

YSP 2022_Aggression and social behavior under risk

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2024-05-08

In Summer 2022, our two YSP students Sophia and Ishita tested whether Amazon mollies altered their 1) aggressive and 2) sociable behavior when under the threat of predation risk. They designed two assays to measure these behaviors and then repeatedly measured individual fish in either or both contexts (with risk, or without risk).

Aggressive behavior: Study measures bites as count data for the duration of an 5 minute trial. Absence of risk (N = 10), Presence of risk (N = 10) were tested 2x each for their specific treatment (testing interval = 8 days). No individual experienced both treatments. 1 individual per treatment died (Total N = 18, or N = 9 per treatment)

Sociability: Study measures association time with conspecific 3N for the duration of an 15 minute trial. N = 10 3N mollies. All fish experienced both treatments in a balanced design. Each fish was tested in control and presence settings (3 trials each, for a total of 6 trials per fish). No deaths.

Load data

```
library(lme4)
```

```
## Loading required package: Matrix
```

```
library(lmerTest)
```

```
##
```

```
## Attaching package: 'lmerTest'
```

```
## The following object is masked from 'package:lme4':
```

```
##
```

```
##      lmer
```

```
## The following object is masked from 'package:stats':
```

```
##
```

```
##      step
```

```
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 3.5.0      v tibble 3.2.1
## v lubridate 1.9.3    v tidyr 1.3.1
## v purrr 1.0.2

## -- Conflicts ----- tidyverse_conflicts() --
## x tidyr::expand() masks Matrix::expand()
## x dplyr::filter() masks stats::filter()
## x dplyr::lag() masks stats::lag()
## x tidyr::pack() masks Matrix::pack()
## x tidyr::unpack() masks Matrix::unpack()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
library(patchwork)
library(ggplot2)
library(dplyr)
library(ggpubr)
library(MuMIn)

#Kate's computer
setwd("C:/Users/katel/Box Sync/YSP data")

agg.data <- read.csv("Aguinaga et al_Risk response data_for deposit.csv")
social.data <- read.csv("Aguinaga et al_Sociability data_for deposit.csv")

head(agg.data)
```

```
##   X   ID trial bites      context
## 1 1 GGGG     1    27 Predator absent
## 2 2 GGGG     2    41 Predator absent
## 3 3 GYYY     1    29 Predator absent
## 4 4 GYYY     2    84 Predator absent
## 5 5 GGYP     1    96 Predator absent
## 6 6 GGYP     2     0 Predator absent
```

```
head(social.data)
```

```
##   X   ID trial total_time      context
## 1 1 GYPP     1   587.512 Predator absent
## 2 2 GYPP     2   322.198 Predator absent
## 3 3 GYPP     3   300.258 Predator absent
## 4 4 GYPP     1   734.612 Predator present
## 5 5 GYPP     2   782.018 Predator present
## 6 6 GYPP     3   596.894 Predator present
```

Data cleaning

Jon provided the data in a few different files. Here I clean up the data to compile each behavioral assay into one data file. This code no longer needs to be run as the cleaned files are loaded up above.

```

dataAbsence <- read.csv("IshitaYSPAbsenceCSV.csv")

data <- dataAbsence %>%
  select(ID, T1A, T2A) %>%
  pivot_longer(!ID, names_to = "trial", values_to = "bites") %>%
  mutate(trial = replace(trial, trial == "T1A", 1),
         trial = replace(trial, trial == "T2A", 2),
         context = "Predator absent")

dataPresence <- read.csv("IshitaYSPPresenceCSV.csv")

data2 <- dataPresence %>%
  pivot_longer(!ID, names_to = "trial", values_to = "bites") %>%
  mutate(trial = replace(trial, trial == "T1P", 1),
         trial = replace(trial, trial == "T2P", 2),
         context = "Predator present")

risk.behav <- rbind(data, data2)
write.csv(risk.behav, file = "Risk response YSP.csv")

####

sophia <- read.csv("SophiaYSPCSV.csv")

sophia2 <- sophia %>%
  pivot_longer(!ID, names_to = "trial", values_to = "total_time") %>%
  mutate(context = ifelse(trial == "T1A" | trial == "T2A" | trial == "T3A", "Predator absent", "Predator present"),
         trial = replace(trial, trial == "T1A" | trial == "T4P", 1),
         trial = replace(trial, trial == "T2A" | trial == "T5P", 2),
         trial = replace(trial, trial == "T3A" | trial == "T6P", 3))

write.csv(sophia2, file = "sociability YSP.csv")

