 3 rows containing non-finite values (stat_smooth).
## Warning: Removed 3 rows containing missing values (geom_point).
  440 -
  400
Osmolality
  360
  320
     Individual Lizard
```

Conclusion

Osmolality seems to be affected by: - SMI (large effect, although not statistically significant) - sex (NS) - whether or not the sample was hemolyzed () ! - week/date of sampling!! () - capture temp & humidity (both *)

So, for the LMM to predict osmolality, we will start with SMI, hemolyzed/not, week/date, and capture temp and absolute humidity as our predictor variables in the model.

Hematocrit was only meaningfully different based on sex, so that's the only statistic we'll include in the paper for what variables affect hematocrit.

What affects evaporative water loss?

Potential relationships: - CEWL ~ date/week, individual, SVL, SMI, gravidity, hct, osml, cloacal temp, ambient temp, ambient RH, measurement temp, measurement RH, body region

$CEWL \sim Body Region$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = reorder(region, TEWL_g_m2h),
                   y = TEWL_g_m2h,
                   color = region
                   ),
               size = 1,
              alpha = 1) +
  scale_x_discrete(labels = c("Dewlap", "Dorsum",
                              "Mite Patch", "Head", "Ventrum")) +
 theme_classic() +
 xlab("Body Region") +
 ylab(bquote('CEWL (g / '*m^2-h*')')) +
  annotate("text", x = 1, y = 65, label = "a", size = 6) +
  annotate("text", x = 2, y = 62, label = "a", size = 6) +
  annotate("text", x = 3, y = 105, label = "b", size = 6) +
  annotate("text", x = 4, y = 92, label = "b", size = 6) +
  annotate("text", x = 5, y = 75, label = "b", size = 6) +
  scale_color_brewer(palette = "Set2") +
 ylim(1, 110) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
       axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 14),
       legend.text.align = 0,
        legend.position = "none"
) -> CEWL_region_fig
CEWL_region_fig
```

```
b
                                                               b
     90
CEWL (g/m^2h)
                                                                              b
                 a
                                 a
     60
     30
       0
              Dewlap
                                          Mite Patch
                                                             Head
                                                                          Ventrum
                             Dorsum
                                       Body Region
# export figure
#ggsave(filename = "CEWL_region_fig.tiff",
        plot = CEWL_region_fig,
        path = "./final_figures",
        device = "tiff",
        dpi = 1200,
     # width = 6, height = 4)
CEWL_lm1 <- lm(TEWL_g_m2h ~ region, data = CEWL_data_full)</pre>
summary(CEWL_lm1)
##
## Call:
## lm(formula = TEWL_g_m2h ~ region, data = CEWL_data_full)
## Residuals:
##
       Min
                1Q Median
                                3Q
## -24.153 -8.364 -2.366
                             5.668
                                    68.934
##
## Coefficients:
               Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                 20.648
                             1.051
                                   19.642 < 2e-16 ***
## regiondors
                  1.255
                             1.481
                                     0.847
                                              0.397
                                     5.161 3.20e-07 ***
                             1.481
## regionhead
                  7.646
## regionmite
                  6.578
                             1.492
                                     4.409 1.20e-05 ***
                                     7.166 1.98e-12 ***
## regionvent
                 10.615
                             1.481
## ---
```

```
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.39 on 694 degrees of freedom
## Multiple R-squared: 0.09502, Adjusted R-squared: 0.0898
## F-statistic: 18.22 on 4 and 694 DF, p-value: 3.059e-14
Also get pairwise statistics:
# one-way ANOVA
CEWL region aov <- aov(data = CEWL data full,
                      TEWL_g_m2h ~ region)
# post-hoc pairwise analysis
TukeyHSD(CEWL_region_aov)
##
    Tukey multiple comparisons of means
##
      95% family-wise confidence level
##
## Fit: aov(formula = TEWL_g_m2h ~ region, data = CEWL_data_full)
##
## $region
##
                 diff
                              lwr
                                        upr
                                                p adj
## dors-dewl 1.254852 -2.79651839 5.306223 0.9156911
## head-dewl 7.645739 3.59436813 11.697109 0.0000032
## mite-dewl 6.578364 2.49773783 10.658990 0.0001172
## vent-dewl 10.615491 6.56411991 14.666861 0.0000000
## head-dors 6.390887 2.35401096 10.427762 0.0001662
## mite-dors 5.323511 1.25727637 9.389746 0.0033630
## vent-dors 9.360638 5.32376273 13.397514 0.0000000
## mite-head -1.067375 -5.13361016 2.998860 0.9523881
## vent-head 2.969752 -1.06712379 7.006627 0.2612293
## vent-mite 4.037127 -0.02910817 8.103362 0.0527256
```

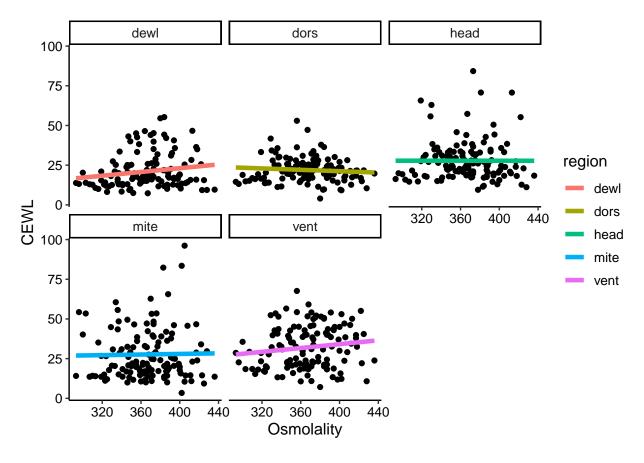
CEWL ~ Osmolality

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = osmolality_mmol_kg,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = osmolality_mmol_kg,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6
              alpha = 1) +
  theme_classic() +
  xlab("Osmolality") +
```

```
ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0,
## Warning: Removed 49 rows containing non-finite values (stat_smooth).
## Warning: Removed 49 rows containing missing values (geom_point).
  100 -
    75
                                                                                region
                                                                                    dewl
                                                                                    dors
    50
                                                                                    head
                                                                                    mite
                                                                                    vent
    25
     0
                    320
                                      360
                                                                          440
                                                        400
                                    Osmolality
# lm
CEWL_lm2 <- lm(TEWL_g_m2h ~ region + osmolality_mmol_kg,</pre>
           data = CEWL_data_full)
summary(CEWL_lm2)
##
## lm(formula = TEWL_g_m2h ~ region + osmolality_mmol_kg, data = CEWL_data_full)
##
## Residuals:
       Min
                1Q Median
                                ЗQ
                                        Max
## -25.283 -8.587 -2.447
                             5.805 67.689
## Coefficients:
```

```
Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                     13.25915
                                5.96986 2.221
                                                   0.0267 *
## regiondors
                                         0.525
                     0.80956
                               1.54206
                                                   0.5998
## regionhead
                                         4.326 1.76e-05 ***
                      6.67027
                                 1.54206
## regionmite
                      6.54184
                               1.55099
                                          4.218 2.82e-05 ***
## regionvent
                     10.99851
                                1.54206
                                         7.132 2.66e-12 ***
## osmolality_mmol_kg 0.02141
                                 0.01601
                                          1.337
                                                   0.1816
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.43 on 644 degrees of freedom
    (49 observations deleted due to missingness)
## Multiple R-squared: 0.1011, Adjusted R-squared: 0.0941
## F-statistic: 14.48 on 5 and 644 DF, p-value: 1.836e-13
# Facet ggplot
ggplot(aes(osmolality_mmol_kg, TEWL_g_m2h), data = CEWL_data_full) +
 geom_point() +
 stat_smooth(aes(x = osmolality_mmol_kg,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             formula = y ~ x,
             method = "lm",
             se = F,
             size = 1.6,
             alpha = 1)+
   theme_classic() +
 xlab("Osmolality") +
 ylab("CEWL") +
 theme(text = element_text(color = "black",
                           family = "sans",
                           size = 12),
       axis.text = element_text(color = "black",
                                family = "sans",
                                size = 10),
       legend.text.align = 0,
)+
 facet_wrap(~ region) # create a facet for each body region
```

- ## Warning: Removed 49 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 49 rows containing missing values (geom_point).



$CEWL \sim Hematocrit$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = hematocrit_percent,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = hematocrit_percent,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Hematocrit") +
  ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
```

```
size = 12),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0,
## Warning: Removed 119 rows containing non-finite values (stat_smooth).
## Warning: Removed 119 rows containing missing values (geom_point).
  100 -
    75
                                                                                region
                                                                                   dewl
                                                                                    dors
    50
                                                                                    head
                                                                                    mite
                                                                                    vent
    25
     0
               20
                                30
                                                 40
                                                                 50
                                    Hematocrit
# lm
CEWL_lm3 <- lm(TEWL_g_m2h ~ region * hematocrit_percent,</pre>
           data = CEWL_data_full)
summary(CEWL_lm3)
##
## lm(formula = TEWL_g_m2h ~ region * hematocrit_percent, data = CEWL_data_full)
##
## Residuals:
##
       Min
                1Q Median
                                ЗQ
                                        Max
## -26.008 -8.565 -2.311
                             5.873 67.969
##
## Coefficients:
##
                                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                  19.06834
                                              7.02497
                                                        2.714 0.00684 **
## regiondors
                                  4.28061
                                              9.92716
                                                       0.431 0.66648
```

```
## regionhead
                    18.41959
                           9.92310 1.856 0.06394 .
## regionmite
                   23.45205 9.94608 2.358 0.01871 *
## regionvent
                   13.34360 9.91989 1.345 0.17912
                    0.08656 0.19541 0.443 0.65795
## hematocrit_percent
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.6 on 570 degrees of freedom
   (119 observations deleted due to missingness)
## Multiple R-squared: 0.1063, Adjusted R-squared: 0.09216
## F-statistic: 7.531 on 9 and 570 DF, p-value: 1.86e-10
```

CEWL ~ Cloacal Temperature

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = cloacal_temp_C,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.4) +
  stat_smooth(aes(x = cloacal_temp_C,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y \sim x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Cloacal Temperature (°C)") +
  ylab(bquote('CEWL (g / '*m^2~h*')')) +
  \#annotate("text", x = 1, y = 65, label = "a", size = 6) +
  scale_color_brewer(palette = "Set2",
                     labels = c("Dewlap", "Dorsum", "Head",
                              "Mite Patch", "Ventrum"),
                     name = "") +
  ylim(1, 100) +
  xlim(20, 28) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
        axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 14),
        legend.text = element_text(color = "black",
                                 family = "sans",
                                  size = 12),
```

```
legend.text.align = 0,
        legend.position = c(0.9, 0.85)
  #guides(color = guide_legend(nrow = 2, byrow = TRUE)
         ) -> CEWL_ctemp_fig
CEWL_ctemp_fig
## Warning: Removed 49 rows containing non-finite values (stat_smooth).
## Warning: Removed 49 rows containing missing values (geom_point).
     100
                                                                             Dewlap
                                                                             Dorsum
                                                                             Head
                                                                             Mite Patch
       75
CEWL (g / m^2 h)
                                                                             Ventrum
      50
      25
        0
             20
                              22
                                                24
                                                                                   28
                              Cloacal Temperature (°C)
# export figure
#ggsave(filename = "CEWL_ctemp_fig.tiff",
       plot = CEWL_ctemp_fig,
       path = "./final_figures",
        device = "tiff",
        dpi = 1200,
     # width = 6, height = 4)
CEWL_lm4 <- lm(TEWL_g_m2h ~ region + cloacal_temp_C,</pre>
          data = CEWL_data_full)
summary(CEWL_lm4)
##
## lm(formula = TEWL_g_m2h ~ region + cloacal_temp_C, data = CEWL_data_full)
## Residuals:
```

```
1Q Median
                              3Q
                                    Max
## -34.079 -7.557 -1.903 5.192 65.103
##
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
                -29.0691 5.8706 -4.952 9.41e-07 ***
## (Intercept)
                 0.7740 1.4630 0.529
## regiondors
                                              0.597
                           1.4630 4.480 8.84e-06 ***
## regionhead
                  6.5538
                                    4.436 1.08e-05 ***
## regionmite
                  6.5272
                            1.4714
## regionvent
                  10.9708
                           1.4630 7.499 2.14e-13 ***
## cloacal_temp_C 2.1439
                             0.2471 8.675 < 2e-16 ***
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 11.79 on 644 degrees of freedom
    (49 observations deleted due to missingness)
## Multiple R-squared: 0.1925, Adjusted R-squared: 0.1862
## F-statistic: 30.71 on 5 and 644 DF, p-value: < 2.2e-16
```

CEWL ~ Capture Temperature

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = temp_C_interpol,
                 y = TEWL_g_m2h,
                 color = region
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                  "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = temp_C_interpol,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Ambient Temperature at Capture") +
  ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 10),
        legend.text.align = 0,
```

Warning: Removed 59 rows containing non-finite values (stat_smooth).

Warning: Removed 59 rows containing missing values (geom_point).

