```r
#Packages
library(dplyr)
library(ggtext)
library(ggpubr)
library(ggplot2)
library(car)
library(emmeans)
library(multcomp)
library(DHARMa)
library(lme4)
library(performance)
library(glmmTMB)

# Experiment 1: Parents ------------------------------------------------
f0_df <- read.csv("f0.csv")

f0_df <- f0_df %>%
  mutate(
    Sex    = factor(Sex, levels = c("Female", "Male")),
    Treatment     = factor(Treatment, levels = c("I", "II", "III"))
  )

# Females: pre, ovi, post, fec, long
female_summary <- f0_df %>%
  filter(Sex == "Female") %>%
  group_by(Treatment) %>%
  summarise(
    across(
      c(pre, ovi, post, fec,dai, long),
      list(
        mean = ~ mean(.x, na.rm = TRUE),
        se = ~ sd(.x, na.rm = TRUE) / sqrt(sum(is.finite(.x)))
      ),
      .names = "{.col}_{.fn}"
    )
  )

View(female_summary)

# Males: long only
male_summary <- f0_df %>%
  filter(Sex == "Male") %>%
  group_by(Treatment) %>%
  summarise(
    n_long = sum(is.finite(long)),
    mean_long = mean(long, na.rm = TRUE),
    se_long = sd(long, na.rm = TRUE) / sqrt(n_long)
  )

male_summary

hist(f0_df$long)

# Tests
f0_female <- f0_df %>%
  filter(Sex == "Female")

# Oviposition period
ovi_model <- lmer(
  ovi ~ Treatment + (1 | Block),
  data = f0_female,
  REML = TRUE
)
check_normality(ovi_model)
summary(ovi_model)
car::Anova(ovi_model, type = 2)

emm_ovi <- emmeans(ovi_model, ~ Treatment)
pairs(emm_ovi, adjust = "holm")
```

```
letters_ovi <- cld(
  emm_ovi,
  adjust = "holm",
  Letters = letters
)
letters_ovi

# Post-oviposition period
table(f0_female$post == 0, useNA = "ifany")

post_model <- glmmTMB(
  post ~ Treatment + (1 | Block),
  data = f0_female,
  family = tweedie(link = "log")
)

post_res_tw <- DHARMa::simulateResiduals(
  fittedModel = post_model,
  n = 1000
) # model check
plot(post_res_tw)
DHARMa::testDispersion(post_res_tw)
DHARMa::testUniformity(post_res_tw)
DHARMa::testOutliers(post_res_tw)

summary(post_model)
car::Anova(post_model, type = 2)

emm_post <- emmeans(
  post_model,
  ~ Treatment,
  type = "response"
)
pairs(
  emm_post,
  adjust = "holm"
)
letters_post <- multcomp::cld(
  emmeans(post_model, ~ Treatment),
  adjust = "holm",
  Letters = letters
)
letters_post

# Daily oviposition
dai_model <- lmer(
  dai ~ Treatment + (1 | Block),
  data = f0_female,
  REML = TRUE
)
check_normality(dai_model)
summary(dai_model)
car::Anova(dai_model, type = 2)

emm_dai <- emmeans(dai_model, ~ Treatment)
pairs(emm_dai, adjust = "holm")
letters_dai <- cld(
  emm_dai,
  adjust = "holm",
  Letters = letters
)
letters_dai

# Fecundity
fec_model <- lmer(
  log(fec) ~ Treatment + (1 | Block),
  data = f0_female,
  REML = TRUE
)
```

```
check_normality(fec_model)
summary(fec_model)
car::Anova(fec_model, type = 2)

emm_fec <- emmeans(fec_model, ~ Treatment)
pairs(emm_fec, adjust = "holm")
letters_fec <- cld(
  emm_dai,
  adjust = "holm",
  Letters = letters
)
letters_fec

# Longevity
long_model <- lmer(
  log(long) ~ Treatment * Sex + (1 | Block),
  data = f0_df,
  REML = TRUE
)
check_normality(long_model)
summary(long_model)
car::Anova(long_model, type = 3)

emm_long <- emmeans(long_model, ~ Treatment * Sex)
pairs(emm_long, adjust = "holm")
letters_long <- cld(
  emm_long,
  adjust = "holm",
  Letters = letters
)
letters_long

# Plot for longevity
n_labels <- f0_df %>%
