Sleep Bout Analysis

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```
bouts <- read.csv("../data/WT_McKO_combined_bouts.csv",
                    fileEncoding = 'UTF-8-BOM')
bouts <- bouts %>% mutate(Time = mdy_hms(Time),
                          Sleep.Stage = as.factor(Sleep.Stage),
                          Genotype = as.factor(Genotype),
                          Transmitter = as.factor(Transmitter))

bouts <- bouts %>% mutate(Hour = hour(Time))
bouts <- bouts %>% mutate(ZT = Time + hours(18))
bouts <- bouts %>% mutate(ZT = hour(ZT))

bouts <- bouts %>% dplyr::mutate(phase = as.factor(ifelse(ZT>11, "dark", "light")))

head(bouts)
```

```
##   Bout Epoch                Time Sleep.Stage Count Duration Genotype
## 1    1  2216 2019-02-10 18:00:00           S    36      360     McKO
## 2    2  2252 2019-02-10 18:06:00           W     2       20     McKO
## 3    3  2254 2019-02-10 18:06:20           S     6       60     McKO
## 4    4  2260 2019-02-10 18:07:20           W     4       40     McKO
## 5    5  2264 2019-02-10 18:08:00           S     4       40     McKO
## 6    6  2268 2019-02-10 18:08:40           W    17      170     McKO
##   Transmitter Day Hour ZT phase
## 1      988823   1   18 12  dark
## 2      988823   1   18 12  dark
## 3      988823   1   18 12  dark
## 4      988823   1   18 12  dark
## 5      988823   1   18 12  dark
## 6      988823   1   18 12  dark
```

```
SumStats_daily <- bouts %>% group_by(Genotype, Transmitter, Sleep.Stage, Day, phase) %>%
  summarise(n_bouts = n(),
            total_duration = sum(Duration),
            min_bout_length = min(Duration),
            mean_bout_length = mean(Duration),
            max_bout_length = max(Duration),
            sd_bout_length = sd(Duration))
```

```
## `summarise()` has grouped output by 'Genotype', 'Transmitter', 'Sleep.Stage', 'Day'. You can overrid
```

```r
head(SumStats_daily)
```

```
## # A tibble: 6 x 11
## # Groups:   Genotype, Transmitter, Sleep.Stage, Day [3]
##    Genotype Transmitter Sleep.Stage   Day phase n_bouts total_duration
##    <fct>    <fct>       <fct>       <int> <fct>   <int>          <int>
## 1 McKO     1050240     P               1 dark       52           1770
## 2 McKO     1050240     P               1 light      86           2990
## 3 McKO     1050240     P               2 dark       37           1290
## 4 McKO     1050240     P               2 light     146           4050
## 5 McKO     1050240     P               3 dark      111           1690
## 6 McKO     1050240     P               3 light     118           3220
## # ... with 4 more variables: min_bout_length <int>, mean_bout_length <dbl>,
## #   max_bout_length <int>, sd_bout_length <dbl>
```

```r
SumStats_phase <- SumStats_daily %>% group_by(Genotype, Transmitter, Sleep.Stage, phase) %>%
  summarise(daily_bouts = mean(n_bouts),
            daily_duration = mean(total_duration),
            min_bout = min(min_bout_length),
            mean_bout = mean(mean_bout_length),
            max_bout = max(max_bout_length))
```

```
## `summarise()` has grouped output by 'Genotype', 'Transmitter', 'Sleep.Stage'. You can override using
```

```r
head(SumStats_phase)
```

```
## # A tibble: 6 x 9
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##    Genotype Transmitter Sleep.Stage phase daily_bouts daily_duration min_bout
##    <fct>    <fct>       <fct>       <fct>       <dbl>          <dbl>    <int>
## 1 McKO     1050240     P           dark         66.7          1583.       10
## 2 McKO     1050240     P           light       117.           3420        10
## 3 McKO     1050240     S           dark         90.3         15800        10
## 4 McKO     1050240     S           light       169           29763.       10
## 5 McKO     1050240     W           dark        108.          25880        10
## 6 McKO     1050240     W           light       138            9907.       10
## # ... with 2 more variables: mean_bout <dbl>, max_bout <int>
```