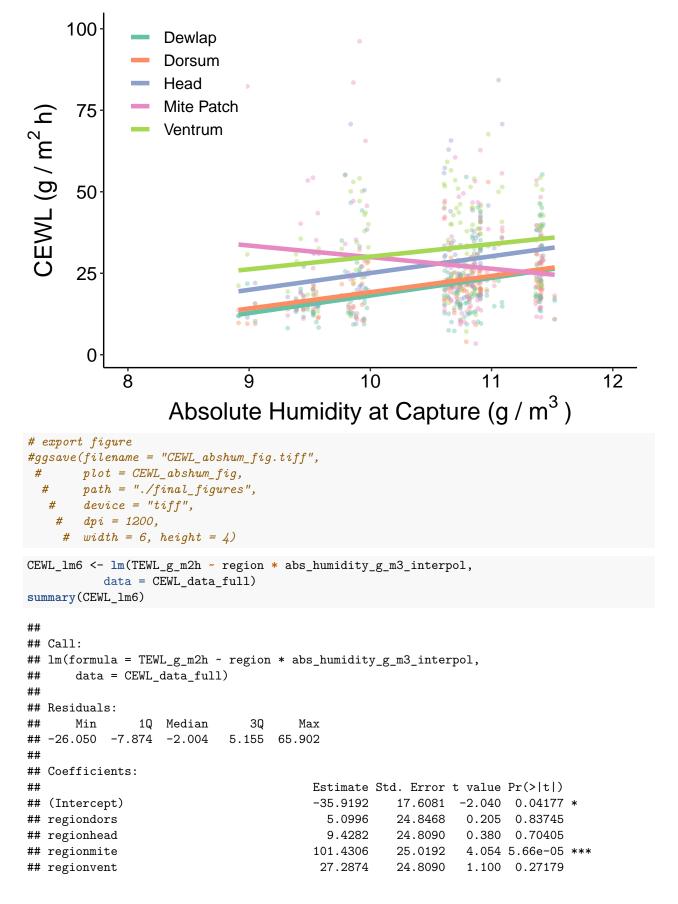
```
100 -
    75
                                                                                 region
                                                                                     dewl
                                                                                     dors
    50
                                                                                     head
                                                                                     mite
                                                                                     vent
    25
     0
                                                                            <u>.</u>24
            16
                            18
                                            20
                                                            22
                         Ambient Temperature at Capture
# lm: CEWL ~ region + capture temp
CEWL_lm5 <- lm(TEWL_g_m2h ~ region * temp_C_interpol,</pre>
           data = CEWL_data_full)
summary(CEWL_lm5)
##
## Call:
  lm(formula = TEWL_g_m2h ~ region * temp_C_interpol, data = CEWL_data_full)
##
## Residuals:
                                 3Q
##
       Min
                1Q Median
                                        Max
           -8.223
                   -1.995
                              5.634
                                     66.793
##
## Coefficients:
##
                               Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                               -11.4129
                                            8.0818
                                                    -1.412 0.158394
## regiondors
                                39.3213
                                           11.3626
                                                      3.461 0.000575 ***
## regionhead
                                35.8369
                                           11.4268
                                                      3.136 0.001791 **
## regionmite
                                15.4923
                                           11.3692
                                                      1.363 0.173478
## regionvent
                                27.6451
                                           11.4268
                                                      2.419 0.015831 *
## temp_C_interpol
                                 1.7418
                                            0.4270
                                                      4.079 5.11e-05 ***
## regiondors:temp_C_interpol
                               -2.0550
                                            0.5997
                                                    -3.427 0.000650 ***
## regionhead:temp_C_interpol
                                -1.5535
                                            0.6036
                                                     -2.574 0.010281 *
## regionmite:temp_C_interpol
                                -0.4746
                                            0.6004
                                                    -0.791 0.429509
## regionvent:temp_C_interpol
                                -0.8876
                                            0.6036
                                                    -1.471 0.141900
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 12.22 on 630 degrees of freedom
## (59 observations deleted due to missingness)
## Multiple R-squared: 0.1406, Adjusted R-squared: 0.1283
## F-statistic: 11.45 on 9 and 630 DF, p-value: < 2.2e-16</pre>
```

CEWL ~ Capture Humidity

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_jitter(aes(x = abs_humidity_g_m3_interpol,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.4) +
  stat_smooth(aes(x = abs_humidity_g_m3_interpol,
                 y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab('Absolute Humidity at Capture (g / '*m^3~')') +
  ylab(bquote('CEWL (g / '*m^2~h*')')) +
  \#annotate("text", x = 1, y = 65, label = "a", size = 6) +
  scale_color_brewer(palette = "Set2",
                     labels = c("Dewlap", "Dorsum",
                              "Head", "Mite Patch", "Ventrum"),
                     name = "") +
  ylim(1, 100) +
  xlim(8, 12) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 14),
        legend.text = element_text(color = "black",
                                 family = "sans",
                                 size = 12),
        legend.text.align = 0,
        legend.position = c(0.15, 0.85)
        ) -> CEWL_abshum_fig
CEWL_abshum_fig
```

- ## Warning: Removed 59 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 59 rows containing missing values (geom_point).



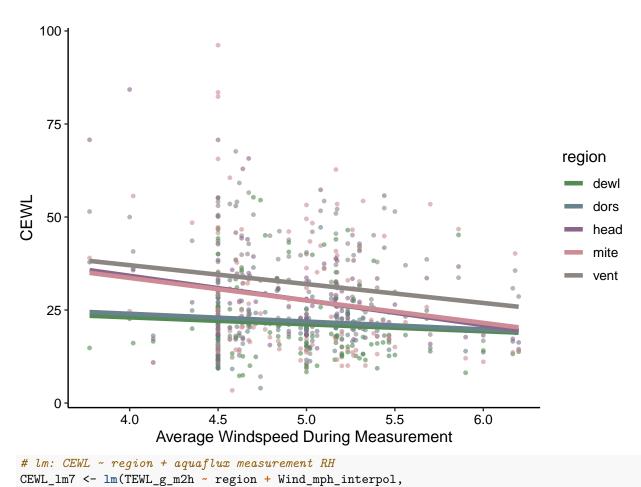
```
## abs_humidity_g_m3_interpol
                                         5.4090
                                                   1.6627 3.253 0.00120 **
## regiondors:abs_humidity_g_m3_interpol -0.4091
                                                   2.3465 -0.174 0.86167
## regionhead:abs_humidity_g_m3_interpol -0.2532
                                                   2.3438 -0.108 0.91399
## regionmite:abs_humidity_g_m3_interpol -8.9659
                                                   2.3611 -3.797 0.00016 ***
## regionvent:abs_humidity_g_m3_interpol -1.5364
                                                   2.3438 -0.656 0.51237
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.14 on 630 degrees of freedom
    (59 observations deleted due to missingness)
## Multiple R-squared: 0.1522, Adjusted R-squared: 0.1401
## F-statistic: 12.57 on 9 and 630 DF, p-value: < 2.2e-16
```

$CEWL \sim Wind Speed$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = Wind_mph_interpol,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = Wind_mph_interpol,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme classic() +
  xlab("Average Windspeed During Measurement") +
  vlab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```

Warning: Removed 59 rows containing non-finite values (stat_smooth).

Warning: Removed 59 rows containing missing values (geom_point).



```
data = CEWL_data_full)
summary(CEWL_lm7)
##
## Call:
  lm(formula = TEWL_g_m2h ~ region + Wind_mph_interpol, data = CEWL_data_full)
##
##
  Residuals:
##
       Min
                1Q Median
                                3Q
                                       Max
           -8.470
                    -2.166
                             5.884
                                    66.328
##
## Coefficients:
##
                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                      42.7246
                                  5.5870
                                           7.647 7.65e-14 ***
## regiondors
                       0.7384
                                  1.5405
                                           0.479
                                                     0.632
## regionhead
                       6.6732
                                  1.5405
                                           4.332 1.72e-05 ***
## regionmite
                       6.6311
                                  1.5495
                                           4.279 2.16e-05 ***
## regionvent
                      10.9815
                                  1.5405
                                           7.129 2.77e-12 ***
## Wind_mph_interpol
                                          -3.919 9.87e-05 ***
                      -4.3387
                                  1.1071
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.32 on 634 degrees of freedom
     (59 observations deleted due to missingness)
```

Multiple R-squared: 0.1204, Adjusted R-squared: 0.1135

```
## F-statistic: 17.36 on 5 and 634 DF, p-value: 3.977e-16
```

$CEWL \sim Solar\ Rad$

```
CEWL_data_full %>%
  ggplot(data = .) +
 geom_point(aes(x = Solar_rad_Wm2_interpol,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = Solar_rad_Wm2_interpol,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
 theme_classic() +
 xlab("Solar Radiation W/m^2") +
 ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
       axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```

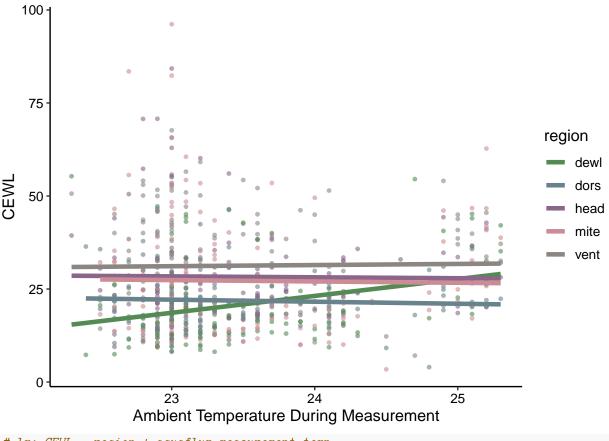
- ## Warning: Removed 59 rows containing non-finite values (stat_smooth).
- ## Warning: Removed 59 rows containing missing values (geom_point).

```
100 -
    75
                                                                                         region
                                                                                              dewl
                                                                                              dors
    50
                                                                                              head
                                                                                              mite
                                                                                              vent
    25
     0
                           700
                                           800
           600
                                                          900
                                                                          1000
                                 Solar Radiation W/m^2
# lm: CEWL ~ region + aquaflux measurement RH
 \label{eq:cewl_lm8} $$ \leftarrow \lim(\text{TEWL\_g\_m2h - region + Solar\_rad\_Wm2\_interpol,} 
            data = CEWL_data_full)
summary(CEWL_lm8)
##
## Call:
  lm(formula = TEWL_g_m2h ~ region + Solar_rad_Wm2_interpol, data = CEWL_data_full)
##
```

```
##
  Residuals:
##
      Min
                1Q Median
                                3Q
                                       Max
           -8.072 -2.283
                             5.790
                                    70.440
##
## Coefficients:
##
                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                           8.829372
                                      3.005574
                                                 2.938 0.00343 **
## regiondors
                                      1.535357
                                                 0.500 0.61760
                           0.766911
## regionhead
                           6.735880
                                      1.535375
                                                 4.387 1.35e-05 ***
## regionmite
                           6.564058
                                      1.544438
                                                 4.250 2.46e-05 ***
## regionvent
                          11.044136
                                      1.535375
                                                 7.193 1.79e-12 ***
## Solar_rad_Wm2_interpol 0.013920
                                      0.003138
                                                 4.436 1.08e-05 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.28 on 634 degrees of freedom
##
     (59 observations deleted due to missingness)
## Multiple R-squared: 0.1262, Adjusted R-squared: 0.1194
```

$CEWL \sim Measurement\ Temperature$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = ambient_temp_C,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = ambient_temp_C,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Ambient Temperature During Measurement") +
  ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
        legend.text.align = 0,
```



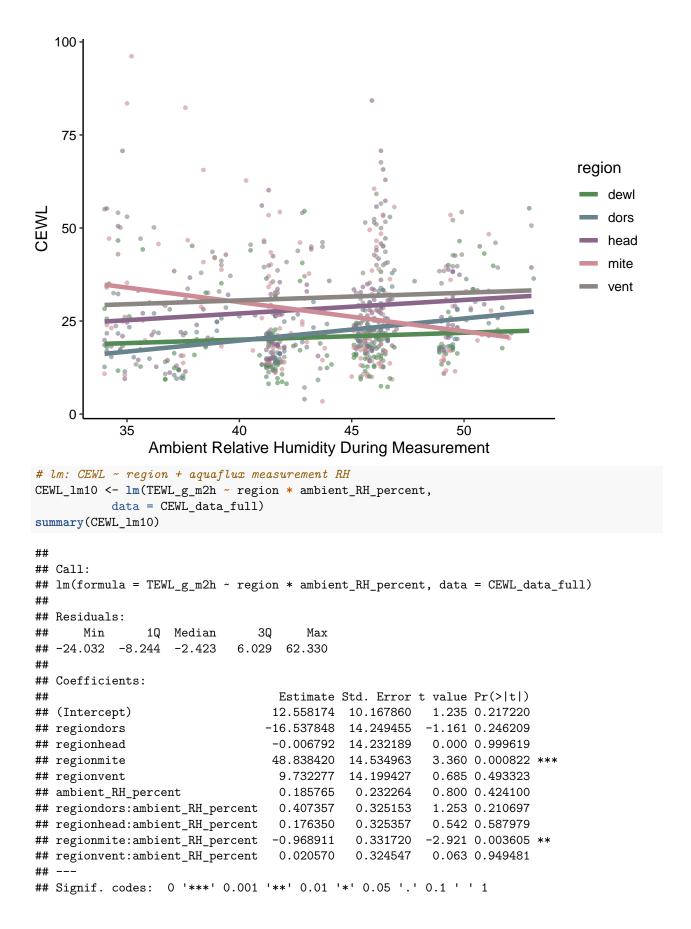
```
## Call:
  lm(formula = TEWL_g_m2h ~ region * ambient_temp_C, data = CEWL_data_full)
##
##
  Residuals:
##
                                 3Q
       Min
                1Q Median
                                        Max
           -8.438
                    -2.262
                              5.727
                                     68.764
##
## Coefficients:
##
                              Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                               -85.690
                                           35.629
                                                   -2.405
                                                           0.01643 *
## regiondors
                               120.409
                                           49.906
                                                     2.413
                                                            0.01609 *
## regionhead
                               119.786
                                           49.830
                                                     2.404
                                                            0.01648 *
## regionmite
                               121.357
                                           49.735
                                                     2.440
                                                            0.01493 *
## regionvent
                               109.812
                                           49.651
                                                     2.212
                                                            0.02732 *
## ambient_temp_C
                                 4.535
                                            1.519
                                                     2.986
                                                            0.00293 **
## regiondors:ambient_temp_C
                                -5.082
                                            2.128
                                                    -2.388
                                                            0.01721 *
## regionhead:ambient_temp_C
                                -4.783
                                            2.125
                                                    -2.250
                                                            0.02474 *
                                                    -2.310
## regionmite:ambient_temp_C
                                -4.894
                                            2.119
                                                            0.02120 *
## regionvent:ambient_temp_C
                                -4.230
                                            2.117
                                                   -1.998
                                                            0.04614 *
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 12.36 on 689 degrees of freedom
## Multiple R-squared: 0.1069, Adjusted R-squared: 0.09525
## F-statistic: 9.165 on 9 and 689 DF, p-value: 3.517e-13
```

CEWL ~ Measurement Humidity

Very interesting relationship! Mite patch CEWL decreases as ambient humidity increases, but every other location appears to increase. In this case, an interaction term is warranted.

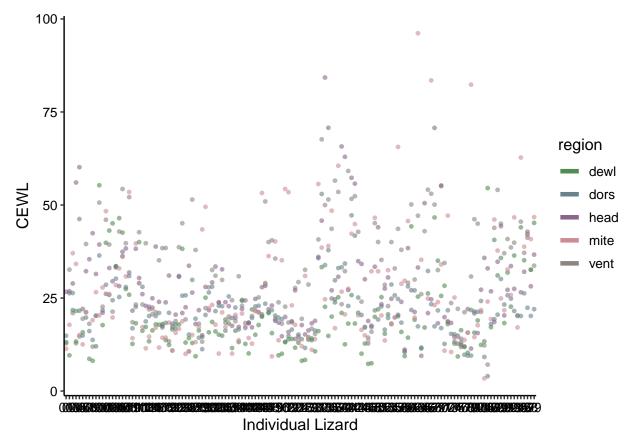
```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = ambient_RH_percent,
                 y = TEWL_g_m2h,
                 color = region
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = ambient_RH_percent,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme classic() +
  xlab("Ambient Relative Humidity During Measurement") +
  ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
       legend.text.align = 0,
```



