

Sehner_ex1_code

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This code is part of the data analyses for the manuscript titled "Timing of nanoplastic exposure during infection impacts viability of host in an aquatic host-parasite system" by Kristel F. S´anchez, Kyla Sehner, Vanderville Villegas, Amruta Rajarajan, Justyna Wolinska.

```
setwd("U://K Sehner MS/code")
```

Packages needed:

```
library(ggplot2)
library(ggpubr)
library(ggrepel)
library(ggsci)
library(ggsignif)
library(ggtext)
library(ggthemes)
library(Rmisc)
```

```
## Loading required package: lattice
```

```
## Loading required package: plyr
```

```
##
## Attaching package: 'plyr'
```

```
## The following object is masked from 'package:ggpubr':
##
## mutate
```

```
library(survival)
library(survminer)
```

```
## Warning: package 'survminer' was built under R version 4.4.2
```

```
##
## Attaching package: 'survminer'
```

```
## The following object is masked from 'package:survival':  
##  
## myeloma
```

```
library(kableExtra)
```

```
## Warning: package 'kableExtra' was built under R version 4.4.2
```

Experiment one data analyses

```
dat1<-read.csv('Ex1_stat.csv')
```

Does exposure of parasite to nanoplastics reduce infectivity?

```
infglm <- glm(infectivity ~ NP, family = binomial, data = dat1)  
summary(infglm)
```

```
##  
## Call:  
## glm(formula = infectivity ~ NP, family = binomial, data = dat1)  
##  
## Coefficients:  
##             Estimate Std. Error z value Pr(>|z|)  
## (Intercept)  2.708      1.033   2.622 0.00874 **  
## NP           0.456      1.189   0.383 0.70136  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
##  
## (Dispersion parameter for binomial family taken to be 1)  
##  
## Null deviance: 32.728  on 89  degrees of freedom  
## Residual deviance: 32.591  on 88  degrees of freedom  
## AIC: 36.591  
##  
## Number of Fisher Scoring iterations: 6
```

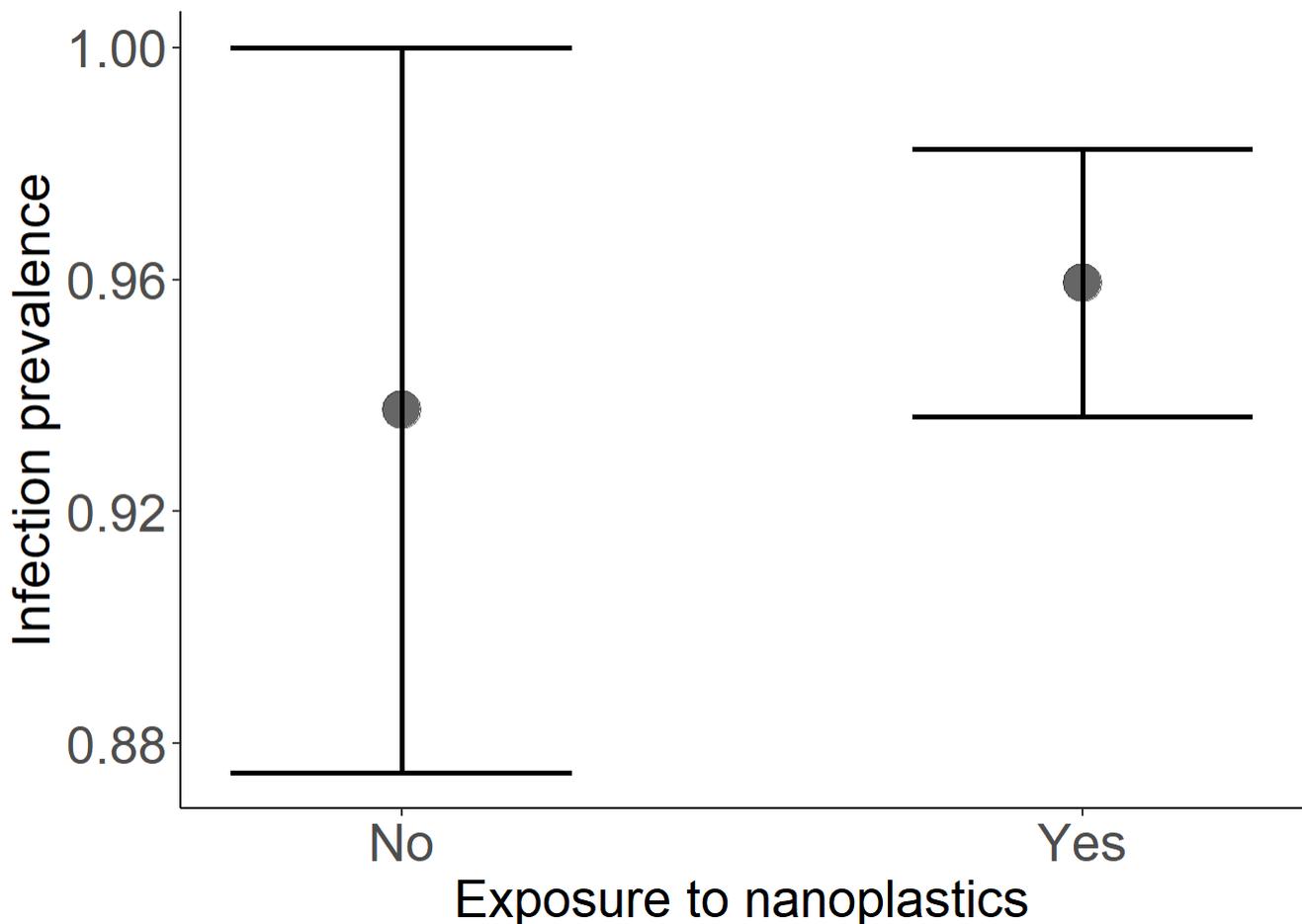
```
infSE<-summarySE(dat1, measurevar= 'infectivity', groupvars = c('NP'), na.rm=TRUE)  
infSE
```

```
##   NP  N infectivity      sd      se      ci  
## 1  0 16  0.9375000 0.2500000 0.06250000 0.13321560  
## 2  1 74  0.9594595 0.1985695 0.02308323 0.04600482
```

```
p1<-ggplot()+
  geom_point(data=infSE, aes(x=NP, y=infectivity), size=7, stroke=0, alpha=.6) +
  geom_errorbar(data=infSE, aes(x=NP, ymin=infectivity-se, ymax=infectivity+se), size=1, width
h=0.5) +
  scale_x_continuous(breaks = c(0, 1),
                    label = c("No", "Yes")) +
  ylab("Infection prevalence") +
  xlab("Exposure to nanoplastics") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 20),
        axis.text.y = element_text(size = 20),
        axis.title.x = element_text(size = 20),
        axis.title.y = element_text(size = 20))
```

```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

p1