```r
SumStats_phase_grps <- SumStats_phase %>% group_by(Genotype, Sleep.Stage, phase) %>%
  summarise(n = n(),
            daily_bout_len = mean(daily_bouts),
            daily_dur = mean(daily_duration),
            avg_bout_length = mean(min_bout),
            min_bout_length = min(min_bout),
            max_bout_length = max(max_bout))
```

```
## `summarise()` has grouped output by 'Genotype', 'Sleep.Stage'. You can override using the `.groups`
```

```r
SumStats_phase_grps
```

```
## # A tibble: 16 x 9
## # Groups:   Genotype, Sleep.Stage [8]
##    Genotype Sleep.Stage phase     n daily_bout_len daily_dur avg_bout_length
##    <fct>    <fct>       <fct> <int>          <dbl>     <dbl>           <dbl>
##  1 McKO     P           dark      9           83.8     1636.              10
##  2 McKO     P           light     9           96.5     3061.              10
##  3 McKO     S           dark      9          191.     18047.              10
##  4 McKO     S           light     9          169.     30235.              10
##  5 McKO     W           dark      9          222.     23622.              10
##  6 McKO     W           light     9          146.      9634.              10
##  7 McKO     X           dark      9           10.9      109.              10
##  8 McKO     X           light     9            5.72      57.2             10
##  9 WT       P           dark     10           88.3     1881.              10
## 10 WT       P           light    10           85.6     3210.              10
## 11 WT       S           dark     10          196.     17024.              10
## 12 WT       S           light    10          174.     28213.              10
## 13 WT       W           dark     10          232.     24289.              10
## 14 WT       W           light    10          151.     11502.              10
## 15 WT       X           dark     10           17.3      173.              10
## 16 WT       X           light    10            9.32      93.2             10
## # ... with 2 more variables: min_bout_length <int>, max_bout_length <int>
```

```
head(SumStats_phase)
```

```
## # A tibble: 6 x 9
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##   Genotype Transmitter Sleep.Stage phase daily_bouts daily_duration min_bout
##   <fct>    <fct>       <fct>       <fct>       <dbl>          <dbl>    <int>
## 1 McKO     1050240     P           dark         66.7          1583.       10
## 2 McKO     1050240     P           light       117.           3420        10
## 3 McKO     1050240     S           dark         90.3         15800        10
## 4 McKO     1050240     S           light       169           29763.       10
## 5 McKO     1050240     W           dark        108.          25880        10
## 6 McKO     1050240     W           light       138            9907.       10
## # ... with 2 more variables: mean_bout <dbl>, max_bout <int>
```