  group_by(Treatment, Sex) %>%
  summarise(
    n = sum(!is.na(long)),
    .groups = "drop"
  )
fig_long <- ggplot(f0_df, aes(x = Treatment, y = long, fill = Sex)) +
  geom_bar(
    stat = "summary",
    fun = "mean",
    position = position_dodge(width = 0.5),
    color = "black",
    width = 0.5
  ) +
  stat_summary(
    fun.data = mean_se,
    geom = "errorbar",
    position = position_dodge(width = 0.5),
    width = 0.25
  ) +  geom_text(
    data = data.frame(
      Treatment = rep(c("I", "II", "III"), each = 2),
      Sex = rep(c("Female", "Male"), 3),
      label = c("b", "d", "bc", "c", "a", "d"),
      y = c(54, 77, 58, 62, 46, 72)
    ),
    aes(x = Treatment, y = y, label = label, group = Sex),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 8
  ) +
  geom_text(
    data = n_labels,
    aes(
      x = Treatment,
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      y = -Inf,
      label = paste0("", n),
      group = Sex
    ),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 6,
    inherit.aes = FALSE
  ) +
  theme_classic2() +
  theme(
    text = element_text(family = "Helvetica", size = 26),
    panel.spacing = unit(1.5, "lines"),
    plot.margin = margin(0.5, 0.5, 0.5, 0.5, "cm"),
    axis.title.x = element_text(margin = margin(t = 10)),
    axis.title.y = element_text(margin = margin(r = 10))
  ) +
  labs(
    x = "Treatment",
    y = "Longevity (days)",
    fill = "Sex"
  ) +
  scale_fill_manual(
    values = c("Female" = "#FA8072", "Male" = "#0072B2"),
    breaks = c("Female", "Male")
  ) +
  coord_cartesian(ylim = c(0, 80))

print(fig_long)


# Experiment 2: Offspring survival ----------------------------------------
f1sur_df <- read.csv("f1sur.csv")

f1sur_df <- f1sur_df %>%
  mutate(
    Sex    = factor(Sex, levels = c("Female", "Male")),
    Treatment     = factor(Treatment, levels = c("I", "II", "III")),
    Age   = factor(Age, levels = c("Young", "Mid", "Old"))
  )

f1sur_summary <- f1sur_df %>%
  group_by(Treatment, Age) %>%
  summarise(
    n = sum(!is.na(sur)),
    survived = sum(sur == 1, na.rm = TRUE),
    survival_pct = mean(sur, na.rm = TRUE) * 100,
    .groups = "drop"
  )

View(f1sur_summary)

model_sur <- glmer(
  sur ~ Treatment * Age + (1 | Block),
  data = f1sur_df,
  family = binomial(link = "logit")
)
summary(model_sur)

dispersion_ratio <- deviance(model_sur) / df.residual(model_sur)
dispersion_ratio

car::Anova(model_sur, type = 2)

emm_sur <- emmeans(model_sur, ~ Treatment)
pairs(emm_sur, adjust = "holm")
letters_sur <- cld(
  emm_sur,
  adjust = "holm",
```

```
  Letters = letters
)
letters_sur

emm_sur <- emmeans(
  model_sur,
  ~ Treatment,
  type = "response"
)
summary(emm_sur, infer = c(TRUE, TRUE))

f1p_dev_summary <- f1sur_df %>%
  filter(is.finite(dev)) %>%
  group_by(Treatment, Age, Sex) %>%
  summarise(
    across(
      dev,
      list(
        mean = ~ mean(.x, na.rm = TRUE),
        se = ~ sd(.x, na.rm = TRUE) / sqrt(sum(is.finite(.x))),
        n = ~ sum(is.finite(.x))
      ),
      .names = "{.col}_{.fn}"
    ),
    .groups = "drop"
  )
View(f1p_dev_summary)

hist(f1sur_df$dev)

f1dev_model <- glmer(
  dev ~ Treatment * Sex * Age + (1 | Block),
  data = f1sur_df,
  family = poisson(link = "log")
)

dispersion_ratio <- deviance(f1dev_model) / df.residual(f1dev_model)
dispersion_ratio

summary(f1dev_model)
