What are the effects of invasive species on a native aquatic community?

Background

Invasive species can alter the ecology and evolution of native species and are important drivers of extinction (Vitousek et al. 1997, Clavero and Garcia-Berthou 2005). Yet, when species introductions occur alongside other types of environmental change, it can be challenging to disentangle the ecological impacts of individual nonnative species relative to other stressors (Didham et al. 2005, Light and Marchetti 2007). This challenge becomes amplified when multiple nonnative species co-occur. Understanding the individual and combined effects of multiple invasive species is especially important to natural resource managers, who are often pressed to prioritize which invasive species to manage (Simberloff et al. 2005).

Two common invaders in freshwater habitats in western North America are the mosquitofish (*Gambusia affinis* and *G. holbrooki*) and the American bullfrog (*Lithobates catesbeianus* or *Rana catesbeiana*). Both species are native to the eastern United States. Mosquitofish are the most widespread freshwater fish and have been introduced on every continent besides Antarctica, while bullfrogs are invasive throughout western North America and regions of South America, Europe, and Asia (Lever 2003, Pyke 2008). Both species have deleterious effects on native aquatic communities; mosquitofish prey on a wide diversity of aquatic invertebrates, amphibians, and other fish (Goodsell and Kats 1999, Leyse et al. 2004), and bullfrogs have negative effects on other aquatic amphibians through competition, predation, and disease transmission (Kiesecker et al. 2001, Pearl et al. 2004).

In this activity, you will use data from an outdoor mesocosm experiment to explore how these two common invasive species, mosquitofish and the American bullfrog, influence the native amphibian community and other aquatic taxa (snails, zooplankton, and phytoplankton). Bullfrog larvae are herbivores and mosquitofish prey on treefrogs, newts, and zooplankton. You will examine both the direct and indirect effects of two invasive species on native amphibians.
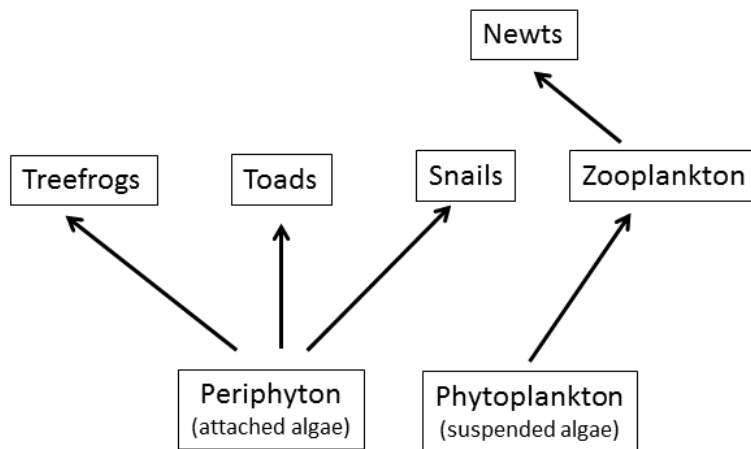
Research Questions

1. How does the presence of bullfrogs and mosquitofish influence the survival of native amphibian species?

2. How does the presence of bullfrogs and mosquitofish influence the mass (an indicator of amphibian growth) of the native amphibians that survived?

3. How does the presence of bullfrogs and mosquitofish influence the density of snails, zooplankton (*Daphnia* and copepods), and phytoplankton (i.e. relative phytoplankton fluorescence)?

What are the null hypotheses?

Data

A total of 20 outdoor pond mesocosms were established by placing 370 L of well water, 45 ml of pond mud, 15 g of rabbit chow, 25 g of dry leaf litter, and 1.25 L of pond water containing concentrated zooplankton into each plastic tank (1.3 m Length × 0.79 m Width × 0.64 m Height).

Photo 2. This photo is of the outdoor mesocosms used to experimentally evaluate the effects of mosquitofish and bullfrog tadpoles on native aquatic communities. Photo Credit: D.L. Preston

Mesocosms are small-scale representations of larger systems, which make it possible to do controlled experiments in a semi-realistic setting. The experiment involved a 2 x 2 factorial design (4 total treatments) that manipulated the presence of mosquitofish and bullfrogs within outdoor mesocosms.

Mosquitofish

		−	**+**
Bullfrogs	**I**	<u>Native Treatment (Control)</u> Native Amphibians Only	<u>Mosquitofish Treatment</u> Native Amphibians + Mosquitofish
	+	<u>Bullfrog Treatment</u> Native Amphibians + Bullfrog	<u>Mosquitofish + Bullfrog Treatment</u> Native Amphibians + Bullfrog + Mosquitofish

Figure 1. A diagram depicting the 2 X 2 factorial design of the experiment. The positive sign (+) indicates that the particular invasive species was added to that treatment while the negative sign (-) indicates that the particular invasive species was not added to that treatment.

The pond mud was added to introduce algae cells, the rabbit chow provided a source of nutrients to fuel growth of primary producers, and the dry leaf litter served as a source of cover for the amphibians. In each mesocosm, 10 native snails (*Helisoma sp.*), 15 native Pacific tree frog (*Pseudacris regilla*) tadpoles, 15 native western toad (*Bufo boreas*) tadpoles and 10 native California newts (*Taricha torosa*) were added. Five of the mesocosms only had the three native amphibian species (Native treatment). For the other 15 mesocosms, five of the mesocosms had 3 invasive American bullfrog tadpoles added in addition to the native species (Bullfrog treatment), five of the mesocosms had 5 invasive mosquitofish added in addition to native species (Mosquitofish treatment), and 5 of the mesocosms had 3 invasive American bullfrog tadpoles and 5 mosquitofish added in addition to the native species (Bullfrog + Mosquitofish treatment).

