

Selection pressures in *Syngnathus floridae*

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```
#This is a cohesive list of all the libraries used in this document
library(ggplot2)
library(cowplot)
```

```
#MomIDs and embryo counts for each section of the male's brood pouch
em_dat <- read.csv("data/EmbryoParentage.csv")
```

```
#Metadata for males and females from the mesocosm experiments
fem_meso <- read.csv("data/all_fem_meso_floridae.csv")
mal_meso <- read.csv("data/all_mal_meso_floridae.csv")
```

Calculating the degree of sexual dimorphism

Other papers have reported varying levels of significant or non-significant size differences between males and females in this species. I want to compare the sizes that I have to see what sexual size dimorphism is like for this population. I am doing this across all fish that were used, including those trials that had no successful matings.

```
#Testing to see if the variances are equal
var.test(fem_meso$length, mal_meso$length)
```

```
##
## F test to compare two variances
##
## data: fem_meso$length and mal_meso$length
## F = 1.4609, num df = 85, denom df = 85, p-value = 0.08239
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.9521741 2.2413401
## sample estimates:
## ratio of variances
##          1.460872
```

```
var.test(fem_meso$depth, mal_meso$depth)
```

```
##
## F test to compare two variances
##
```

```
## data: fem_meso$depth and mal_meso$depth
## F = 145.24, num df = 84, denom df = 85, p-value < 2.2e-16
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
## 94.57063 223.18630
## sample estimates:
## ratio of variances
## 145.242
```

```
var.test(fem_meso$svl, mal_meso$svl)
```

```
##
## F test to compare two variances
##
## data: fem_meso$svl and mal_meso$svl
## F = 3.7553, num df = 85, denom df = 85, p-value = 4.107e-09
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
## 2.447628 5.761515
## sample estimates:
## ratio of variances
## 3.755269
```

```
t.test(fem_meso$length, mal_meso$length, var.equal = TRUE)
```

```
##
## Two Sample t-test
##
## data: fem_meso$length and mal_meso$length
## t = 0.86463, df = 170, p-value = 0.3885
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -3.662412 9.371226
## sample estimates:
## mean of x mean of y
## 177.9724 175.1180
```

```
t.test(fem_meso$depth, mal_meso$depth, var.equal = FALSE)
```

```
##
## Welch Two Sample t-test
##
## data: fem_meso$depth and mal_meso$depth
## t = 1.7947, df = 85.143, p-value = 0.07625
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.297791 5.822011
## sample estimates:
## mean of x mean of y
## 8.487471 5.725360
```

```
t.test(fem_meso$svl, mal_meso$svl, var.equal = FALSE)
```

```
##
## Welch Two Sample t-test
##
## data: fem_meso$svl and mal_meso$svl
## t = 2.0687, df = 127.27, p-value = 0.0406
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.1724569 7.7613105
## sample estimates:
## mean of x mean of y
##  77.94264 73.97576
```

```
#Checking the power
d_mean <- abs(mean(fem_meso$svl) - mean(mal_meso$svl))
pool_sd <- sqrt((var(fem_meso$svl) + var(mal_meso$svl))/ 2)
d <- d_mean/pool_sd
library(pwr)
```

```
## Warning: package 'pwr' was built under R version 4.3.2
```

```
pwr.t.test(n = nrow(fem_meso),
           d = d,
           sig.level = 0.05,
           type = 'one.sample',
           alternative = 'two.sided')
```

```
##
##      One-sample t test power calculation
##
##              n = 86
##              d = 0.315476
##      sig.level = 0.05
##      power = 0.8244444
##      alternative = two.sided
```

Calculating mating and reproductive success for individuals who mated

Syngnathus floridae (dusky pipefish) were sampled from three distinct seagrass beds around Tampa Bay in Tampa, Florida. Sexually mature females (standard length ≥ 120 mm) and pregnant males were collected and brought back to the University of Tampa for mesocosm experiments. In these mesocosms, 8 males and 8 females were housed together in a 140L tank for a period of 14-days and allowed to mate freely. Parentage analysis was done with all of the pregnant males from the trials to figure out how many times each male and female mated, and the number of eggs that were transferred. The results of that are here.

First I had to calculate the mating and reproductive success for each male and female who mated based on the assigned mom for each genotyped embryo.