```
infglm2 <- glm (infectivity ~ Conc * Size, family = binomial, data=dat1)
summary(infglm2)
```

```
##
## Call:
## glm(formula = infectivity ~ Conc * Size, family = binomial, data = dat1)
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.1554102  1.1821153   2.669  0.0076 **
## Conc        -0.8363530  0.7838678  -1.067  0.2860
## Size        -0.0008566  0.0220329  -0.039  0.9690
## Conc:Size    0.0129993  0.0155215   0.838  0.4023
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##    Null deviance: 32.728  on 89  degrees of freedom
## Residual deviance: 30.389  on 86  degrees of freedom
## AIC: 38.389
##
## Number of Fisher Scoring iterations: 8
```

```
inf2SE<-summarySE(dat1, measurevar= 'infectivity', groupvars = c('Conc', 'Size'), na.rm=TRUE)
inf2SE
```

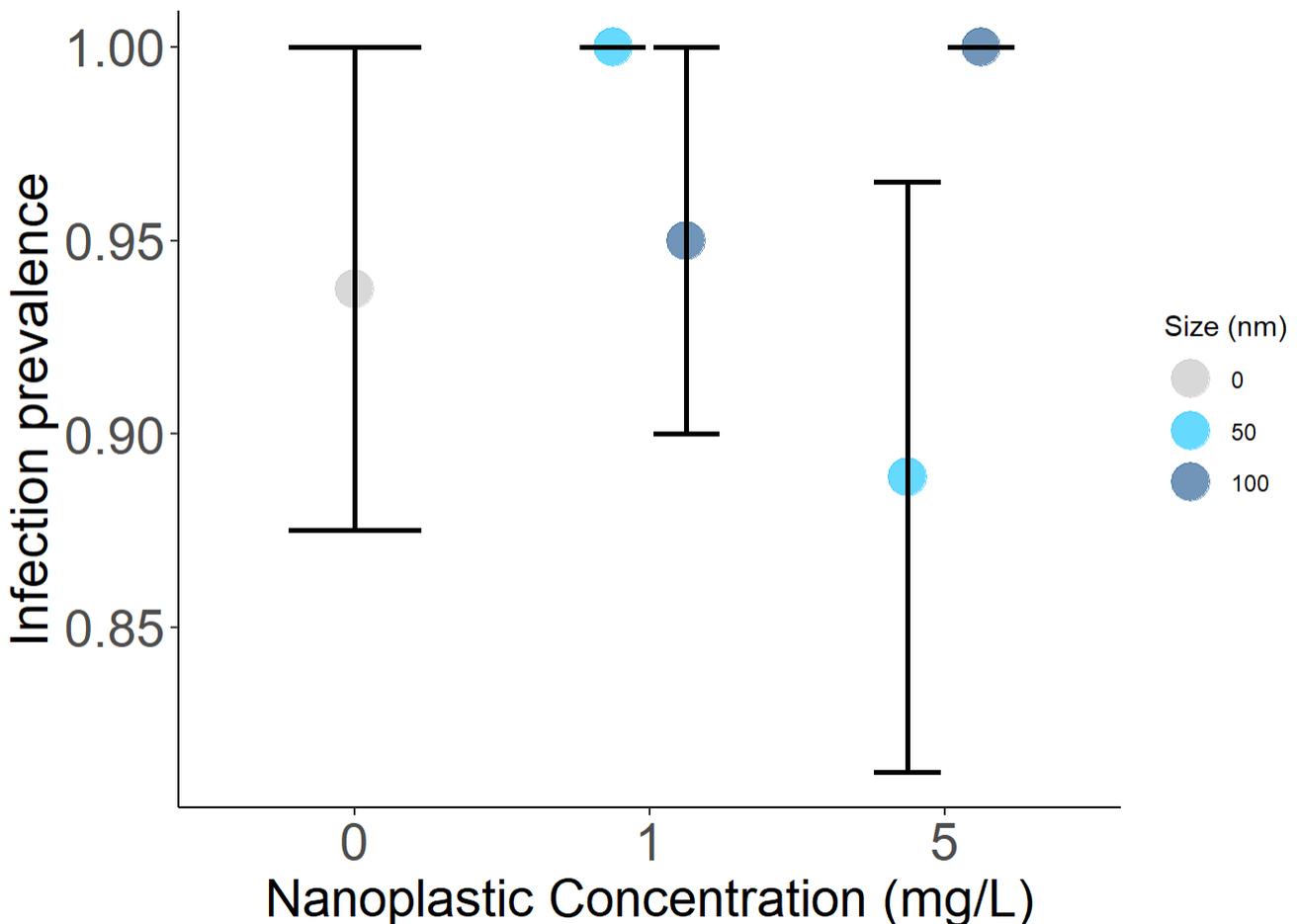
```
##   Conc Size  N infectivity      sd      se      ci
## 1    0    0 16  0.9375000 0.2500000 0.06250000 0.1332156
## 2    1   50 17  1.0000000 0.0000000 0.00000000 0.0000000
## 3    1  100 20  0.9500000 0.2236068 0.05000000 0.1046512
## 4    5   50 18  0.8888889 0.3233808 0.07622159 0.1608135
## 5    5  100 19  1.0000000 0.0000000 0.00000000 0.0000000
```