```

Aggression data

Testing to see whether number of bites differs based on the context (pred present, pred absent) or across trials.

```
mod1.agg <- lmer(bites ~ trial * context + (1|ID), data = agg.data)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
ranova(mod1.agg) # no evidence of individual differences here
```

```
## ANOVA-like table for random-effects: Single term deletions
##
## Model:
## bites ~ trial + context + (1 | ID) + trial:context
##      npar logLik   AIC      LRT Df Pr(>Chisq)
## <none>    6 -178.54 369.08
## (1 | ID)   5 -178.54 367.08 5.6843e-14 1          1
```

```
anova(mod1.agg) # no evidence of strong differences across context, or trial
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## trial          521.36   521.36     1    32  0.1670 0.6855
## context        295.21   295.21     1    32  0.0946 0.7604
## trial:context  318.03   318.03     1    32  0.1019 0.7517
```

```
summary(mod1.agg)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: bites ~ trial * context + (1 | ID)
##   Data: agg.data
##
## REML criterion at convergence: 357.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.3364 -0.8114  0.2029  0.6071  1.9032
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   ID       (Intercept)          0        0.00
##   Residual                    3122      55.87
## Number of obs: 36, groups: ID, 18
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)      65.667    41.644  32.000   1.577   0.125
## trial             1.667    26.338  32.000   0.063   0.950
## contextPredator present -18.111    58.893  32.000  -0.308   0.760
## trial:contextPredator present 11.889    37.247  32.000   0.319   0.752
##
## Correlation of Fixed Effects:
##              (Intr) trial  cntxPp
## trial        -0.949
## cntxtPrdtrp -0.707  0.671
## tr1:cntxtPp  0.671 -0.707 -0.949
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

Because we have such a small sample size though and we didn't necessary expect there to be a strong interaction between context and trial, let's remove that interaction and re-run the model

```
mod2.agg <- lmer(bites ~ trial + context + (1|ID), data = agg.data)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
ranova(mod2.agg)
```

```
## ANOVA-like table for random-effects: Single term deletions
##
## Model:
## bites ~ trial + context + (1 | ID)
##      npar  logLik    AIC LRT Df Pr(>Chisq)
## <none>    5 -183.12 376.24
## (1 | ID)   4 -183.12 374.24   0  1         1
```

```
anova(mod2.agg)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##      Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## trial  521.36  521.36     1    33  0.1717 0.6813
## context    0.69    0.69     1    33  0.0002 0.9880
```

```
summary(mod2.agg)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: bites ~ trial + context + (1 | ID)
## Data: agg.data
##
## REML criterion at convergence: 366.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.3061 -0.8766  0.2057  0.6694  1.9836
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 0 0.00
## Residual 3037 55.11
## Number of obs: 36, groups: ID, 18
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)    56.7500    30.4605 33.0000   1.863   0.0714 .
## trial           7.6111    18.3684 33.0000   0.414   0.6813
## contextPredator present -0.2778    18.3684 33.0000  -0.015   0.9880
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trial
## trial        -0.905
## cntxtPrdtrp -0.302  0.000
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
r.squaredGLMM(mod2.agg)
```

```
## Warning: 'r.squaredGLMM' now calculates a revised statistic. See the help page.
```

```
##           R2m           R2c
## [1,] 0.004888064 0.004888064
```

Getting lots of singular fit warnings because ID accounts for no variance so will re-run without ID results still the same - no strong difference in bites across trials or contexts

```
mod1.agglm <- lm(bites ~ trial*context, data = agg.data)
anova(mod1.agglm)
```

```
## Analysis of Variance Table
##
## Response: bites
##           Df Sum Sq Mean Sq F value Pr(>F)
## trial      1    521  521.36  0.1670 0.6855
## context    1      1    0.69  0.0002 0.9882
## trial:context 1    318  318.03  0.1019 0.7517
## Residuals  32  99889 3121.53
```

Sociability data

Now let's compare whether total time spent associating with a conspecific differed across contexts or trials

```
mod1.soc <- lmer(total_time ~ trial * context + (1|ID), data = social.data)
ranova(mod1.soc) # significant effect of individual
```

