

# 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 override
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>

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
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>
```

```
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(mean_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`
SumStats_phase_grps
```

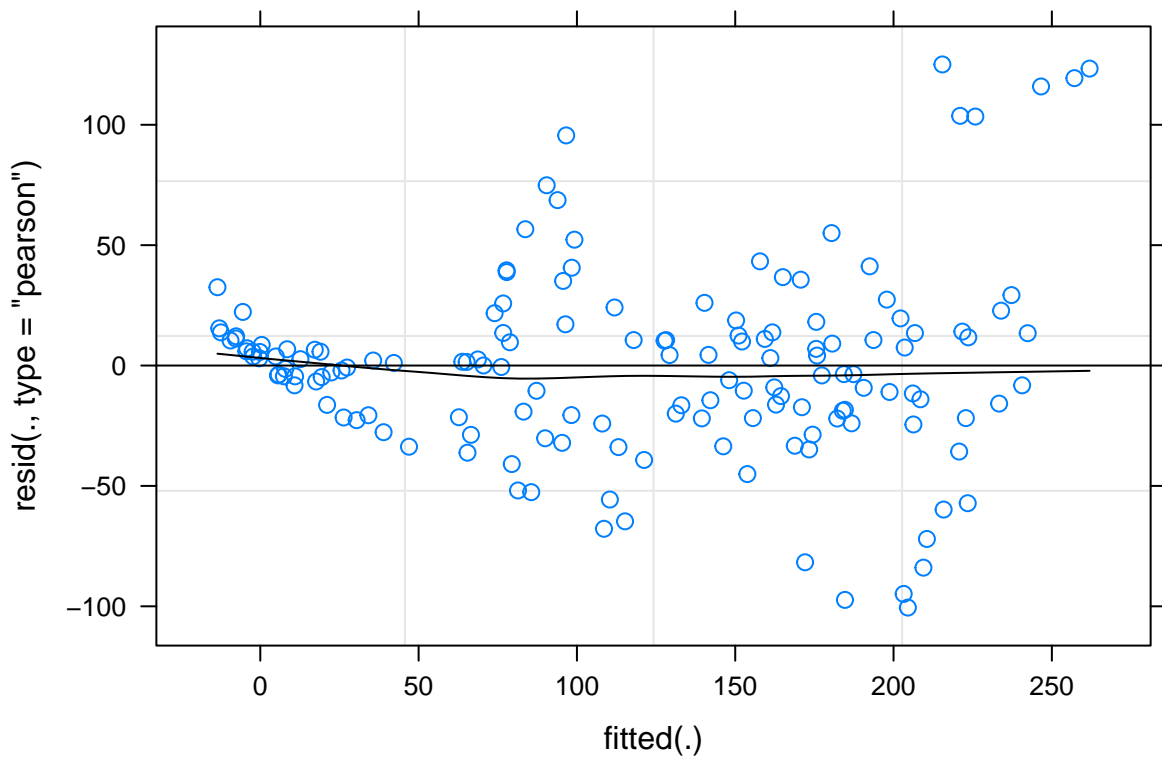
```
## # A tibble: 16 x 9
## # Groups:   Genotype, Sleep.Stage [8]
##   Genotype Sleep.Stage phase   n daily_bout_len daily_dur avg_bout_length
##   <fct>    <fct>      <fct> <int>    <dbl>          <dbl>    <dbl>
## 1 McKO     P         dark    9      83.8          1636.     21.1
## 2 McKO     P         light    9      96.5          3061.     31.0
## 3 McKO     S         dark    9     191.          18047.    111.
## 4 McKO     S         light    9     169.          30235.    191.
## 5 McKO     W         dark    9     222.          23622.    130.
## 6 McKO     W         light    9     146.          9634.     68.8
## 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.     26.2
## 10 WT       P      light     10     85.6     3210.     36.8
## 11 WT       S      dark     10    196.     17024.    101.
## 12 WT       S      light     10    174.     28213.    166.
## 13 WT       W      dark     10    232.     24289.    129.
## 14 WT       W      light     10    151.     11502.    81.3
## 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>
```

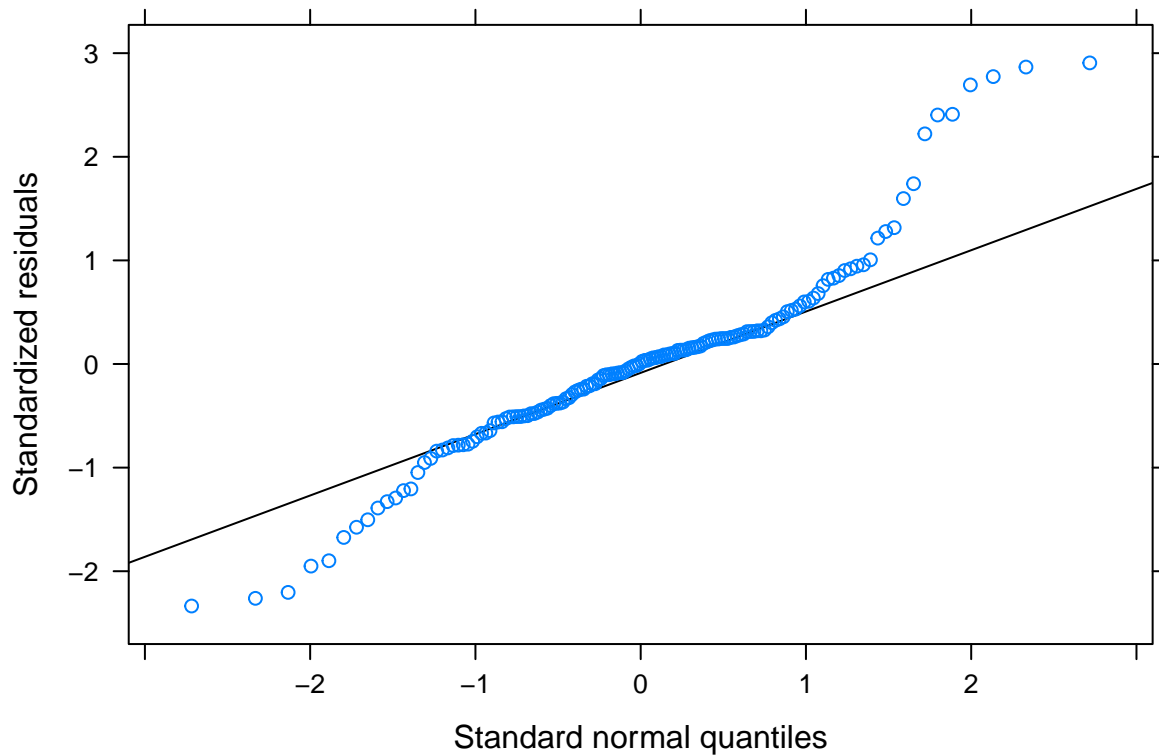
```
head(SumStats_phase)
```

