mrot diapause - oxidative stress

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This is an analysis of oxidative stress in newly emerged females who either went through diapause or developed directly. We used protein carbonylation in the thorax as a measure of oxidative stress.

Set-up

```r
library(tidyverse)
```

```
## -- Attaching core tidyverse packages ----------------------- tidyverse 2.0.0 --
## v dplyr     1.1.4     v readr     2.1.5
## v forcats   1.0.0     v stringr   1.5.1
## v ggplot2   3.5.1     v tibble    3.2.1
## v lubridate 1.9.3     v tidyr     1.3.1
## v purrr     1.0.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become error
```

```r
library(nortest)
library(car)
```

```
## Loading required package: carData
##
## Attaching package: 'car'
##
## The following object is masked from 'package:dplyr':
##
##     recode
##
## The following object is masked from 'package:purrr':
##
##     some
```

```r
library(lme4)
```

```
## Warning: package 'lme4' was built under R version 4.3.3
```

```
## Loading required package: Matrix
##
## Attaching package: 'Matrix'
##
## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
```

```r
library(ggsignif)
```

Get data

```r
oxstress <- read_csv("MROT_protcarb_bodysize_data.csv",
                     col_select = c(1:9),
                     col_names = TRUE,
                     col_types = cols(
                       Bee_ID = col_character(),
                       Diapause_NonDiapause = col_character(),
                       Sex = col_character(),
                       Date_emerged = col_date("%m/%d/%y"),
                       Thorax_length_mm = col_double(),
                       Assay_number = col_character(),
                       C = col_double(),
                       P = col_double(),
                       CP = col_double()
                     ))
problems(oxstress)
```
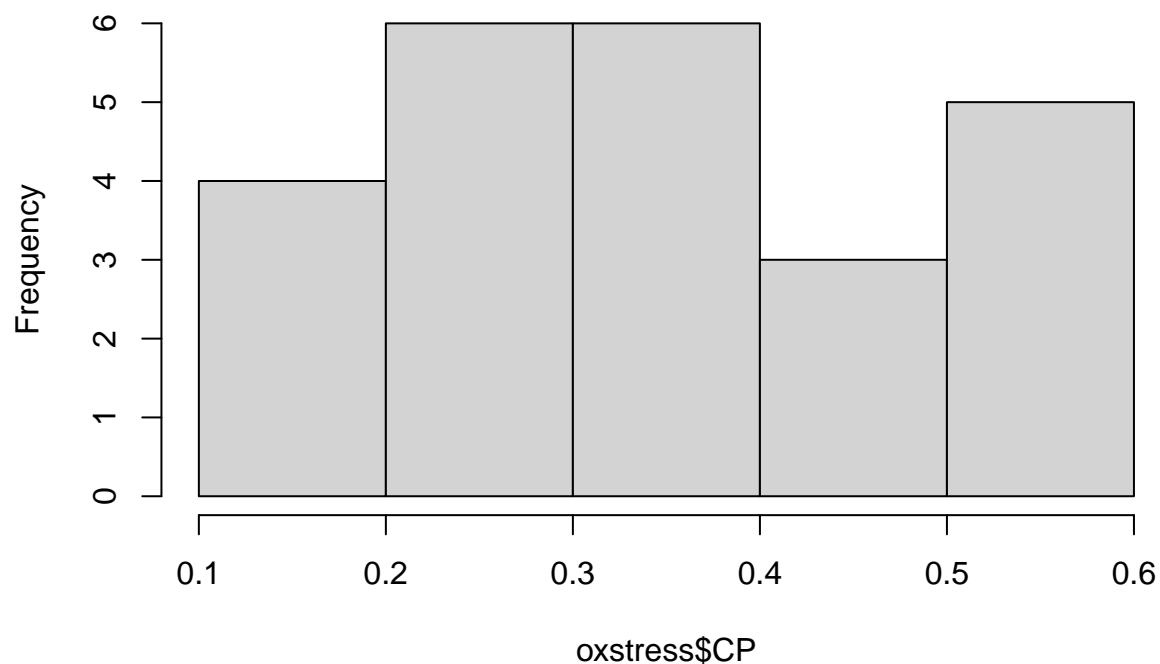
Summary stats

```r
hist(oxstress$CP)
```