```

p2<-ggplot()+
  geom_point(data=inf2SE, aes(x=factor(Conc), y=infectivity, color=factor(Size)), size=7, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=inf2SE, aes(x=factor(Conc), ymin=infectivity-se, ymax=infectivity+se), size=1, width=0.5, position= position_dodge2()) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
                    name = c("Size (nm)"))+
  ylab("Infection prevalence") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 20),
        axis.text.y = element_text(size = 20),
        axis.title.x = element_text(size = 20),
        axis.title.y = element_text(size = 20))

```

p2



Does parasite pre-exposure to nanoplastics impacts parasite fitness (parasite reproduction within the host)?

In data set there are two columns with spore data. The column labeled "spores" is total spore reproduction per hosts, while "Parasite.Reproduction" is a rate, total spores / number of days hosts survived post-parasite exposure, so it would be daily spore production or reproduction rate.

Within host-reproduction

First, remove non-infected individuals

```
dat1.2 <- subset( dat1, infectivity == 1)
```

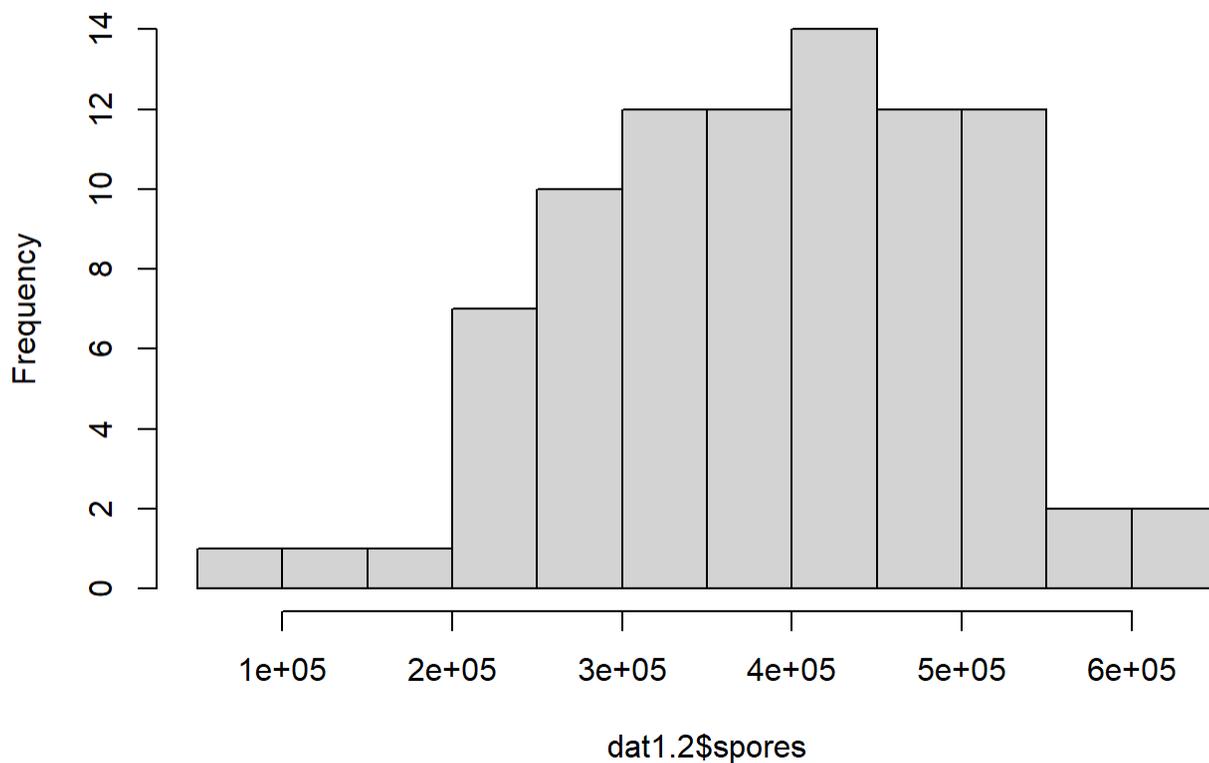
Data distribution

```
shapiro.test(dat1.2$spores)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data:  dat1.2$spores  
## W = 0.9827, p-value = 0.3054
```

```
hist(dat1.2$spores)
```

Histogram of dat1.2\$spores



Data looks normal

```
sporeglm <- glm( spores ~ Conc * Size, family= Gamma, data=dat1.2)  
summary(sporeglm)
```

```
##
## Call:
## glm(formula = spores ~ Conc * Size, family = Gamma, data = dat1.2)
##
## Coefficients:
##           Estimate Std. Error t value Pr(>|t|)
## (Intercept) 2.390e-06 1.684e-07 14.192 <2e-16 ***
## Conc        5.768e-09 9.146e-08  0.063  0.950
## Size        1.415e-09 2.818e-09  0.502  0.617
## Conc:Size   4.774e-10 1.232e-09  0.388  0.699
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Gamma family taken to be 0.08404538)
##
## Null deviance: 8.3661 on 85 degrees of freedom
## Residual deviance: 8.1298 on 82 degrees of freedom
## AIC: 2257.6
##
## Number of Fisher Scoring iterations: 5
```