Spreadsheet information

1. Species codes (used on many of the spreadsheets):
   - PSRE = Pacific Tree Frog (*Pseudacris regilla*)
   - BUBO = western Toad (*Bufo boreas*)
   - TATO = California newt (*Taricha torosa*)

2. Number of Zooplankton: Number in a sample of 6.9 L of water for the mesocosm.

3. Run Number (phytoplankton): Each sample was run five times. Use the mean of the five runs as the value for that mesocosm.

4. Phytoplankton Fluorescence = A relative value that measures the amount of light absorbed by chlorophyll in the sample. These numbers do not have units because they are relative to a sample blank, rather than absolute measurements.

Exercise

Note that code is not provided for commands that have been provided previously. Refer to prior exercises if necessary.

Load package `tidyverse`

```
library(tidyverse)
```

Load data and view it

The data can be found at the following paths relative to your home folder:
"../shared/treatments.csv"
"../shared/Amphib_survival.csv"
"../shared/Amphib_mass.csv"
"../shared/phytoplankton.csv"
"../shared/snails_zoopl.csv"

```
treatments <- read.csv("treatments.csv")
amphib_survival <- read.csv("Amphib_survival.csv")
amphib_mass <- read.csv("Amphib_mass.csv")
snails_and_zoopl <- read.csv("snails_zoopl.csv")
phytoplankton <- read.csv("phytoplankton.csv")
```

How does the presence of bullfrogs and mosquitofish influence the survival of native amphibian species?

Stop and think: what is missing from the survival data frame to answer this question?

To put two data frames together based on a shared variable use the `left_join` function. This function takes two data frames and adds information from the right frame into the left frame while retaining the entire left frame.

```
amphib_survival2 <- left_join(amphib_survival,treatments)
```

Now create a plot to examine the question.

```
ggplot(amphib_survival2, aes(Treatment,No_of_Survivors, color=Species))+
  geom_boxplot()+
  theme(axis.text.x=element_text(angle = 45, hjust = 1))+
  ylab("# of survivors")
```
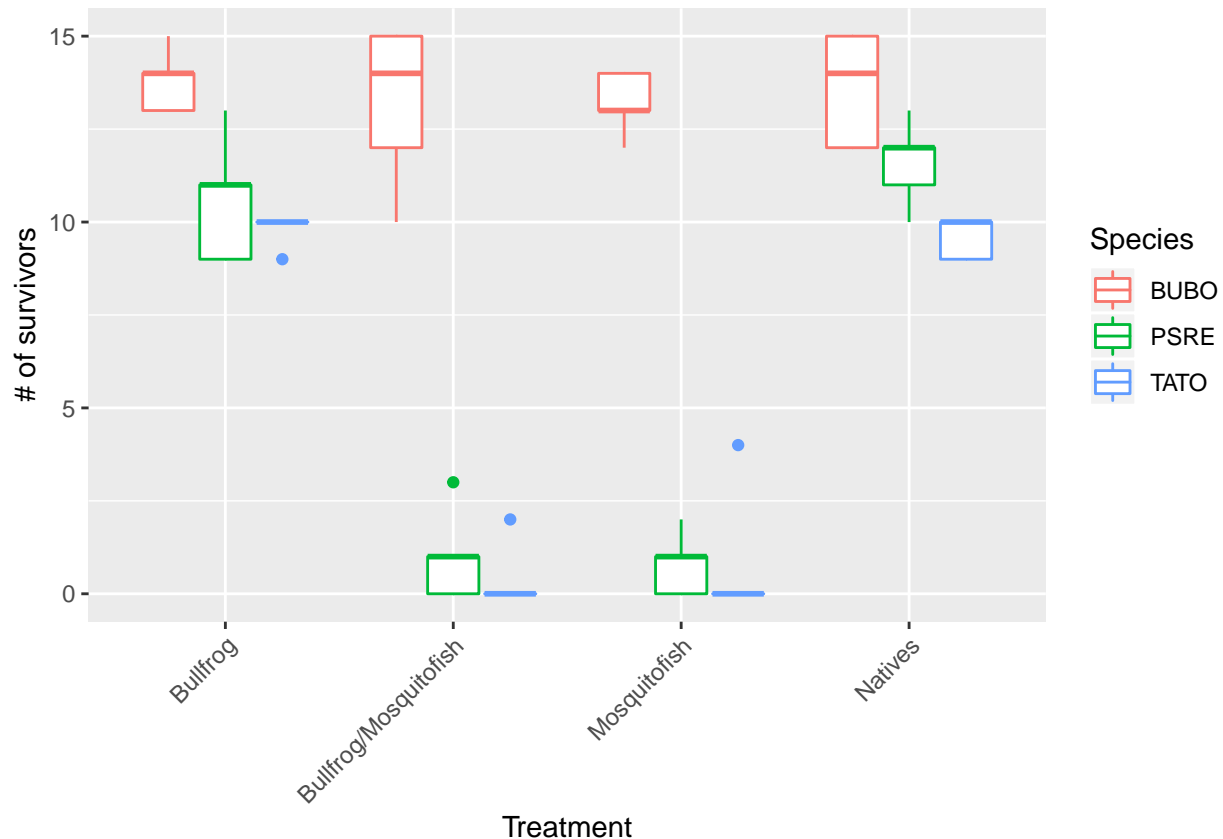
```
#mosquitofish strongly reduced treefrog and newt survival
#toads are highly toxic and distasteful as larvae
```

How would you answer the question?