## Histogram of oxstress$CP


```
oxstress %>%
  group_by(Diapause_NonDiapause) %>%
  summarise(mean_CP = mean(CP),
            se_CP = sd(CP)/sqrt(n()),
            mean_size = mean(Thorax_length_mm),
            se_size = sd(Thorax_length_mm)/sqrt(n()),
            n = n()
            )
```

```
## # A tibble: 2 x 6
##   Diapause_NonDiapause mean_CP  se_CP mean_size se_size     n
##   <chr>                  <dbl>  <dbl>     <dbl>    <dbl> <int>
## 1 D                      0.389 0.0412      2.67   0.0681    12
## 2 ND                     0.309 0.0343      2.56   0.0632    12
```

Diapausing females have 26% higher levels of protein carbonylation. Diapausing females are also 4% larger than nondiapausing females.

```r
qqp(oxstress$CP, "norm")
```



```
## [1] 16 19
```
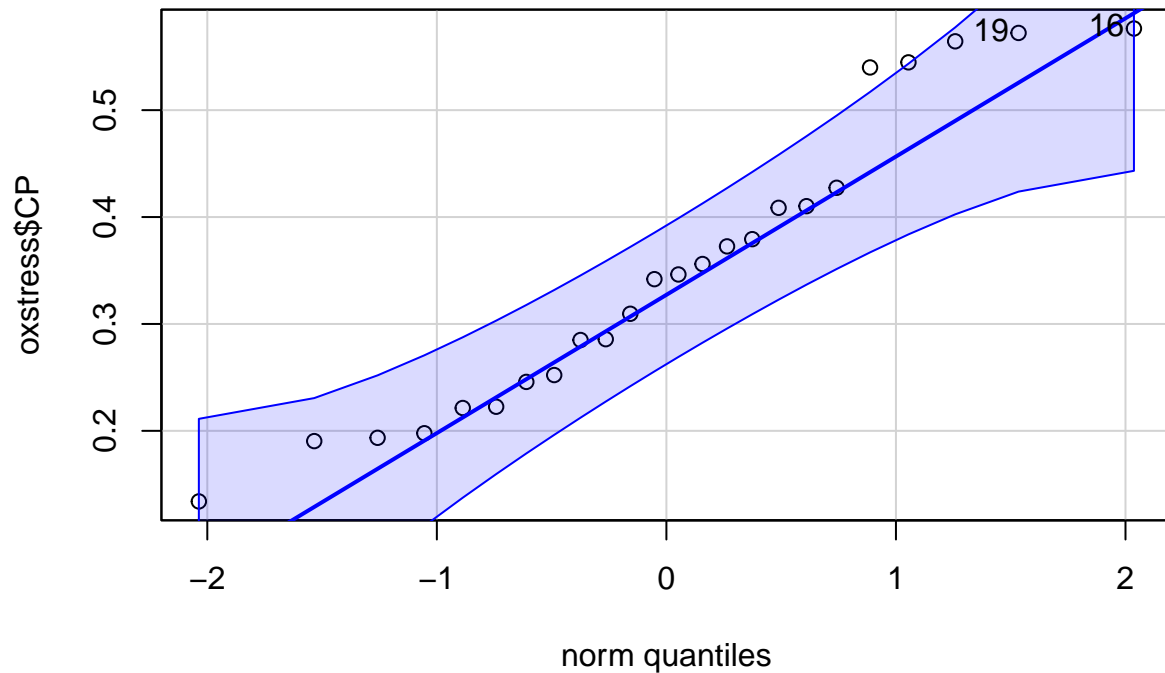
```r
ad.test(oxstress$CP)
```

```
##
##  Anderson-Darling normality test
##
## data:  oxstress$CP
## A = 0.51867, p-value = 0.1691
```

