

RT-qPCR

2024-07-08

```
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 errors
```

```
library(ggplot2)
```

```
library(ggpubr)
```

```
# Read the data from a CSV file
data <- read.csv("rt_qpcr_R_input.csv")
data
```

	Samples	PTTH	RPL8	RPS18	Treatment
## 1	24	25.18426	19.04945	18.27096	D
## 2	26	25.68535	19.13377	19.02566	D
## 3	29	26.04118	19.44330	18.54108	D
## 4	30	25.62088	19.43956	19.28410	D
## 5	31	25.70196	18.93281	18.01337	D
## 6	34	25.02376	18.75440	18.39305	D
## 7	37	26.51769	19.26831	19.20132	D
## 8	38	25.00886	18.70748	18.23315	D
## 9	40	25.94472	19.22576	19.16862	D
## 10	44	25.04067	19.20457	18.58692	D
## 11	46	26.26230	19.53128	19.40272	D
## 12	50	24.31316	19.05853	18.14212	D
## 13	53	26.66308	19.10026	18.72548	ND
## 14	54	25.83588	19.32724	19.46378	ND
## 15	55	25.14047	19.08484	18.51597	ND
## 16	56	26.45697	19.42642	19.37390	ND
## 17	57	24.78331	19.25137	18.26798	ND
## 18	58	25.89390	19.32516	18.45864	ND
## 19	59	26.06732	19.32498	19.61401	ND
## 20	60	25.58068	19.14378	18.14956	ND
## 21	61	26.93015	18.97198	18.23836	ND
## 22	62	26.47673	19.12938	19.25552	ND

```
## 23      63 25.56481 18.87340 18.18393      ND
## 24      64 27.47589 19.42036 19.53596      ND
```

```
# Calculate the geometric mean of the reference genes for each sample
data <- data %>%
  mutate(Ref_mean = rowMeans(select(., RPL8, RPS18)))

# Calculate deltaCt (deltaCt = Ct_target - Ct_reference_mean)
data <- data %>%
  mutate(Delta_Ct = PTH - Ref_mean)

# Calculate mean deltaCt for the control group
mean_Delta_Ct_ND <- mean(data %>% filter(Treatment == "ND") %>% pull(Delta_Ct))

# Calculate double DeltaCt (double DeltaCt = deltaCt_sample - mean_deltaCt_ND)
data <- data %>%
  mutate(Delta_Delta_Ct = Delta_Ct - mean_Delta_Ct_ND)

# Calculate fold change
data <- data %>%
  mutate(Fold_Change = 2^(-Delta_Delta_Ct))
data
```