```
sporeSE<-summarySE(dat1.2, measurevar= 'spores', groupvars = c('Conc', 'Size'), na.rm=TRUE)
sporeSE
```

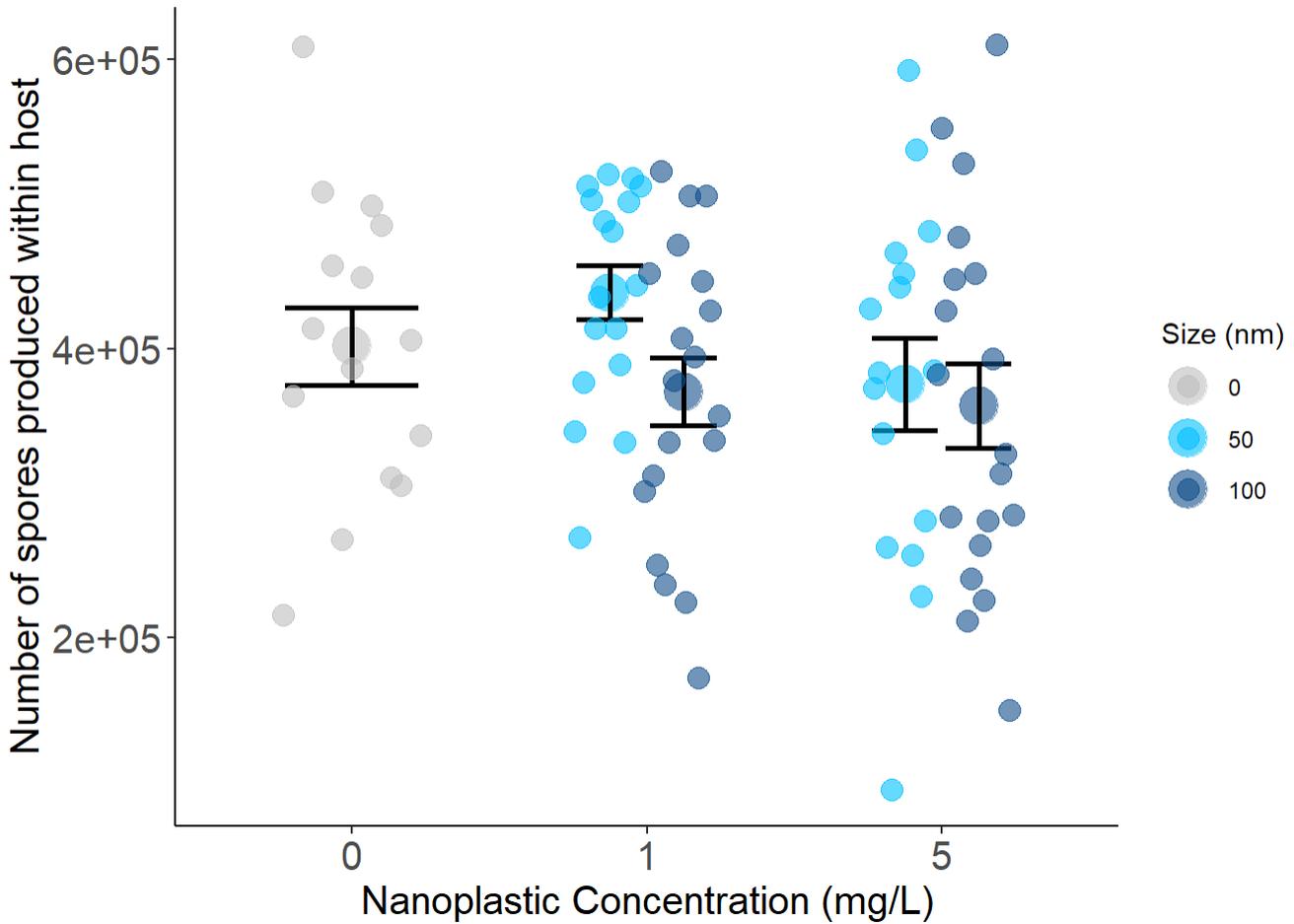
```
##   Conc Size  N  spores      sd      se      ci
## 1    0    0 15 401500.0 104137.5 26888.19 57669.44
## 2    1   50 17 438867.6  76108.2 18458.95 39131.23
## 3    1  100 19 370164.5 103233.5 23683.39 49756.95
## 4    5   50 16 375375.0 127420.3 31855.07 67897.48
## 5    5  100 19 360611.8 127576.4 29268.02 61489.84
```

```

p3<-ggplot()+
  geom_point(data=sporeSE, aes(x=factor(Conc), y=spores, color=factor(Size)), size=7, stroke=
0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=sporeSE, aes(x=factor(Conc), ymin=spores-se, ymax=spores+se), size=1, wi
dth=0.5, position= position_dodge2()) +
  geom_point(data=dat1.2, aes(x=factor(Conc), y=spores, color=factor(Size)), size=4, stroke=
0, alpha=.6, position=position_dodge2(width=0.5)) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
  name = c("Size (nm)"))+
  ylab("Number of spores produced within host") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 15),
  axis.text.y = element_text(size = 15),
  axis.title.x = element_text(size = 15),
  axis.title.y = element_text(size = 15))

```

p3



```

sporerepglm <- glm( Parasite.Reproduction ~ Conc * Size, data=dat1.2)
summary(sporerepglm)

```

```
##
## Call:
## glm(formula = Parasite.Reproduction ~ Conc * Size, data = dat1.2)
##
## Coefficients:
##           Estimate Std. Error t value Pr(>|t|)
## (Intercept) 24880.3483  1315.0648  18.919  <2e-16 ***
## Conc        -98.9976   661.4471  -0.150   0.881
## Size         0.1237    21.1891   0.006   0.995
## Conc:Size    -5.0682     8.7345  -0.580   0.563
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 29629926)
##
## Null deviance: 2538284199  on 85  degrees of freedom
## Residual deviance: 2429653910  on 82  degrees of freedom
## AIC: 1729.5
##
## Number of Fisher Scoring iterations: 2
```