```
lm_n_bouts <- lmer(daily_bouts ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_phase
plot(lm_n_bouts, type=c("p","smooth"), col.line=1)
```
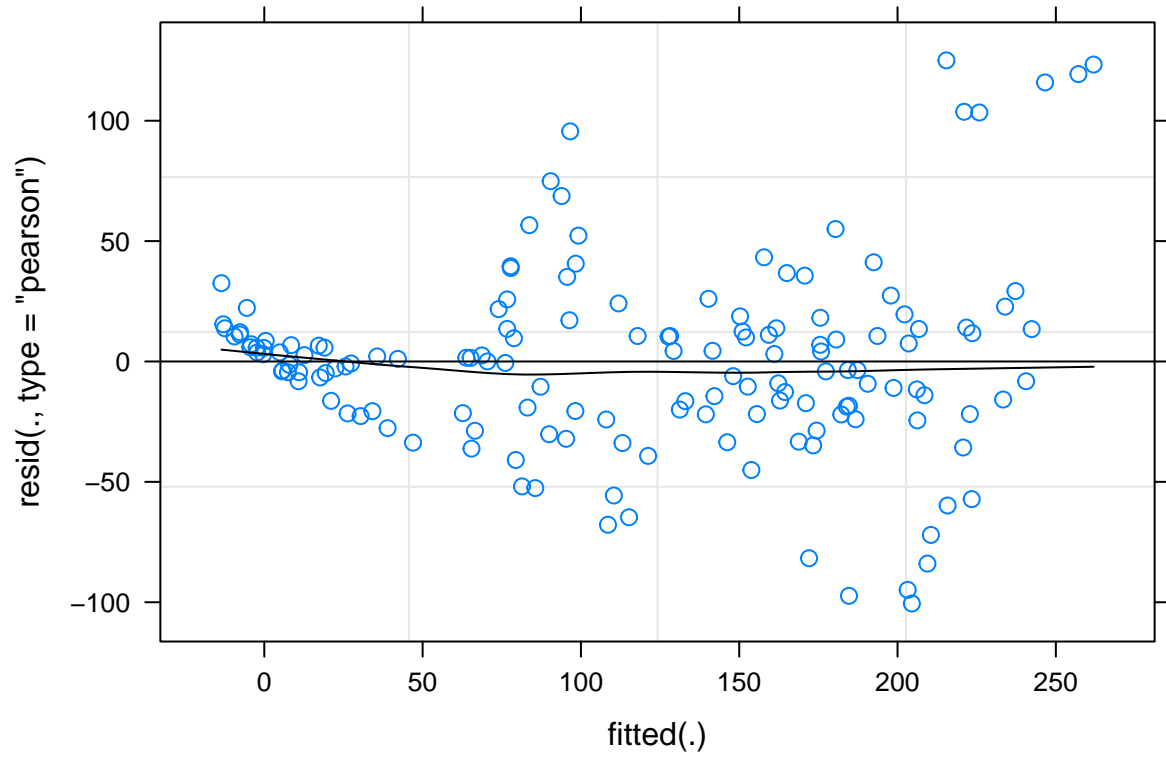
```
lattice::qqmath(lm_n_bouts)
```
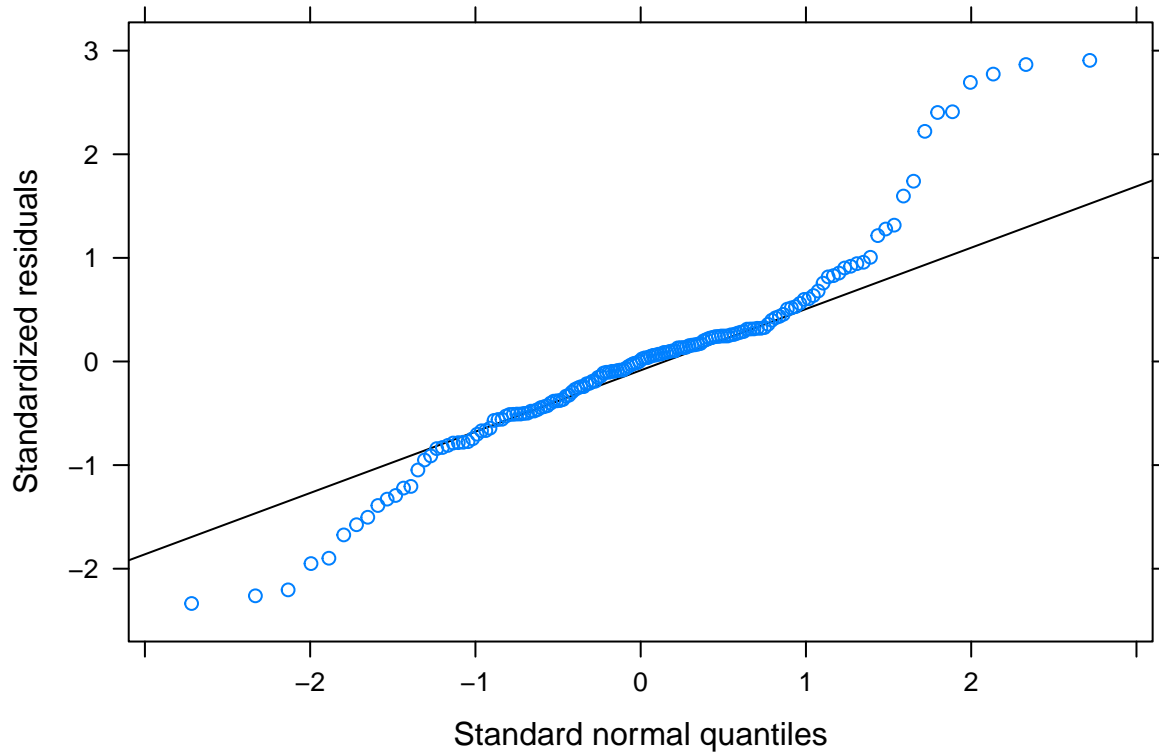
```
anova(lm_n_bouts)
```
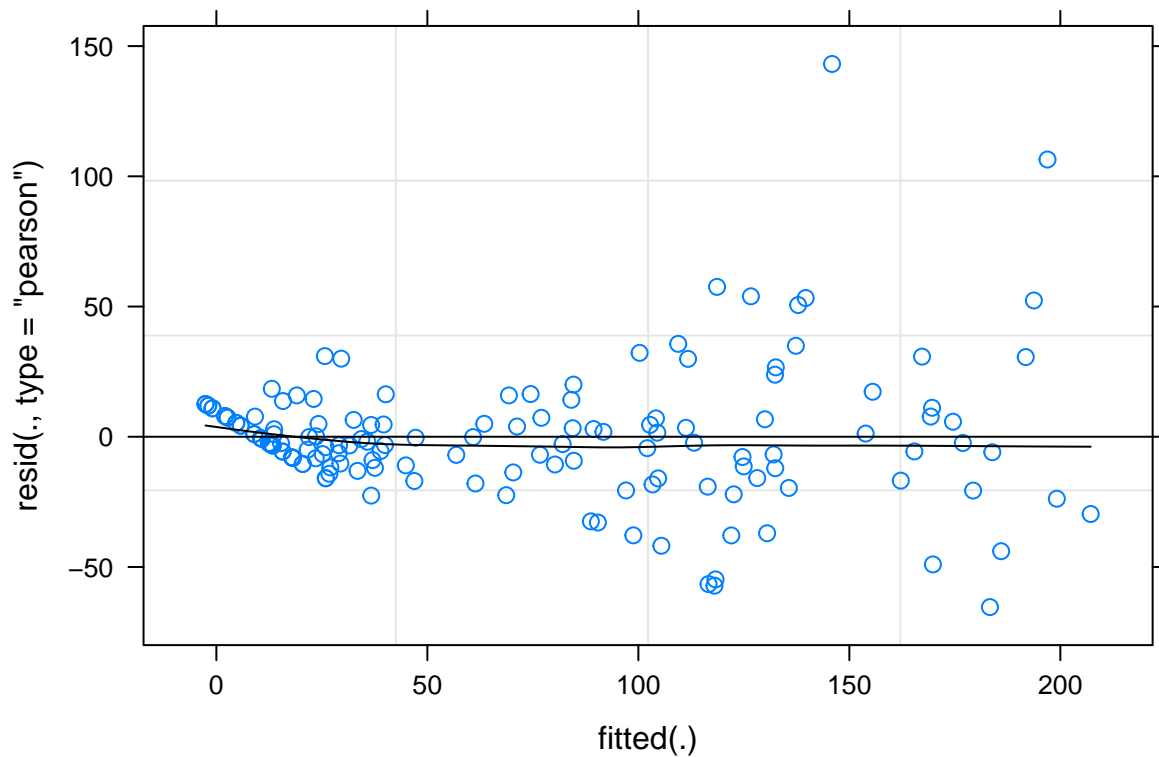
```
## Analysis of Variance Table
##                         npar Sum Sq Mean Sq  F value
## Sleep.Stage                3 813578  271193 146.4365
## phase                      1  25066   25066  13.5350
## Genotype                   1    176     176   0.0948
## Sleep.Stage:phase          3  38793   12931   6.9824
## Sleep.Stage:Genotype       3    634     211   0.1142
## phase:Genotype             1    354     354   0.1913
## Sleep.Stage:phase:Genotype 3    304     101   0.0547
```