To compare the means of more than two groups (i.e. the three treatments here compared to the control) we use an Analysis of Variance (ANOVA). Note that because this experiment follows a factorial design (i.e. two interacting factors: bullfrogs, mosquitofish, and both), we not only need to look at the impacts of the individual factors, but their interaction. Because of this interaction we will change our data table a bit so that each row is coded for whether bullfrogs are present or absent, and whether mosquitofish are present or absent.

```
treatments2 <- read.csv("treatments2.csv")
amphib_survival3 <- left_join(amphib_survival,treatments2)
```

Now you can construct the linear model relating the number of survivors to the treatment, and output the ANOVA table. Note that this looks similar to the linear regression we conducted earlier in the semester, but for this analysis we have multiple categorical explanatory variables (the * in the arguments allow for the interaction term).

```
lm_survivors <- lm(No_of_Survivors ~ Bullfrog * Mosquitofish, data = amphib_survival3)
anova(lm_survivors)
```

```
## Analysis of Variance Table
##
## Response: No_of_Survivors
##                     Df  Sum Sq Mean Sq F value    Pr(>F)
## Bullfrog             1    0.27    0.27  0.0125    0.9114
## Mosquitofish         1  653.40  653.40 30.5974 8.673e-07 ***
```

```
## Bullfrog:Mosquitofish  1    0.07     0.07  0.0031     0.9556
## Residuals              56 1195.87    21.35
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Look at the column `Pr(>F)`. This is the p-value, which is the probability that the observed F value (the ratio of the group mean square and the error mean squre) would have occured by chance if the null hypothesis (the means for all treatments are equal) was true.

Which explanatory variable(s) impact the number of survivors? Is this consistent with your plot?

Now output the R^2 value from the summary of the linear model. R^2 is the contribution of the differences among groups to the total variation in the data.

```
summary(lm_survivors)
```

```
##
## Call:
## lm(formula = No_of_Survivors ~ Bullfrog * Mosquitofish, data = amphib_survival3)
##
## Residuals:
##    Min     1Q Median    3Q    Max
## -4.933 -3.867 -1.400  2.450 10.133
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)            11.6000     1.1932   9.722 1.26e-13 ***
## Bullfrog               -0.2000     1.6874  -0.119  0.90608
## Mosquitofish           -6.6667     1.6874  -3.951  0.00022 ***
## Bullfrog:Mosquitofish   0.1333     2.3863   0.056  0.95564
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.621 on 56 degrees of freedom
## Multiple R-squared:  0.3534, Adjusted R-squared:  0.3188
## F-statistic:  10.2 on 3 and 56 DF,  p-value: 1.845e-05
```

Note: The adjusted R-squared is a modified version of R-squared that has been adjusted for the number of predictors in the model. The adjusted R-squared increases only if the new term improves the model more than would be expected by chance. It decreases when a predictor improves the model by less than expected by chance.

Note that in this analysis we have treated survivors of all species equally even though we observe in the graph that the treatment effect varied with species. Repeat the analysis for each species separately.

```
lm_survivors_BUBO <- lm(No_of_Survivors ~ Bullfrog * Mosquitofish,
                        data = filter(amphib_survival3, Species == "BUBO"))
anova(lm_survivors_BUBO)
```

```
## Analysis of Variance Table
##
## Response: No_of_Survivors
##                       Df Sum Sq Mean Sq F value Pr(>F)
## Bullfrog               1   0.05    0.05  0.0238 0.8793
## Mosquitofish           1   1.25    1.25  0.5952 0.4516
## Bullfrog:Mosquitofish  1   0.05    0.05  0.0238 0.8793
## Residuals             16  33.60    2.10
```

```
lm_survivors_TATO <- lm(No_of_Survivors ~ Bullfrog * Mosquitofish,
                        data = filter(amphib_survival3, Species == "TATO"))
anova(lm_survivors_TATO)
```

```
## Analysis of Variance Table
##
## Response: No_of_Survivors
##                      Df Sum Sq Mean Sq  F value     Pr(>F)
## Bullfrog              1   0.05    0.05   0.0444     0.8357
## Mosquitofish          1 414.05  414.05 368.0444 1.816e-12 ***
## Bullfrog:Mosquitofish 1   0.45    0.45   0.4000     0.5360
## Residuals            16  18.00    1.12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_survivors_TATO)
```