```
sporerepSE<-summarySE(dat1.2, measurevar= 'Parasite.Reproduction', groupvars = c('Conc', 'Size'), na.rm=TRUE)
sporerepSE
```

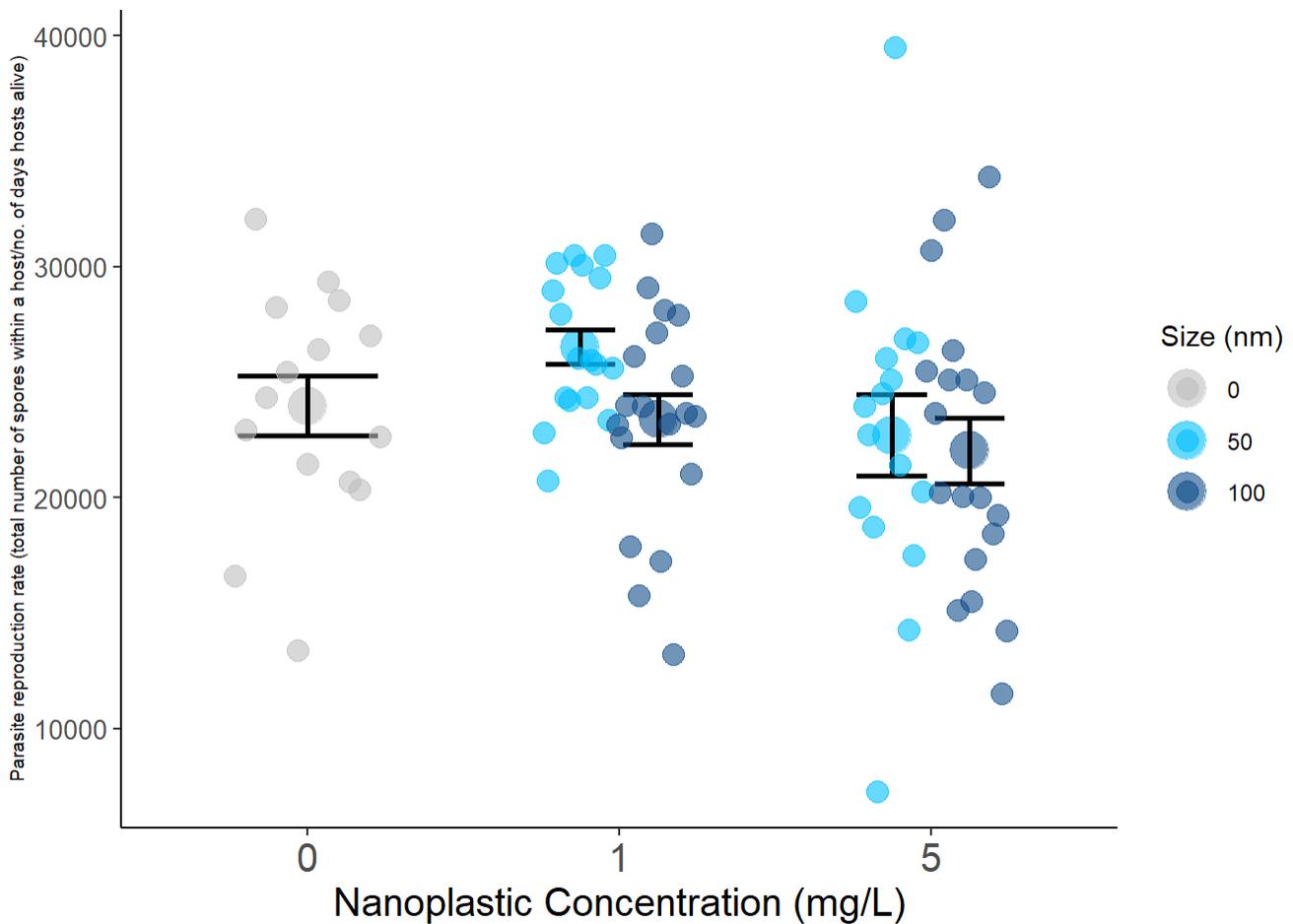
```
##   Conc Size  N Parasite.Reproduction      sd      se      ci
## 1    0    0  15                23975.94 5002.796 1291.7165 2770.456
## 2    1   50  17                26529.14 3045.980  738.7586 1566.098
## 3    1  100  19                23394.39 4725.988 1084.2161 2277.853
## 4    5   50  16                22700.55 7022.907 1755.7268 3742.243
## 5    5  100  19                22042.13 6193.275 1420.8348 2985.063
```

```

p4<-ggplot()+
  geom_point(data=sporerepSE, aes(x=factor(Conc), y=Parasite.Reproduction, color=factor(Size)), size=7, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=sporerepSE, aes(x=factor(Conc), ymin=Parasite.Reproduction-se, ymax=Parasite.Reproduction+se), size=1, width=0.5, position= position_dodge2()) +
  geom_point(data=dat1.2, aes(x=factor(Conc), y=Parasite.Reproduction, color=factor(Size)), size=4, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
                    name = c("Size (nm)"))+
  ylab("Parasite reproduction rate (total number of spores within a host/no. of days hosts alive)") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 15),
        axis.text.y = element_text(size = 10),
        axis.title.x = element_text(size = 15),
        axis.title.y = element_text(size = 7))

```

p4



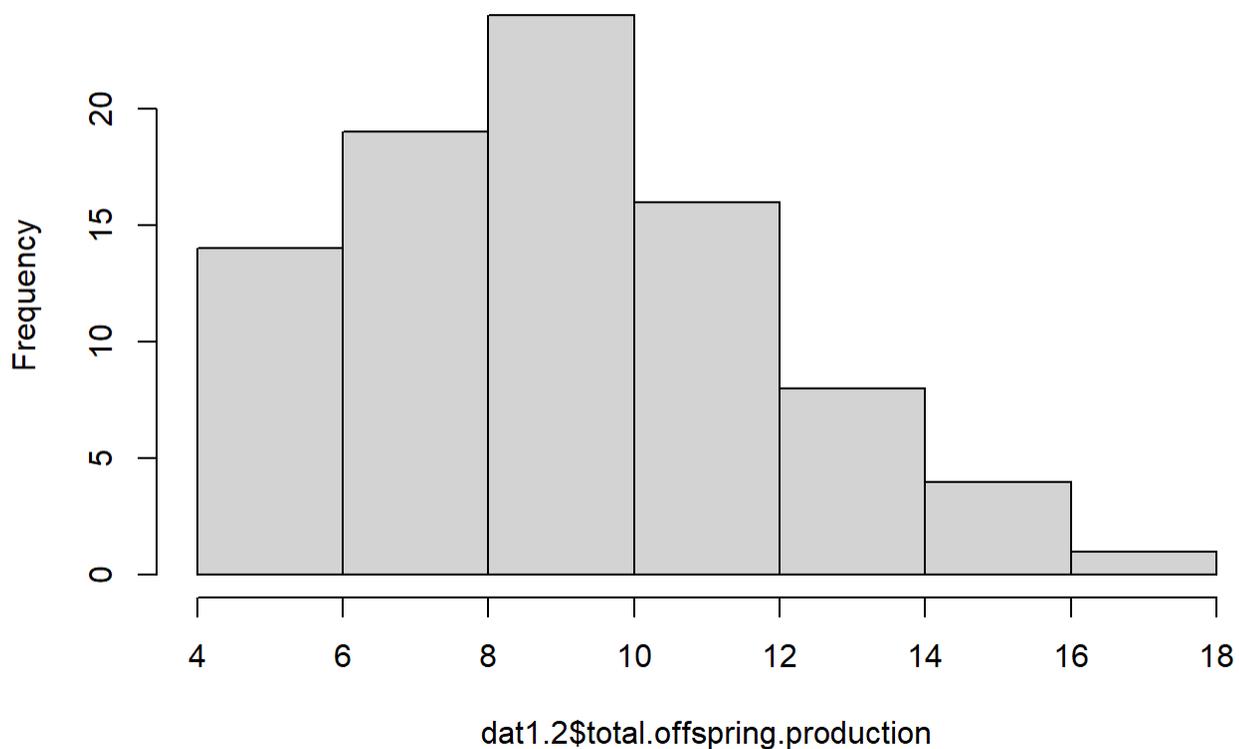
host fitness aka changes in virulence