```

#Row-by-Row analysis of parentage data by male brood pouch section

#Read in the data
#em_dat <- read.csv("~/EmbryoParentage.csv")

#For each row in the dataset(each section of the pouch) apply this function
mom_counts <- do.call(rbind,apply(em_dat, 1, function(one_section){

  #Save all of the momIDs into an object
  mom_ids<-c(one_section[grepl("momID",names(one_section))])

  #Calculate the number of eggs that belongs to each potential mom based on
  #the proportions and total number of developed and undeveloped embryos
  mom_props<-c(as.numeric(one_section[grepl("prop",names(one_section))]))
  mom_counts_dev<-mom_props*as.numeric(one_section["num_embryos_dev"])
  mom_counts_und<-mom_props*as.numeric(one_section["num_embryos_non_dev"])

  #Create a dataframe that contains the maleID, pouch section number and the
  #number of eggs that belongs to each momID
  this_section<-data.frame(
    maleID=one_section["maleID"],
    section_num=one_section["section_num"],
    mom_ids[which((mom_counts_dev + mom_counts_und) > 0)],
    mom_counts_dev[which((mom_counts_dev + mom_counts_und)>0)],
    mom_counts_und[which((mom_counts_dev + mom_counts_und)>0)]
  )

  #Rename the columns
  colnames(this_section)[3:5]<-c("momID","num_dev","num_und")

  return(this_section)
}))

#Calculate female fitness
fem_fitness<-do.call(rbind,by(mom_counts, mom_counts$momID,function(dat){

  mom_fitness<-data.frame(
    momID=unique(dat$momID),
    MatingSuccess=length(unique(dat$maleID)),
    NumDeveloped=round(sum(dat$num_dev)),
    NumUndeveloped=round(sum(dat$num_und))
  )
  return(mom_fitness)
}))

fem_fitness$totalEggs <- fem_fitness$NumDeveloped + fem_fitness$NumUndeveloped

#Calculate Male Fitness
mal_fitness<-do.call(rbind,by(mom_counts, mom_counts$maleID,function(dat){

  dad_fitness<-data.frame(
    maleID=unique(dat$maleID),

```

```

    MatingSuccess=length(unique(dat$momID)),
    NumDeveloped_Calc=round(sum(dat$num_dev)),
    NumUndeveloped_Calc=round(sum(dat$num_und))
  )
  return(dad_fitness)
}))

mal_fitness$totalEggs <- mal_fitness$NumDeveloped_Calc + mal_fitness$NumUndeveloped_Calc

```

After running the above R script we have two datasets, `mal_fitness` and `fem_fitness`. These datasets include information about the mating success (number of mates) and reproductive success (Number of embryos transferred). We can split reproductive success up further later if we want to from the total number of embryos transferred to the number of embryos developed and the number that were undeveloped.

I want to include all of the other metadata that I have for these individuals (traits, collection location, latency to pregnancy, etc.) as well as tack on all of the information for the individuals who did not mate. To do that I am going to need to merge the fitness datasets with `fem_meso` and `mal_meso`.

```

#Make a column in *_meso that contains the full fishID (i.e. FL1M3) to match the
#formatting in the fitness datasets (make sure they have the same name for merging purposes)
fem_meso$momID <- paste0("FL", fem_meso$trial_num, "F", fem_meso$fishID)
mal_meso$maleID <- paste0("FL", mal_meso$trial_num, "M", mal_meso$fishID)

#Merge the datasets based on the columns created above
fem_all <- merge(fem_meso, fem_fitness, by = "momID", all.x = TRUE, all.y = TRUE)
mal_all <- merge(mal_meso, mal_fitness, by = "maleID", all.x = TRUE, all.y = TRUE)

```

There are a few trials that I want to remove from the analysis:

1. All trials where there were no successful matings (7, 9, 10, 11).
2. Trial 1, a male gave birth and the babies were immediately eaten by the adults so the trial was ended early and therefore I was unable to get any parentage information for that trial.

I also want to replace the NAs that were automatically added to the columns from the fitness dataset (MatingSuccess, NumDeveloped, NumUndeveloped, totalEggs) with 0s and add a column to the female dataset that tells me whether or not the female mated (with 1 or 0).

```

#Subset the merged datasets to remove trials without successful matings and Trial 1
fem_succ <- subset(fem_all, !(trial_num %in% c(7, 9, 10, 11, 1)))
mal_succ <- subset(mal_all, !(trial_num %in% c(7, 9, 10, 11, 1)))

#Replace NAs with 0s in the columns related to fitness
mal_succ[,15:18] <- sapply(mal_succ[,15:18],
                           function(x)
                             ifelse(is.na(x), 0, x))

fem_succ[,11:14] <- sapply(fem_succ[,11:14],
                           function(x)
                             ifelse(is.na(x), 0, x))