```
## # 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)
```



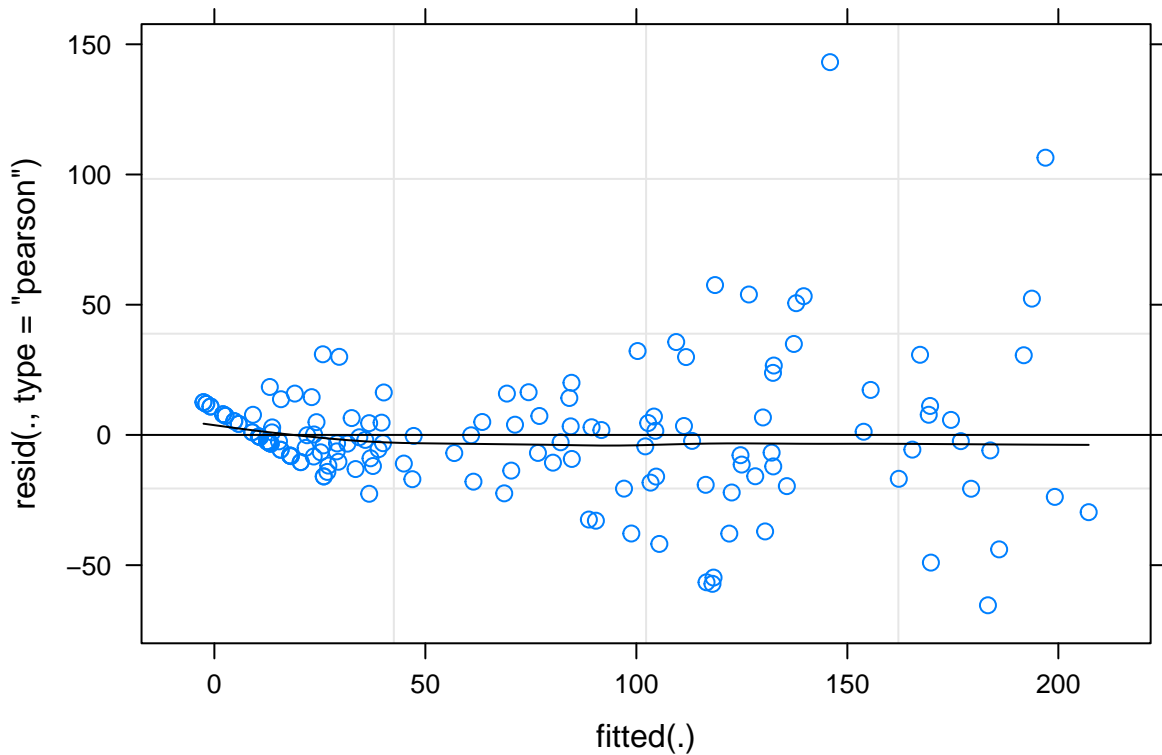
```
anova(lm_n_bouts)
```