```
##
## Call:
## lm(formula = No_of_Survivors ~ Bullfrog * Mosquitofish, data = filter(amphib_survival3,
##     Species == "TATO"))
##
## Residuals:
##    Min     1Q Median     3Q    Max
##  -0.80  -0.65  -0.40   0.25   3.20
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)             9.6000     0.4743  20.239 7.97e-13 ***
## Bullfrog                0.2000     0.6708   0.298    0.769
## Mosquitofish           -8.8000     0.6708 -13.118 5.60e-10 ***
## Bullfrog:Mosquitofish  -0.6000     0.9487  -0.632    0.536
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.061 on 16 degrees of freedom
## Multiple R-squared:  0.9584, Adjusted R-squared:  0.9506
## F-statistic: 122.8 on 3 and 16 DF,  p-value: 2.946e-11
```

```
lm_survivors_PSRE <- lm(No_of_Survivors ~ Bullfrog * Mosquitofish,
                        data = filter(amphib_survival3, Species == "PSRE"))
anova(lm_survivors_PSRE)
```

```
## Analysis of Variance Table
##
## Response: No_of_Survivors
##                      Df Sum Sq Mean Sq  F value     Pr(>F)
## Bullfrog              1    0.8    0.80   0.5079     0.4863
## Mosquitofish          1  520.2  520.20 330.2857 4.166e-12 ***
## Bullfrog:Mosquitofish 1    1.8    1.80   1.1429     0.3009
## Residuals            16   25.2    1.58
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_survivors_PSRE)
```

```
## 
## Call:
## lm(formula = No_of_Survivors ~ Bullfrog * Mosquitofish, data = filter(amphib_survival3,
##      Species == "PSRE"))
## 
## Residuals:
##    Min     1Q Median     3Q    Max
##  -1.60  -0.85   0.10   0.40   2.40
## 
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)            11.6000     0.5612  20.668 5.76e-13 ***
## Bullfrog               -1.0000     0.7937  -1.260    0.226
## Mosquitofish          -10.8000     0.7937 -13.607 3.27e-10 ***
## Bullfrog:Mosquitofish   1.2000     1.1225   1.069    0.301
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 1.255 on 16 degrees of freedom
## Multiple R-squared:  0.954,  Adjusted R-squared:  0.9454
## F-statistic: 110.6 on 3 and 16 DF,  p-value: 6.538e-11
```

Are there differences in impacts on different species?

How does the presence of bullfrogs and mosquitofish influence the mass (an indicator of amphibian growth) of the native amphibians that survived?

Multiple measurements within a mesocosm (e.g. amphibian mass) are not independent of one another. Calculate the mean value per mesocosm and use this as the response variable. Hint: you'll need to make a new table.

```
amphib_mean_mass <- group_by(amphib_mass, Tank, Species) %>%
  summarise(mean_mass = mean(Mass))
```

Add the treatment information to the new table.

```
amphib_mean_mass2 <- left_join(amphib_mean_mass,treatments)
amphib_mean_mass3 <- left_join(amphib_mean_mass,treatments2)
```

Answer the question using a plot and ANOVA.

```
ggplot(amphib_mean_mass2, aes(Species,mean_mass,color=Treatment )) +
  geom_boxplot()+ geom_point(alpha=.4,position=position_jitterdodge())+
  theme(axis.text.x=element_text(angle = 45, hjust = 1))+ylab("mean mass (g)")+
  scale_x_discrete(labels=c("Toads", "Treefrogs", "Newts"))
```
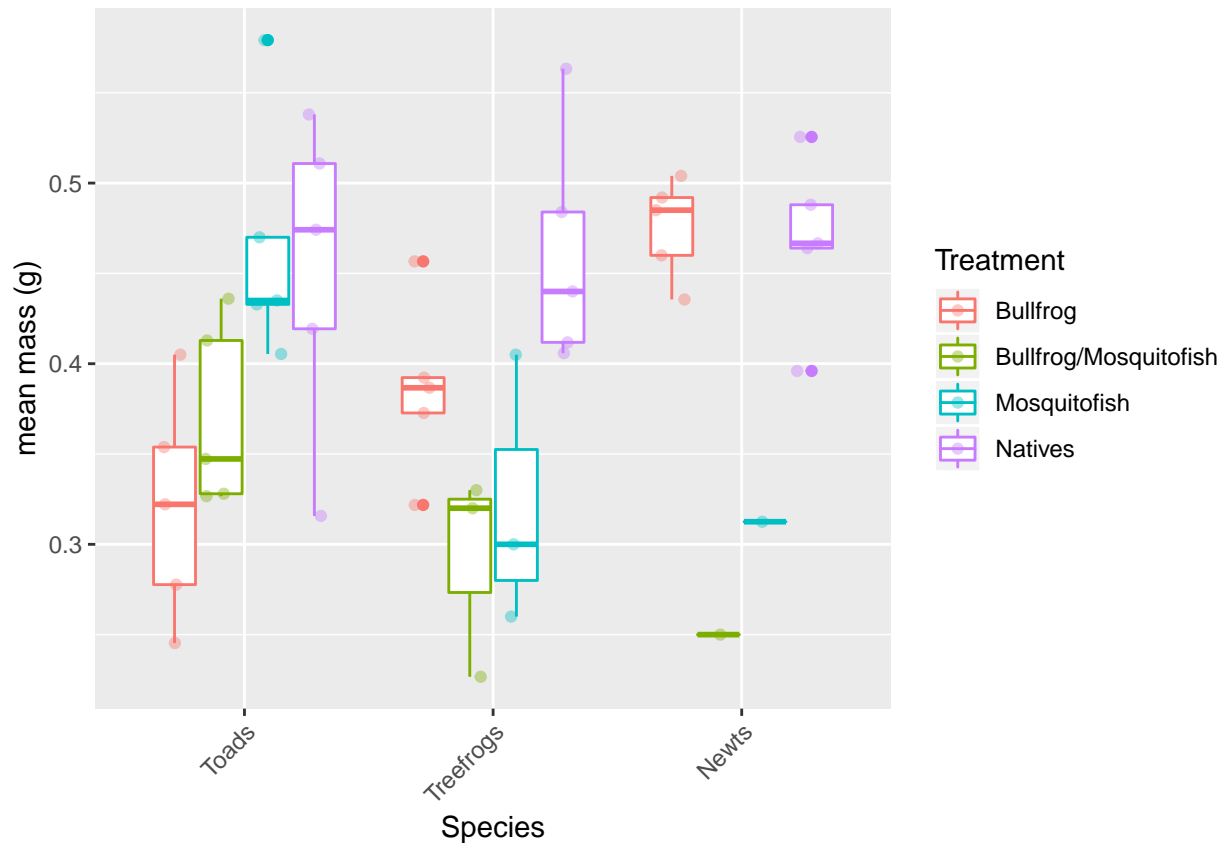
```
#BUBO
# Toads compete with bullfrogs for resources (algae), so the presence of bullfrogs
# reduced their growth rates.
#PSRE
# Treefrogs compete with bullfrogs for algae, so the presence of bullfrogs
# reduced their growth rates. They are also preyed upon by mosquitofish,
# so the ones that survive have spent more time hiding and less time foraging.
#TATO
# Newts are carnivores so they do not compete with the bullfrogs.
# Newts that survive with mosquitofish were smaller because they also had to spend
# less time foraging to avoid predation.
```