car::Anova(f1dev_model, type = 2)

emm_f1dev <- emmeans(f1dev_model, ~  Age)
pairs(emm_f1dev, adjust = "holm")
letters_f1dev <- cld(
  emm_f1dev,
  adjust = "holm",
  Letters = letters
)
letters_f1dev

emm_age <- emmeans(
  f1dev_model,
  ~ Age,
  type = "response"
)

summary(emm_age, infer = c(TRUE, TRUE))

# Experiment 2: Offspring reproduction ------------------------------------

f1p_df <- read.csv("f1pair.csv")

f1p_df <- f1p_df %>%
  mutate(
    Sex    = factor(Sex, levels = c("Female", "Male")),
    Treatment     = factor(Treatment, levels = c("I", "II", "III")),
    Age   = factor(Age, levels = c("Young", "Mid"))
  )
```

```
f1p_female_summary <- f1p_df %>%
  filter(Sex == "Female") %>%
  group_by(Treatment, Age) %>%
  summarise(
    across(
      c(pre, ovi, post, fec, dai),
      list(
        mean = ~ mean(.x, na.rm = TRUE),
        se = ~ sd(.x, na.rm = TRUE) / sqrt(sum(is.finite(.x))),
        n = ~ sum(is.finite(.x))
      ),
      .names = "{.col}_{.fn}"
    )
  )

View(f1p_female_summary)

# Tests
f1p_female <- f1p_df %>%
  filter(Sex == "Female")

hist(f1p_female$ovi)

# Oviposition period
f1ovi_model <- lmer(
  log(ovi) ~ Treatment * Age + (1 | Block),
  data = f1p_female,
  REML = TRUE
)
check_normality(f1ovi_model)
summary(f1ovi_model)
car::Anova(f1ovi_model, type = 2)

emm_f1ovi <- emmeans(f1ovi_model, ~ Treatment)
pairs(emm_f1ovi, adjust = "holm")
letters_f1ovi <- cld(
  emm_f1ovi,
  adjust = "holm",
  Letters = letters
)
letters_f1ovi

emm_ovi <- emmeans(
  f1ovi_model,
  ~ Treatment,
  type = "response"
)

summary(emm_ovi, infer = c(TRUE, TRUE))

# Post-oviposition period
table(f1p_female$post == 0, useNA = "ifany")

f1post_model <- glmer(
  post ~ Treatment * Age + (1 | Block),
  data = f1p_female,
  family = poisson(link = "log")
)

dispersion_ratio <- deviance(f1post_model) / df.residual(f1post_model)
dispersion_ratio

summary(f1post_model)
car::Anova(f1post_model, type = 2)

emm_f1post <- emmeans(f1post_model, ~ Treatment * Age)
pairs(emm_f1post, adjust = "holm")
letters_f1post <- cld(
```

```
  emm_f1post,
  adjust = "holm",
  Letters = letters
)
letters_f1post

# Daily oviposition
f1dai_model <- lmer(
  log(dai) ~ Treatment * Age + (1 | Block),
  data = f1p_female,
  REML = TRUE
)
check_normality(f1dai_model)
par(mfrow = c(1, 2))
plot(fitted(f1dai_model), resid(f1dai_model))
qqnorm(resid(f1dai_model))
qqline(resid(f1dai_model))
summary(f1dai_model)
car::Anova(f1dai_model, type = 2)

emm_f1dai <- emmeans(f1dai_model, ~  Age)
pairs(emm_f1dai, adjust = "holm")
letters_f1dai <- cld(
  emm_f1dai,
  adjust = "holm",
  Letters = letters
)
letters_f1dai

emm_dai <- emmeans(
  f1dai_model,
  ~ Age,
  type = "response"
)
summary(emm_dai, infer = c(TRUE, TRUE))

# Fecundity
f1fec_model <- lmer(
  log(fec) ~ Treatment * Age + (1 | Block),
  data = f1p_female,
  REML = TRUE
)

check_normality(f1fec_model)
summary(f1fec_model)
car::Anova(f1fec_model, type = 2)

emm_f1fec <- emmeans(f1fec_model, ~ Treatment)
pairs(emm_f1fec, adjust = "holm")
letters_f1fec <- cld(
  emm_f1fec,
  adjust = "holm",
  Letters = letters
)
letters_f1fec

emm_fec <- emmeans(
  f1fec_model,
  ~ Treatment,
  type = "response"
)

summary(emm_fec, infer = c(TRUE, TRUE))

# Longevity
hist(f1p_df$long)

f1long_model <- lmer(
  log(long) ~ Treatment * Age * Sex + (1 | Block),