```
shapiro.test(dat1.2$total.offspring.production)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data:  dat1.2$total.offspring.production  
## W = 0.97467, p-value = 0.08888
```

```
hist(dat1.2$total.offspring.production)
```

Histogram of dat1.2\$total.offspring.production



```
hoffsglm <- glm (total.offspring.production ~ Conc * Size, family=poisson, data=dat1.2)  
summary(hoffsglm)
```

```
##
## Call:
## glm(formula = total.offspring.production ~ Conc * Size, family = poisson,
##      data = dat1.2)
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  2.2951696  0.0769926  29.810  <2e-16 ***
## Conc         0.0008927  0.0389204   0.023   0.982
## Size        -0.0013532  0.0012735  -1.063   0.288
## Conc:Size    0.0001798  0.0005180   0.347   0.729
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for poisson family taken to be 1)
##
## Null deviance: 75.600  on 85  degrees of freedom
## Residual deviance: 74.297  on 82  degrees of freedom
## AIC: 431
##
## Number of Fisher Scoring iterations: 4
```

```
hoffSE<-summarySE(dat1.2, measurevar= 'total.offspring.production', groupvars = c('Conc', 'Si
ze'), na.rm=TRUE)
hoffSE
```

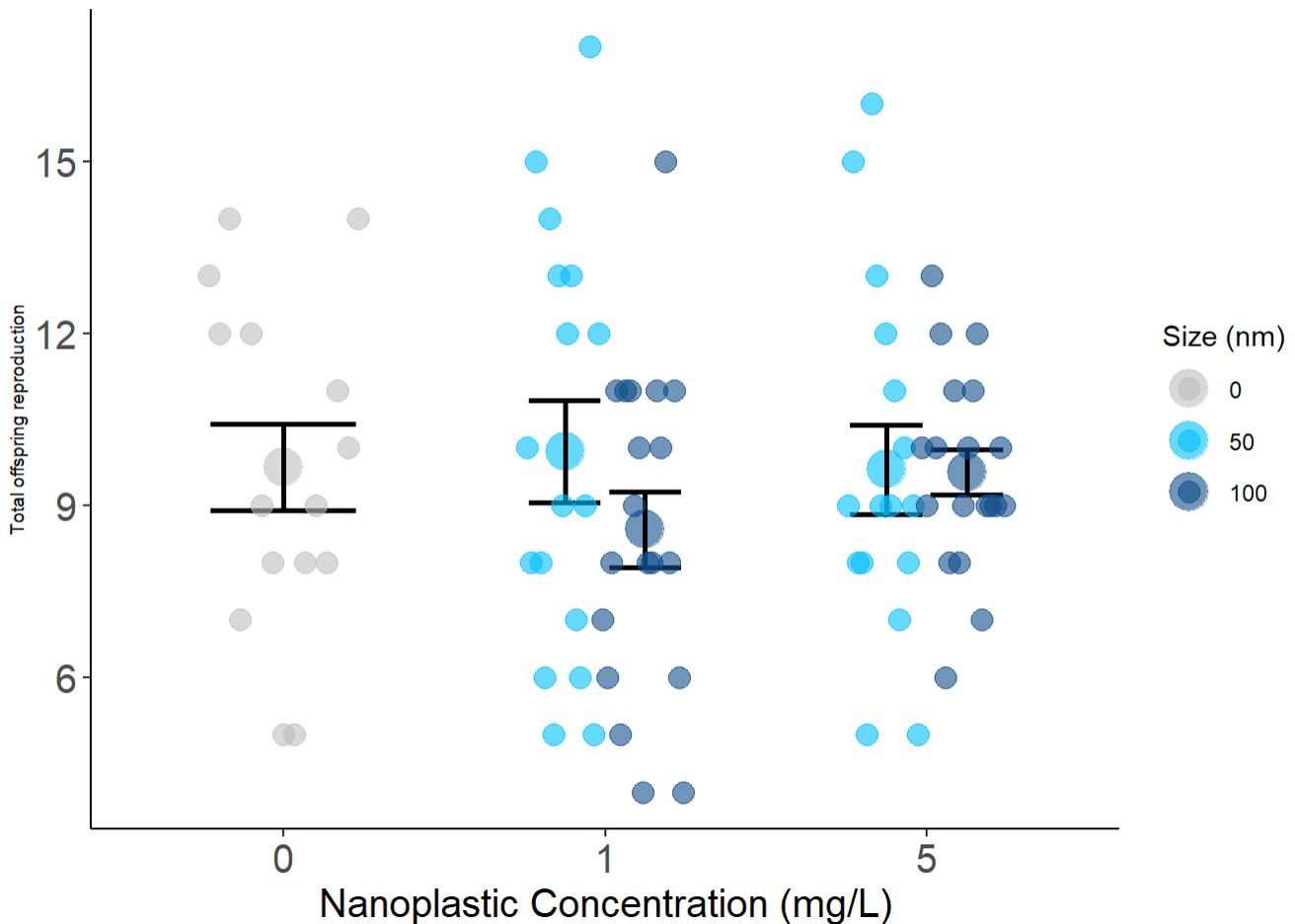
```
##   Conc Size  N total.offspring.production      sd      se      ci
## 1    0    0  15          9.666667  2.943920  0.7601170  1.6302887
## 2    1    50  17          9.941176  3.682231  0.8930721  1.8932284
## 3    1   100  19          8.578947  2.854155  0.6547880  1.3756585
## 4    5    50  16          9.625000  3.138471  0.7846177  1.6723731
## 5    5   100  19          9.578947  1.742150  0.3996767  0.8396896
```