```
emmeans(lm_n_bouts, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```
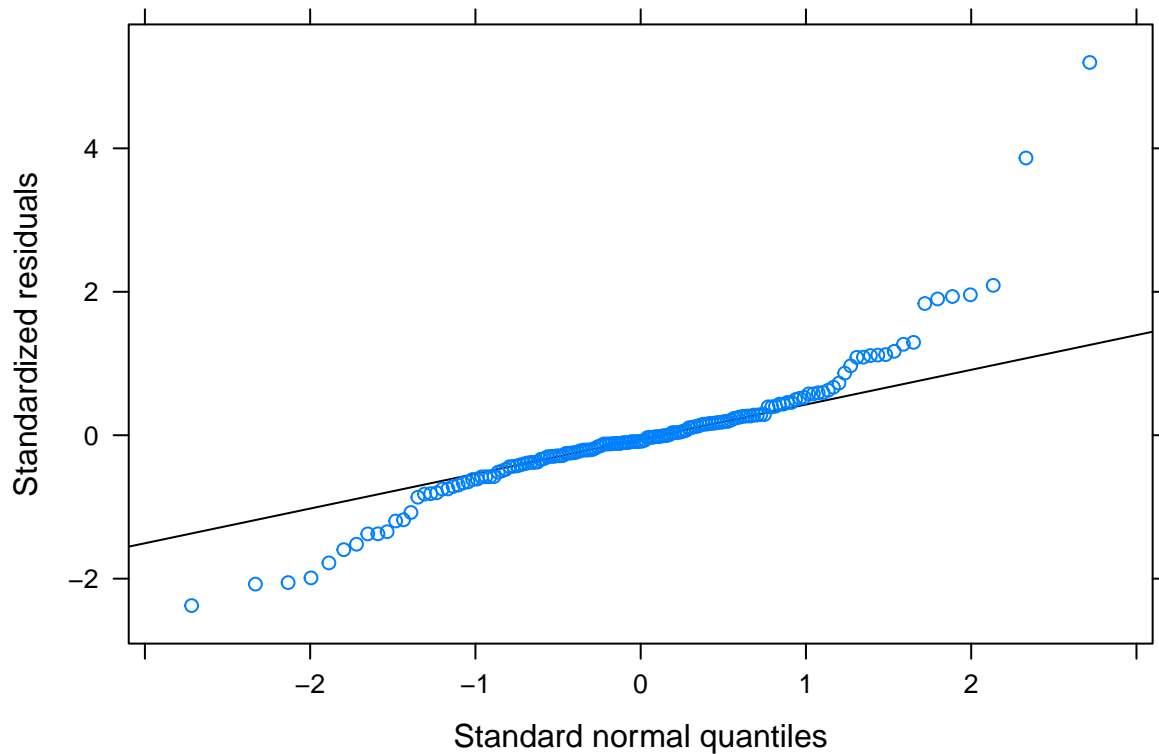
```
## Sleep.Stage = P, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -4.46 21.9 109  -0.203  0.8395
##
## Sleep.Stage = S, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -5.49 21.9 109  -0.250  0.8030
##
## Sleep.Stage = W, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT   -10.43 21.9 109  -0.475  0.6356
```

```
## 
## Sleep.Stage = X, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -6.40 21.9 109  -0.292  0.7711
## 
## Sleep.Stage = P, phase = light:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    10.93 21.9 109   0.498  0.6195
## 
## Sleep.Stage = S, phase = light:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -4.96 21.9 109  -0.226  0.8215
## 
## Sleep.Stage = W, phase = light:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -4.68 21.9 109  -0.213  0.8316
## 
## Sleep.Stage = X, phase = light:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    -3.60 21.9 109  -0.164  0.8699
## 
## Degrees-of-freedom method: kenward-roger
```

```r
lm_mean_length <- lmer(mean_bout ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_pha
plot(lm_mean_length, type=c("p","smooth"), col.line=1)
```

```
lattice::qqmath(lm_mean_length)
```



```
anova(lm_mean_length)
```

```
## Analysis of Variance Table
##                         npar Sum Sq Mean Sq  F value
## Sleep.Stage                3 437815  145938 192.4372
## phase                      1   1922    1922   2.5345
## Genotype                   1     35      35   0.0462
## Sleep.Stage:phase          3  76820   25607  33.7653
## Sleep.Stage:Genotype       3   3306    1102   1.4533
## phase:Genotype             1      1       1   0.0018
## Sleep.Stage:phase:Genotype 3    978     326   0.4298
```

```
emmeans(lm_mean_length, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT   -5.146 13.6 120  -0.378  0.7062
##
## Sleep.Stage = S, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT    9.419 13.6 120   0.692  0.4905
##
```

```