```
## 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
```

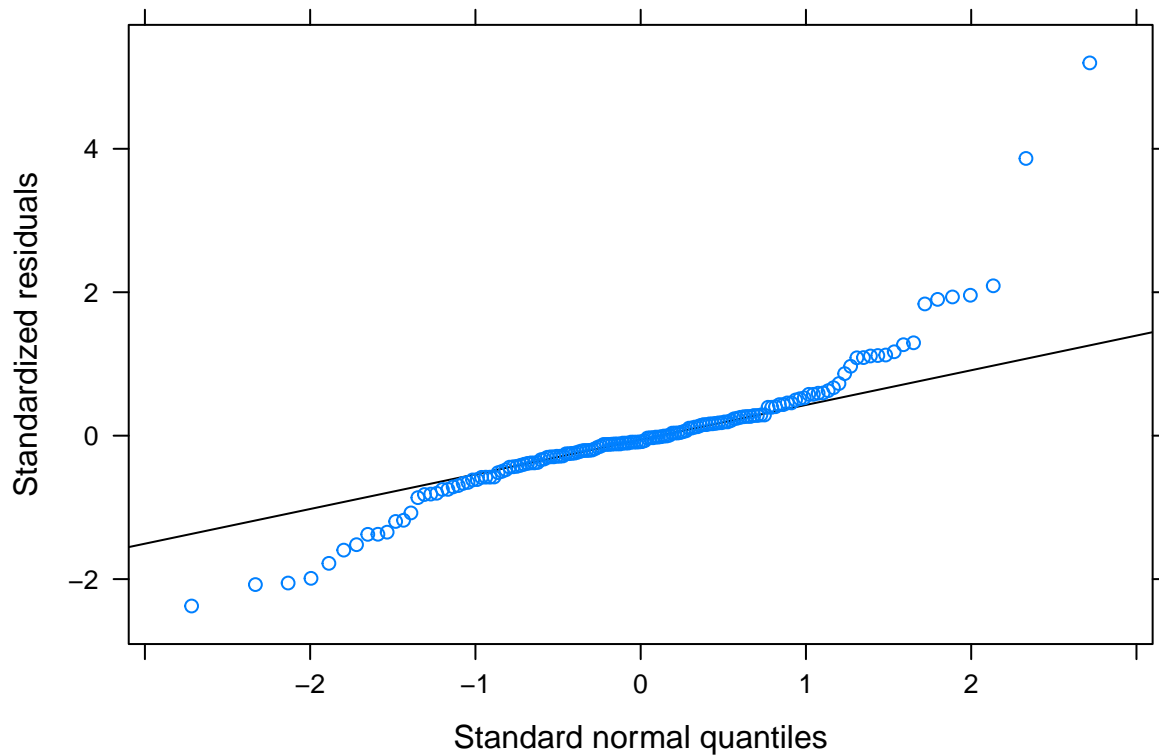
```
emmeans(lm_n_bouts, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