```
##
## Residual standard error: 12.25 on 689 degrees of freedom
## Multiple R-squared: 0.1227, Adjusted R-squared: 0.1112
## F-statistic: 10.7 on 9 and 689 DF, p-value: 1.218e-15
```

$CEWL \sim Individual$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = individual_ID,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                 "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = individual_ID,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Individual Lizard") +
 ylab("CEWL") +
  # just to get a better look
  # ylim(5, 40) +
 theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
       axis.text = element_text(color = "black",
                                 family = "sans",
                                 size = 10),
       legend.text.align = 0,
```



```
## Call:
  lm(formula = TEWL_g_m2h ~ region + individual_ID, data = CEWL_data_full)
##
##
  Residuals:
##
       Min
                1Q Median
                                 3Q
                                        Max
           -5.066
                    -0.605
                              3.882
                                     50.951
##
## Coefficients:
##
                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                     10.6186
                                  4.6593
                                           2.279 0.023047 *
## regiondors
                       1.2460
                                  1.2314
                                           1.012 0.312063
## regionhead
                      7.5112
                                  1.2299
                                           6.107 1.91e-09 ***
## regionmite
                      6.5149
                                  1.2421
                                           5.245 2.23e-07 ***
## regionvent
                     10.5050
                                  1.2312
                                           8.532 < 2e-16 ***
## individual_ID02
                      6.8620
                                  6.4959
                                           1.056 0.291264
## individual_ID03
                     11.2260
                                  6.4959
                                           1.728 0.084516 .
## individual ID04
                                  6.4959
                     11.8020
                                           1.817 0.069782 .
## individual_ID05
                     15.7600
                                  6.4959
                                           2.426 0.015579 *
## individual_ID06
                      1.1740
                                  6.4959
                                           0.181 0.856646
## individual_ID07
                     10.9940
                                  6.4959
                                           1.692 0.091122 .
## individual_ID08
                                  6.8927
                                           0.600 0.548729
                      4.1359
```

```
## individual_ID09
                       3.2900
                                            0.506 0.612725
                                  6.4959
                       6.3380
                                  6.4959
## individual_ID10
                                            0.976 0.329642
## individual ID100
                      24.6420
                                  6.4959
                                            3.793 0.000165 ***
## individual_ID101
                       6.4260
                                  6.4959
                                            0.989 0.322977
## individual_ID102
                      22.0060
                                  6.4959
                                            3.388 0.000755 ***
## individual ID103
                      17.7180
                                  6.4959
                                            2.728 0.006583 **
## individual ID104
                      12.2440
                                  6.4959
                                            1.885 0.059969
## individual_ID105
                      16.0240
                                  6.4959
                                            2.467 0.013935 *
## individual_ID106
                      19.3860
                                  6.4959
                                            2.984 0.002967 **
## individual_ID107
                      25.5560
                                  6.4959
                                            3.934 9.41e-05 ***
## individual_ID108
                      17.0740
                                  6.4959
                                            2.628 0.008816 **
## individual_ID109
                      23.0830
                                  6.4959
                                            3.553 0.000413 ***
## individual_ID11
                       0.9960
                                  6.4959
                                            0.153 0.878196
## individual_ID110
                      12.6240
                                  6.4959
                                            1.943 0.052477 .
## individual_ID111
                      18.4400
                                  6.4959
                                            2.839 0.004696 **
## individual_ID112
                       6.3660
                                  6.4959
                                            0.980 0.327512
## individual_ID113
                       6.2300
                                  6.4959
                                            0.959 0.337943
## individual ID114
                       2.7780
                                  6.4959
                                            0.428 0.669068
## individual_ID115
                       5.4820
                                            0.844 0.399079
                                  6.4959
## individual_ID116
                      7.2460
                                  6.4959
                                            1.115 0.265131
## individual_ID117
                       6.1460
                                  6.4959
                                            0.946 0.344491
## individual ID118
                       5.2920
                                  6.4959
                                            0.815 0.415612
## individual_ID119
                       5.9640
                                  6.4959
                                            0.918 0.358956
## individual ID12
                       3.4740
                                  6.4959
                                            0.535 0.593003
## individual ID120
                      -1.0340
                                  6.4959
                                           -0.159 0.873587
## individual_ID122
                       2.1560
                                  6.4959
                                            0.332 0.740088
## individual_ID123
                      11.2120
                                  6.4959
                                            1.726 0.084903
## individual_ID124
                      13.9580
                                  6.4959
                                            2.149 0.032088 *
## individual_ID125
                       2.3280
                                  6.4959
                                            0.358 0.720195
## individual_ID126
                       1.1020
                                  6.4959
                                            0.170 0.865351
## individual_ID127
                      11.3820
                                  6.4959
                                            1.752 0.080296 .
## individual_ID128
                       3.7980
                                  6.4959
                                            0.585 0.559002
## individual_ID129
                       1.7460
                                  6.4959
                                            0.269 0.788195
## individual_ID13
                       3.7280
                                  6.4959
                                            0.574 0.566267
## individual_ID130
                      13.2940
                                  6.4959
                                            2.047 0.041177 *
## individual_ID131
                      7.0620
                                  6.4959
                                            1.087 0.277444
## individual ID132
                      10.5240
                                  6.4959
                                            1.620 0.105780
## individual_ID133
                      8.1840
                                  6.4959
                                            1.260 0.208246
## individual_ID134
                       3.3780
                                  6.4959
                                            0.520 0.603256
## individual_ID135
                                  6.4959
                                            1.309 0.191240
                       8.5000
## individual ID136
                       3.2040
                                  6.4959
                                            0.493 0.622042
## individual_ID137
                       5.3940
                                  6.4959
                                            0.830 0.406687
## individual_ID138
                       0.8740
                                  6.4959
                                            0.135 0.893019
## individual_ID139
                       3.7500
                                  6.4959
                                            0.577 0.563979
## individual_ID14
                       2.5180
                                  6.4959
                                            0.388 0.698439
## individual_ID140
                       7.1280
                                  6.4959
                                            1.097 0.272983
                                            0.332 0.739856
## individual_ID141
                       2.1580
                                  6.4959
## individual_ID142
                       0.0960
                                  6.4959
                                            0.015 0.988214
## individual_ID143
                       2.5460
                                  6.4959
                                            0.392 0.695253
## individual_ID144
                       5.4120
                                  6.4959
                                            0.833 0.405123
## individual_ID145
                       5.8540
                                  6.4959
                                            0.901 0.367881
## individual_ID146
                      12.6000
                                  6.4959
                                            1.940 0.052925 .
## individual_ID147
                      16.3360
                                  6.4959
                                            2.515 0.012192 *
## individual ID148
                      12.3080
                                  6.4959
                                            1.895 0.058648 .
```

```
## individual_ID149
                       6.2480
                                            0.962 0.336550
                                   6.4959
## individual_ID15
                                   6.4959
                      11.0700
                                            1.704 0.088913 .
## individual ID150
                       1.2660
                                   6.4959
                                            0.195 0.845548
## individual_ID16
                       3.6880
                                   6.4959
                                            0.568 0.570439
## individual_ID17
                       8.8720
                                   6.4959
                                            1.366 0.172560
## individual ID18
                                   6.4959
                       7.5120
                                            1.156 0.248007
## individual ID19
                       2.3800
                                   6.4959
                                            0.366 0.714217
## individual_ID20
                       3.8700
                                   6.4959
                                            0.596 0.551578
## individual_ID21
                       1.3540
                                   6.4959
                                            0.208 0.834962
## individual_ID24
                      -1.5180
                                   6.4959
                                           -0.234 0.815315
## individual_ID26
                       3.9620
                                   6.4959
                                            0.610 0.542162
## individual_ID27
                       1.6940
                                   6.4959
                                            0.261 0.794359
## individual_ID29
                                   6.4959
                      -1.0100
                                           -0.155 0.876497
                                           -0.117 0.906661
## individual_ID30
                      -0.7620
                                   6.4959
## individual_ID31
                      21.0780
                                   6.4959
                                            3.245 0.001246 **
## individual_ID32
                      31.8780
                                   6.4959
                                            4.907 1.22e-06 ***
## individual_ID33
                                   6.4959
                                            4.204 3.06e-05 ***
                      27.3060
## individual ID34
                      27.0060
                                   6.4959
                                            4.157 3.73e-05 ***
## individual_ID35
                      17.0280
                                            2.621 0.008999 **
                                   6.4959
## individual_ID36
                      14.5640
                                   6.4959
                                            2.242 0.025355 *
## individual_ID37
                      24.8320
                                   6.4959
                                            3.823 0.000147 ***
## individual ID38
                      18.2340
                                   6.4959
                                            2.807 0.005177 **
## individual_ID39
                      16.7400
                                   6.4959
                                            2.577 0.010224 *
## individual ID40
                      15.1860
                                   6.4959
                                            2.338 0.019754 *
## individual ID41
                      29.0760
                                   6.4959
                                            4.476 9.24e-06 ***
## individual_ID42
                      26.8000
                                   6.4959
                                            4.126 4.27e-05 ***
## individual_ID43
                      13.0740
                                   6.4959
                                            2.013 0.044635
## individual_ID44
                       5.2440
                                   6.4959
                                            0.807 0.419852
## individual_ID45
                       2.6480
                                   6.4959
                                            0.408 0.683694
## individual_ID46
                      -2.3720
                                           -0.365 0.715136
                                   6.4959
## individual_ID47
                       2.3020
                                   6.4959
                                            0.354 0.723190
## individual_ID48
                      17.5700
                                   6.4959
                                            2.705 0.007045 **
## individual_ID50
                      15.7140
                                   6.4959
                                            2.419 0.015882
## individual_ID51
                       9.6060
                                            1.479 0.139768
                                   6.4959
## individual_ID52
                       4.5420
                                   6.4959
                                            0.699 0.484713
## individual_ID53
                       9.4440
                                   6.4959
                                            1.454 0.146557
## individual ID54
                      10.1540
                                   6.4959
                                            1.563 0.118591
## individual_ID55
                                  7.5076
                       9.1074
                                            1.213 0.225613
## individual_ID57
                      19.1340
                                   6.4959
                                            2.946 0.003359 **
## individual_ID58
                                   6.4959
                                            1.421 0.155820
                       9.2320
## individual ID59
                      -1.4960
                                   6.4959
                                           -0.230 0.817944
## individual_ID60
                      15.3640
                                   6.4959
                                            2.365 0.018365
## individual_ID61
                      20.3884
                                   6.8927
                                            2.958 0.003229 **
## individual_ID62
                       7.6660
                                   6.4959
                                            1.180 0.238455
## individual_ID63
                      28.0760
                                   6.4959
                                            4.322 1.83e-05 ***
## individual_ID64
                       3.0020
                                   6.4959
                                            0.462 0.644163
## individual_ID65
                      20.2227
                                  7.5076
                                            2.694 0.007282 **
## individual_ID66
                      15.9060
                                   6.4959
                                            2.449 0.014650 *
## individual_ID67
                      29.0647
                                   6.8927
                                            4.217 2.90e-05 ***
## individual_ID68
                      25.8240
                                   6.4959
                                            3.975 7.96e-05 ***
## individual_ID69
                                   6.4959
                      10.4900
                                            1.615 0.106909
## individual_ID70
                      21.5900
                                   6.4959
                                            3.324 0.000947 ***
## individual_ID71
                       5.4480
                                   6.4959
                                            0.839 0.402008
## individual ID72
                       9.0560
                                   6.4959
                                            1.394 0.163843
```

```
## individual_ID73
                     -0.1003
                                 6.8927 -0.015 0.988390
                                        0.067 0.946511
## individual_ID74
                     0.4360
                                 6.4959
## individual ID75
                     -1.0780
                                 6.4959 -0.166 0.868256
## individual_ID76
                     -0.9610
                                 6.5006 -0.148 0.882526
## individual_ID77
                     -2.0700
                                 6.4959 -0.319 0.750102
                                 6.4959 -0.174 0.861963
## individual ID78
                    -1.1300
## individual ID79
                     15.3960
                                 6.4959
                                         2.370 0.018125 *
## individual ID81
                     7.9560
                                 6.4959
                                          1.225 0.221181
## individual ID82
                     7.9280
                                 6.4959
                                          1.220 0.222808
## individual_ID83
                     5.6060
                                 6.4959
                                          0.863 0.388507
## individual_ID84
                    -0.9820
                                 6.4959
                                         -0.151 0.879895
## individual_ID85
                     8.7580
                                 6.4959
                                          1.348 0.178133
## individual_ID86
                     5.8934
                                 6.8926
                                         0.855 0.392907
                     17.9160
## individual_ID87
                                 6.4959
                                          2.758 0.006007 **
## individual_ID88
                                 6.4959
                                          3.057 0.002341 **
                     19.8600
## individual_ID89
                     20.2420
                                 6.4959
                                          3.116 0.001928 **
## individual_ID90
                     8.3440
                                 6.4959
                                          1.285 0.199503
## individual ID91
                     15.8880
                                 6.4959
                                          2.446 0.014761 *
## individual_ID92
                     13.3280
                                 6.4959
                                          2.052 0.040663 *
## individual_ID93
                     18.6180
                                 6.4959
                                          2.866 0.004313 **
## individual_ID94
                     2.5028
                                 7.5076
                                         0.333 0.738981
## individual ID95
                     24.0200
                                 6.4959
                                          3.698 0.000239 ***
## individual_ID96
                     18.1100
                                 6.4959
                                          2.788 0.005487 **
## individual ID97
                                 6.4959
                     22.3080
                                          3.434 0.000639 ***
## individual ID98
                     15.1060
                                 6.4959
                                          2.325 0.020407 *
## individual_ID99
                     21.0840
                                 6.4959
                                          3.246 0.001242 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 10.27 on 553 degrees of freedom
## Multiple R-squared: 0.5047, Adjusted R-squared: 0.3749
## F-statistic: 3.887 on 145 and 553 DF, p-value: < 2.2e-16
```

$CEWL \sim SVL$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = SVL_mm,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                  "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = SVL_mm,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y \sim x,
              method = "lm",
              se = F,
              size = 1.6.
              alpha = 1) +
```

```
theme_classic() +
  xlab("SVL") +
  ylab("CEWL") +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 12),
        axis.text = element_text(color = "black",
                                 family = "sans",
                                  size = 10),
        legend.text.align = 0,
## Warning: Removed 44 rows containing non-finite values (stat_smooth).
## Warning: Removed 44 rows containing missing values (geom_point).
   100 -
    75
                                                                                region
                                                                                    dewl
                                                                                    dors
   50
                                                                                    head
                                                                                    mite
                                                                                    vent
    25
     0
                  50
                              55
                                          60
                                                     65
                                                                 70
      45
                                       SVL
# lm
CEWL_lm12 <- lm(TEWL_g_m2h ~ region + SVL_mm,</pre>
           data = CEWL_data_full)
summary(CEWL_lm12)
##
## Call:
## lm(formula = TEWL_g_m2h ~ region + SVL_mm, data = CEWL_data_full)
##
## Residuals:
       Min
                1Q Median
                                ЗQ
                                        Max
## -22.778 -8.230 -2.224 5.569 67.956
```