```
lm_mass <- lm(mean_mass ~ Bullfrog * Mosquitofish, data = amphib_mean_mass3)
anova(lm_mass)
```

```
## Analysis of Variance Table
##
## Response: mean_mass
##                      Df   Sum Sq  Mean Sq F value   Pr(>F)
## Bullfrog              1 0.054334 0.054334  9.2688 0.003926 **
## Mosquitofish          1 0.042861 0.042861  7.3116 0.009703 **
## Bullfrog:Mosquitofish 1 0.000025 0.000025  0.0043 0.948008
## Residuals            44 0.257930 0.005862
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_mass)
```

```
##
## Call:
## lm(formula = mean_mass ~ Bullfrog * Mosquitofish, data = amphib_mean_mass3)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.148717 -0.049895  0.004397  0.053616  0.179177
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)          0.460217   0.019769  23.280   <2e-16 ***
## Bullfrog            -0.066167   0.027957  -2.367   0.0224 *
## Mosquitofish        -0.060227   0.032282  -1.866   0.0688 .
## Bullfrog:Mosquitofish -0.002994   0.045654  -0.066   0.9480
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.07656 on 44 degrees of freedom
## Multiple R-squared:  0.2737, Adjusted R-squared:  0.2242
## F-statistic: 5.528 on 3 and 44 DF,  p-value: 0.002611
```

```
lm_TATOmass <- lm(mean_mass ~ Bullfrog * Mosquitofish,
                  data = filter(amphib_mean_mass3,Species=="TATO"))
anova(lm_TATOmass)
```

```
## Analysis of Variance Table
##
## Response: mean_mass
##                      Df   Sum Sq  Mean Sq F value     Pr(>F)
## Bullfrog              1 0.000057 0.000057  0.0383 0.8497630
## Mosquitofish          1 0.060438 0.060438 40.5461 0.0002165 ***
## Bullfrog:Mosquitofish 1 0.002028 0.002028  1.3606 0.2770297
## Residuals             8 0.011925 0.001491
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
lm_PSREmass <- lm(mean_mass ~ Bullfrog * Mosquitofish,
                  data = filter(amphib_mean_mass3,Species=="PSRE"))
anova(lm_PSREmass)
```

```
## Analysis of Variance Table
##
## Response: mean_mass
##                      Df   Sum Sq  Mean Sq F value    Pr(>F)
## Bullfrog              1 0.013406 0.013406  3.6605 0.079870 .
## Mosquitofish          1 0.050959 0.050959 13.9149 0.002873 **
## Bullfrog:Mosquitofish 1 0.001942 0.001942  0.5303 0.480442
## Residuals            12 0.043947 0.003662
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
lm_BUBOmass <- lm(mean_mass ~ Bullfrog * Mosquitofish,
                  data = filter(amphib_mean_mass3,Species=="BUBO"))
anova(lm_BUBOmass)
```

```
## Analysis of Variance Table
##
## Response: mean_mass
##                      Df   Sum Sq   Mean Sq F value   Pr(>F)
## Bullfrog              1 0.063354 0.063354 13.4044 0.002108 **
## Mosquitofish          1 0.004840 0.004840  1.0241 0.326597
## Bullfrog:Mosquitofish 1 0.001664 0.001664  0.3520 0.561254
## Residuals            16 0.075622 0.004726
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

