

Figure 2d

```
library(readxl)
library(lubridate)

##
## Attaching package: 'lubridate'
## The following objects are masked from 'package:base':
##
##   date, intersect, setdiff, union
library(mgcv)

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

```
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
```

```
#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
```

```
#model data with generalized linear mixed modes
```

```
mod <- gam(Expulsion ~ Algae + s(Well_ID, bs="re"),data=dat,family=binomial())
S <- summary(mod)$p.table
S
```

```
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.9459097 0.4780983 -4.070103 4.699226e-05
## AlgaeM. gaditana 2.9873634 0.5842341 5.113298 3.165820e-07
## AlgaeN. oculata 2.9675662 0.6161945 4.815957 1.464962e-06
## AlgaeC. velia 0.9808267 0.6334066 1.548495 1.215033e-01
```

```
#model pairwise comparison (Tukey)
```

```
mod.emm.s <- emmeans(mod, "Algae")
```

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

```
## Well_ID %in% Algae
```

```
mod.emm.s <- emmeans(mod, "Algae")
```

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

```
## Well_ID %in% Algae
```

```
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"), " - ")
```

```
write_csv(pairwise, "2d_pairwise.csv")
```

```
#clean up results for graph
```

```
result <- data.frame(mod.emm.s)
summary <- data.frame(Algae=result$Algae)
#Transform data (linkfunction)
summary$mean<- plogis(result$emmean)
summary$down <- plogis(result$lower.CL)
summary$up <- plogis(result$upper.CL)
head(summary)
```

```
##           Algae      mean      down      up
## 1          SSB01 0.1250001 0.05260831 0.2687486
## 2 M. gaditana 0.7391304 0.59334409 0.8461997
## 3 N. oculata 0.7352952 0.56300006 0.8569232
## 4    C. velia 0.2758617 0.14353276 0.4640837
```

```
write_csv(summary, "2d_summary.csv")
```

```
# 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(0.975, length.out=nrow(signi), by=-0.025)
signi
```

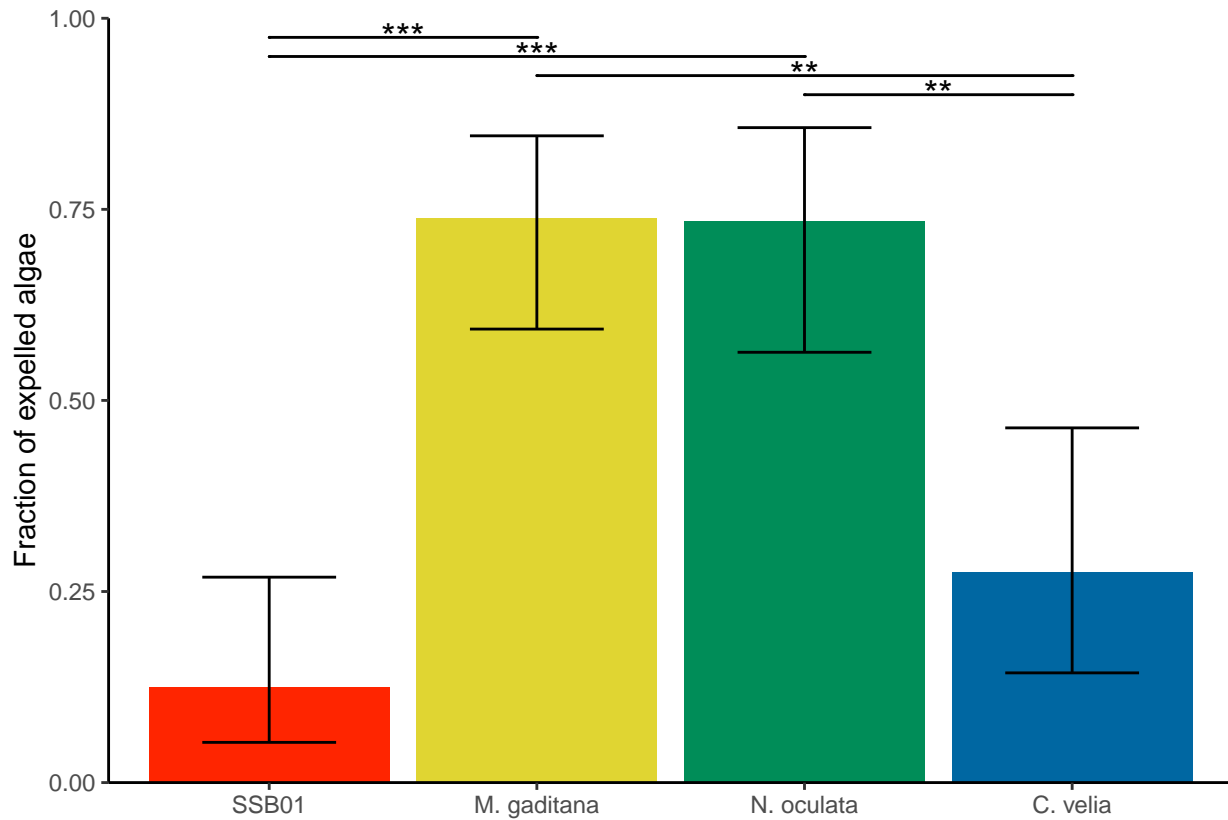
```
##           start      end label      y
## 1          SSB01 M. gaditana *** 0.975
## 2          SSB01 N. oculata *** 0.950
## 3 M. gaditana    C. velia ** 0.925
## 4 N. oculata     C. velia ** 0.900
```

```
#Plot graph
```

```
P1<-ggplot(data = summary) +
  geom_bar(aes(x = Algae, y = mean),stat = "identity", fill = c("#FF2500","#E0D532","#008D58","#0067A2"),
  geom_errorbar(aes(x = Algae, y = mean, ymin=down, ymax=up), width=.5) +
  theme_classic() +
  geom_signif(data = signi , aes(xmin = start, xmax = end, annotations = label, y_position = y), tip_l
  labs(x = "", y = "Fraction of expelled algae") +
  scale_y_continuous(expand = c(0, 0), limits = c(0,1))
```

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

```
plot(P1)
```



```
ggsave("Figure_2d.pdf",width=7.6,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, "2d_n.csv")
```