Analysis

```r
m1 <- lmer(CP ~ Diapause_NonDiapause + Thorax_length_mm + (1|Assay_number),
           data = oxstress)
summary(m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: CP ~ Diapause_NonDiapause + Thorax_length_mm + (1 | Assay_number)
##    Data: oxstress
##
```

```
## REML criterion at convergence: -33.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.3166 -0.6245 -0.2681  0.9101  2.0095
##
## Random effects:
##  Groups        Name         Variance Std.Dev.
##  Assay_number (Intercept) 0.010171 0.10085
##  Residual                 0.006902 0.08308
## Number of obs: 24, groups:  Assay_number, 4
##
## Fixed effects:
##                          Estimate Std. Error t value
## (Intercept)              0.96087    0.24182   3.973
## Diapause_NonDiapauseND  -0.12916    0.03661  -3.528
## Thorax_length_mm        -0.20922    0.08777  -2.384
##
## Correlation of Fixed Effects:
##             (Intr) D_NDND
## Dps_NnDpsND -0.371
## Thrx_lngth_ -0.973  0.311
```

```r
Anova(m1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: CP
##                        Chisq Df Pr(>Chisq)
## Diapause_NonDiapause 12.4480  1  0.0004184 ***
## Thorax_length_mm      5.6822  1  0.0171382 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
AIC(m1)
```

```
## [1] -23.23202
```

```r
m0 <- lmer(CP ~ 0 + (1|Assay_number), data = oxstress)
summary(m0)
```

```
## Linear mixed model fit by maximum likelihood  ['lmerMod']
## Formula: CP ~ 0 + (1 | Assay_number)
##    Data: oxstress
##
##      AIC      BIC   logLik deviance df.resid
##    -18.7    -16.4     11.4    -22.7       22
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.8392 -0.4677  0.1778  0.5894  2.1326
##
```

```
## Random effects:
##  Groups         Name        Variance Std.Dev.
##  Assay_number (Intercept) 0.12808  0.3579
##  Residual                 0.01119  0.1058
## Number of obs: 24, groups:  Assay_number, 4
```

```r
anova(m1, m0)
```

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

```
## Data: oxstress
## Models:
## m0: CP ~ 0 + (1 | Assay_number)
## m1: CP ~ Diapause_NonDiapause + Thorax_length_mm + (1 | Assay_number)
##    npar     AIC     BIC logLik deviance Chisq Df Pr(>Chisq)
## m0    2 -18.745 -16.389 11.373  -22.745
## m1    5 -35.435 -29.545 22.718  -45.435 22.69  3  4.686e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Plot