How does the presence of bullfrogs and mosquitofish influence the density of zooplankton (Daphnia and copepods)?

```
zoopl <- snails_and_zoopl %>%
  filter(Species != "Snails") %>%
  group_by(Tank) %>%
  summarize(zooplankton = sum(No_of_Individuals))
zoopl2 <- left_join(zoopl,treatments)
zoopl2$zooplankton_density <- zoopl2$zooplankton / 6.9
ggplot(zoopl2, aes(Treatment,zooplankton_density)) +
  geom_boxplot()+
  theme(axis.text.x=element_text(angle = 45, hjust = 1))
```
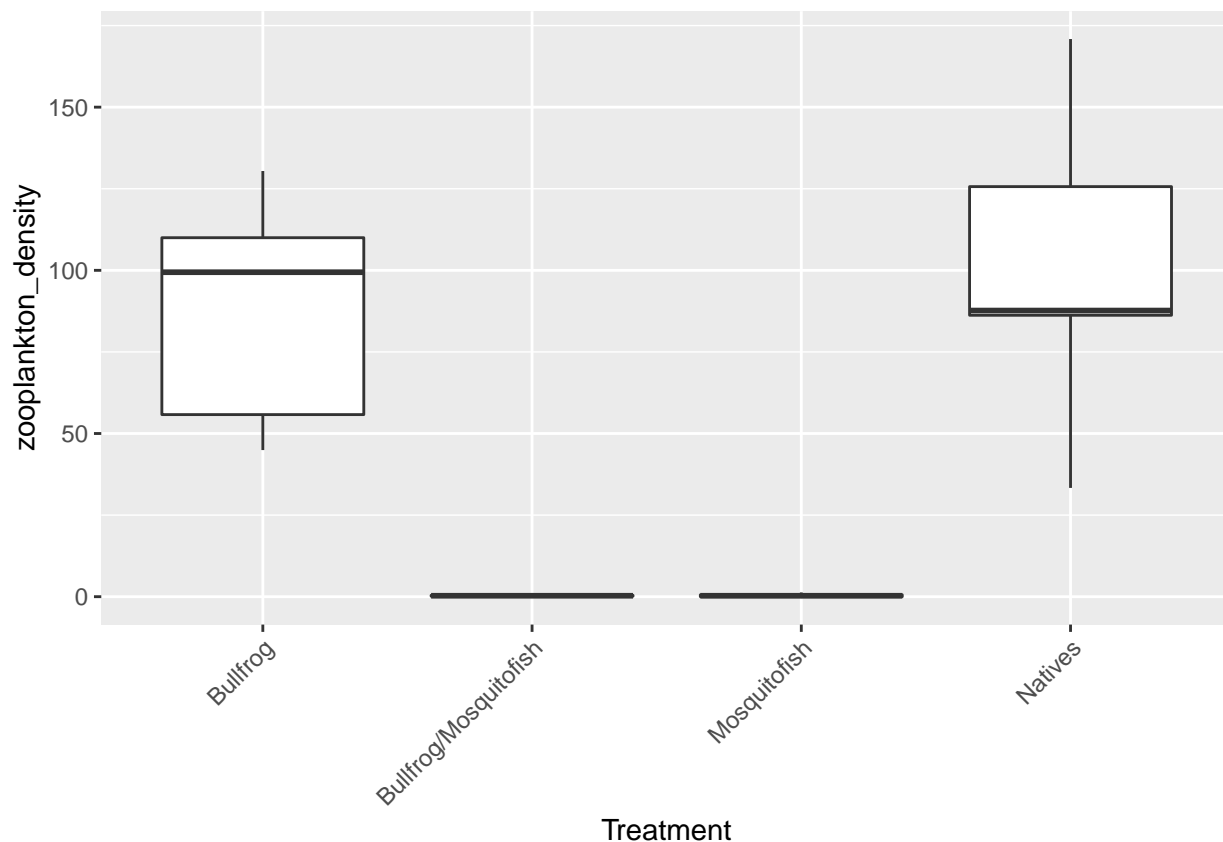
```
zoopl3 <- left_join(zoopl2,treatments2)
lm_zoopl <- lm(zooplankton_density ~ Bullfrog * Mosquitofish, data = zoopl3)
anova(lm_zoopl)
```

```
## Analysis of Variance Table
##
## Response: zooplankton_density
##                      Df Sum Sq Mean Sq F value    Pr(>F)
## Bullfrog              1    202     202  0.2053    0.6565
## Mosquitofish          1  44221   44221 44.8596 5.122e-06 ***
## Bullfrog:Mosquitofish 1    197     197  0.1997    0.6609
## Residuals            16  15772     986
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_zoopl)
```

```
##
## Call:
## lm(formula = zooplankton_density ~ Bullfrog * Mosquitofish, data = zoopl3)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -67.420  -3.594  -0.101   3.478  70.116
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)             100.75      14.04   7.176 2.21e-06 ***
## Bullfrog                -12.64      19.86  -0.636 0.533495
## Mosquitofish           -100.32      19.86  -5.052 0.000118 ***
## Bullfrog:Mosquitofish    12.55      28.08   0.447 0.660917
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 31.4 on 16 degrees of freedom
## Multiple R-squared:  0.7388, Adjusted R-squared:  0.6899
## F-statistic: 15.09 on 3 and 16 DF,  p-value: 6.33e-05
```