```
##
## Coefficients:
##
              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.8945 6.3736 0.297 0.76638
## regiondors
                                  0.547 0.58465
               0.8336
                          1.5243
## regionhead
                       1.5244 4.405 1.24e-05 ***
               6.7145
## regionmite
              6.5225
                         1.5331 4.255 2.40e-05 ***
                         1.5243 7.205 1.61e-12 ***
## regionvent
               10.9831
                          0.0954 3.050 0.00238 **
## SVL_mm
                0.2910
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.34 on 649 degrees of freedom
    (44 observations deleted due to missingness)
## Multiple R-squared: 0.1109, Adjusted R-squared: 0.1041
## F-statistic: 16.2 on 5 and 649 DF, p-value: 4.529e-15
```

$CEWL \sim SMI$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = SMI,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1.
             alpha = 0.4) +
  #scale_colour_manual(values = c("palegreen4", "lightblue4",
                                   "plum4", "lightpink3", "seashell4")) +
  stat smooth(aes(x = SMI,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y ~ x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Scaled Mass Index (g)") +
  ylab(bquote('CEWL (g / '*m^2-h*')')) +
  \#annotate("text", x = 1, y = 65, label = "a", size = 6) +
  scale_color_brewer(palette = "Set2",
                     labels = c("Dewlap", "Dorsum",
                              "Head", "Mite Patch", "Ventrum"),
                     name = "") +
  ylim(1, 100) +
 xlim(2, 16) +
  scale_x_continuous(breaks = c(seq(2, 16, by = 2))) +
  theme(text = element_text(color = "black",
                            family = "sans",
                            size = 18),
        axis.text = element_text(color = "black",
                                 family = "sans",
```

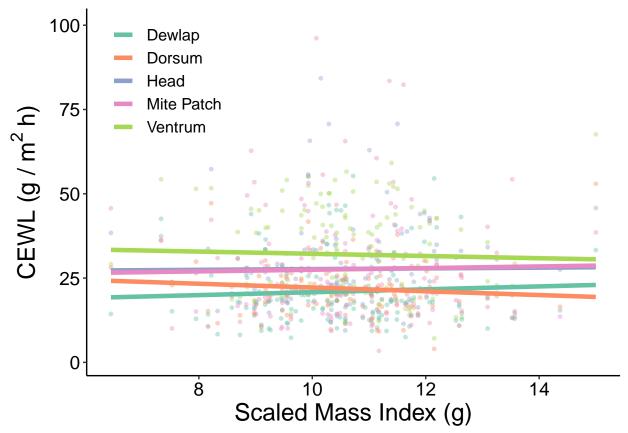
```
size = 14),
        legend.text = element_text(color = "black",
                                 family = "sans",
                                 size = 12),
        legend.text.align = 0,
        legend.position = c(0.15, 0.85)
         ) -> CEWL_SMI_fig
## Scale for 'x' is already present. Adding another scale for 'x', which will
```

replace the existing scale.

```
CEWL_SMI_fig
```

Warning: Removed 44 rows containing non-finite values (stat_smooth).

Warning: Removed 44 rows containing missing values (geom_point).



```
# export figure
#qgsave(filename = "CEWL_mass_fig.tiff",
        plot = CEWL_mass_fig,
        path = "./final_figures",
        device = "tiff",
        dpi = 1200,
        width = 6, height = 4)
CEWL_lm13 <- lm(TEWL_g_m2h ~ region + SMI,</pre>
```

```
data = CEWL_data_full)
summary(CEWL_lm13)
##
## Call:
## lm(formula = TEWL_g_m2h ~ region + SMI, data = CEWL_data_full)
##
## Residuals:
##
      Min
                               3Q
               1Q Median
                                      Max
## -24.867 -8.436 -2.543
                            5.906 68.539
##
## Coefficients:
              Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) 21.27550
                          3.85593
                                   5.518 4.97e-08 ***
## regiondors
              0.82970
                          1.53515
                                    0.540
                                             0.589
## regionhead
               6.66447
                          1.53515
                                    4.341 1.64e-05 ***
## regionmite
               6.55811
                          1.54398
                                    4.248 2.48e-05 ***
## regionvent 10.95754
                                    7.138 2.55e-12 ***
                          1.53515
## SMI
              -0.02108
                          0.34976 -0.060
                                             0.952
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.42 on 649 degrees of freedom
    (44 observations deleted due to missingness)
## Multiple R-squared: 0.09821,
                                   Adjusted R-squared: 0.09126
## F-statistic: 14.14 on 5 and 649 DF, p-value: 3.836e-13
```

CEWL ~ Mass

Head has an opposite trend from all the other body regions, so we need an interaction term.

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = mass_g,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.4) +
  #scale_colour_manual(values = c("palegreen4", "lightblue4",
                                   "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = mass_g,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y \sim x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Mass (g)") +
  ylab(bquote('CEWL (g / '*m^2~h*')')) +
  \#annotate("text", x = 1, y = 65, label = "a", size = 6) +
```

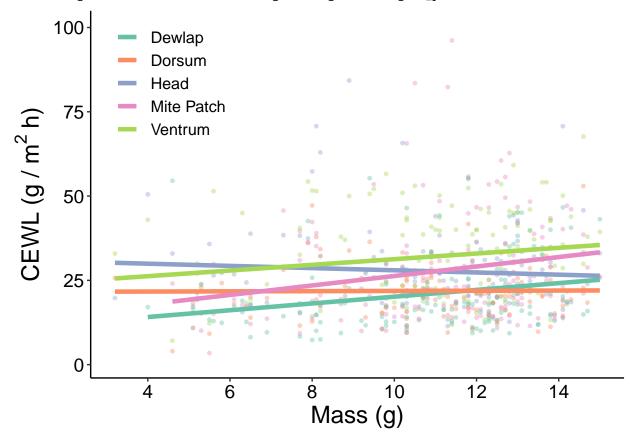
```
scale_color_brewer(palette = "Set2",
                   labels = c("Dewlap", "Dorsum",
                            "Head", "Mite Patch", "Ventrum"),
                   name = "") +
ylim(1, 100) +
xlim(2, 16) +
scale_x_continuous(breaks = c(seq(2, 16, by = 2))) +
theme(text = element_text(color = "black",
                          family = "sans",
                          size = 18),
      axis.text = element_text(color = "black",
                               family = "sans",
                               size = 14),
      legend.text = element_text(color = "black",
                               family = "sans",
                               size = 12),
      legend.text.align = 0,
      legend.position = c(0.15, 0.85)
       ) -> CEWL_mass_fig
```

Scale for 'x' is already present. Adding another scale for 'x', which will ## replace the existing scale.

CEWL_mass_fig

Warning: Removed 44 rows containing non-finite values (stat_smooth).

Warning: Removed 44 rows containing missing values (geom_point).



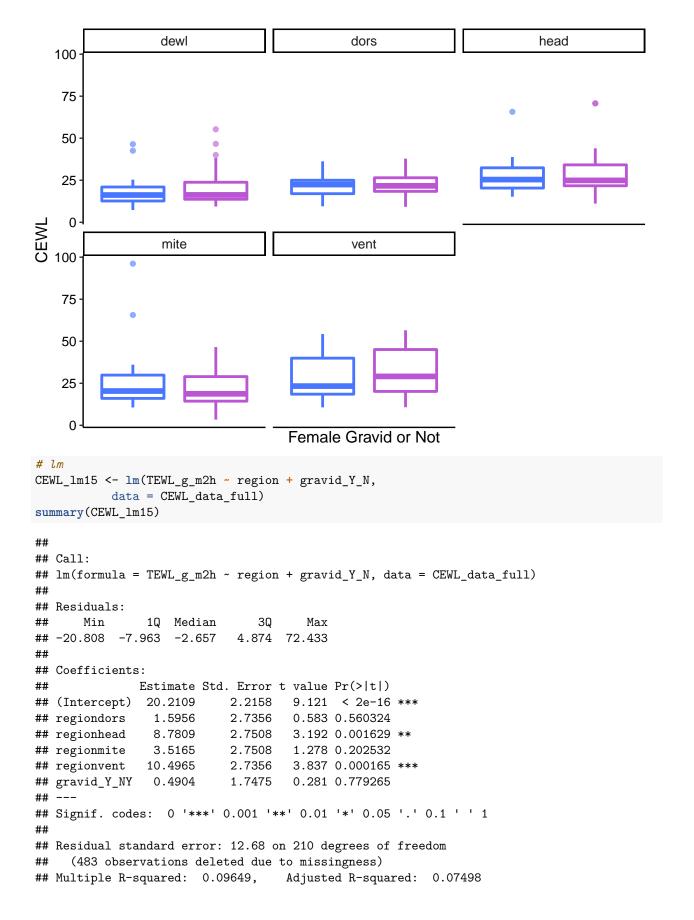
```
# export figure
#ggsave(filename = "CEWL_mass_fig.tiff",
        plot = CEWL_mass_fig,
        path = "./final_figures",
      device = "tiff",
    # dpi = 1200,
     # width = 6, height = 4)
# lm
CEWL_lm13.1 <- lm(TEWL_g_m2h ~ region*mass_g,
           data = CEWL_data_full)
summary(CEWL_lm13.1)
##
## Call:
## lm(formula = TEWL_g_m2h ~ region * mass_g, data = CEWL_data_full)
## Residuals:
##
       Min
                1Q Median
                                 3Q
## -22.116 -8.402 -2.377
                              6.152 67.902
##
## Coefficients:
##
                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                     10.0978 4.7889 2.109 0.03536 *
                     11.4597 6.7379 1.701 0.08947 .
21.1479 6.6404 3.185 0.00152 **
## regiondors
## regionhead
## regionmite
                      2.1432 6.8771 0.312 0.75541
## regionvent 12.7721 6.6883 1.910 0.05662 .
## mass_g 1.0055 0.4283 2.348 0.01920 *
## regiondors:mass_g -0.9757 0.6029 -1.618 0.10606
                                0.5954 -2.237 0.02565 *
## regionhead:mass_g -1.3316
                                            0.650 0.51571
## regionmite:mass_g 0.3995
                                   0.6142
## regionvent:mass_g -0.1646
                                   0.5986 -0.275 0.78336
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 12.27 on 645 degrees of freedom
     (44 observations deleted due to missingness)
## Multiple R-squared: 0.1258, Adjusted R-squared: 0.1136
## F-statistic: 10.31 on 9 and 645 DF, p-value: 5.958e-15
CEWL \sim Sex
CEWL data full %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = sex_M_F,
                   y = TEWL_g_m2h,
                    color = sex_M_F
                    ),
                size = 1,
                alpha = 0.6) +
  facet_wrap(~region) +
```

```
scale_color_manual(values = c("royalblue1", "mediumorchid")) +
  scale_x_discrete(breaks = c(1,2,3)) +
  theme_classic() +
  xlab("Sex") +
  ylab("CEWL") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
)
                  dewl
                                              dors
                                                                          head
   100
    75
    50
    25
O CEWL
     0
                   mite
                                              vent
    75
    50
    25
                                              Sex
CEWL_lm14 <- lm(TEWL_g_m2h ~ region + sex_M_F,</pre>
           data = CEWL_data_full)
summary(CEWL_lm14)
##
## Call:
## lm(formula = TEWL_g_m2h ~ region + sex_M_F, data = CEWL_data_full)
## Residuals:
       Min
                1Q Median
                                 3Q
                                        Max
## -25.238 -8.527 -2.446
                              6.048 69.226
##
## Coefficients:
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) 20.3658
                            1.2936 15.743 < 2e-16 ***
```

```
0.548
## regiondors
               0.8403
                          1.5340
                                           0.584
## regionhead
               6.6673
                          1.5340 4.346 1.61e-05 ***
## regionmite
                          1.5429
             6.5686
                                  4.257 2.37e-05 ***
                                   7.150 2.35e-12 ***
## regionvent 10.9678
                          1.5340
## sex_M_FM
               1.0146
                          1.0319
                                  0.983
                                           0.326
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.41 on 649 degrees of freedom
    (44 observations deleted due to missingness)
## Multiple R-squared: 0.09955,
                                  Adjusted R-squared: 0.09261
## F-statistic: 14.35 on 5 and 649 DF, p-value: 2.418e-13
```

CEWL ~ Gravidity

```
CEWL_data_full %>%
  dplyr::filter(sex_M_F == "F") %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = gravid_Y_N,
                   y = TEWL_g_m2h,
                   color = gravid_Y_N
                   ),
               size = 1,
               alpha = 0.6) +
  facet_wrap(~region) +
  scale_color_manual(values = c("royalblue1", "mediumorchid")) +
  scale_x_discrete(breaks = c(1,2,3)) +
  theme_classic() +
  xlab("Female Gravid or Not") +
 ylab("CEWL") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
       legend.text.align = 0,
       legend.position = "none"
```



```
## F-statistic: 4.486 on 5 and 210 DF, p-value: 0.000663
```

$CEWL \sim Week$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_boxplot(aes(x = as.factor(date),
                   y = TEWL_g_m2h,
                   color = as.factor(date)
                    ),
               size = 1,
               alpha = 0.6) +
  facet_wrap(~region) + # could not figure out how to change facet labels without changing underlying d
  scale_color_manual(values = c("royalblue1", "mediumorchid", "seagreen4",
                                 "royalblue1", "mediumorchid", "seagreen4")) +
  scale_x_discrete(breaks = c(1,2,3)) +
  theme_classic() +
  xlab("Date") +
  ylab("CEWL") +
  theme(text = element_text(color = "black", family = "sans", size = 12),
        axis.text = element_text(color = "black", family = "sans", size = 10),
        legend.text.align = 0,
        legend.position = "none"
                  dewl
                                               dors
                                                                          head
   100
    75
    50
    25
O CEWL
                   mite
                                               vent
    75
    50
    25
                                              Date
```

CEWL_lm16 <- $lm(TEWL_g_m2h \sim region + date,$

```
data = CEWL_data_full)
summary(CEWL_lm16)
##
## lm(formula = TEWL_g_m2h ~ region + date, data = CEWL_data_full)
##
## Residuals:
##
      Min
               1Q Median
                              3Q
                                     Max
## -24.299 -8.269 -2.413 5.549 69.013
##
## Coefficients:
                Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) -559.70526 607.60731 -0.921
## regiondors
                1.25433
                          1.48142
                                     0.847
                                              0.397
## regionhead
                 7.64676
                         1.48142
                                     5.162 3.20e-07 ***
## regionmite
                 6.57099
                         1.49214
                                     4.404 1.23e-05 ***
                         1.48142
                                    7.166 1.98e-12 ***
## regionvent
             10.61651
                           0.03241 0.955
## date
                 0.03096
                                             0.340
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 12.39 on 693 degrees of freedom
## Multiple R-squared: 0.09621,
                                  Adjusted R-squared: 0.08968
## F-statistic: 14.75 on 5 and 693 DF, p-value: 9.094e-14
```