```
## ANOVA-like table for random-effects: Single term deletions
##
## Model:
## total_time ~ trial + context + (1 | ID) + trial:context
##           npar logLik    AIC    LRT Df Pr(>Chisq)
## <none>        6 -367.80 747.61
## (1 | ID)      5 -370.69 751.38 5.7678 1 0.01632 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(mod1.soc)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## trial      105829  105829      1    47  5.3510 0.02513 *
## context      5157    5157      1    47  0.2608 0.61199
## trial:context  7393    7393      1    47  0.3738 0.54389
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(mod1.soc)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: total_time ~ trial * context + (1 | ID)
## Data: social.data
##
## REML criterion at convergence: 735.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.3288 -0.5248  0.1425  0.7145  1.9128
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 6633    81.45
## Residual          19777   140.63
## Number of obs: 60, groups: ID, 10
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)      613.34      72.65  55.69   8.442 1.52e-11 ***
## trial            -65.03      31.45  47.00  -2.068  0.0442 *
## contextPredator present      49.06      96.07  47.00   0.511  0.6120
## trial:contextPredator present  27.19      44.47  47.00   0.611  0.5439
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trial  cntxPp
## trial          -0.866
## cntxtPrdtrp -0.661  0.655
## tr1:cntxtPp  0.612 -0.707 -0.926
```

Again because our sample size is so small and we didn't really expect a strong interaction, let's remove the interaction and re-run the model

We see a significant effect of individual and context - fish spend more time associating with their conspecific when a predator is present (which is exactly we expect it). We should report the parameter estimates, se and t-values from the summary and then the F-stats and p-values from teh anova()

```
mod2.soc <- lmer(total_time ~ trial + context + (1|ID), data = social.data)
ranova(mod2.soc)
```

```
## ANOVA-like table for random-effects: Single term deletions
##
## Model:
## total_time ~ trial + context + (1 | ID)
##      npar logLik   AIC   LRT Df Pr(>Chisq)
## <none>     5 -372.70 755.41
## (1 | ID)    4 -375.68 759.36 5.9563 1 0.01467 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(mod2.soc)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##      Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## trial  105829  105829     1    48   5.4218 0.024146 *
## context 160486  160486     1    48   8.2220 0.006131 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(mod2.soc)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: total_time ~ trial + context + (1 | ID)
## Data: social.data
##
## REML criterion at convergence: 745.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.3383 -0.6264  0.1412  0.6503  1.9219
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 6676    81.71
## Residual          19519   139.71
## Number of obs: 60, groups: ID, 10
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)      586.15      57.19  49.15  10.250 8.51e-14 ***
## trial            -51.44      22.09  48.00  -2.328  0.02415 *
## contextPredator present  103.44      36.07  48.00   2.867  0.00613 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trial
## trial          -0.773
## cntxtPrdtrp -0.315  0.000
```

```
r.squaredGLMM(mod2.soc)
```

```
##              R2m      R2c
## [1,] 0.1469848 0.3643898
```

We can also estimate the repeatability of individual - about 0.25

```
summary(mod2.soc)
```



```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: total_time ~ trial + context + (1 | ID)
## Data: social.data
##
## REML criterion at convergence: 745.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.3383 -0.6264  0.1412  0.6503  1.9219
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 6676      81.71
## Residual          19519    139.71
## Number of obs: 60, groups: ID, 10
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)      586.15      57.19  49.15  10.250 8.51e-14 ***
## trial            -51.44      22.09  48.00   -2.328  0.02415 *
## contextPredator present  103.44      36.07  48.00    2.867  0.00613 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trial
## trial          -0.773
## cntxtPrdtrp -0.315  0.000
```

```
6676/(6676+19519)
```

```
## [1] 0.2548578
```

While the interaction between trial and context is not significant, the graph makes it appear that there could be a slight trend for greater habituation in the control fish, compared to the predator cue fish. As such, we'll perform a post hoc to look at this in more detail. We recognize that these results should be interpreted very cautiously and are more just descriptive of the patterns we're seeing rather than a proper test.

```
social.control <- social.data %>%
  filter(context == "Predator absent")

social.pred <- social.data %>%
  filter(context == "Predator present")

control1 <- lmer(total_time ~ trial + (1|ID), data = social.control)
anova(control1)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##      Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## trial  84581   84581     1    19  4.2765 0.05253 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(control1)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: total_time ~ trial + (1 | ID)
## Data: social.control
##
## REML criterion at convergence: 368
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.53853 -0.45990 -0.01597  0.41489  1.99979
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 5069    71.2
## Residual          19778   140.6
## Number of obs: 30, groups: ID, 10
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)   613.34      71.57  26.92   8.570 3.57e-09 ***
## trial         -65.03      31.45  19.00  -2.068  0.0525 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr)
## trial -0.879
```