How does the presence of bullfrogs and mosquitofish influence the density of phytoplankton (i.e. relative phytoplankton fluorescence)?

```
phytoplankton2 <- group_by(phytoplankton, Tank) %>%
  summarise(Fluorescence = mean(Phytoplankton_Fluorescence))
phytoplankton3 <- left_join(phytoplankton2,treatments)
ggplot(phytoplankton3, aes(Treatment,Fluorescence)) +
  geom_boxplot()+
  theme(axis.text.x=element_text(angle = 45, hjust = 1))
```
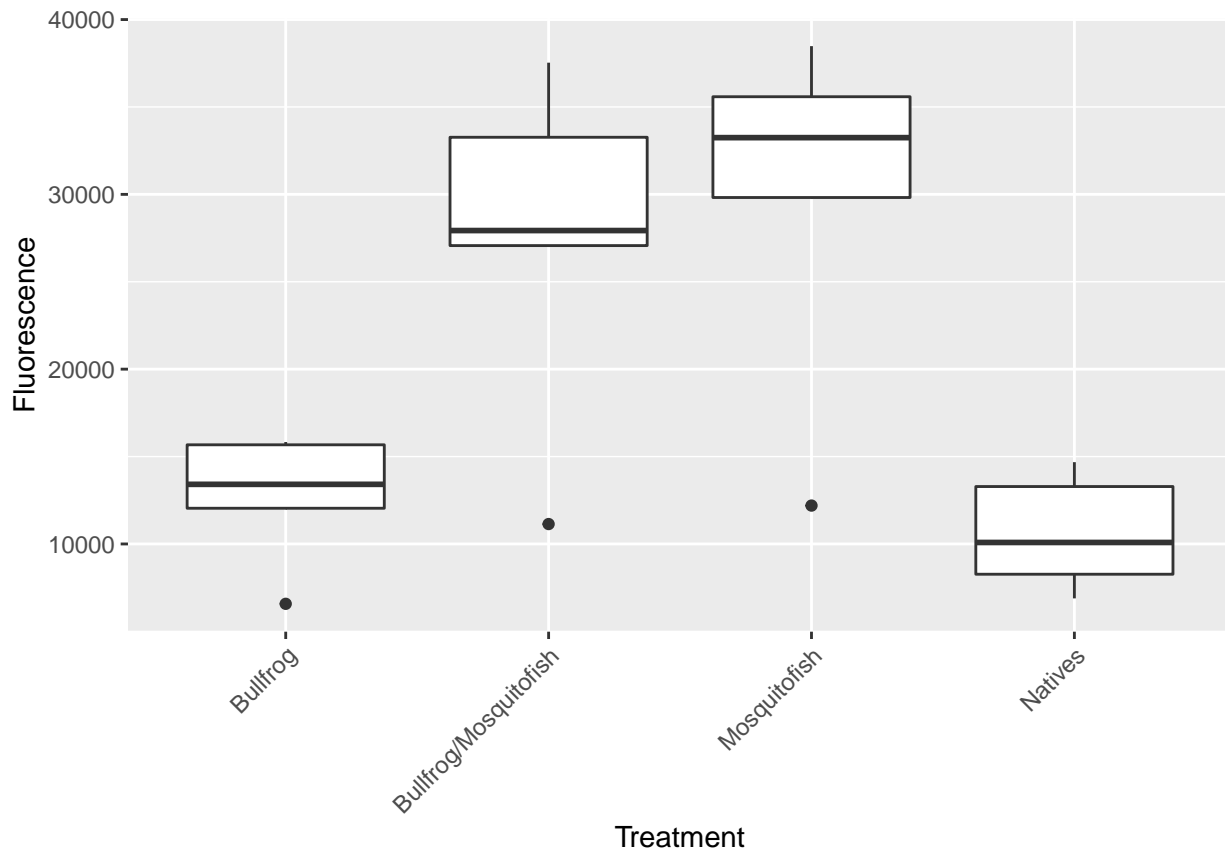
```
#grazing by zooplankton controlled the abundance of phytoplankton in the mesocosms
#mosquitofish impact zooplankton

phytoplankton4 <- left_join(phytoplankton3,treatments2)
lm_phyto <- lm(Fluorescence ~ Bullfrog * Mosquitofish, data = phytoplankton4)
anova(lm_phyto)
```

```
## Analysis of Variance Table
##
## Response: Fluorescence
##                     Df     Sum Sq     Mean Sq F value    Pr(>F)
## Bullfrog             1     211768      211768  0.0036 0.9526872
## Mosquitofish         1 1436831178  1436831178 24.6458 0.0001406 ***
## Bullfrog:Mosquitofish 1   25796290    25796290  0.4425 0.5154020
## Residuals           16  932786026    58299127
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_phyto)
```

```
##
## Call:
## lm(formula = Fluorescence ~ Bullfrog * Mosquitofish, data = phytoplankton4)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -17668.5  -1091.2    622.9   3544.4  10145.7
##
```

```
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)             10641       3415   3.116  0.00665 **
## Bullfrog                 2066       4829   0.428  0.67454
## Mosquitofish            19223       4829   3.981  0.00107 **
## Bullfrog:Mosquitofish   -4543       6829  -0.665  0.51540
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7635 on 16 degrees of freedom
## Multiple R-squared:  0.6106, Adjusted R-squared:  0.5376
## F-statistic: 8.364 on 3 and 16 DF,  p-value: 0.001426
```