$CEWL \sim holding \ time$

```
CEWL_data_full %>%
  ggplot(data = .) +
  geom_point(aes(x = hold_time,
                 y = TEWL_g_m2h,
                 color = region
                 ),
             size = 1,
             alpha = 0.6) +
  scale_colour_manual(values = c("palegreen4", "lightblue4",
                                  "plum4", "lightpink3", "seashell4")) +
  stat_smooth(aes(x = hold_time,
                  y = TEWL_g_m2h,
                  color = region
                  ),
              formula = y \sim x,
              method = "lm",
              se = F,
              size = 1.6,
              alpha = 1) +
  theme_classic() +
  xlab("Holding Time (minutes)") +
  ylab("CEWL (g/m^2/hr)") +
  theme(text = element_text(color = "black",
                             family = "sans",
                             size = 12),
```

```
axis.text = element_text(color = "black",
                                  family = "sans",
                                  size = 10),
        legend.text.align = 0,
## Warning: Removed 69 rows containing non-finite values (stat_smooth).
## Warning: Removed 69 rows containing missing values (geom_point).
   100 -
    75
                                                                                  region
CEWL (g/m^2/hr)
                                                                                      dewl
                                                                                       dors
    50
                                                                                       head
                                                                                       mite
                                                                                      vent
    25
      0
                              100
                                                        200
                               Holding Time (minutes)
CEWL_lm17 <- glm(data = CEWL_data_full,</pre>
                      TEWL_g_m2h ~ hold_time + region)
summary(CEWL_lm17)
##
  glm(formula = TEWL_g_m2h ~ hold_time + region, data = CEWL_data_full)
##
## Deviance Residuals:
##
       Min
                 1Q
                      Median
                                    3Q
                                             Max
## -28.477
                       -2.076
                                 5.613
                                          67.028
             -8.346
##
## Coefficients:
                Estimate Std. Error t value Pr(>|t|)
##
## (Intercept) 26.958624
                            1.534599
                                     17.567 < 2e-16 ***
## hold_time
               -0.039935
                            0.007613 -5.246 2.14e-07 ***
                                                 0.633
## regiondors
                0.739762
                            1.546876
                                       0.478
                                      4.255 2.42e-05 ***
## regionhead
                6.581377
                            1.546876
```

```
6.566046
                           1.556123
                                      4.219 2.81e-05 ***
## regionmite
              11.034295
                                      7.133 2.73e-12 ***
## regionvent
                           1.546868
##
                   0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Signif. codes:
##
##
   (Dispersion parameter for gaussian family taken to be 150.7369)
##
##
       Null deviance: 108878
                             on 629
                                      degrees of freedom
## Residual deviance: 94060
                             on 624
                                      degrees of freedom
##
     (69 observations deleted due to missingness)
## AIC: 4955.6
##
## Number of Fisher Scoring iterations: 2
```

Conclusion

The following variables should be included in the full LMM: - body region - cloacal temperature at measurement - capture temp (and try an interaction with region) - capture wind speed and solar radiation - absolute humidity (and try an interaction with region) - measurement temperature (and try an interaction with region) - individual ID (as a random effect) - SVL and mass - hold time

LMMs

Hydration

Based on the simple linear models and figures above, osmolality should be predicted by sample eye, hemolysis, date/week, individual, capture temperature, and capture absolute humidity.

Prep dataframe for computing models:

```
##
         date
                          individual_ID osmolality_mmol_kg temp_C_interpol
##
    Min.
           :2021-04-05
                                 :
                                    1
                                         Min.
                                                :293.0
                                                             Min.
                                                                     :14.58
##
    1st Qu.:2021-04-19
                          2
                                    1
                                         1st Qu.:338.0
                                                             1st Qu.:16.95
##
   Median :2021-04-26
                          3
                                    1
                                         Median :370.0
                                                             Median :19.46
##
    Mean
           :2021-04-25
                          4
                                         Mean
                                                :365.5
                                                             Mean
                                                                    :19.08
                                    1
##
    3rd Qu.:2021-05-03
                          5
                                     1
                                         3rd Qu.:389.0
                                                             3rd Qu.:20.14
##
    Max.
           :2021-05-17
                          6
                                         Max.
                                                :436.0
                                                                     :23.61
                                     1
                                                             Max.
##
                          (Other):115
##
    abs_humidity_g_m3_interpol
                                      SMI
                                                  hemolyzed
   Min.
           : 8.504
                                        : 6.450
                                                  N:82
##
                                Min.
   1st Qu.: 9.820
                                1st Qu.: 9.929
                                                  Y:39
##
  Median :10.666
                                Median :10.583
##
## Mean
           :10.377
                                Mean
                                        :10.714
```

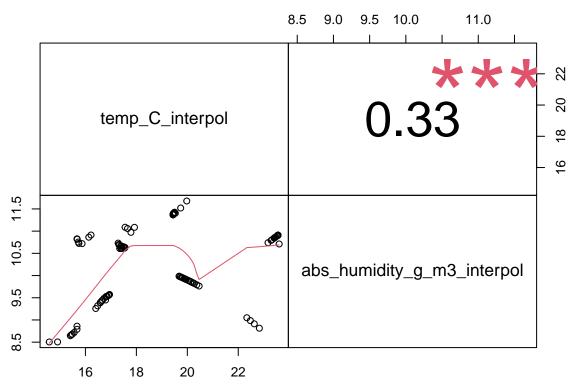
```
## 3rd Qu.:10.908 3rd Qu.:11.501
## Max. :11.679 Max. :14.999
```

Multicollinearity

First, check for multicollinearity among independent variables:

```
pairs(hydrat_mod_dat)
```

```
0 50
                                         16
                                             20
                        150
                                                                  8
                                                                     12
                 individual_ID
                                                                                        300 420
                             nolality_mmol_
                                         temp_C_interpo
                                                                              hemolyzed
    Apr 15
                           300 380
                                                   8.5 10.0
                                                                            1.0
                                                                                 1.6
\# also make another plot with r-sq values
# only continuous numeric variables work for this one
hydrat_mod_dat %>%
  # select cont variables
  dplyr::select(temp_C_interpol,
                  abs_humidity_g_m3_interpol) %>%
  # multicollinearity plot
  chart.Correlation(., histogram = F, pch = 19)
```



Date and individual_ID are collinear and should not both be used as fixed effects. But, individual_ID is a factor and there is not repetition in the dataset, so that's not feasible as a variable. Temperature and absolute humidity are the only continuous variables, and they are not badly collinear.

Models & Selection

Start with all the variables that were significant individually in SLRs.

```
# model 1
hydrat_mod1 <- lme4::lmer(data = hydrat_mod_dat,</pre>
                           # response variable
                           osmolality_mmol_kg ~
                             # start with interaction + singular effect
                            abs_humidity_g_m3_interpol*temp_C_interpol +
                             # other potentially important factor
                             SMI +
                             # random effects
                             (1|date) + (1|hemolyzed))
summary(hydrat_mod1)
## Linear mixed model fit by REML ['lmerMod']
\verb|## Formula: osmolality_mmol_kg ~ abs_humidity_g_m3_interpol * temp_C_interpol + \\
       SMI + (1 | date) + (1 | hemolyzed)
##
##
      Data: hydrat_mod_dat
##
## REML criterion at convergence: 1035.4
##
## Scaled residuals:
##
        Min
                  1Q
                       Median
                                     3Q
                                              Max
## -2.54027 -0.58267 0.07275 0.63884 2.96793
##
## Random effects:
```

```
## Groups
                          Variance Std.Dev.
## date
                                   28.690
              (Intercept) 823.13
## hemolyzed (Intercept) 26.43
                                    5.141
                                  17.532
## Residual
                          307.36
## Number of obs: 121, groups: date, 6; hemolyzed, 2
##
## Fixed effects:
##
                                               Estimate Std. Error t value
## (Intercept)
                                               865.3682
                                                          329.7647
                                                                     2.624
## abs_humidity_g_m3_interpol
                                              -57.7664
                                                           36.5767 -1.579
## temp_C_interpol
                                              -27.6566
                                                           17.7098 -1.562
                                                -0.8364
                                                            1.2377 -0.676
## abs_humidity_g_m3_interpol:temp_C_interpol
                                                3.2251
                                                            1.9630
                                                                     1.643
##
## Correlation of Fixed Effects:
##
               (Intr) ab___3 tmp_C SMI
## abs_hmd__3_ -0.989
## tmp_C_ntrpl -0.980
                      0.977
               -0.043 0.019 -0.010
## ab__3:_C_ 0.971 -0.987 -0.990 -0.008
drop1(hydrat_mod1)
## Single term deletions
## Model:
## osmolality_mmol_kg ~ abs_humidity_g_m3_interpol * temp_C_interpol +
       SMI + (1 | date) + (1 | hemolyzed)
##
                                              npar
                                                       AIC
## <none>
                                                    1072.2
## SMI
                                                  1 1070.7
## abs_humidity_g_m3_interpol:temp_C_interpol
                                                  1 1072.8
The model would improve (based on lower AIC) if we drop SMI as a predictor.
# model 2
hydrat_mod2 <- lme4::lmer(data = hydrat_mod_dat,</pre>
                          osmolality_mmol_kg ~
                           abs_humidity_g_m3_interpol*temp_C_interpol +
                            (1|date) + (1|hemolyzed))
summary(hydrat_mod2)
## Linear mixed model fit by REML ['lmerMod']
## Formula: osmolality_mmol_kg ~ abs_humidity_g_m3_interpol * temp_C_interpol +
##
       (1 | date) + (1 | hemolyzed)
##
      Data: hydrat_mod_dat
## REML criterion at convergence: 1038.1
##
## Scaled residuals:
                  1Q
                      Median
## -2.47326 -0.62565 0.07308 0.64959
                                        2.99129
## Random effects:
                          Variance Std.Dev.
## Groups
              Name
              (Intercept) 844.50
## date
                                   29.060
```

```
## hemolyzed (Intercept) 24.79
                                    4.978
                          305.73
                                   17.485
## Residual
## Number of obs: 121, groups: date, 6; hemolyzed, 2
## Fixed effects:
                                              Estimate Std. Error t value
##
## (Intercept)
                                                          330.658
                                               861.250
                                                                    2.605
## abs_humidity_g_m3_interpol
                                               -57.895
                                                           36.763 -1.575
## temp_C_interpol
                                               -28.063
                                                           17.782 -1.578
## abs_humidity_g_m3_interpol:temp_C_interpol
                                                 3.247
                                                           1.974
                                                                   1.645
## Correlation of Fixed Effects:
               (Intr) ab___3_ tmp_C_
## abs_hmd__3_ -0.989
## tmp_C_ntrpl -0.982 0.978
## ab__3:_C_ 0.972 -0.987 -0.991
# compare to full model
anova(hydrat_mod1, hydrat_mod2)
## refitting model(s) with ML (instead of REML)
## Data: hydrat mod dat
## Models:
## hydrat_mod2: osmolality_mmol_kg ~ abs_humidity_g_m3_interpol * temp_C_interpol +
## hydrat_mod2:
                    (1 | date) + (1 | hemolyzed)
## hydrat_mod1: osmolality_mmol_kg ~ abs_humidity_g_m3_interpol * temp_C_interpol +
## hydrat_mod1:
                    SMI + (1 | date) + (1 | hemolyzed)
                              BIC logLik deviance Chisq Df Pr(>Chisq)
##
                       AIC
              npar
## hydrat_mod2
                 7 1070.7 1090.3 -528.37
                                            1056.7
## hydrat_mod1
                  8 1072.2 1094.6 -528.11
                                            1056.2 0.5164 1
                                                                 0.4724
```

We do not decrease our predictive power significantly by dropping SMI, so we will consider the reduced model (2) to be better than the full (1).

Check whether to continue dropping terms:

```
drop1(hydrat_mod2)
```

Dropping the current predictors will not improve AIC any more than its current value, so hydrat_mod2 is the best model to predict osmolality.

Best Model

```
# save model 2 summary object
osml_best_mod <- summary(hydrat_mod2)
# extract stats table from summary object
osml_best_mod_vals <- data.frame(osml_best_mod$coefficients)</pre>
```

```
# export
write.csv(osml_best_mod_vals, "osml_best_mod_vals.csv")
```

Check LM Assumptions (Hydration Model)

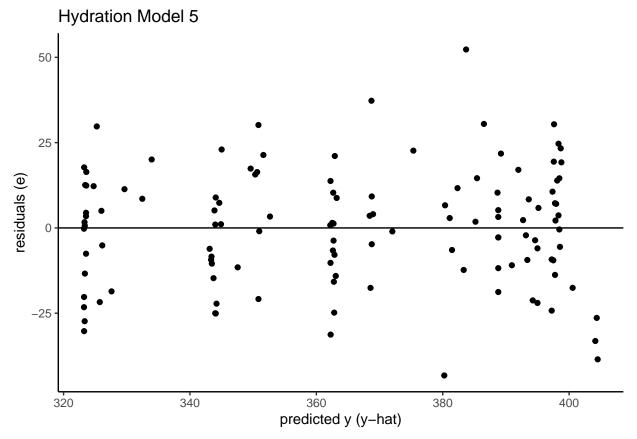
First, get residuals:

Linearity and Equal Variance

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). We don't care if there is a relationship between the residuals \sim dependent variable (actual y).

Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

```
ggplot(data = res_hydrat_mod, aes(x = y_hat, y = e)) +
  geom_point() +
  theme_classic() +
  xlab("predicted y (y-hat)") +
  ylab("residuals (e)") +
  ggtitle("Hydration Model 5") +
  geom_hline(yintercept = 0)
```



It looks pretty evenly distributed. No clear patterning, so linearity and equal error variance should be satisfied.