##	Samples	PTH	RPL8	RPS18	Treatment	Ref_mean	Delta_Ct
## 1	24	25.18426	19.04945	18.27096	D	18.66020	6.524054
## 2	26	25.68535	19.13377	19.02566	D	19.07972	6.605637
## 3	29	26.04118	19.44330	18.54108	D	18.99219	7.048984
## 4	30	25.62088	19.43956	19.28410	D	19.36183	6.259049
## 5	31	25.70196	18.93281	18.01337	D	18.47309	7.228876
## 6	34	25.02376	18.75440	18.39305	D	18.57373	6.450029
## 7	37	26.51769	19.26831	19.20132	D	19.23481	7.282880
## 8	38	25.00886	18.70748	18.23315	D	18.47031	6.538550
## 9	40	25.94472	19.22576	19.16862	D	19.19719	6.747533
## 10	44	25.04067	19.20457	18.58692	D	18.89574	6.144925
## 11	46	26.26230	19.53128	19.40272	D	19.46700	6.795300
## 12	50	24.31316	19.05853	18.14212	D	18.60032	5.712839
## 13	53	26.66308	19.10026	18.72548	ND	18.91287	7.750212
## 14	54	25.83588	19.32724	19.46378	ND	19.39551	6.440372
## 15	55	25.14047	19.08484	18.51597	ND	18.80040	6.340066
## 16	56	26.45697	19.42642	19.37390	ND	19.40016	7.056811
## 17	57	24.78331	19.25137	18.26798	ND	18.75967	6.023638
## 18	58	25.89390	19.32516	18.45864	ND	18.89190	7.002003
## 19	59	26.06732	19.32498	19.61401	ND	19.46949	6.597828
## 20	60	25.58068	19.14378	18.14956	ND	18.64667	6.934016
## 21	61	26.93015	18.97198	18.23836	ND	18.60517	8.324981
## 22	62	26.47673	19.12938	19.25552	ND	19.19245	7.284281
## 23	63	25.56481	18.87340	18.18393	ND	18.52866	7.036149
## 24	64	27.47589	19.42036	19.53596	ND	19.47816	7.997724
##	Delta_Delta_Ct	Fold_Change					
## 1	-0.541619359	1.4556055					
## 2	-0.460036434	1.3755766					
## 3	-0.016688954	1.0116351					
## 4	-0.806624679	1.7491144					
## 5	0.163202946	0.8930402					

```
## 6      -0.615644239    1.5322421
## 7       0.217206246    0.8602296
## 8      -0.527123829    1.4410534
## 9      -0.318139989    1.2467222
## 10     -0.920748724    1.8930975
## 11     -0.270373489    1.2061200
## 12     -1.352834299    2.5541341
## 13       0.684538831    0.6222047
## 14     -0.625301769    1.5425334
## 15     -0.725607419    1.6535967
## 16     -0.008862209    1.0061617
## 17     -1.042035014    2.0591301
## 18     -0.063670629    1.0451215
## 19     -0.467844929    1.3830420
## 20     -0.131657914    1.0955520
## 21       1.259307401    0.4177445
## 22       0.218607666    0.8593944
## 23     -0.029524834    1.0206759
## 24       0.932050821    0.5241128
```

```
# Perform a t-test to compare the treatment groups
```

```
#F-test for equal variance (check the assumption that variance is equal between the two groups)
```

```
fctest <- var.test(Delta_Ct ~ Treatment, data = data)
fctest
```

```
##
## F test to compare two variances
##
## data: Delta_Ct by Treatment
## F = 0.43412, num df = 11, denom df = 11, p-value = 0.1821
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.1249737 1.5080055
## sample estimates:
## ratio of variances
##      0.4341209
```

```
# One-tailed
```

```
t_test1 <- t.test(Delta_Ct ~ Treatment, alternative = "less", var.equal = TRUE, data = data)
t_test1
```

```
##
## Two Sample t-test
##
## data: Delta_Ct by Treatment
## t = -1.9077, df = 22, p-value = 0.03478
## alternative hypothesis: true difference in means between group D and group ND is less than 0
## 95 percent confidence interval:
##      -Inf -0.04536523
## sample estimates:
## mean in group D mean in group ND
##      6.611555      7.065673
```

```

# Two-tailed
t_test2 <- t.test(Delta_Ct ~ Treatment, alternative = "two.sided", var.equal = TRUE, data = data)
t_test2

##
## Two Sample t-test
##
## data: Delta_Ct by Treatment
## t = -1.9077, df = 22, p-value = 0.06957
## alternative hypothesis: true difference in means between group D and group ND is not equal to 0
## 95 percent confidence interval:
## -0.9477889 0.0395514
## sample estimates:
## mean in group D mean in group ND
## 6.611555 7.065673

```

```

# Plot the relative expression levels (fold change)

#labs(title = "Fold Change of PTH Expression",
boxplot <- ggplot(data, aes(x=Treatment, y=Fold_Change, fill=Treatment)) +
  geom_boxplot(position = position_dodge(width = 0.75), alpha = 0.8, outlier.color=NA)+
  geom_jitter(position = position_jitter(width = 0.20)) +
  geom_signif(data = data, stat = "signif", position = "identity",
comparisons = list(c("D", "ND")), map_signif_level = TRUE,
annotations = "0.07", textsize = 6) +
  coord_cartesian(ylim = c(0.5, 2.8)) +
  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("Fold Change")

ggsave("RTqPCR_boxplot.png", width = 7, height = 5)

# Calculate means and standard errors for the bar plot
summary_data <- data %>%
  group_by(Treatment) %>%
  summarise(mean_Fold_Change = mean(Fold_Change),
            se_Fold_Change = sd(Fold_Change) / sqrt(n()))
summary_data

```

```

## # A tibble: 2 x 3
##   Treatment mean_Fold_Change se_Fold_Change
##   <chr>          <dbl>          <dbl>
## 1 D             1.43            0.137
## 2 ND            1.10            0.140

```

```

# Plot the bar plot with standard error bars
barplot <- ggplot(summary_data, aes(x = Treatment, y = mean_Fold_Change, fill = Treatment)) +
  geom_bar(stat = "identity", width = 0.5) +