```

```
  data = f1p_df,
  REML = TRUE
)
check_normality(f1long_model)
summary(f1long_model)
car::Anova(f1long_model, type = 3)

emm_f1long <- emmeans(f1long_model, ~ Treatment * Sex)
pairs(emm_f1long, adjust = "holm")
letters_f1long <- cld(
  emm_f1long,
  adjust = "holm",
  Letters = letters
)
letters_f1long

f1long_model <- glmer(
  long ~ Treatment * Age * Sex + (1 | Block),
  data = f1p_df,
  family = poisson(link = "log")
)

dispersion_ratio <- deviance(f1long_model) / df.residual(f1long_model)
dispersion_ratio

summary(f1long_model)
car::Anova(f1long_model, type = 2)

emm_f1long <- emmeans(f1long_model, ~ Treatment * Age * Sex)
pairs(emm_f1long, adjust = "holm")
letters_f1long <- cld(
  emm_f1long,
  adjust = "holm",
  Letters = letters
)
letters_f1long

# Plot for longevity
f1pn_labels <- f1p_df %>%
  group_by(Treatment, Sex, Age) %>%
  summarise(
    n = sum(!is.na(long)),
    .groups = "drop"
  )

letter_labels <- data.frame(
  Treatment = c("I", "II", "III", "I", "II", "III",
                "I", "II", "III", "I", "II", "III"),
  Age = c(rep("Young", 3), rep("Mid", 3),
          rep("Young", 3), rep("Mid", 3)),
  Sex = c(rep("Female", 6), rep("Male", 6)),
  label = c("cd", "abc", "ab", "bc", "ab", "a",
            "g", "ef", "ef", "fg", "de", "e")
)

letter_labels$y <- c(
  55, 49, 48, 54, 48, 47,
  79, 70, 68, 77, 63, 64
)

f1p_df$Age <- factor(f1p_df$Age, levels = c("Young", "Mid"))
f1pn_labels$Age <- factor(f1pn_labels$Age, levels = c("Young", "Mid"))
letter_labels$Age <- factor(letter_labels$Age, levels = c("Young", "Mid"))

fig_f1plong <- ggplot(
  f1p_df,
  aes(x = Treatment, y = long, fill = Sex)
) +
  geom_bar(
```

```
    stat = "summary",
    fun = "mean",
    position = position_dodge(width = 0.5),
    color = "black",
    width = 0.5
  ) +
  stat_summary(
    fun.data = mean_se,
    geom = "errorbar",
    position = position_dodge(width = 0.5),
    width = 0.25
  ) +
  geom_text(
    data = f1pn_labels,
    aes(
      x = Treatment,
      y = -Inf,
      label = n,
      group = Sex
    ),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 6,
    inherit.aes = FALSE
  ) +
  geom_text(
    data = letter_labels,
    aes(
      x = Treatment,
      y = y,
      label = label,
      group = Sex
    ),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 7,
    inherit.aes = FALSE
  ) +
  facet_wrap(~ Age) +
  theme_classic2() +
  theme(
    text = element_text(family = "Helvetica", size = 26),
    panel.spacing = unit(1.5, "lines"),
    plot.margin = margin(0.5, 0.5, 0.5, 0.5, "cm"),
    axis.title.x = element_text(margin = margin(t = 10)),
    axis.title.y = element_text(margin = margin(r = 10))
  ) +
  labs(
    x = "Treatment",
    y = "Longevity (days)",
    fill = "Sex"
  ) +
  scale_fill_manual(
    values = c("Female" = "#FA8072", "Male" = "#0072B2"),
    breaks = c("Female", "Male")
  ) +
  coord_cartesian(ylim = c(0, 80))

print(fig_f1plong)


# Experiment 2: Offspring longevity (individually reared) -----------------
f1i_df <- read.csv("f1ind.csv")

f1i_df <- f1i_df %>%
  mutate(
    Sex    = factor(Sex, levels = c("Female", "Male")),
    Treatment     = factor(Treatment, levels = c("I", "II", "III")),
    Age   = factor(Age, levels = c("Young", "Mid", "Old"))
```

```
  )

hist(f1i_df$long)

f1ilong_model <- lmer(