```
pred1 <- lmer(total_time ~ trial + (1|ID), data = social.pred)
anova(pred1)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##      Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## trial  28640   28640     1    19   1.3487 0.2599
```

```
summary(pred1)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: total_time ~ trial + (1 | ID)
## Data: social.pred
##
## REML criterion at convergence: 370.8
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.9624 -0.4979  0.1934  0.8109  1.1883
##
## Random effects:
```

```
## Groups      Name      Variance Std.Dev.
## ID          (Intercept) 6684      81.76
## Residual                21236    145.73
## Number of obs: 30, groups: ID, 10
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)   662.40      74.99   27.26   8.833 1.74e-09 ***
## trial         -37.84      32.59   19.00  -1.161    0.26
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr)
## trial -0.869
```

Figures

```
agg.data <- read.csv("Aguinaga et al_Risk response data_for deposit.csv")
social.data <- read.csv("Aguinaga et al_Sociability data_for deposit.csv")

agg.data$trial <- factor(agg.data$trial)
social.data$trial <- factor(social.data$trial)

agg.data <- agg.data%>%
  mutate(context = recode(context,
                           "Predator absent" = "Control",
                           "Predator present" = "Risk cue"))

social.data <- social.data%>%
  mutate(context = recode(context,
                           "Predator absent" = "Control",
                           "Predator present" = "Risk cue"))

agg.plot <- ggplot(agg.data, aes(x = trial, y = bites, fill = context)) +
  geom_line(data = agg.data[agg.data$context == "Control",],
            aes(x = trial, y = bites, group = ID, color = context),
            position = position_nudge(x = -0.19),
            linewidth = 0.5, color = "orchid", alpha = 0.7) +
  geom_point(data = agg.data[agg.data$context == "Control",],
             aes(x = trial, y = bites, group = ID, color = context),
             position = position_nudge(x = -0.19),
             size = 2, shape = 21, fill = "orchid") +
  geom_line(data = agg.data[agg.data$context == "Risk cue",],
            aes(x = trial, y = bites, group = ID, color = context),
            position = position_nudge(x = 0.19),
            linewidth = 0.5, color = "turquoise", alpha = 0.7) +
  geom_point(data = agg.data[agg.data$context == "Risk cue",],
             aes(x = trial, y = bites, group = ID, color = context),
             position = position_nudge(x = 0.19),
             size = 2, shape = 21, fill = "turquoise") +
```

```

geom_boxplot(data = agg.data, aes(x = trial, y = bites, fill = context), alpha = 0.7) +
scale_fill_manual(values = c("orchid4", "turquoise3")) +
stat_summary(fun.y = "mean", geom = "point", aes(color=paste("mean", context)), position = position_d
scale_color_manual(values = c("turquoise4", "darkorchid4", "turquoise4", "darkorchid4")) +
ylab("Number of bites") +
xlab("") +
theme_classic() +
theme(legend.title = element_blank(),
      legend.position = c(0.18, 0.8),
      legend.text = element_text(size = 8)) +
annotate("text", label = "a.", x = 0.5, y = 180, size = 6)

```

```

## Warning: The 'fun.y' argument of 'stat_summary()' is deprecated as of ggplot2 3.3.0.
## i Please use the 'fun' argument instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.

```

```

## Warning: A numeric 'legend.position' argument in 'theme()' was deprecated in ggplot2
## 3.5.0.
## i Please use the 'legend.position.inside' argument of 'theme()' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.