```

p5<-ggplot()+
  geom_point(data=hoffSE, aes(x=factor(Conc), y=total.offspring.production, color=factor(Size)), size=7, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=hoffSE, aes(x=factor(Conc), ymin=total.offspring.production-se, ymax=total.offspring.production+se), size=1, width=0.5, position= position_dodge2()) +
  geom_point(data=dat1.2, aes(x=factor(Conc), y=total.offspring.production, color=factor(Size)), size=4, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
                    name = c("Size (nm)"))+
  ylab("Total offspring reproduction") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 15),
        axis.text.y = element_text(size = 15),
        axis.title.x = element_text(size = 15),
        axis.title.y = element_text(size = 7))

```

p5



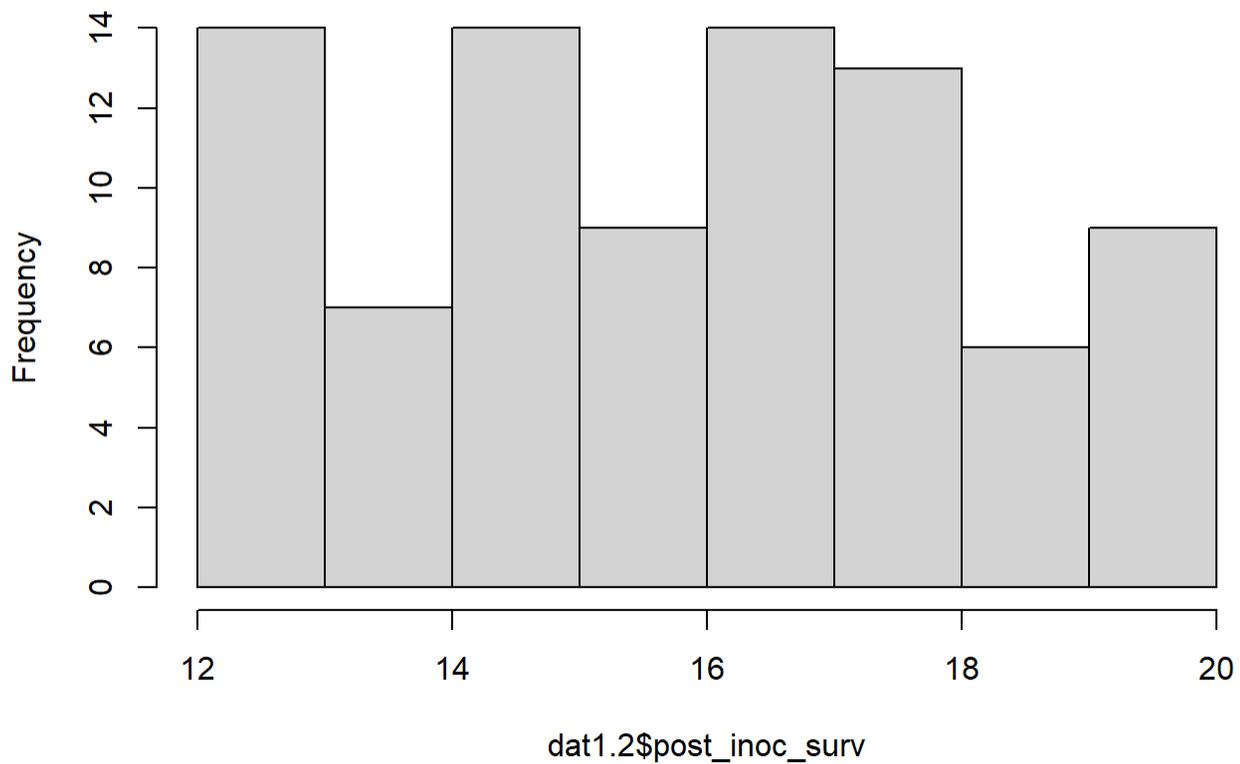
survivability post-infection

```
shapiro.test(dat1.2$post_inoc_surv)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data:  dat1.2$post_inoc_surv  
## W = 0.94606, p-value = 0.001303
```

```
hist(dat1.2$post_inoc_surv)
```

Histogram of dat1.2\$post_inoc_surv



```
survglm <- glm(post_inoc_surv ~ Conc * Size, family=poisson, data=dat1.2)  
summary(survglm)
```

```
##
## Call:
## glm(formula = post_inoc_surv ~ Conc * Size, family = poisson,
##      data = dat1.2)
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  2.820e+00  5.910e-02  47.717  <2e-16 ***
## Conc         -6.419e-04  2.996e-02  -0.021   0.983
## Size         -6.572e-04  9.654e-04  -0.681   0.496
## Conc:Size     5.934e-05  3.974e-04   0.149   0.881
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for poisson family taken to be 1)
##
## Null deviance: 27.273  on 85  degrees of freedom
## Residual deviance: 26.726  on 82  degrees of freedom
## AIC: 432.64
##
## Number of Fisher Scoring iterations: 4
```