How does the presence of bullfrogs and mosquitofish influence the density of snails?

```
snails <- snails_and_zoopl %>% filter(Species == "Snails")
snails2 <- left_join(snails,treatments)
ggplot(snails2, aes(Treatment,No_of_Individuals)) +
  geom_boxplot()+
  theme(axis.text.x=element_text(angle = 45, hjust = 1))
```
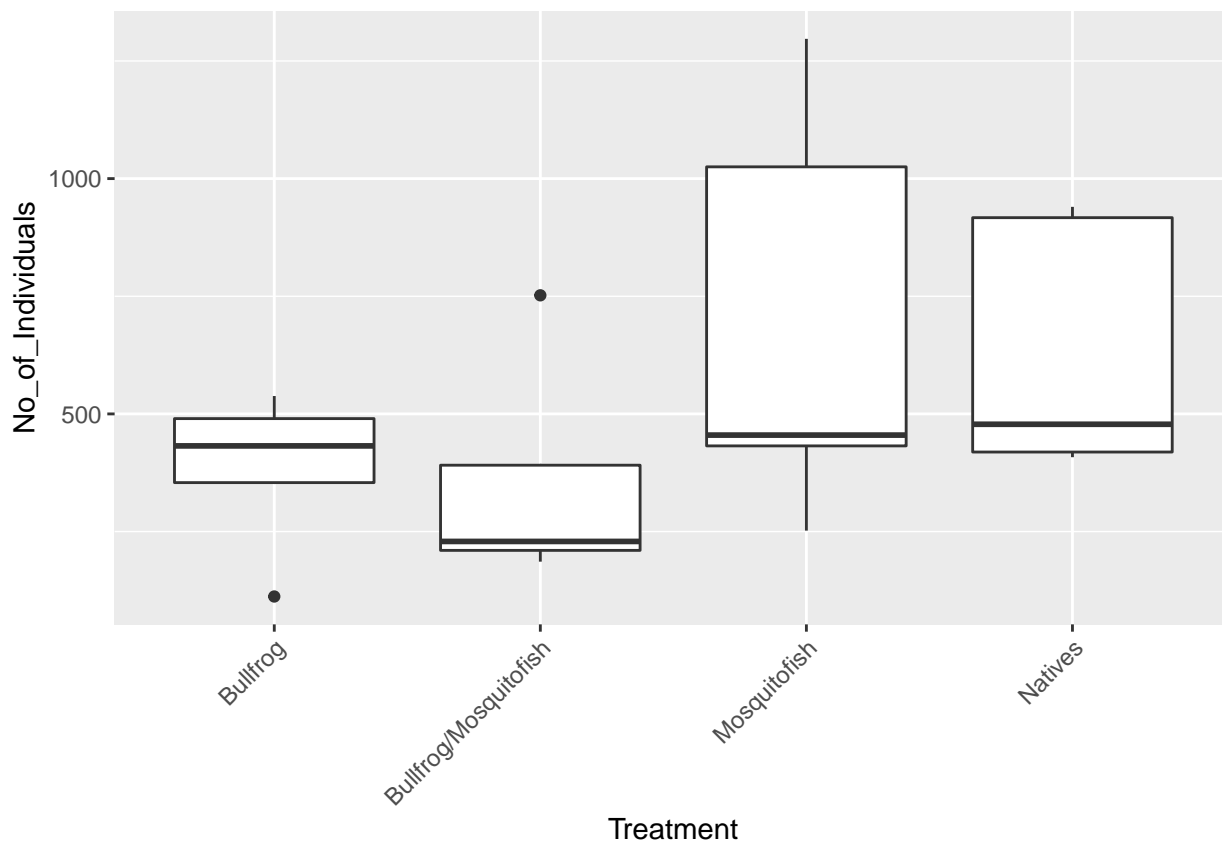


```
#change in snail density is likely due to competition from bullfrogs.
#Snails had less to eat in the presence of bullfrogs and therefore produced fewer offspring.
```

```
snails2 <- left_join(snails2,treatments2)
lm_snail <- lm(No_of_Individuals ~ Bullfrog * Mosquitofish, data = snails2)
```

```
anova(lm_snail)
```

```
## Analysis of Variance Table
##
## Response: No_of_Individuals
##                      Df  Sum Sq Mean Sq F value  Pr(>F)
## Bullfrog              1  428952  428952  4.8125 0.04337 *
## Mosquitofish          1     994     994  0.0112 0.91721
## Bullfrog:Mosquitofish 1   10442   10442  0.1172 0.73659
## Residuals            16 1426122   89133
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_snail)
```

```
##
## Call:
## lm(formula = No_of_Individuals ~ Bullfrog * Mosquitofish, data = snails2)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -440.2 -216.2  -77.9  185.8  604.8
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)              632.4      133.5   4.737 0.000224 ***
## Bullfrog                -247.2      188.8  -1.309 0.208963
## Mosquitofish              59.8      188.8   0.317 0.755563
## Bullfrog:Mosquitofish    -91.4      267.0  -0.342 0.736594
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 298.6 on 16 degrees of freedom
## Multiple R-squared:  0.2359, Adjusted R-squared:  0.09268
## F-statistic: 1.647 on 3 and 16 DF,  p-value: 0.2183
```

Redraw the food web to show bullfrogs and mosquitofish, and their direct and indirect effects.