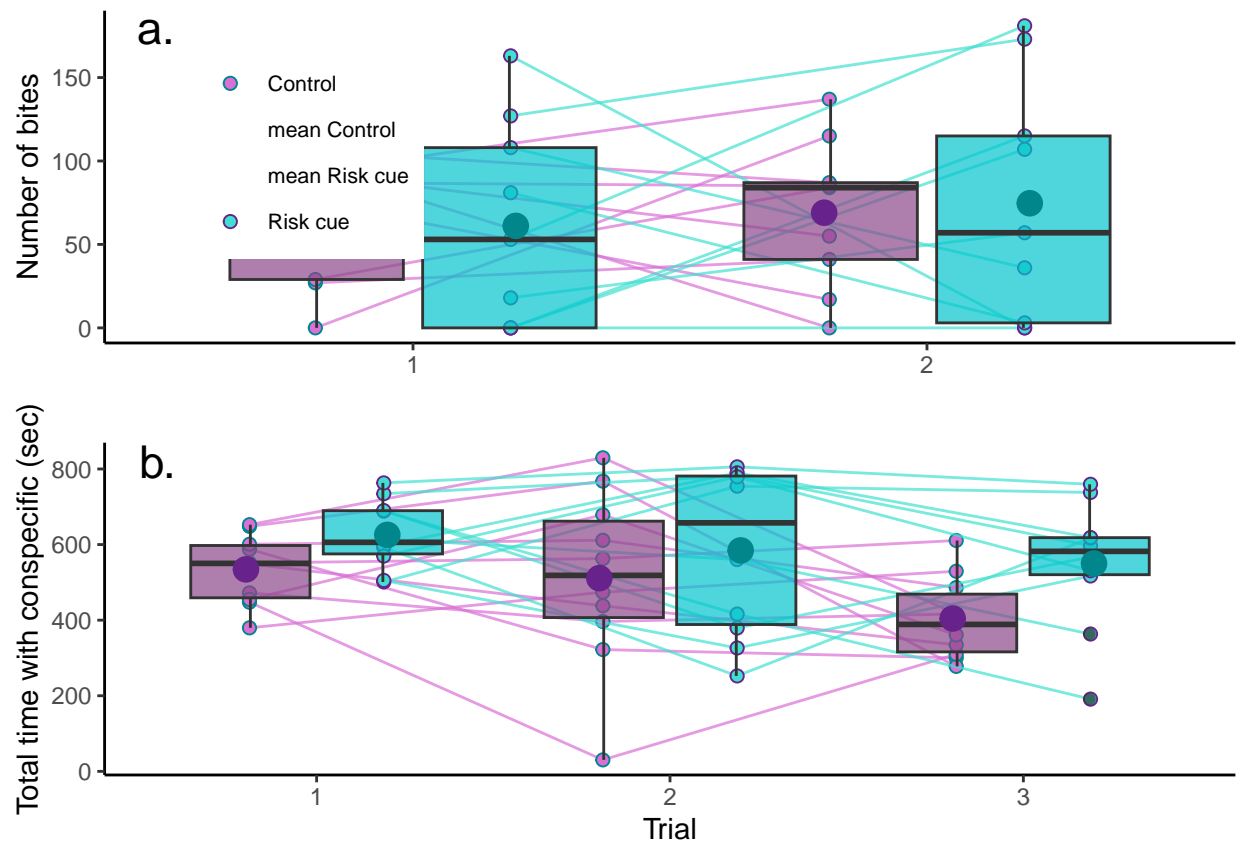
```

```

soc.plot <- ggplot(social.data, aes(x = trial, y = total_time, fill = context)) +
  geom_line(data = social.data[social.data$context == "Control",],
    aes(x = trial, y = total_time, group = ID, color = context),
    position = position_nudge(x = -0.19),
    linewidth = 0.5, color = "orchid", alpha = 0.7) +
  geom_point(data = social.data[social.data$context == "Control",],
    aes(x = trial, y = total_time, group = ID, color = context),
    position = position_nudge(x = -0.19),
    size = 2, shape = 21, fill = "orchid") +
  geom_line(data = social.data[social.data$context == "Risk cue",],
    aes(x = trial, y = total_time, group = ID, color = context),
    position = position_nudge(x = 0.19),
    linewidth = 0.5, color = "turquoise", alpha = 0.7) +
  geom_point(data = social.data[social.data$context == "Risk cue",],
    aes(x = trial, y = total_time, group = ID, color = context),
    position = position_nudge(x = 0.19),
    size = 2, shape = 21, fill = "turquoise") +
  geom_boxplot(data = social.data, aes(x = trial, y = total_time, fill = context), alpha = 0.7) +
  scale_fill_manual(values = c("orchid4", "turquoise3")) +
  stat_summary(fun.y = "mean", geom = "point", aes(color=paste("mean", context)), position = position_d
  scale_color_manual(values = c("turquoise4", "darkorchid4", "turquoise4", "darkorchid4")) +
  ylab("Total time with conspecific (sec)") +
  xlab("Trial") +
  theme_classic() +
  theme(legend.title = element_blank(),
        legend.position = "none") +
  annotate("text", label = "b.", x = 0.55, y = 820, size = 6)

```

```
fig <- ggpubr::ggarrange(agg.plot, soc.plot, ncol = 1)
fig
```



```
# ggsave(fig, file = "YSP figures.pdf", height = 180, width = 120, units = "mm", dpi = 600)
```

```
#NB: I removed one of the legend keys in Adobe after the fact as I could not figure it out in R!
```