## Sleep.Stage = W, phase = dark:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT    0.767 13.6 120   0.056  0.9552
##
## Sleep.Stage = X, phase = dark:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT    0.000 13.6 120   0.000  1.0000
##
## Sleep.Stage = P, phase = light:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT   -5.784 13.6 120  -0.425  0.6718
##
## Sleep.Stage = S, phase = light:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT   24.825 13.6 120   1.823  0.0708
##
## Sleep.Stage = W, phase = light:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT  -12.489 13.6 120  -0.917  0.3610
##
## Sleep.Stage = X, phase = light:
##  contrast  estimate    SE  df t.ratio p.value
##  McKO - WT    0.000 13.6 120   0.000  1.0000
##
## Degrees-of-freedom method: kenward-roger
```

```r
lm_max_length <- lmer(max_bout ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_phase
#plot(lm_max_length, type=c("p","smooth"), col.line=1)
#lattice::qqmath(lm_max_length)
anova(lm_max_length)
```

```
## Analysis of Variance Table
##                        npar   Sum Sq  Mean Sq F value
## Sleep.Stage               3 88814079 29604693 63.6687
## phase                     1   843042   843042  1.8131
## Genotype                  1   170326   170326  0.3663
## Sleep.Stage:phase         3  7416195  2472065  5.3165
## Sleep.Stage:Genotype      3   244168    81389  0.1750
## phase:Genotype            1   192675   192675  0.4144
## Sleep.Stage:phase:Genotype 3  313821   104607  0.2250
```

```r
emmeans(lm_max_length, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT   -18.78  322 133  -0.058  0.9536
##
## Sleep.Stage = S, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
##  McKO - WT  -425.78  322 133  -1.323  0.1881
##
## Sleep.Stage = W, phase = dark:
##  contrast  estimate   SE  df t.ratio p.value
```

```
## McKO - WT  -162.78 322 133  -0.506  0.6139
##
## Sleep.Stage = X, phase = dark:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     0.00 322 133   0.000  1.0000
##
## Sleep.Stage = P, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT   -46.56 322 133  -0.145  0.8852
##
## Sleep.Stage = S, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     4.00 322 133   0.012  0.9901
##
## Sleep.Stage = W, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     5.67 322 133   0.018  0.9860
##
## Sleep.Stage = X, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     0.00 322 133   0.000  1.0000
##
## Degrees-of-freedom method: kenward-roger
```

```r
lm_duration <- lmer(daily_duration ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_
```