  long ~ Treatment * Age * Sex + (1 | Block),
  data = f1i_df,
  REML = TRUE
)

summary(f1ilong_model)
check_normality(f1ovi_model)
par(mfrow = c(1, 2))
plot(fitted(f1ilong_model), resid(f1ilong_model))
qqnorm(resid(f1ilong_model))
qqline(resid(f1ilong_model))
car::Anova(f1ilong_model, type = 2)

emm_f1ilong <- emmeans(f1ilong_model, ~ Treatment * Sex * Age)
pairs(emm_f1ilong, adjust = "holm")
letters_f1ilong <- cld(
  emm_f1ilong,
  adjust = "holm",
  Letters = letters
)
letters_f1ilong

#Plot
f1i_df <- read.csv("f1ind.csv") %>%
  mutate(
    Sex = factor(Sex, levels = c("Female", "Male")),
    Treatment = factor(Treatment, levels = c("I", "II", "III")),
    Age = factor(Age, levels = c("Young", "Mid", "Old"))
  )

f1in_labels <- f1i_df %>%
  group_by(Treatment, Sex, Age) %>%
  summarise(
    n = sum(!is.na(long)),
    .groups = "drop"
  )

letter_labels_i <- data.frame(
  Treatment = c(
    "I", "II", "III", "I", "II", "III",
    "I", "II", "III", "I", "II", "III",
    "I", "II", "III", "I", "II", "III"
  ),
  Sex = c(
    "Female", "Female", "Female",
    "Male", "Male", "Male",
    "Female", "Female", "Female",
    "Male", "Male", "Male",
    "Female", "Female", "Female",
    "Male", "Male", "Male"
  ),
  Age = c(
    rep("Young", 6),
    rep("Mid", 6),
    rep("Old", 6)
  ),
  label = c(
    "d", "bcd", "bcd", "bcd", "abc", "abc",
    "cd", "abcd", "abcd", "abcd", "ab", "ab",
    "bcd", "abc", "ab", "abc", "ab", "a"
  ),
  y = c(
    139, 125, 126, 128, 115, 115,
    132, 120, 116, 125, 108, 111,
```

```
    128, 115, 108, 115, 107, 100
  )
)

f1in_labels$Age <- factor(
  f1in_labels$Age,
  levels = c("Young", "Mid", "Old")
)

letter_labels_i$Age <- factor(
  letter_labels_i$Age,
  levels = c("Young", "Mid", "Old")
)

letter_labels_i$Treatment <- factor(
  letter_labels_i$Treatment,
  levels = c("I", "II", "III")
)

letter_labels_i$Sex <- factor(
  letter_labels_i$Sex,
  levels = c("Female", "Male")
)

fig_f1ilong <- ggplot(
  f1i_df,
  aes(x = Treatment, y = long, fill = Sex)
) +
  geom_bar(
    stat = "summary",
    fun = "mean",
    position = position_dodge(width = 0.5),
    color = "black",
    width = 0.5
  ) +
  stat_summary(
    fun.data = mean_se,
    geom = "errorbar",
    position = position_dodge(width = 0.5),
    width = 0.25
  ) +
  geom_text(
    data = f1in_labels,
    aes(
      x = Treatment,
      y = -Inf,
      label = n,
      group = Sex
    ),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 6,
    inherit.aes = FALSE
  ) +
  geom_text(
    data = letter_labels_i,
    aes(
      x = Treatment,
      y = y,
      label = label,
      group = Sex
    ),
    position = position_dodge(width = 0.5),
    vjust = -0.5,
    size = 7,
    inherit.aes = FALSE
  ) +
  facet_wrap(~ Age, nrow = 1) +
  theme_classic2() +
```

```
  theme(
    text = element_text(family = "Helvetica", size = 26),
    panel.spacing = unit(1.5, "lines"),
    plot.margin = margin(0.5, 0.5, 0.5, 0.5, "cm"),
    axis.title.x = element_text(margin = margin(t = 10)),
    axis.title.y = element_text(margin = margin(r = 10))
  ) +
  labs(
    x = "Treatment",
    y = "Longevity (days)",
    fill = "Sex"
  ) +
  scale_fill_manual(
    values = c("Female" = "#FA8072", "Male" = "#0072B2"),
    breaks = c("Female", "Male")
  ) +
  coord_cartesian(ylim = c(0, 150))

print(fig_f1ilong)
```