Brown-Forsythe test to statistically check equal variance:

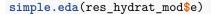
H0: normally distributed (non-sig test is GOOD) HA: NOT normally distributed (reject nul == assumption not satisfied)

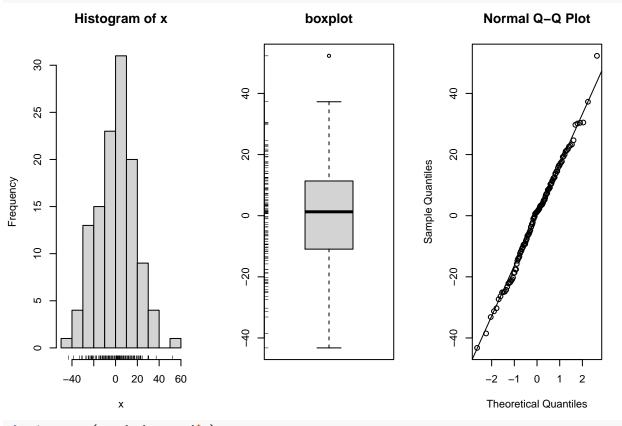
```
# need to create the right data & format first
bf_data_temp <- res_hydrat_mod %>%
 dplyr::filter(complete.cases(temp_C_interpol)) %>%
 dplyr::mutate(middle = median(temp_C_interpol),
               side = temp C interpol > middle)
bf_data_temp$side <- as.factor(bf_data_temp$side)</pre>
# now run test
bf.test(formula = e \sim side, # y\sim x
       data = bf_data_temp, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
##
    data : e and side
##
   statistic : 0.004278733
##
##
    num df : 1
    denom df : 118.0462
##
##
    p.value : 0.9479566
##
              : Difference is not statistically significant.
##
    Result
## -----
# need to create the right data & format first
bf_data_abshum <- res_hydrat_mod %>%
 dplyr::filter(complete.cases(abs_humidity_g_m3_interpol)) %>%
 dplyr::mutate(middle = median(abs_humidity_g_m3_interpol),
               side = abs_humidity_g_m3_interpol > middle)
bf_data_abshum$side <- as.factor(bf_data_abshum$side)</pre>
# now run test
bf.test(formula = e \sim side, # y\sim x
       data = bf_data_abshum, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
##
##
    data : e and side
##
##
    statistic : 0.1445405
##
    num df
              : 1
##
    denom df : 118.9914
```

Equal variance is satisfied.

Now check normality. 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(res_hydrat_mod\$e)

```
##
## Shapiro-Wilk normality test
##
## data: res_hydrat_mod$e
## W = 0.99535, p-value = 0.9629
```

Normality is satisfied.

Conclusion

hydrat_mod2 is the best model to predict osmolality, and the model satisfies all linear regression assumptions.

CEWL

Based on the simple linear models and figures above, CEWL should be predicted by: - body region - cloacal temperature at measurement - capture temperature, absolute humidity, wind speed, and solar radiation -

ambient temperature during measurement - SVL and mass - hold time (time between capture vs measurement) - individual ID (as a random effect)

Prep dataframe for models:

```
CEWL_mod_dat <- CEWL_data_full %>%
  # select variables of interest
  dplyr::select(date,
                hold_time,
                individual_ID,
                mass_g,
                SVL_mm,
                TEWL_g_m2h,
                region,
                cloacal_temp_C,
                temp_C_interpol,
                abs_humidity_g_m3_interpol,
                Wind_mph_interpol,
                Solar_rad_Wm2_interpol,
                ambient_temp_C
                ) %>%
  dplyr::filter(complete.cases(.))
```

Multicollinearity

Check for multicollinearity among independent variables:

```
CEWL_mod_dat %>%
  # get rid of dependent variable
  dplyr::select(-TEWL_g_m2h, individual_ID) %>%
  # multicollinearity plot
  pairs(.)
          50
                         4 12
                                                      16 22
                                                                    4.0
                                                                                  22.5
                                 SVL_mm
                                        region
                                               cal_tem
                                                      o_C_inte
                                                       36,
                                                              dity_g_m
                                                                     _mph_int
                                                                            d_Wm2
                                                                                    ient_tem
                                45 65
                                              20 26
                                                                           600
                  20 140
                                                             9.0 11.5
  Apr 15
```

```
# also make another plot with r-sq values
# non-numeric variables don't work for this
CEWL mod dat %>%
  # select variables of interest
  dplyr::select(-TEWL_g_m2h, -date, -region, -individual_ID) %>%
  # multicollinearity plot
  chart.Correlation(., histogram = F, pch = 19)
              4 8 12
                                  20 24 28
                                                      9.0 10.5
                                                                          600
                                                                                900
                                                                                                  250
                                                   **
                                                                      **
      hold_time
                                     -0.67
                                                                                                  20
                          0.85
                                      -0.15
                                                                     0.065
                                                                                         0.009
                                                                                                  65
                                                                     0.095
                                                                                                  45
26
                                                0.30
                                                                               0.033
                                                                                        0.30
                                                          0.26
                                                                    -0.24
20
                                                                                                  22
                                              mp_C_interpo
                                                                              0.42
                                                                                        0.44
                                                           0.043
                                                                    -0.29
11.5
                                                                                                  9
                                                                                        0.36
                                                                              0.38
                                                                  nd mph inter
                                                                                        0.30
1000
                                                                                         0.16
900
                                                                                                  22.5
    50
         200
                       45
                            60
                                            16
                                                20
                                                                 4.0
                                                                      5.5
                                                                                    22.5
                                                                                          24.5
```

Mass and SVL are very collinear variables that should not be used in combination. Cloacal temp and hold time are pretty collinear, which makes sense; as we held them, they got cooler because they were inside and not basking. Individual ID and absolute humidity are also pretty collinear, but it's less intuitive. Temperature and solar radiation, as well as humidity and solar radiation, are each collinear pairs. We will use model selection to figure out which variable from each collinear pair is better to include in the model.

Models & Selection

This is the full model with every potential variable and interaction based on figures and SLR.

summary(CEWL_mod1)

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: TEWL_g_m2h ~ region * (mass_g + ambient_temp_C + temp_C_interpol +
      abs_humidity_g_m3_interpol) + hold_time + SVL_mm + cloacal_temp_C +
##
      temp_C_interpol:abs_humidity_g_m3_interpol + Wind_mph_interpol +
##
      Solar_rad_Wm2_interpol + (1 | individual_ID)
##
     Data: CEWL_mod_dat
##
## REML criterion at convergence: 4723.3
##
## Scaled residuals:
      Min
##
               1Q Median
                               3Q
                                      Max
## -2.2331 -0.5426 -0.1011 0.4132 5.1591
##
## Random effects:
## Groups
                             Variance Std.Dev.
                 Name
   individual_ID (Intercept) 30.02
                                      5.479
## Residual
                             94.93
                                      9.743
## Number of obs: 630, groups: individual_ID, 128
##
## Fixed effects:
##
                                               Estimate Std. Error t value
## (Intercept)
                                             -1.559e+02 1.576e+02 -0.990
                                              8.701e+01 4.214e+01
## regiondors
                                                                     2.065
## regionhead
                                              9.480e+01 4.184e+01
                                                                     2.266
## regionmite
                                              1.483e+02 4.253e+01
                                                                     3.487
                                              1.091e+02 4.174e+01
## regionvent
                                                                     2.615
## mass_g
                                              1.132e+00 5.892e-01
                                                                     1.921
                                             -4.522e-01 1.893e+00 -0.239
## ambient_temp_C
## temp_C_interpol
                                              3.792e+00 7.363e+00
                                                                    0.515
## abs_humidity_g_m3_interpol
                                              7.795e+00 1.297e+01
                                                                     0.601
## hold time
                                              7.604e-03 1.481e-02
                                                                     0.513
## SVL_mm
                                              1.191e-01 2.461e-01
                                                                     0.484
                                              2.455e+00 5.874e-01
## cloacal_temp_C
                                                                     4.179
                                             -3.363e-01 2.004e+00 -0.168
## Wind_mph_interpol
                                              1.266e-02 7.133e-03
## Solar_rad_Wm2_interpol
                                                                    1.775
## regiondors:mass_g
                                             -9.988e-01 4.884e-01 -2.045
## regionhead:mass_g
                                             -1.300e+00 4.807e-01 -2.705
## regionmite:mass_g
                                              1.113e-01 4.970e-01
                                                                     0.224
## regionvent:mass_g
                                             -1.059e-01 4.844e-01 -0.219
## regiondors:ambient_temp_C
                                             -2.018e+00 2.056e+00 -0.982
## regionhead:ambient_temp_C
                                             -2.673e+00 2.059e+00 -1.298
## regionmite:ambient temp C
                                             -2.372e+00 2.075e+00 -1.143
## regionvent:ambient_temp_C
                                                         2.056e+00 -1.920
                                             -3.947e+00
## regiondors:temp_C_interpol
                                             -1.797e+00 5.403e-01 -3.326
## regionhead:temp_C_interpol
                                             -1.214e+00
                                                         5.431e-01 -2.236
## regionmite:temp_C_interpol
                                             -4.837e-02
                                                         5.479e-01 -0.088
## regionvent:temp_C_interpol
                                             -4.230e-01 5.441e-01 -0.778
## regiondors:abs_humidity_g_m3_interpol
                                              5.372e-01 2.067e+00
                                                                    0.260
## regionhead:abs_humidity_g_m3_interpol
                                              1.070e+00 2.074e+00
                                                                     0.516
## regionmite:abs_humidity_g_m3_interpol
                                             -8.155e+00
                                                         2.083e+00 -3.915
## regionvent:abs_humidity_g_m3_interpol
                                              3.362e-01 2.073e+00
                                                                     0.162
## temp_C_interpol:abs_humidity_g_m3_interpol -2.726e-01 7.050e-01 -0.387
```

```
##
## Correlation matrix not shown by default, as p = 31 > 12.
## Use print(x, correlation=TRUE) or
       vcov(x)
##
                      if you need it
Check which variables to drop:
drop1(CEWL_mod1)
## Single term deletions
##
## Model:
## TEWL_g_m2h ~ region * (mass_g + ambient_temp_C + temp_C_interpol +
       abs_humidity_g_m3_interpol) + hold_time + SVL_mm + cloacal_temp_C +
       temp_C_interpol:abs_humidity_g_m3_interpol + Wind_mph_interpol +
##
##
       Solar_rad_Wm2_interpol + (1 | individual_ID)
##
                                                        AIC
## <none>
                                                     4810.5
## hold_time
                                                   1 4808.8
                                                   1 4808.7
## SVL_mm
## cloacal_temp_C
                                                   1 4826.3
## Wind_mph_interpol
                                                   1 4808.5
## Solar rad Wm2 interpol
                                                   1 4811.9
## region:mass_g
                                                   4 4817.0
## region:ambient temp C
                                                   4 4806.5
## region:temp_C_interpol
                                                   4 4819.7
## region:abs_humidity_g_m3_interpol
                                                   4 4830.6
## temp_C_interpol:abs_humidity_g_m3_interpol
                                                   1 4808.6
Based on AIC, dropping SVL, hold time, temp, humidity, wind speed, region:ambient_temp_C, and
temp\_C\_interpol:abs\_humidity\_g\_m3\_interpol\ would\ result\ in\ a\ better\ model.
Start with region:ambient_temp_C and temp_C_interpol:abs_humidity_g_m3_interpol interactions:
# model 2
CEWL_mod2 <- lme4::lmer(data = CEWL_mod_dat,</pre>
                         TEWL_g_m2h ~
                          region * (mass_g +
                                      temp_C_interpol +
                                      abs_humidity_g_m3_interpol) +
                          hold_time + SVL_mm +
                          cloacal_temp_C +
                          Wind_mph_interpol +
                          Solar_rad_Wm2_interpol +
                          (1|individual_ID))
summary(CEWL_mod2)
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
       hold_time + SVL_mm + cloacal_temp_C + Wind_mph_interpol +
##
##
       Solar_rad_Wm2_interpol + (1 | individual_ID)
##
      Data: CEWL_mod_dat
## REML criterion at convergence: 4747.5
```

Scaled residuals:

```
##
               1Q Median
                               3Q
## -2.2159 -0.5667 -0.0867 0.4067 5.1995
##
## Random effects:
## Groups
                 Name
                             Variance Std.Dev.
  individual ID (Intercept) 30.90
                                      5.559
## Residual
                             95.06
                                       9.750
## Number of obs: 630, groups: individual_ID, 128
##
## Fixed effects:
##
                                          Estimate Std. Error t value
## (Intercept)
                                        -87.058039 24.523264 -3.550
## regiondors
                                         51.945825 22.844519
                                                                2.274
                                         49.495464 22.784245
## regionhead
                                                                2.172
                                        107.535596 23.034315
## regionmite
                                                                4.668
## regionvent
                                         42.587507 22.743292
                                                                1.873
## mass_g
                                                     0.592606
                                                                2.027
                                          1.200986
## temp C interpol
                                          0.710060
                                                     0.439275
                                                               1.616
## abs_humidity_g_m3_interpol
                                          2.421998 1.732231
                                                               1.398
## hold time
                                          0.005608
                                                     0.014493
                                                                0.387
## SVL_mm
                                          0.080709
                                                     0.247390
                                                               0.326
## cloacal_temp_C
                                                     0.562955
                                          2.244956
                                                                3.988
                                                     1.518371 -1.953
## Wind_mph_interpol
                                         -2.965487
## Solar_rad_Wm2_interpol
                                          0.013837
                                                     0.005330
                                                                2.596
## regiondors:mass_g
                                         -1.009311 0.488679 -2.065
## regionhead:mass_g
                                         -1.316901 0.480895 -2.738
                                          0.125620
                                                     0.497154
                                                                0.253
## regionmite:mass_g
## regionvent:mass_g
                                         -0.154379
                                                     0.484223 -0.319
## regiondors:temp_C_interpol
                                         -2.033402
                                                     0.481402 - 4.224
## regionhead:temp_C_interpol
                                         -1.538362
                                                     0.483775 -3.180
## regionmite:temp_C_interpol
                                         -0.355824
                                                     0.482327 - 0.738
## regionvent:temp_C_interpol
                                         -0.904063
                                                     0.485072 - 1.864
## regiondors:abs_humidity_g_m3_interpol -0.191633
                                                     1.919221 -0.100
## regionhead:abs_humidity_g_m3_interpol
                                          0.018035
                                                     1.914084
                                                                0.009
## regionmite:abs_humidity_g_m3_interpol
                                         -9.039969
                                                     1.936276
                                                               -4.669
## regionvent:abs_humidity_g_m3_interpol -1.224058
                                                     1.913883 -0.640
## Correlation matrix not shown by default, as p = 25 > 12.
## Use print(x, correlation=TRUE) or
##
       vcov(x)
                     if you need it
# compare
anova(CEWL_mod2, CEWL_mod1)
## refitting model(s) with ML (instead of REML)
## Data: CEWL mod dat
## Models:
## CEWL_mod2: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
## CEWL_mod2:
                 hold_time + SVL_mm + cloacal_temp_C + Wind_mph_interpol +
## CEWL_mod2:
                 Solar_rad_Wm2_interpol + (1 | individual_ID)
## CEWL_mod1: TEWL_g_m2h ~ region * (mass_g + ambient_temp_C + temp_C_interpol +
## CEWL_mod1:
                 abs_humidity_g_m3_interpol) + hold_time + SVL_mm + cloacal_temp_C +
                 {\tt temp\_C\_interpol:abs\_humidity\_g\_m3\_interpol\ +\ Wind\_mph\_interpol\ +\ }
## CEWL_mod1:
## CEWL_mod1:
                 Solar_rad_Wm2_interpol + (1 | individual_ID)
```

```
## npar AIC BIC logLik deviance Chisq Df Pr(>Chisq)
## CEWL_mod2 27 4807.8 4927.8 -2376.9 4753.8
## CEWL mod1 33 4810.5 4957.2 -2372.2 4744.5 9.3042 6 0.1572
```

AIC improved somewhat and we do not lose a significant amount of predictive power, so the reduced model is better. Continue dropping...

Check drop terms again:

```
drop1(CEWL mod2)
## Single term deletions
##
## Model:
hold_time + SVL_mm + cloacal_temp_C + Wind_mph_interpol +
     Solar_rad_Wm2_interpol + (1 | individual_ID)
##
##
                               npar
## <none>
                                   4807.8
                                  1 4805.9
## hold_time
## SVL_mm
                                  1 4805.9
## cloacal_temp_C
                                  1 4821.9
                                  1 4809.8
## Wind_mph_interpol
## Solar_rad_Wm2_interpol
                                 1 4812.8
## region:mass_g
                                 4 4814.4
## region:temp_C_interpol
                                  4 4824.0
```

Hold time, SVL, and wind should still be deleted.

region:abs_humidity_g_m3_interpol

4 4832.5

```
## Linear mixed model fit by REML ['lmerMod']
## Formula:
## TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
##
       cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
##
      Data: CEWL mod dat
##
## REML criterion at convergence: 4746.5
##
## Scaled residuals:
##
       Min
                1Q Median
                                3Q
                                       Max
## -2.2165 -0.5681 -0.0845 0.3897 5.2348
##
## Random effects:
## Groups
                              Variance Std.Dev.
                  Name
## individual ID (Intercept) 31.28
                                       5.593
## Residual
                              95.06
                                       9.750
```

```
## Number of obs: 630, groups: individual_ID, 128
##
## Fixed effects:
                                          Estimate Std. Error t value
##
## (Intercept)
                                        -99.785318 19.355118 -5.156
## regiondors
                                        51.879222 22.844484
                                                               2.271
## regionhead
                                        49.554223 22.783334
                                                              2.175
                                      107.365993 23.033169 4.661
## regionmite
## regionvent
                                       42.581631 22.742456 1.872
## mass_g
                                         1.312125 0.398001 3.297
## temp_C_interpol
                                         0.875234 0.425830 2.055
                                                   1.663082 1.585
## abs_humidity_g_m3_interpol
                                          2.635599
                                                              5.758
## cloacal_temp_C
                                         2.210436
                                                    0.383892
                                         0.012051
## Solar_rad_Wm2_interpol
                                                    0.005007
                                                               2.407
                                        -1.007466
                                                    0.488673 -2.062
## regiondors:mass_g
## regionhead:mass_g
                                        -1.317803
                                                    0.480831 -2.741
                                         0.126792
## regionmite:mass_g
                                                    0.497084
                                                               0.255
## regionvent:mass g
                                        -0.153665 0.484142 -0.317
## regiondors:temp_C_interpol
                                        -2.030832 0.481401 -4.219
## regionhead:temp_C_interpol
                                         -1.537802 0.483770 -3.179
## regionmite:temp_C_interpol
                                        -0.354780 0.482324 -0.736
## regionvent:temp_C_interpol
                                         -0.901930
                                                    0.485071 -1.859
## regiondors:abs_humidity_g_m3_interpol -0.191317
                                                    1.919226 -0.100
## regionhead:abs_humidity_g_m3_interpol
                                          0.012735
                                                    1.914041
                                                               0.007
## regionmite:abs_humidity_g_m3_interpol -9.027196
                                                    1.936220 -4.662
## regionvent:abs_humidity_g_m3_interpol -1.227483 1.913844 -0.641
## Correlation matrix not shown by default, as p = 22 > 12.
## Use print(x, correlation=TRUE)
##
      vcov(x)
                     if you need it
# compare
anova(CEWL_mod3, CEWL_mod1)
## refitting model(s) with ML (instead of REML)
## Data: CEWL_mod_dat
## Models:
## CEWL_mod3: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
                 cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
## CEWL mod3:
## CEWL_mod1: TEWL_g_m2h ~ region * (mass_g + ambient_temp_C + temp_C_interpol +
                 abs_humidity_g_m3_interpol) + hold_time + SVL_mm + cloacal_temp_C +
## CEWL mod1:
## CEWL mod1:
                 temp_C_interpol:abs_humidity_g_m3_interpol + Wind_mph_interpol +
                 Solar_rad_Wm2_interpol + (1 | individual_ID)
## CEWL mod1:
##
                    AIC
                           BIC logLik deviance Chisq Df Pr(>Chisq)
            npar
## CEWL mod3
              24 4805.9 4912.6 -2379.0
                                         4757.9
              33 4810.5 4957.2 -2372.2
                                         4744.5 13.45 9
## CEWL mod1
                                                            0.1433
anova(CEWL_mod3, CEWL_mod2)
## refitting model(s) with ML (instead of REML)
## Data: CEWL_mod_dat
## Models:
## CEWL_mod3: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
## CEWL mod3:
                cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
```

```
## CEWL_mod2: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
                 hold_time + SVL_mm + cloacal_temp_C + Wind_mph_interpol +
## CEWL mod2:
## CEWL mod2:
                  Solar_rad_Wm2_interpol + (1 | individual_ID)
                           BIC logLik deviance Chisq Df Pr(>Chisq)
##
                     AIC
            npar
## CEWL mod3
              24 4805.9 4912.6 -2379.0
                                          4757.9
## CEWL mod2
              27 4807.8 4927.8 -2376.9
                                          4753.8 4.1461 3
                                                               0.2461
```

Once again, the AIC is slightly lower, and our model fit is not significantly worse by going to model 3, so the reduced model is better.

```
Check drop terms again to make sure nothing else could potentially be removed:
drop1(CEWL_mod3)
## Single term deletions
##
## Model:
## TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
       cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
##
##
                                      npar
                                              AIC
                                           4805.9
## <none>
## cloacal_temp_C
                                         1 4834.7
## Solar_rad_Wm2_interpol
                                         1 4809.9
## region:mass_g
                                         4 4812.6
## region:temp_C_interpol
                                         4 4822.1
## region:abs_humidity_g_m3_interpol
                                         4 4830.5
Check whether removing Solar_rad_Wm2_interpol is okay:
# model 4
CEWL_mod4 <- lme4::lmer(data = CEWL_mod_dat,</pre>
                        TEWL_g_m2h ~
                          region * (mass_g +
                                      temp_C_interpol +
                                      abs_humidity_g_m3_interpol) +
                          cloacal_temp_C +
                          (1|individual_ID))
summary(CEWL_mod4)
## Linear mixed model fit by REML ['lmerMod']
## TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
##
       cloacal_temp_C + (1 | individual_ID)
##
      Data: CEWL_mod_dat
##
## REML criterion at convergence: 4743.4
##
## Scaled residuals:
                1Q Median
##
       Min
                                 3Q
                                        Max
## -2.2684 -0.5593 -0.0981 0.3787 5.1696
##
## Random effects:
## Groups
                  Name
                               Variance Std.Dev.
## individual_ID (Intercept) 33.36
                                        5.776
                                        9.747
## Residual
                               95.01
## Number of obs: 630, groups: individual_ID, 128
```

##

```
## Fixed effects:
##
                                          Estimate Std. Error t value
## (Intercept)
                                        -103.22837 19.46944 -5.302
                                                    22.84083 2.270
## regiondors
                                          51.84649
## regionhead
                                          49.38419
                                                    22.77852
                                                              2.168
                                        107.53197 23.02916 4.669
## regionmite
                                         42.41691 22.73810 1.865
## regionvent
                                                     0.40108 3.292
## mass g
                                           1.32056
## temp_C_interpol
                                          1.21248
                                                     0.40537 2.991
## abs_humidity_g_m3_interpol
                                          3.84425
                                                     1.59777 2.406
## cloacal_temp_C
                                          1.99607
                                                      0.38092 5.240
                                                      0.48865 -2.065
## regiondors:mass_g
                                          -1.00927
## regionhead:mass_g
                                         -1.31378
                                                     0.48076 - 2.733
## regionmite:mass_g
                                          0.12224
                                                     0.49701
                                                              0.246
                                                      0.48408 -0.305
## regionvent:mass_g
                                          -0.14769
## regiondors:temp_C_interpol
                                          -2.03061
                                                      0.48130 -4.219
## regionhead:temp_C_interpol
                                         -1.53934
                                                     0.48365 -3.183
## regionmite:temp_C_interpol
                                          -0.35235
                                                      0.48223 -0.731
                                                      0.48498 -1.865
## regionvent:temp_C_interpol
                                          -0.90458
## regiondors:abs_humidity_g_m3_interpol
                                         -0.18693
                                                      1.91886 -0.097
## regionhead:abs_humidity_g_m3_interpol
                                         0.02664
                                                      1.91363
                                                              0.014
## regionmite:abs_humidity_g_m3_interpol
                                          -9.04168
                                                      1.93588 -4.671
                                                      1.91344 -0.635
## regionvent:abs_humidity_g_m3_interpol
                                          -1.21423
##
## Correlation matrix not shown by default, as p = 21 > 12.
## Use print(x, correlation=TRUE) or
      vcov(x)
                     if you need it
# compare
anova(CEWL_mod4, CEWL_mod1)
## refitting model(s) with ML (instead of REML)
## Data: CEWL_mod_dat
## Models:
## CEWL_mod4: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
## CEWL mod4:
                 cloacal_temp_C + (1 | individual_ID)
## CEWL_mod1: TEWL_g_m2h ~ region * (mass_g + ambient_temp_C + temp_C_interpol +
                 abs_humidity_g_m3_interpol) + hold_time + SVL_mm + cloacal_temp_C +
## CEWL_mod1:
## CEWL mod1:
                 temp_C_interpol:abs_humidity_g_m3_interpol + Wind_mph_interpol +
## CEWL mod1:
                 Solar_rad_Wm2_interpol + (1 | individual_ID)
            npar
                    AIC
                          BIC logLik deviance Chisq Df Pr(>Chisq)
## CEWL_mod4 23 4809.9 4912.1 -2381.9
                                         4763.9
## CEWL mod1
              33 4810.5 4957.2 -2372.2
                                         4744.5 19.382 10
                                                             0.03567 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
anova(CEWL_mod4, CEWL_mod3)
## refitting model(s) with ML (instead of REML)
## Data: CEWL_mod_dat
## Models:
## CEWL_mod4: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
## CEWL_mod4: cloacal_temp_C + (1 | individual_ID)
## CEWL_mod3: TEWL_g_m2h ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
```

We do lose significant predictive power when we go from model 3 to 4... So model 3 is the best model to predict CEWL.

Best Model

The best model is CEWL predicted by: body region and its interaction with mass and capture temp and humidity, cloacal temperature, solar radiation at capture time, and individual ID as a random effect.

```
# save best model summary object
CEWL_best_mod <- summary(CEWL_mod3)
# extract stats table from summary object
CEWL_best_mod_vals <- data.frame(CEWL_best_mod$coefficients)
# export
write.csv(CEWL_best_mod_vals, "CEWL_best_mod_vals.csv")</pre>
```

Check LM Assumptions

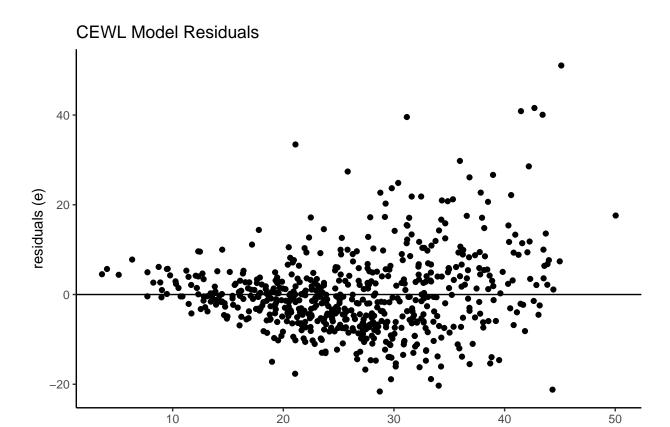
First, get residuals:

Linearity and Equal Variance

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). We don't care if there is a relationship between the residuals \sim dependent variable (actual y).

Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

```
ggplot(data = CEWL_mod_res, aes(x = y_hat, y = e)) +
  geom_point() +
  theme_classic() +
  xlab("predicted y (y-hat)") +
  ylab("residuals (e)") +
  ggtitle("CEWL Model Residuals") +
  geom_hline(yintercept = 0)
```



It's definitely making a fan shape. :(

Brown-Forsythe test to statistically check equal variance, for each continuous predictor variable:

H0: normally distributed (non-sig test is GOOD) HA: NOT normally distributed (reject nul == assumption not satisfied)

predicted y (y-hat)

```
# need to create the right data & format first
bf_data_CEWL <- CEWL_mod_res %>%
  dplyr::mutate(middle_mass = median(mass_g), # mass
                side_mass = as.factor(mass_g > middle_mass),
                # solar radiation
                middle_sorad = median(Solar_rad_Wm2_interpol),
                side_sorad = as.factor(Solar_rad_Wm2_interpol > middle_sorad),
                # temperature
                middle_temp = median(temp_C_interpol),
                side_temp = as.factor(temp_C_interpol > middle_temp),
                # absolute humidity
                middle_absh = median(abs_humidity_g_m3_interpol),
                side_absh = as.factor(abs_humidity_g_m3_interpol > middle_absh),
                middle_ct = median(cloacal_temp_C),
                side_ct = as.factor(cloacal_temp_C > middle_ct)
# now run test
bf.test(formula = e ~ side_absh, # y~x
        data = bf_data_CEWL, # dataframe
```

```
alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_absh
##
    statistic : 0.08139769
##
##
    num df
             : 1
    denom df : 615.6298
##
    p.value : 0.7755081
##
##
##
    Result
            : Difference is not statistically significant.
bf.test(formula = e ~ side_temp, # y~x
       data = bf_data_CEWL, # dataframe
       alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_temp
##
    statistic : 0.03237498
##
##
    num df : 1
##
    denom df : 585.0611
    p.value : 0.8572694
##
##
##
            : Difference is not statistically significant.
## -----
bf.test(formula = e ~ side_sorad, # y~x
      data = bf_data_CEWL, # dataframe
       alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_sorad
##
##
    statistic : 0.0762158
##
    num df
            : 1
    denom df : 626.0987
##
##
    p.value : 0.7825838
##
##
    Result : Difference is not statistically significant.
```

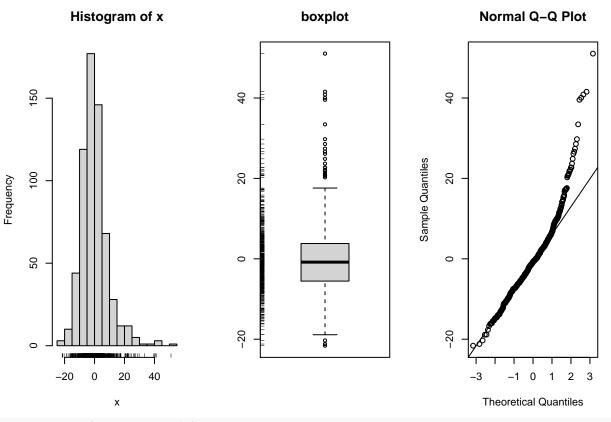
```
bf.test(formula = e ~ side_ct, # y~x
       data = bf_data_CEWL, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
  ______
##
##
    data : e and side_ct
##
##
    statistic : 0.951633
##
    num df : 1
    denom df : 575.2982
##
##
    p.value : 0.329714
##
##
    Result
              : Difference is not statistically significant.
bf.test(formula = e ~ side_mass, # y~x
       data = bf_data_CEWL, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
       )
##
##
    Brown-Forsythe Test (alpha = 0.05)
##
##
    data : e and side_mass
##
    statistic : 0.1110193
##
##
    num df : 1
    denom df : 627.9847
##
##
    p.value : 0.7390978
##
##
             : Difference is not statistically significant.
```

Equal variance is satisfied for all 5 continuous predictor variables.

Now check normality. 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(CEWL_mod_res$e)
```