```

```

geom_errorbar(aes(ymin = mean_Fold_Change - se_Fold_Change, ymax = mean_Fold_Change +
                  se_Fold_Change), width = 0.1) +
theme_classic() +
scale_fill_manual(values = c("#D84727", "#94D2BD")) +
theme(legend.position = "none",
      axis.text.x = element_text(size = 14),
      axis.text.y = element_text(size = 14)) +
xlab("") +
scale_x_discrete(labels = c("Diapause", "Non-diapause")) +
ylab("Fold Change")

ggsave("RTqPCR_barplot.png")

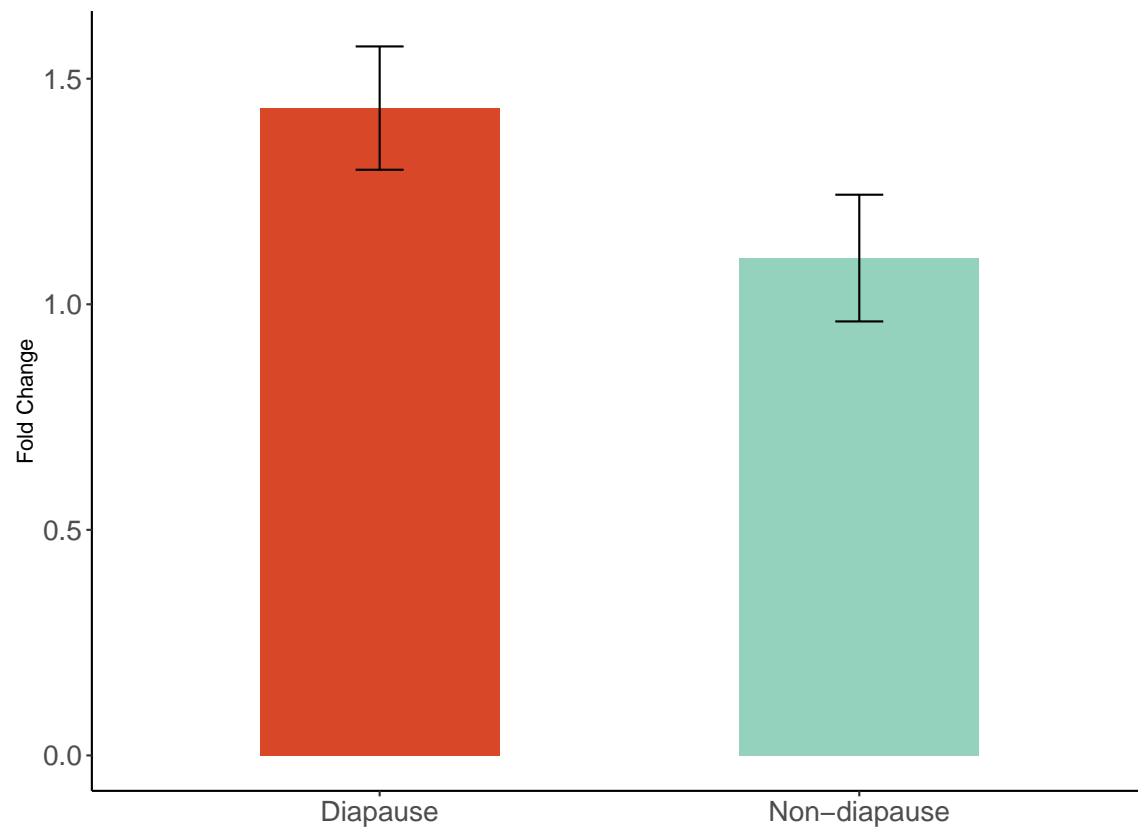
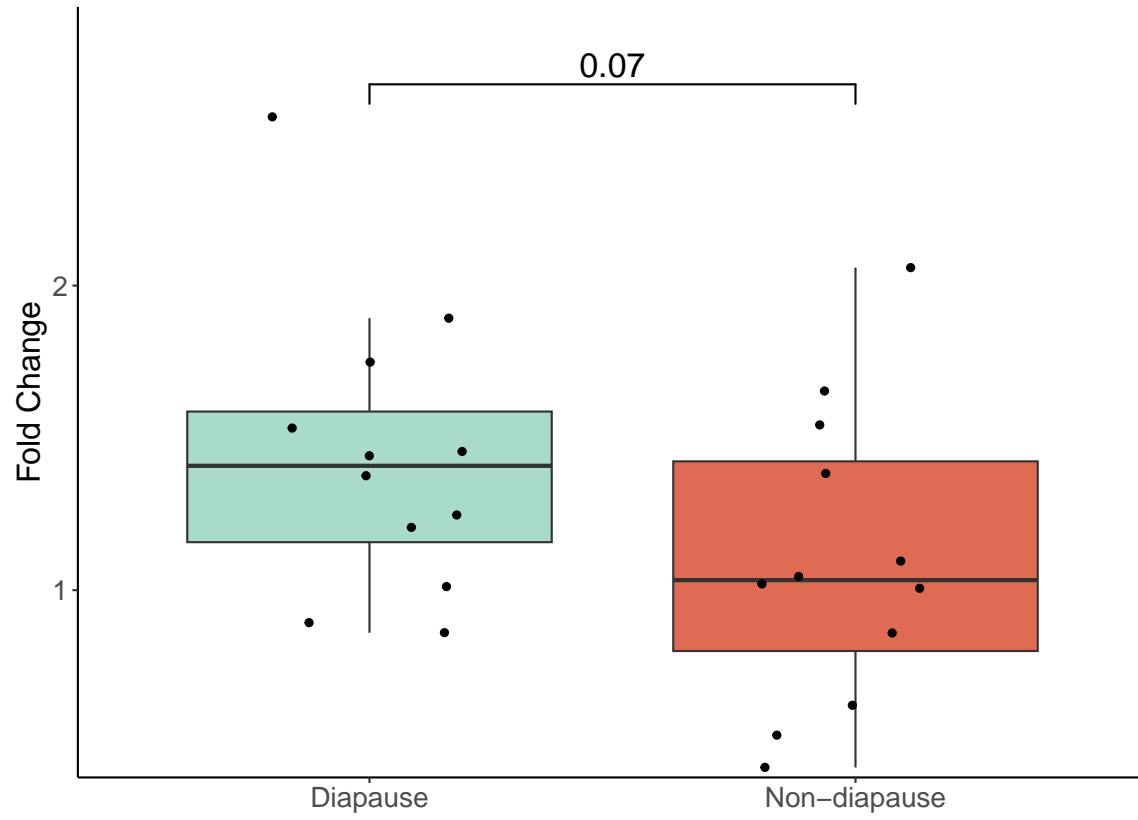
```