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



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



```
anova(lm_mean_length)
```

```
## Analysis of Variance Table
```

	npair	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
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

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

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

```



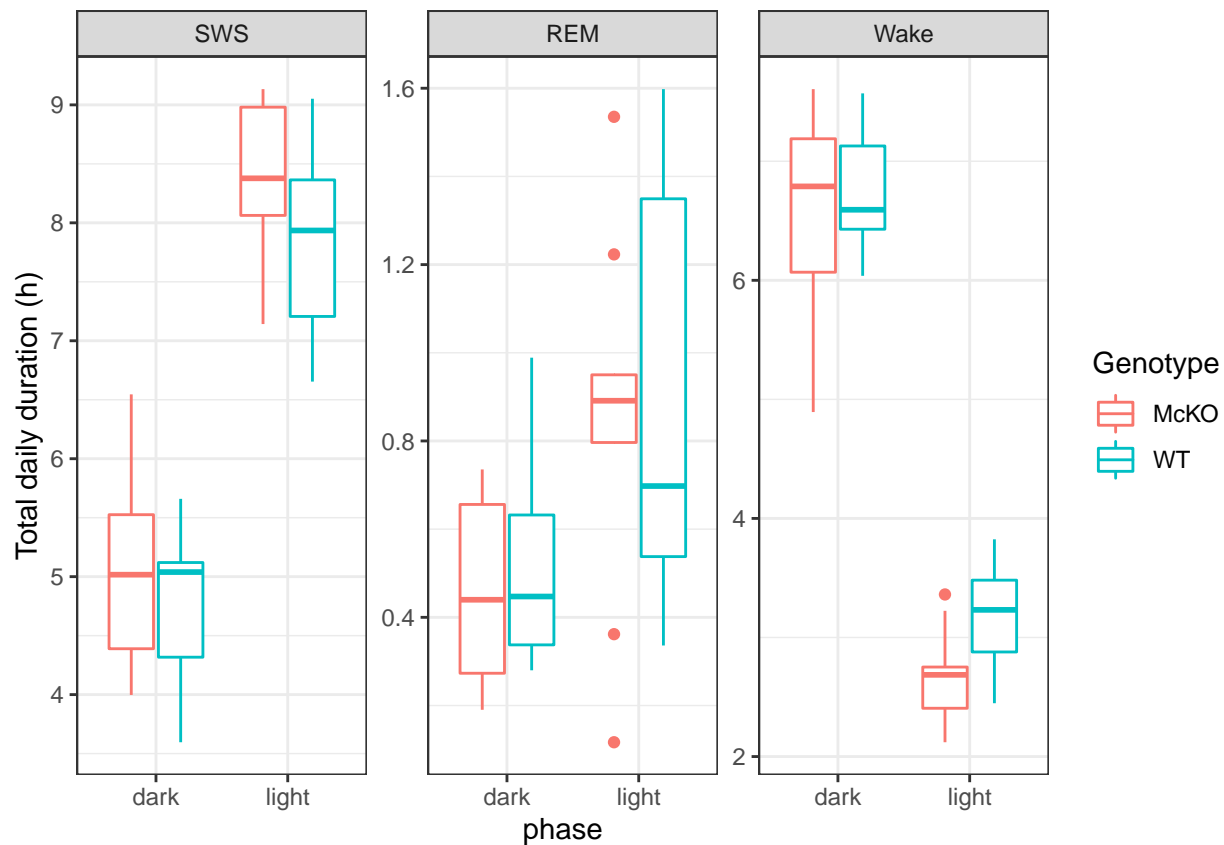
```