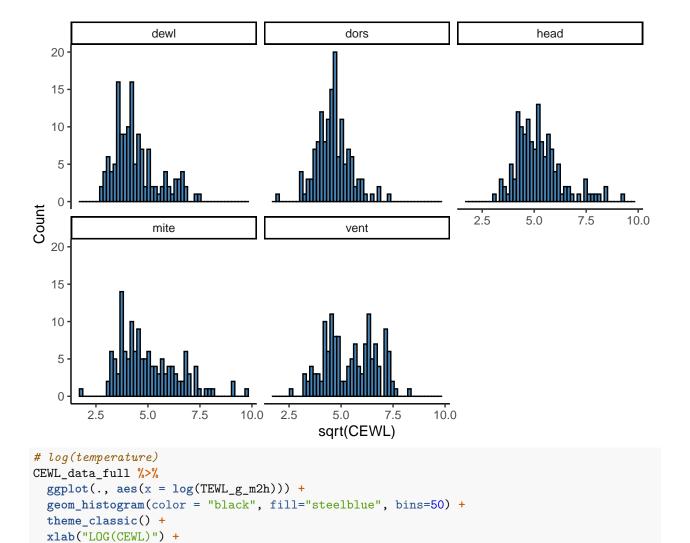
```
shapiro.test(CEWL_mod_res$e)
```

```
##
## Shapiro-Wilk normality test
##
## data: CEWL_mod_res$e
## W = 0.92375, p-value < 2.2e-16
not normal!</pre>
```

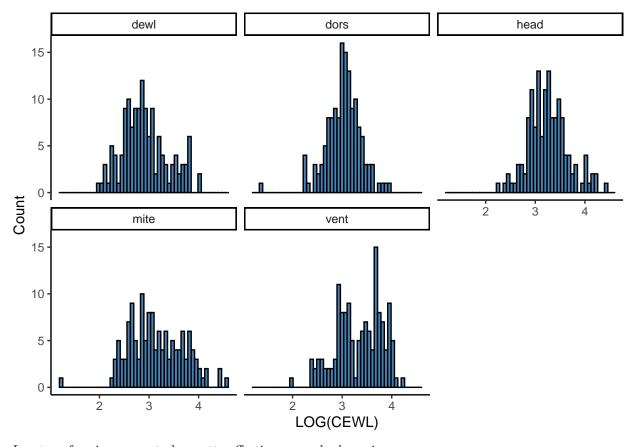
Test Transformations

Can I improve satisfaction of LM assumptions by transforming the dependent variable?

```
# sqrt(TEWL)
CEWL_data_full %>%
    ggplot(., aes(x = sqrt(TEWL_g_m2h))) +
    geom_histogram(color = "black", fill="steelblue", bins=50) +
    theme_classic() +
    xlab("sqrt(CEWL)") +
    ylab("Count") +
    facet_wrap(~region)
```



ylab("Count") +
facet_wrap(~region)



Log transforming seems to be pretty effective across body regions.

Transform & Re-Model

I will log-transform CEWL and see whether it makes the models satisfy LMM assumptions better.

Run CEWL model 4 with log-transformed CEWL:

```
# log-transformed model 3
CEWL_mod3_t <- lme4::lmer(data = CEWL_mod_dat,</pre>
                           log(TEWL_g_m2h) ~
                            region * (mass_g +
                                      temp_C_interpol +
                                      abs_humidity_g_m3_interpol) +
                             cloacal_temp_C + Solar_rad_Wm2_interpol +
                             (1|individual_ID))
summary(CEWL_mod3_t)
## Linear mixed model fit by REML ['lmerMod']
## Formula:
  log(TEWL_g_m2h) ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
       cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
##
##
      Data: CEWL_mod_dat
##
## REML criterion at convergence: 638.8
##
## Scaled residuals:
##
       Min
                1Q Median
                                 3Q
                                        Max
## -4.3124 -0.5294 -0.0337 0.4981
                                   3.8402
```

```
##
## Random effects:
## Groups
                 Name
                             Variance Std.Dev.
## individual_ID (Intercept) 0.0434
                                      0.2083
## Residual
                             0.1078
                                      0.3283
## Number of obs: 630, groups: individual_ID, 128
## Fixed effects:
##
                                          Estimate Std. Error t value
## (Intercept)
                                        -2.4491457 0.6712670 -3.649
## regiondors
                                         2.1145282 0.7695182
                                                                2.748
## regionhead
                                         2.2521822 0.7673448
                                                                2.935
## regionmite
                                         4.1254137 0.7758522
                                                                5.317
## regionvent
                                         2.6094520 0.7660117
                                                                3.407
                                         0.0635451 0.0137578
                                                               4.619
## mass_g
## temp_C_interpol
                                         0.0380952 0.0148088
                                                                2.572
## abs_humidity_g_m3_interpol
                                         0.1505318 0.0577753
                                                                2.605
## cloacal temp C
                                        0.0865716 0.0137910
                                                                6.277
## Solar_rad_Wm2_interpol
                                         0.0004175 0.0001799
                                                                2.320
## regiondors:mass g
                                        -0.0422830 0.0164664 -2.568
## regionhead:mass_g
                                        -0.0579755 0.0161973 -3.579
## regionmite:mass_g
                                        0.0011536 0.0167446
                                                                0.069
                                        -0.0172561 0.0163107 -1.058
## regionvent:mass_g
                                        -0.0905381 0.0162139 -5.584
## regiondors:temp_C_interpol
## regionhead:temp_C_interpol
                                        -0.0640707 0.0162917 -3.933
## regionmite:temp_C_interpol
                                        -0.0274852 0.0162453 -1.692
## regionvent:temp_C_interpol
                                        -0.0455998 0.0163389 -2.791
## regiondors:abs_humidity_g_m3_interpol 0.0125548 0.0646434
                                                                0.194
## regionhead:abs_humidity_g_m3_interpol -0.0119491 0.0644646 -0.185
## regionmite:abs_humidity_g_m3_interpol -0.3212611 0.0652199 -4.926
## regionvent:abs_humidity_g_m3_interpol -0.1073542 0.0644585 -1.665
##
## Correlation matrix not shown by default, as p = 22 > 12.
## Use print(x, correlation=TRUE) or
      vcov(x)
##
                     if you need it
# compare
anova(CEWL mod3 t, CEWL mod3)
## refitting model(s) with ML (instead of REML)
## Data: CEWL mod dat
## Models:
## CEWL_mod3_t: log(TEWL_g_m2h) ~ region * (mass_g + temp_C_interpol + abs_humidity_g_m3_interpol) +
## CEWL_mod3_t:
                 cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
## CEWL mod3: TEWL g m2h ~ region * (mass g + temp C interpol + abs humidity g m3 interpol) +
## CEWL mod3:
                 cloacal_temp_C + Solar_rad_Wm2_interpol + (1 | individual_ID)
              npar
                      AIC
                             BIC
                                   logLik deviance Chisq Df Pr(>Chisq)
                24 549.8 656.5 -250.92
                                             501.8
## CEWL_mod3_t
                24 4805.9 4912.6 -2378.96
## CEWL_mod3
                                            4757.9
                                                       0 0
```

Re-Check Assumptions (transformed model 3)

First, get residuals:

```
CEWL_t_mod_res <- CEWL_mod_dat %>%
mutate(y_hat = predict(CEWL_mod3_t),
    e = residuals(CEWL_mod3_t))
```

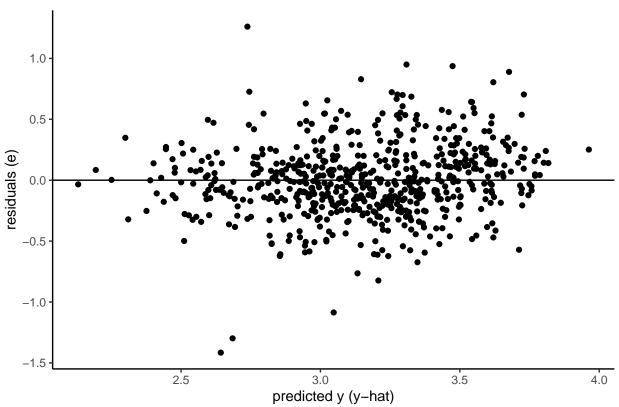
Linearity and Equal Variance

Is the function **linear**? Is there **equal** variance of the residuals? The residuals should be homoskedactic relative to y_hat (or x). We don't care if there is a relationship between the residuals \sim dependent variable (actual y).

Plotting residuals shows us whether the data meets linearity and equal variance assumptions:

```
ggplot(data = CEWL_t_mod_res, aes(x = y_hat, y = e)) +
  geom_point() +
  theme_classic() +
  xlab("predicted y (y-hat)") +
  ylab("residuals (e)") +
  ggtitle("CEWL Model 13 Residuals") +
  geom_hline(yintercept = 0)
```

CEWL Model 13 Residuals



It looks much much better. :) Equal error variance doesn't look perfect, but there's no more fan shape.

Brown-Forsythe test to statistically check equal variance:

H0: normally distributed (non-sig test is GOOD) HA: NOT normally distributed (reject nul == assumption not satisfied)

```
# need to create the right data & format first
bf_data_CEWL_t <- CEWL_t_mod_res %>%
dplyr::mutate(middle_mass = median(mass_g), # mass
```

```
side_mass = as.factor(mass_g > middle_mass),
               # solar radiation
               middle_sorad = median(Solar_rad_Wm2_interpol),
               side_sorad = as.factor(Solar_rad_Wm2_interpol > middle_sorad),
               # temperature
               middle_temp = median(temp_C_interpol),
               side_temp = as.factor(temp_C_interpol > middle_temp),
               # absolute humidity
               middle_absh = median(abs_humidity_g_m3_interpol),
               side_absh = as.factor(abs_humidity_g_m3_interpol > middle_absh),
               middle_ct = median(cloacal_temp_C),
               side_ct = as.factor(cloacal_temp_C > middle_ct)
# now run test
bf.test(formula = e ~ side_absh, # y~x
       data = bf_data_CEWL_t, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_absh
##
    statistic : 0.1208918
##
##
    num df : 1
##
    denom df : 626.2323
    p.value : 0.7281862
##
##
    Result : Difference is not statistically significant.
bf.test(formula = e ~ side_temp, # y~x
       data = bf_data_CEWL_t, # dataframe
       alpha = 0.05, # default 0.05
       na.rm = TRUE, # remove missing data before running?
       verbose = TRUE # print output to console?
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_temp
##
##
    statistic : 0.04963674
             : 1
##
    num df
    denom df : 579.6971
##
##
    p.value : 0.8237751
##
##
             : Difference is not statistically significant.
```

```
bf.test(formula = e ~ side_sorad, # y~x
      data = bf_data_CEWL_t, # dataframe
      alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
      verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side sorad
##
##
    statistic : 0.04627073
##
    num df : 1
    denom df : 627.9542
##
##
    p.value : 0.8297543
##
##
    Result : Difference is not statistically significant.
bf.test(formula = e ~ side_ct, # y~x
      data = bf_data_CEWL_t, # dataframe
      alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
      verbose = TRUE # print output to console?
##
##
    Brown-Forsythe Test (alpha = 0.05)
## -----
##
    data : e and side_ct
##
    statistic : 0.7705684
##
    num df
##
##
    denom df : 614.2173
##
    p.value : 0.3803845
##
##
    Result : Difference is not statistically significant.
## -----
bf.test(formula = e ~ side mass, # y~x
      data = bf_data_CEWL_t, # dataframe
      alpha = 0.05, # default 0.05
      na.rm = TRUE, # remove missing data before running?
      verbose = TRUE # print output to console?
##
    Brown-Forsythe Test (alpha = 0.05)
##
## -----
##
    data : e and side_mass
##
##
    statistic : 0.1630792
##
    num df : 1
    denom df : 623.8621
##
```

```
## p.value : 0.6864756
```

##

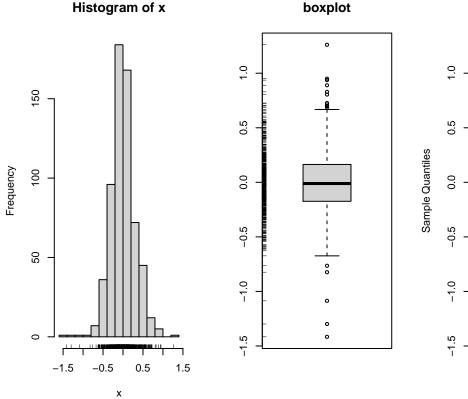
Result : Difference is not statistically significant.

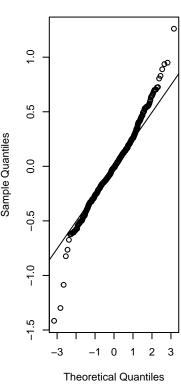
Equal variance is still satisfied for all continuous predictor variables.

Now check normality. 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(CEWL_t_mod_res$e)
```





Normal Q-Q Plot

```
shapiro.test(CEWL_t_mod_res$e)
```

```
##
## Shapiro-Wilk normality test
##
## data: CEWL_t_mod_res$e
## W = 0.98182, p-value = 4.674e-07
```

Still not statistically normal... but the distribution looks a lot better.

Conclusion

The best CEWL model should use log-transformed CEWL because this greatly improves the model based on AIC and it allows the model to satisfy the linearity assumption of LMM.

```
# save log model 3 summary object
CEWL_best_t_mod <- summary(CEWL_mod3_t)
# extract stats table from summary object</pre>
```

```
CEWL_best_t_mod_vals <- data.frame(CEWL_best_t_mod$coefficients)
# export
write.csv(CEWL_best_t_mod_vals, "CEWL_best_mod_vals.csv")</pre>
```

What to Present in the Paper

- figures (exported)
- $\bullet \ \, \mathrm{hct} \,\, \mathrm{SLR}$
- best osml mod (transformed?)
- best CEWL mod (CEWL transformed)