Saving 8 x 12 in image

```

fig1 <- ggarrange(boxplot, barplot,
                  nrow = 2, ncol = , widths = 7, heights = 14)
fig1

```



```
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] ggpubr_0.6.0    lubridate_1.9.3 forcats_1.0.0  stringr_1.5.1
## [5] dplyr_1.1.4     purrr_1.0.2     readr_2.1.5    tidyr_1.3.1
## [9] tibble_3.2.1    ggplot2_3.5.1    tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
## [1] utf8_1.2.4      generics_0.1.3   rstatix_0.7.2    stringi_1.8.4
## [5] hms_1.1.3       digest_0.6.36    magrittr_2.0.3    evaluate_0.24.0
## [9] grid_4.3.2      timechange_0.3.0 fastmap_1.2.0     backports_1.5.0
## [13] fansi_1.0.6     scales_1.3.0     textshaping_0.4.0 abind_1.4-5
## [17] cli_3.6.3       rlang_1.1.4      cowplot_1.1.3     munsell_0.5.1
## [21] withr_3.0.0     yaml_2.3.10      tools_4.3.2       tzdb_0.4.0
## [25] ggsignif_0.6.4  colorspace_2.1-1 broom_1.0.6       vctrs_0.6.5
## [29] R6_2.5.1        lifecycle_1.0.4  car_3.1-2         ragg_1.3.2
## [33] pkgconfig_2.0.3 pillar_1.9.0      gtable_0.3.5      glue_1.7.0
## [37] systemfonts_1.1.0 xfun_0.46        tidymodels_1.2.1 rstudioapi_0.16.0
## [41] knitr_1.48      farver_2.1.2     htmltools_0.5.8.1 labeling_0.4.3
## [45] rmarkdown_2.27  carData_3.0-5    compiler_4.3.2
```

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

```
## To cite package 'tidyverse' in publications use:
##
## Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R,
## Golemund 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'Agostini
##   year = {2019},
##   journal = {Journal of Open Source Software},
##   volume = {4},
##   number = {43},
##   pages = {1686},
##   doi = {10.21105/joss.01686},
## }
```

```
citation("ggplot2")
```

```
## To cite ggplot2 in publications, please use
##
##   H. Wickham. ggplot2: Elegant Graphics for Data Analysis.
##   Springer-Verlag New York, 2016.
##
## A BibTeX entry for LaTeX users is
##
##   @Book{,
##     author = {Hadley Wickham},
##     title = {ggplot2: Elegant Graphics for Data Analysis},
##     publisher = {Springer-Verlag New York},
##     year = {2016},
##     isbn = {978-3-319-24277-4},
##     url = {https://ggplot2.tidyverse.org},
##   }
```

```
citation("ggpubr")
```

```
## To cite package 'ggpubr' in publications use:
##
##   Kassambara A (2023). _ggpubr: 'ggplot2' Based Publication Ready
##   Plots_. R package version 0.6.0,
##   <https://CRAN.R-project.org/package=ggpubr>.
##
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
##     title = {ggpubr: 'ggplot2' Based Publication Ready Plots},
##     author = {Alboukadel Kassambara},
##     year = {2023},
##     note = {R package version 0.6.0},
##     url = {https://CRAN.R-project.org/package=ggpubr},
##   }
```