Figure 2e

```r
library(readxl)
library(lubridate)
```

```
##
## Attaching package: 'lubridate'
```

```
## The following objects are masked from 'package:base':
##
##     date, intersect, setdiff, union
```

```r
library(mgcv)
```

```
## Loading required package: nlme
```

```
## This is mgcv 1.8-34. For overview type 'help("mgcv-package")'.
```

```r
library(ggplot2)
library(ggsignif)
library(readr)
library(emmeans)
library(tidyr)
library(plyr)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:plyr':
##
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize
```

```
## The following object is masked from 'package:nlme':
##
##     collapse
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```r
#load in raw data
dat <- data.frame(read_csv("2d+e_data.csv"))
```

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   `Repeat(Date)` = col_character(),
##   Algae = col_character(),
```

```
##   Treatment = col_character(),
##   Well_ID = col_character(),
##   Larvae = col_character(),
##   Time_to_first_expulsion = col_double()
## )
```

```r
head(dat)
```

```
##   Repeat.Date. Algae Treatment Well_ID Larvae Time_to_first_expulsion
## 1            I SSB01        No      S1      1                     720
## 2            I SSB01        No      S1      2                     720
## 3            I SSB01        No      S1      3                     720
## 4            I SSB01        No      S1    4.1                     720
## 5            I SSB01        No      S1    4-2                     720
## 6            I SSB01        No      S1      5                     385
```

```r
#clean up raw data
dat$Algae <- factor(dat$Algae, levels= c("SSB01", "M. gaditana", "N. oculata", "C. velia"))
dat$Well_ID <- factor(dat$Well_ID)
dat$Treatment <- factor(dat$Treatment)
dat$Repeat.Date. <- factor(as.character(dat$Repeat.Date.))
dat$Expulsion <- ifelse(dat$Time_to_first_expulsion<720,1,0)
head(dat)
```

```
##   Repeat.Date. Algae Treatment Well_ID Larvae Time_to_first_expulsion Expulsion
## 1            I SSB01        No      S1      1                     720         0
## 2            I SSB01        No      S1      2                     720         0
## 3            I SSB01        No      S1      3                     720         0
## 4            I SSB01        No      S1    4.1                     720         0
## 5            I SSB01        No      S1    4-2                     720         0
## 6            I SSB01        No      S1      5                     385         1
```

```r
#model data with generalized linear mixed modes
mod <- gam(Time_to_first_expulsion ~ Algae + s(Well_ID, bs="re"),data=dat,family=nb())
S <- summary(mod)$p.table
S
```

```
##                    Estimate Std. Error    z value      Pr(>|z|)
## (Intercept)       6.4737059  0.1366815 47.3634436 0.0000000000
## AlgaeM. gaditana -0.6526006  0.1872723 -3.4847677 0.0004925644
## AlgaeN. oculata  -0.6616846  0.2012795 -3.2873916 0.0010112011
## AlgaeC. velia    -0.1112702  0.2100024 -0.5298521 0.5962144953
```

```r
#model pairwise comparison (Tukey)
mod.emm.s <-  emmeans(mod, "Algae")
```

```
## NOTE: A nesting structure was detected in the fitted model:
##     Well_ID %in% Algae
```

```r
pairwise <- data.frame(pairs(mod.emm.s))
pairwise$label <- ifelse(pairwise$p.value < 0.001, "***",ifelse(pairwise$p.value < 0.01, "**",ifelse(pa
pairwise <- pairwise %>% separate(contrast, c("start", "end"), " - ")
head(pairwise)
```

```
##   start          end    estimate        SE       df   t.ratio
## 1 SSB01 M. gaditana 0.652600600 0.1817317 144.3942 3.59101147
## 2 SSB01  N. oculata 0.661684554 0.1961350 144.3942 3.37361878
## 3 SSB01    C. velia 0.111270217 0.2050768 144.3942 0.54257827
```

```
## 4 M. gaditana  N. oculata   0.009083953 0.1902004 144.3942  0.04775991
## 5 M. gaditana    C. velia -0.541330383 0.1994085 144.3942 -2.71468003
## 6  N. oculata    C. velia -0.550414337 0.2126177 144.3942 -2.58875154
##       p.value label
## 1 0.002517742    **
## 2 0.005214989    **
## 3 0.948401975
## 4 0.999960703
## 5 0.036969151     *
## 6 0.051351993
```

```r
write_csv(pairwise, "2e_pairwise.csv")
```

```r
#clean up results for graph
result <- data.frame(mod.emm.s)
results <- data.frame(Algae=result$Algae)
#Transform data (linkfunction)
results$mean<- exp(result$emmean)
results$down <- exp(result$lower.CL)
results$up <- exp(result$upper.CL)
head(results)
```

```
##        Algae     mean     down       up
## 1      SSB01 647.8803 498.2223 842.4932
## 2 M. gaditana 337.3447 264.0369 431.0060
## 3  N. oculata 334.2942 251.3630 444.5865
## 4    C. velia 579.6565 425.6900 789.3107
```

```r
write_csv(results, "2e_summary.csv")
```

```r
# prepair for geom_signif
signi <- data.frame(start = factor(pairwise$start))
signi$end <- factor(pairwise$end)
signi$label <- factor(pairwise$label)
signi <- filter(signi, label != "")
signi$y <- seq(780, length.out=nrow(signi), by=-20)
signi
```

