

Figure 2b

```
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("2b_data.csv"))

##
## -- Column specification -----
## cols(
##   `Repeat(Date)` = col_datetime(format = ""),
##   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 2018-05-04 SSB01 No S1 1 2880
## 2 2018-05-04 SSB01 No S1 5-1 2880
## 3 2018-05-04 SSB01 No S1 5-2 2880
## 4 2018-05-04 SSB01 No S1 5-3 2880
## 5 2018-05-04 SSB01 No S1 5-4 2880
## 6 2018-05-04 SSB01 No S1 5-5 2880

#clean up raw data
dat$Algae <- factor(dat$Algae, levels= c("SSB01", "M. gaditana", "N. oculata", "C. velia", "Beads"))
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<2880,1,0)
head(dat)

## Repeat.Date. Algae Treatment Well_ID Larvae Time_to_first_expulsion Expulsion
## 1 2018-05-04 SSB01 No S1 1 2880 0
## 2 2018-05-04 SSB01 No S1 5-1 2880 0
## 3 2018-05-04 SSB01 No S1 5-2 2880 0
## 4 2018-05-04 SSB01 No S1 5-3 2880 0
## 5 2018-05-04 SSB01 No S1 5-4 2880 0
## 6 2018-05-04 SSB01 No S1 5-5 2880 0

#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) -3.223580 2.475677 -1.3021006 0.1928820
## AlgaeM. gaditana 2.905127 3.904348 0.7440747 0.4568313
## AlgaeN. oculata 5.708487 4.013896 1.4221812 0.1549737
## AlgaeC. velia 3.560053 3.920572 0.9080442 0.3638549
## AlgaeBeads 3.080480 3.895076 0.7908651 0.4290227

#model pairwise comparison (Tukey)
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$p.value < 0.01, "**",ifelse(pairwise$p.value < 0.05, "*",)))
pairwise <- pairwise %>% separate(contrast, c("start", "end"), " - ")
head(pairwise)

## start end estimate SE df t.ratio p.value
## 1 SSB01 M. gaditana -2.905127 1.3767208 93.33559 -2.1101786 0.224504469
## 2 SSB01 N. oculata -5.708487 1.6621625 93.33559 -3.4343735 0.007729999

```

```
## 3      SSB01      C. velia -3.560053 1.4220788 93.33559 -2.5034144 0.098610939
## 4      SSB01      Beads -3.080480 1.3502003 93.33559 -2.2814983 0.160143558
## 5 M. gaditana N. oculata -2.803360 1.1398432 93.33559 -2.4594264 0.109005916
## 6 M. gaditana C. velia -0.654926 0.7475067 93.33559 -0.8761473 0.904969030
## label
## 1
## 2      **
## 3
## 4
## 5
## 6
```

```
write_csv(pairwise, "2b_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 0.03828793 0.003027665 0.3429378
## 2 M. gaditana 0.42105263 0.224238823 0.6466225
## 3 N. oculata 0.92307692 0.603032463 0.9895608
## 4 C. velia 0.58333333 0.304440869 0.8174527
## 5 Beads 0.46428571 0.289965876 0.6477921
```

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

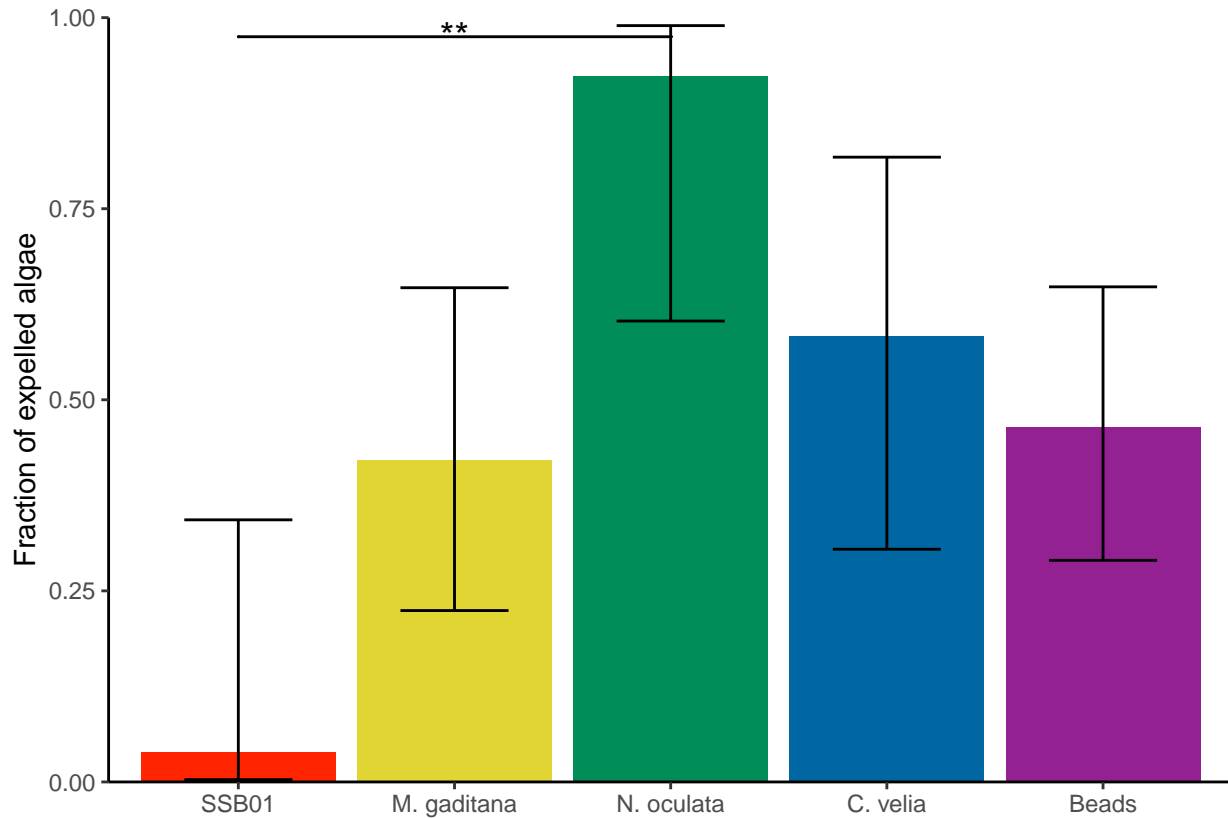
```
# prepair for geom_signif
signi <- data.frame(start = factor(pairwise$start))
signi$end <- factor(pairwise$end)
signi$label <- factor(pairwise$label)
signi <- filter(signi, label != "")
signi$y <- seq(0.975, length.out=nrow(signi), by=-0.025)
signi
```

```
## start      end label      y
## 1 SSB01 N. oculata      ** 0.975
```

```
#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_2b.pdf",width=9,height=11,units="cm")
```

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

```
##      Algae Treatment  N
## 1      SSB01        No 27
## 2 M. gaditana        No 19
## 3 N. oculata         No 13
## 4   C. velia        No 12
## 5      Beads        No 28
```

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