## 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
SumStats_phase$Sleep.Stage <- recode_factor(SumStats_phase$Sleep.Stage, S = "SWS", P = "REM", X = "Artifact")

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

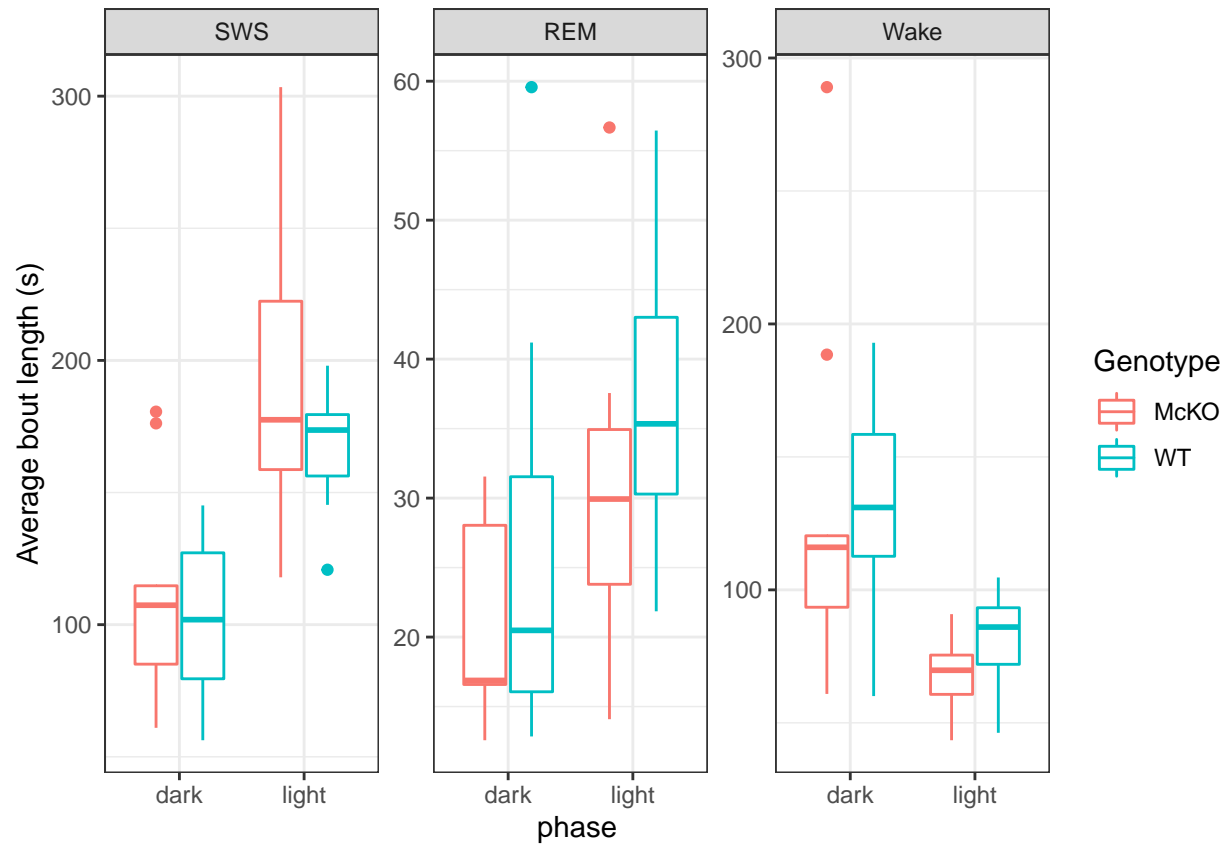
```



```
#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()
```

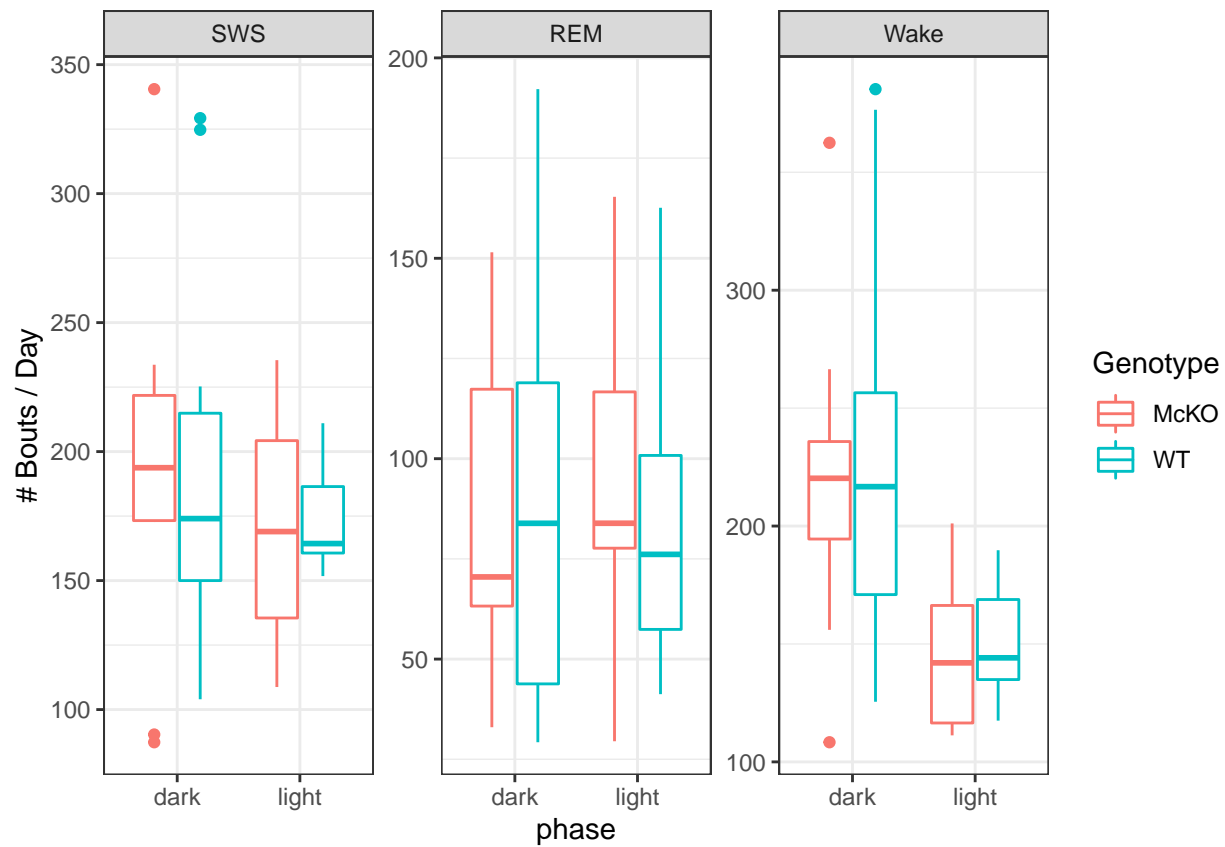
```
length_phase_plot
```



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

```
daily_n_bout_plot <- SumStats_phase %>% filter(Sleep.Stage != "Artifact") %>%
  ggplot(aes(x=phase, y=daily_bouts)) +
  facet_wrap(~Sleep.Stage, scales="free_y") +
  geom_boxplot(aes(color=Genotype)) +
  ylab("# Bouts / Day") +
  theme_bw()
```

```
daily_n_bout_plot
```



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