```
##         start         end label   y
## 1       SSB01 M. gaditana    ** 780
## 2       SSB01  N. oculata    ** 760
## 3 M. gaditana    C. velia     * 740
```

```r
#Plot graph
formatter60 <- function(x){
x/60
}
P1 <- ggplot() +
  geom_dotplot(data = dat, aes(x=Algae, y=Time_to_first_expulsion, color = Algae, fill = Algae), binwidt
  scale_color_manual(breaks = c("SSB01", "M. gaditana", "N. oculata", "C. velia"),
  values=c("#FF2500","#E0D532","#008D58","#0067A2")) +
  scale_fill_manual(breaks = c("SSB01", "M. gaditana", "N. oculata", "C. velia"),
  values=c("#FF2500","#E0D532","#008D58","#0067A2")) +
  theme_classic() +
  labs(x = "", y = "Time to first expulsion (h)") +
  geom_signif(data = signi , aes(xmin = start, xmax = end, annotations = label, y_position = y),  tip_le
  scale_y_continuous(expand = c(0, 0), limits = c(0,800), labels = formatter60, breaks=c(120,240,360,48
```

```
## Warning: Ignoring unknown parameters: size
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
plot(P1)
```
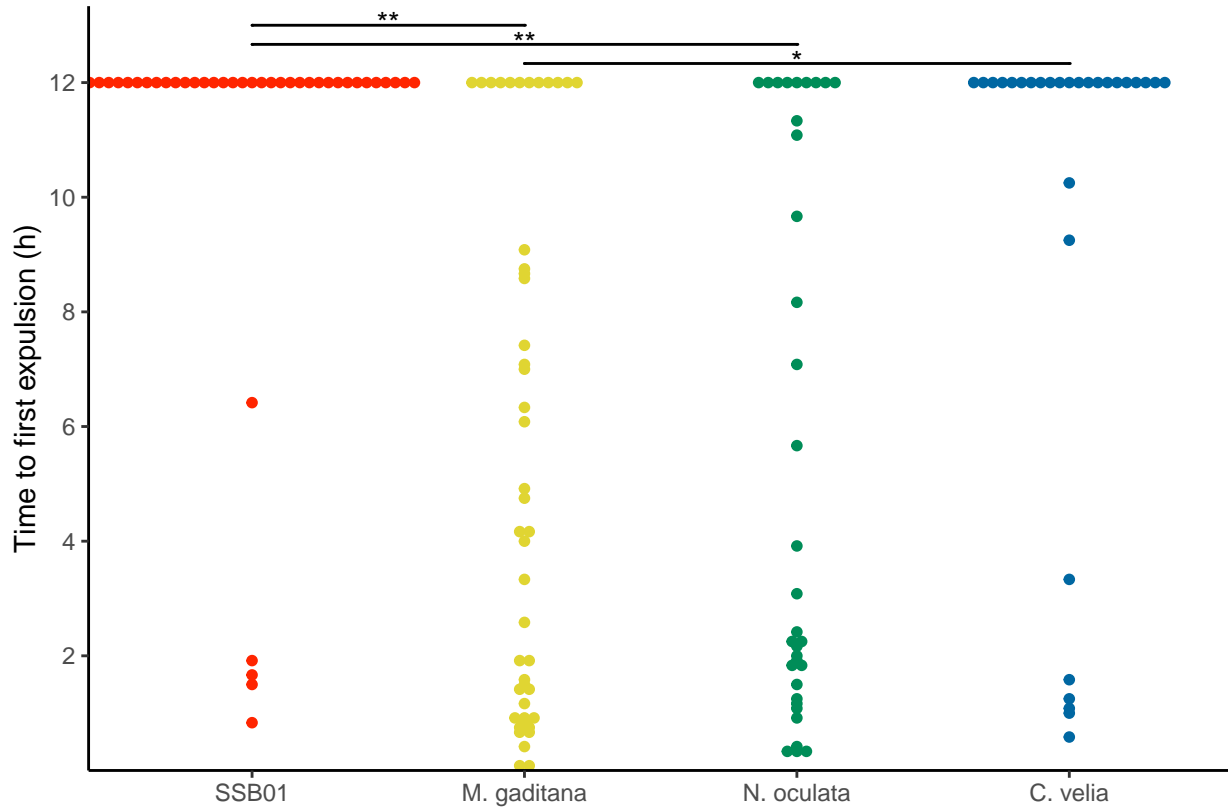


```
ggsave("Figure_2e.pdf",width=16,height=11,units="cm")
```

```
# get n count per treatment
ndat <- ddply(dat, c("Algae", "Treatment", "Repeat.Date."), summarise,
              N     = length(Larvae))
n_Figure <-  ddply(ndat, c("Algae", "Treatment"), summarise,
              N     = length(Repeat.Date.))
n_Figure
```

```
##          Algae Treatment N
## 1        SSB01        No 4
## 2 M. gaditana        No 4
## 3  N. oculata        No 4
## 4    C. velia        No 4
```

```
write_csv(n_Figure, "2e_n.csv")
```