```r
ggplot(oxstress, aes(x = Diapause_NonDiapause, y = CP,
                     fill = Diapause_NonDiapause)) +
  geom_boxplot(alpha = 0.8, outlier.color=NA) +
  geom_jitter(position = position_jitter(width = 0.20)) +
  geom_signif(data = oxstress, stat = "signif", position = "identity",
              comparisons = list(c("D", "ND")), map_signif_level = TRUE,
              annotations = "***", textsize = 8) +
  coord_cartesian(ylim = c(0.1, 0.7)) +
  theme_classic() +
  scale_fill_manual(values = c("#94D2BD","#D84727")) +
  theme(legend.position = "none",
        axis.title=element_text(size=16), axis.text=element_text(size=14)) +
  xlab("") +
  scale_x_discrete(labels = c("Diapause", "Non-diapause")) +
  ylab("Protein carbonyl / Total protein (nmol/mg)")
```
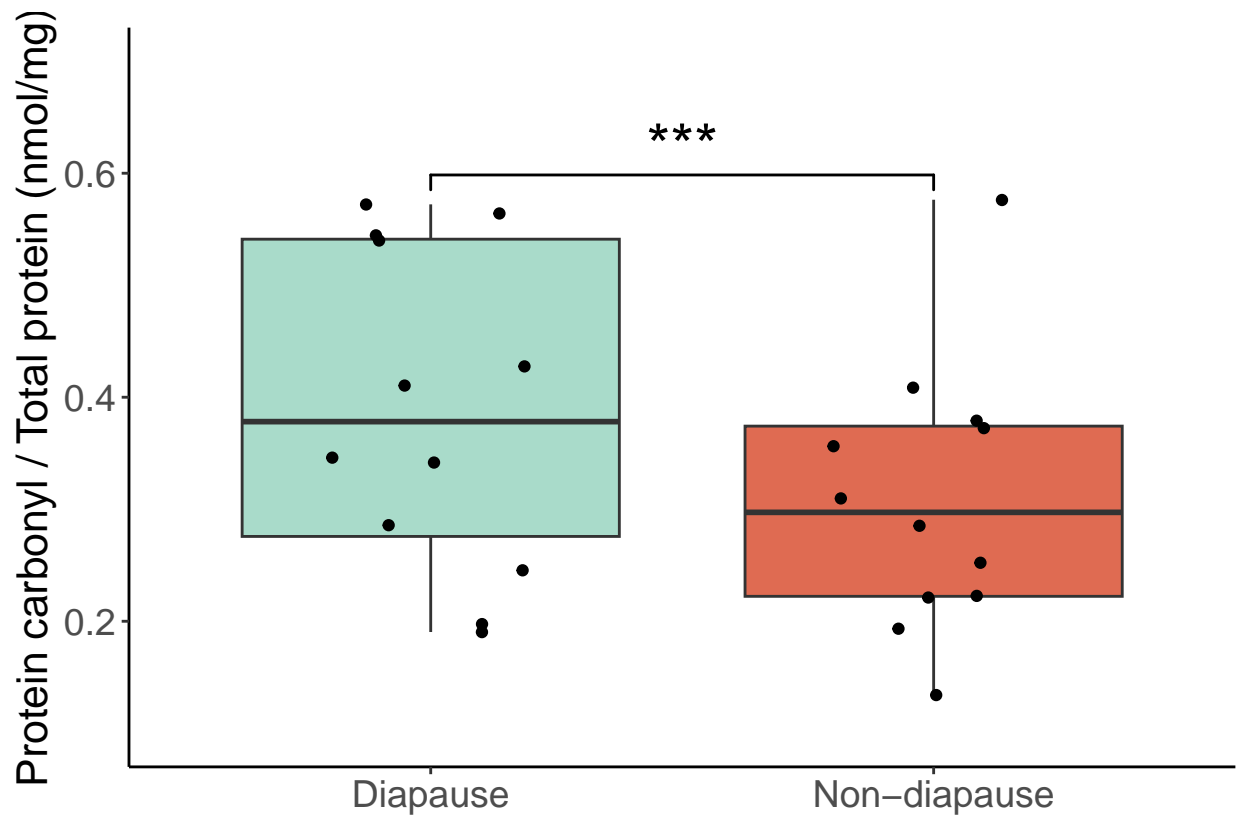
```r
ggsave("oxstress.png", width = 7, height = 5)
```

There is an outlier in the non-diapause group. This is MRE053. It is extremely small, based on thorax width, and Addie had a note that the protein precipitate was hardly visible for 53 and 54. Therefore, it is likely that the CP value is exaggerated due to the very small amount of protein in this individual due to its small size.

bookkeeping

```r
sessionInfo()
```

```
## R version 4.3.2 (2023-10-31)
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS Sonoma 14.2.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib;  LAPACK v
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/Denver
```

```
## tzcode source: internal
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] ggsignif_0.6.4   lme4_1.1-35.5   Matrix_1.6-5    car_3.1-2
##  [5] carData_3.0-5    nortest_1.0-4   lubridate_1.9.3 forcats_1.0.0
##  [9] stringr_1.5.1    dplyr_1.1.4     purrr_1.0.2     readr_2.1.5
## [13] tidyr_1.3.1      tibble_3.2.1    ggplot2_3.5.1   tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##  [1] gtable_0.3.5     xfun_0.46       lattice_0.22-6   tzdb_0.4.0
##  [5] vctrs_0.6.5      tools_4.3.2     generics_0.1.3   parallel_4.3.2
##  [9] fansi_1.0.6      highr_0.11      pkgconfig_2.0.3  lifecycle_1.0.4
## [13] compiler_4.3.2   farver_2.1.2    textshaping_0.4.0 munsell_0.5.1
## [17] htmltools_0.5.8.1 yaml_2.3.10    pillar_1.9.0     nloptr_2.1.1
## [21] crayon_1.5.3     MASS_7.3-60.0.1 boot_1.3-30      abind_1.4-5
## [25] nlme_3.1-165     tidyselect_1.2.1 digest_0.6.36   stringi_1.8.4
## [29] labeling_0.4.3   splines_4.3.2   fastmap_1.2.0    grid_4.3.2
## [33] colorspace_2.1-1 cli_3.6.3       magrittr_2.0.3   utf8_1.2.4
## [37] withr_3.0.0      scales_1.3.0    bit64_4.0.5      timechange_0.3.0
## [41] rmarkdown_2.27   bit_4.0.5       ragg_1.3.2       hms_1.1.3
## [45] evaluate_0.24.0  knitr_1.48      rlang_1.1.4      Rcpp_1.0.13
## [49] glue_1.7.0       rstudioapi_0.16.0 vroom_1.6.5     minqa_1.2.7
## [53] R6_2.5.1         systemfonts_1.1.0
```