```
survSE<-summarySE(dat1.2, measurevar= 'post_inoc_surv', groupvars = c('Conc', 'Size'), na.rm=
TRUE)
survSE
```

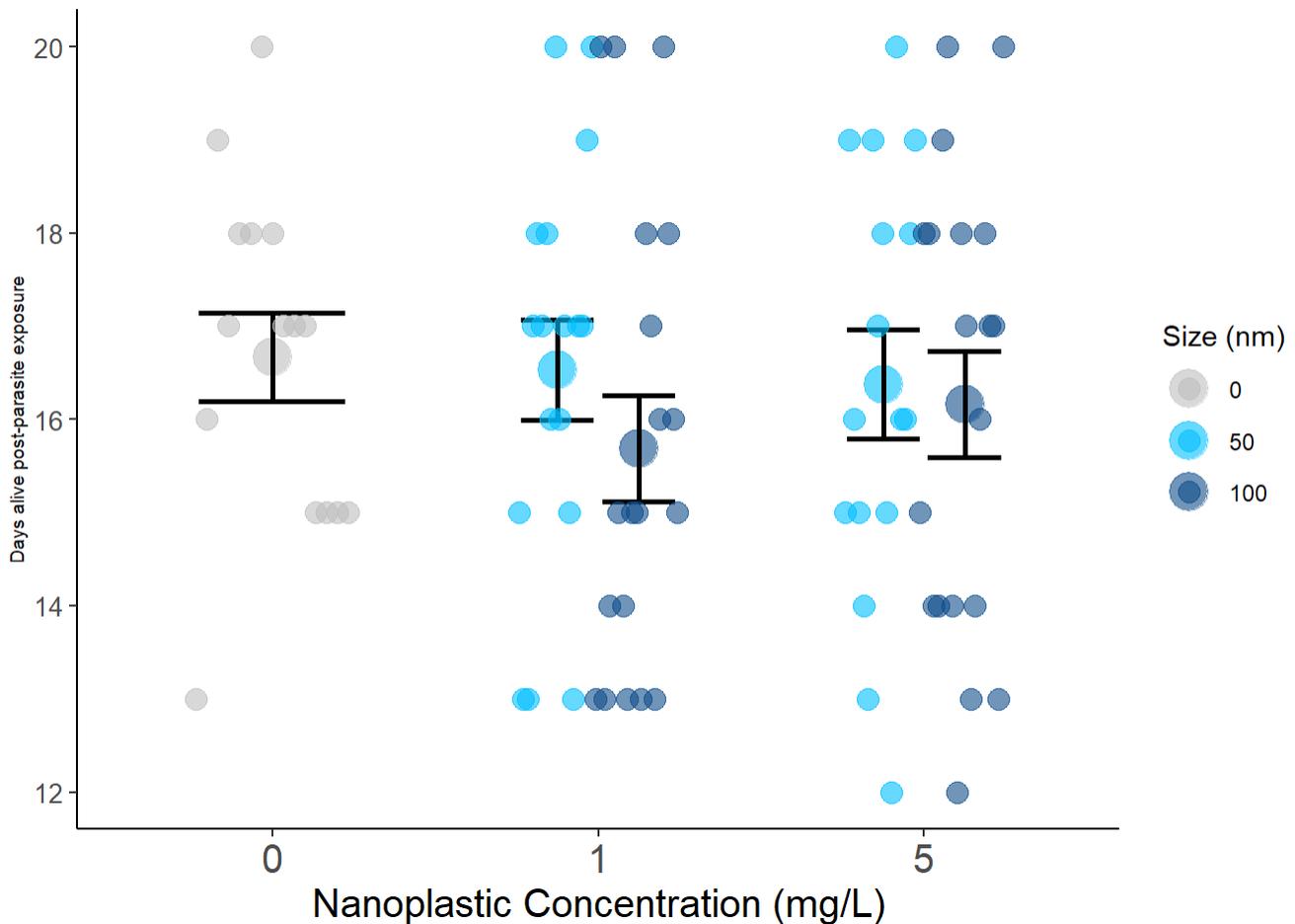
```
##   Conc Size  N post_inoc_surv      sd      se      ci
## 1    0    0  15      16.66667  1.838737  0.4747598  1.018258
## 2    1   50  17      16.52941  2.211268  0.5363113  1.136929
## 3    1  100  19      15.68421  2.495610  0.5725322  1.202846
## 4    5   50  16      16.37500  2.334524  0.5836309  1.243980
## 5    5  100  19      16.15789  2.477973  0.5684860  1.194345
```

```

p5<-ggplot()+
  geom_point(data=survSE, aes(x=factor(Conc), y=post_inoc_surv, color=factor(Size)), size=7,
stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=survSE, aes(x=factor(Conc), ymin=post_inoc_surv-se, ymax=post_inoc_surv
+se), size=1, width=0.5, position= position_dodge2()) +
  geom_point(data=dat1.2, aes(x=factor(Conc), y=post_inoc_surv, color=factor(Size)), size=4,
stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
name = c("Size (nm)"))+
  ylab("Days alive post-parasite exposure") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 15),
axis.text.y = element_text(size = 10),
axis.title.x = element_text(size = 15),
axis.title.y = element_text(size = 7))

```

p5



proportion of animals that died:

```

survbinglm <- glm(complete.survival ~ Conc * Size, family=binomial, data=dat1.2)

```

```
## Warning: glm.fit: fitted probabilities numerically 0 or 1 occurred
```

```
summary(survbinglm)
```

```
##
## Call:
## glm(formula = complete.survival ~ Conc * Size, family = binomial,
##      data = dat1.2)
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -2.639e+00  1.035e+00  -2.550  0.0108 *
## Conc         1.702e+01  2.946e+03   0.006  0.9954
## Size        -2.993e-03  3.346e-02  -0.089  0.9287
## Conc:Size    -3.400e-01  5.893e+01  -0.006  0.9954
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##      Null deviance: 26.029  on 85  degrees of freedom
## Residual deviance: 22.436  on 82  degrees of freedom
## AIC: 30.436
##
## Number of Fisher Scoring iterations: 21
```

```
survbinSE<-summarySE(dat1.2, measurevar= 'complete.survival', groupvars = c('Conc', 'Size'),
na.rm=TRUE)
survbinSE
```

```
##   Conc Size  N complete.survival      sd      se      ci
## 1    0    0 15      0.06666667 0.2581989 0.06666667 0.1429858
## 2    1    50 17      0.05882353 0.2425356 0.05882353 0.1247003
## 3    1   100 19      0.00000000 0.0000000 0.00000000 0.0000000
## 4    5    50 16      0.06250000 0.2500000 0.06250000 0.1332156
## 5    5   100 19      0.00000000 0.0000000 0.00000000 0.0000000
```

```

p6<-ggplot()+
  geom_point(data=survbinSE, aes(x=factor(Conc), y=complete.survival, color=factor(Size)), si
  ze=7, stroke=0, alpha=.6, position=position_dodge2(width=0.5)) +
  geom_errorbar(data=survbinSE, aes(x=factor(Conc), ymin=complete.survival-se, ymax=complet
  e.survival+se), size=1, width=0.5, position= position_dodge2()) +
  scale_color_manual(values = c('grey',"deepskyblue", "dodgerblue4"),
    name = c("Size (nm)"))+
  scale_y_continuous(limits = c(0, 0.5))+
  ylab("Proportion of infected animals that survived to dat 20 post-parasite exposure") +
  xlab("Nanoplastic Concentration (mg/L)") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 15),
    axis.text.y = element_text(size = 10),
    axis.title.x = element_text(size = 15),
    axis.title.y = element_text(size = 7))

```

p6