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

```r
#plot(lm_duration, type=c("p","smooth"), col.line=1)
#lattice::qqmath(lm_duration)
anova(lm_duration)
```

```
## Analysis of Variance Table
##                         npar    Sum Sq    Mean Sq    F value
## Sleep.Stage                3 1.4569e+10 4856432337 1443.3593
## phase                      1 3.5464e+05     354638    0.1054
## Genotype                   1 1.5500e+02        155    0.0000
## Sleep.Stage:phase          3 3.0043e+09 1001440234  297.6337
## Sleep.Stage:Genotype       3 3.7574e+07   12524544    3.7224
## phase:Genotype             1 3.6430e+03       3643    0.0011
## Sleep.Stage:phase:Genotype 3 5.7953e+06    1931773    0.5741
```

```r
emmeans(lm_duration, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     -245 843 136  -0.290  0.7719
##
## Sleep.Stage = S, phase = dark:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     1023 843 136   1.214  0.2268
##
```

```
## Sleep.Stage = W, phase = dark:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     -667 843 136  -0.792  0.4300
##
## Sleep.Stage = X, phase = dark:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT      -64 843 136  -0.076  0.9396
##
## Sleep.Stage = P, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     -149 843 136  -0.177  0.8596
##
## Sleep.Stage = S, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT     2022 843 136   2.399  0.0178
##
## Sleep.Stage = W, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT    -1868 843 136  -2.216  0.0284
##
## Sleep.Stage = X, phase = light:
##  contrast  estimate  SE  df t.ratio p.value
##  McKO - WT      -36 843 136  -0.043  0.9660
##
## Degrees-of-freedom method: kenward-roger
```

```r
SumStats_phase$Sleep.Stage <- recode_factor(SumStats_phase$Sleep.Stage, S = "SWS", P = "REM", X = "Arti

daily_duration_plot <- SumStats_phase %>% filter(Sleep.Stage != "Artifact") %>%
  ggplot(aes(x=phase, y=(daily_duration/3600))) +
  facet_wrap(~Sleep.Stage, scales="free_y") +
  geom_boxplot(aes(color=Genotype)) +
  ylab("Total daily duration (h)") +
  theme_bw()

daily_duration_plot
```
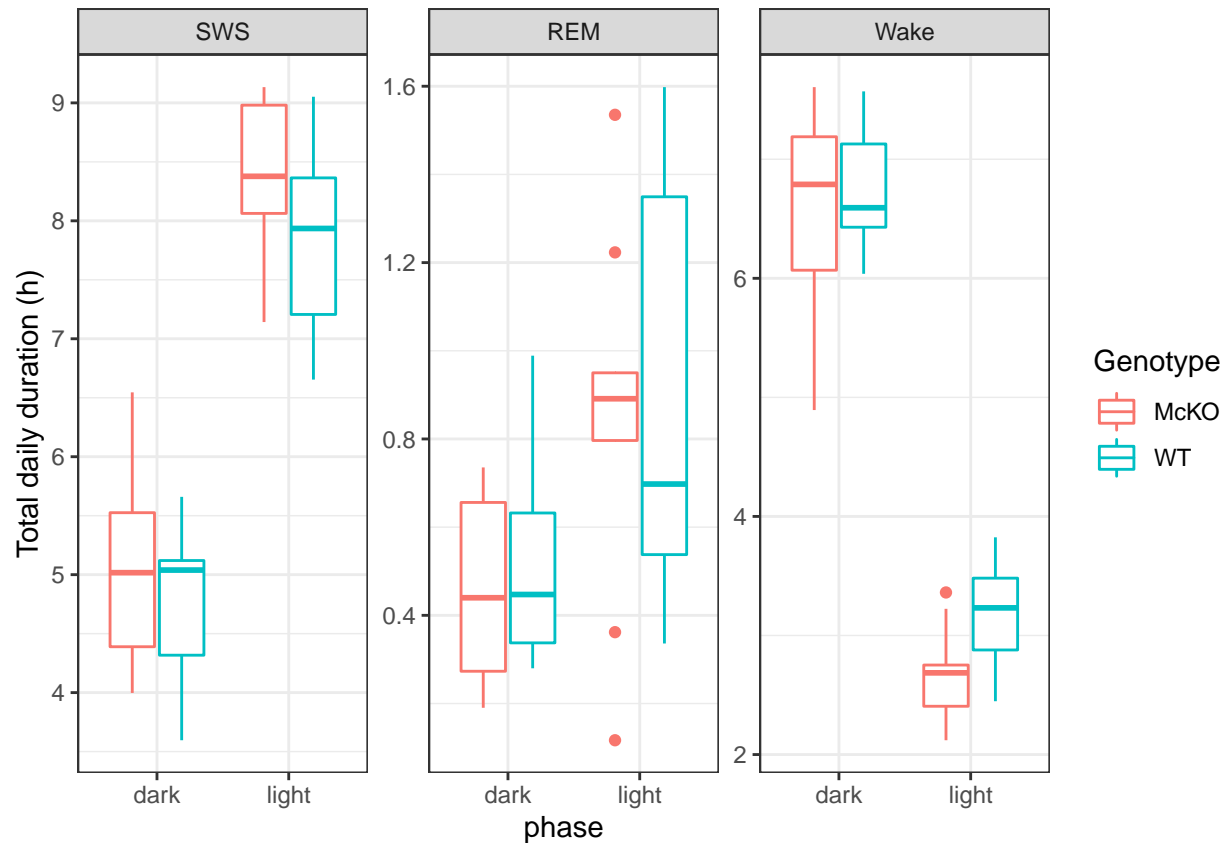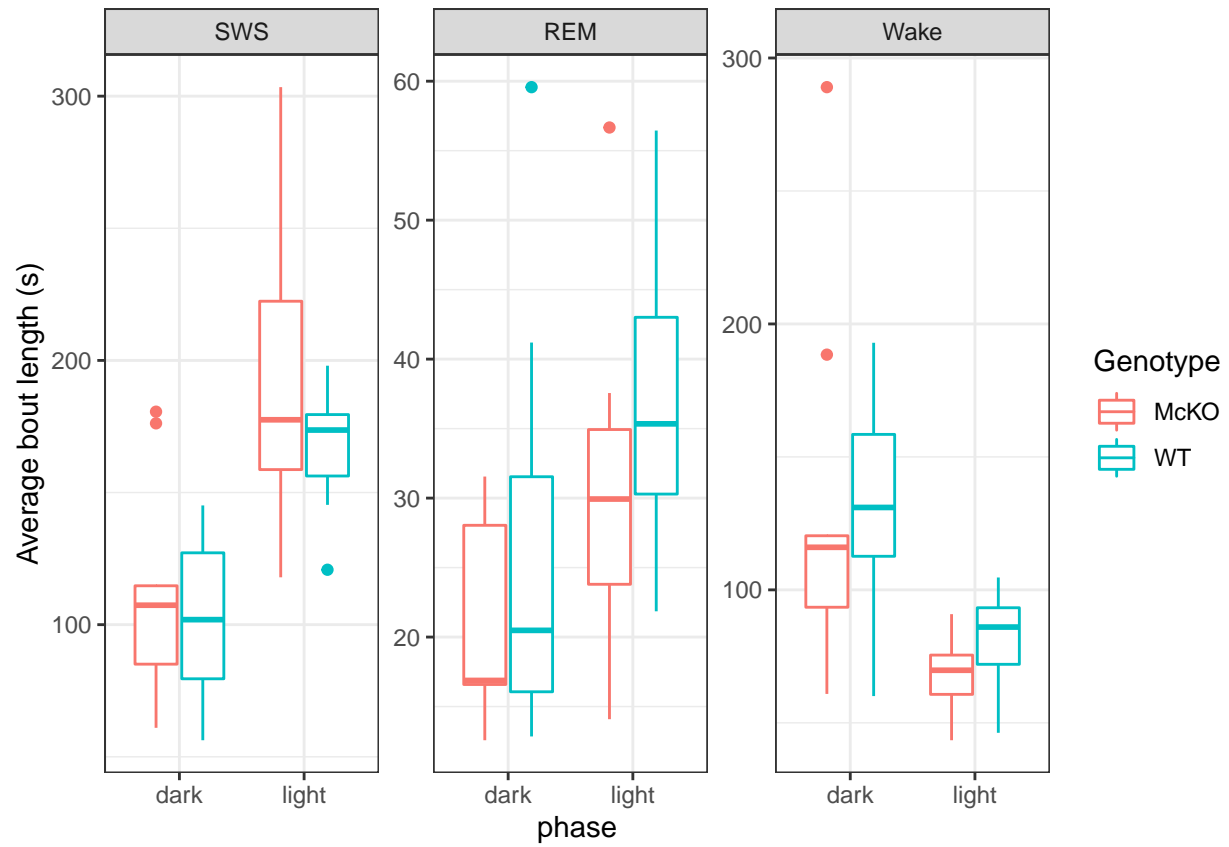
```
#ggsave(filename="../figures/daily_duration_plot.svg", plot=daily_duration_plot, height=4, width=7)

length_phase_plot <- SumStats_phase %>% filter(Sleep.Stage != "Artifact") %>%
  ggplot(aes(x=phase, y=mean_bout)) +
  facet_wrap(~Sleep.Stage, scales="free_y") +
  geom_boxplot(aes(color=Genotype)) +
  ylab("Average bout length (s)") +
  theme_bw()

length_phase_plot
```

```
#ggsave(filename="../figures/length_phase_plot.svg", plot=length_phase_plot, height=4, width=7)
```