```r
citation("tidyverse")
```

```
## To cite package 'tidyverse' in publications use:
##
##   Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R,
##   Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller
##   E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V,
##   Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). "Welcome to
##   the tidyverse." _Journal of Open Source Software_, *4*(43), 1686.
##   doi:10.21105/joss.01686 <https://doi.org/10.21105/joss.01686>.
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     title = {Welcome to the {tidyverse}},
##     author = {Hadley Wickham and Mara Averick and Jennifer Bryan and Winston Chang and Lucy D'Agostin
##     year = {2019},
##     journal = {Journal of Open Source Software},
##     volume = {4},
##     number = {43},
##     pages = {1686},
##     doi = {10.21105/joss.01686},
##   }
```

```r
citation("car")
```

```
## To cite the car package in publications use:
```

```
##
##   Fox J, Weisberg S (2019). _An R Companion to Applied Regression_,
##   Third edition. Sage, Thousand Oaks CA.
##   <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
##
## A BibTeX entry for LaTeX users is
##
##   @Book{,
##     title = {An {R} Companion to Applied Regression},
##     edition = {Third},
##     author = {John Fox and Sanford Weisberg},
##     year = {2019},
##     publisher = {Sage},
##     address = {Thousand Oaks {CA}},
##     url = {https://socialsciences.mcmaster.ca/jfox/Books/Companion/},
##   }
```

```r
citation("nortest")
```

```
## To cite package 'nortest' in publications use:
##
##   Gross J, Ligges U (2015). _nortest: Tests for Normality_. R package
##   version 1.0-4, <https://CRAN.R-project.org/package=nortest>.
##
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
##     title = {nortest: Tests for Normality},
##     author = {Juergen Gross and Uwe Ligges},
##     year = {2015},
##     note = {R package version 1.0-4},
##     url = {https://CRAN.R-project.org/package=nortest},
##   }
```

```r
citation("lme4")
```

```
## To cite lme4 in publications use:
##
##   Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015).
##   Fitting Linear Mixed-Effects Models Using lme4. Journal of
##   Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     title = {Fitting Linear Mixed-Effects Models Using {lme4}},
##     author = {Douglas Bates and Martin M{\"a}chler and Ben Bolker and Steve Walker},
##     journal = {Journal of Statistical Software},
##     year = {2015},
##     volume = {67},
##     number = {1},
##     pages = {1--48},
##     doi = {10.18637/jss.v067.i01},
##   }
```

```r
citation("ggsignif")
```

```
## To cite 'ggsignif' in publications use:
##
##   Ahlmann-Eltze, C., & Patil, I. (2021). ggsignif: R Package for
##   Displaying Significance Brackets for 'ggplot2'. PsyArxiv.
##   doi:10.31234/osf.io/7awm6
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     title = {{ggsignif}: R Package for Displaying Significance Brackets for {'ggplot2'}},
##     author = {Ahlmann-Eltze Constantin and Indrajeet Patil},
##     year = {2021},
##     journal = {PsyArxiv},
##     url = {https://psyarxiv.com/7awm6},
##     doi = {10.31234/osf.io/7awm6},
##   }
```