#Add a column for females to denote mated or unmated
fem_succ$mated <- ifelse(fem_succ$MatingSuccess > 0, 1, 0)

```

Summary statistics for successfully mated individuals

Males

Across all 7 trials and 56 total males, there were 24 males that mated at least one time and 6 of those males had two mates.

Looking across all males, including the ones that did not mate, this is what we find as the mean, sd, and se for the number of embryos transferred and how many of those developed versus didn't:

	mean	SD	SE	max	min
Number of Embryos	108.5	155.1902059	20.7381636	500	0
Developed Embryos	94.1964286	139.7347383	18.6728398	463	0
Undeveloped Embryos	14.3035714	27.7907242	3.7136917	131	0

These values will be influenced by the number of 0s coming from males who did not mate. So let's look at the same thing, but this time for only males who had at least one successful mating:

	mean	SD	SE	max	min
Number of Embryos	253.1666667	139.1941611	28.4128892	500	9
Developed Embryos	219.7916667	133.7427704	27.3001287	463	7
Undeveloped Embryos	33.375	34.3901054	7.0198509	131	0

We can see from the bottom table that even when we only include males who mated there is still a wide range in the brood size. I want to see what relationship there is between brood pouch size (in terms of both total area and length) and brood size (total number of embryos).

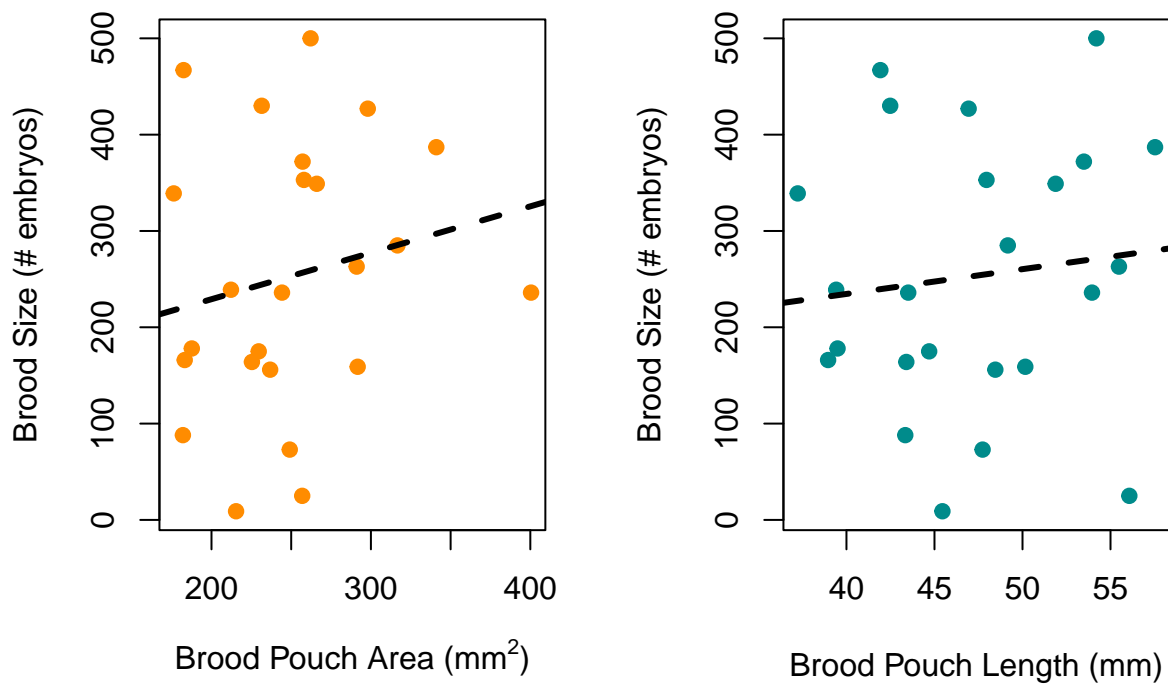


Figure 1: Scatterplot of the relationship between brood pouch size metrics and the number of embryos a male had.

There may be some correlation happening here, but it doesn't look particularly strong (Fig. 1). Let's run some correlations tests to see what they say.

```
##
## Pearson's product-moment correlation
##
## data: as.numeric(mated_mal$bp_area) and mated_mal$totalEggs
## t = 0.90485, df = 22, p-value = 0.3753
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.2316769 0.5507364
## sample estimates:
## cor
## 0.1894228

##
## Pearson's product-moment correlation
##
## data: as.numeric(mated_mal$bp_length) and mated_mal$totalEggs
## t = 0.51929, df = 22, p-value = 0.6087
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
```

```
## -0.3069838 0.4916139
## sample estimates:
##      cor
## 0.1100398
```

There is not a significant correlation between the number of eggs and size of the brood pouch when we look at brood pouch area OR brood pouch length.

Multiple of the wild study papers looked at correlations between body size in terms of standard length and the number of embryos and found significant positive correlations.

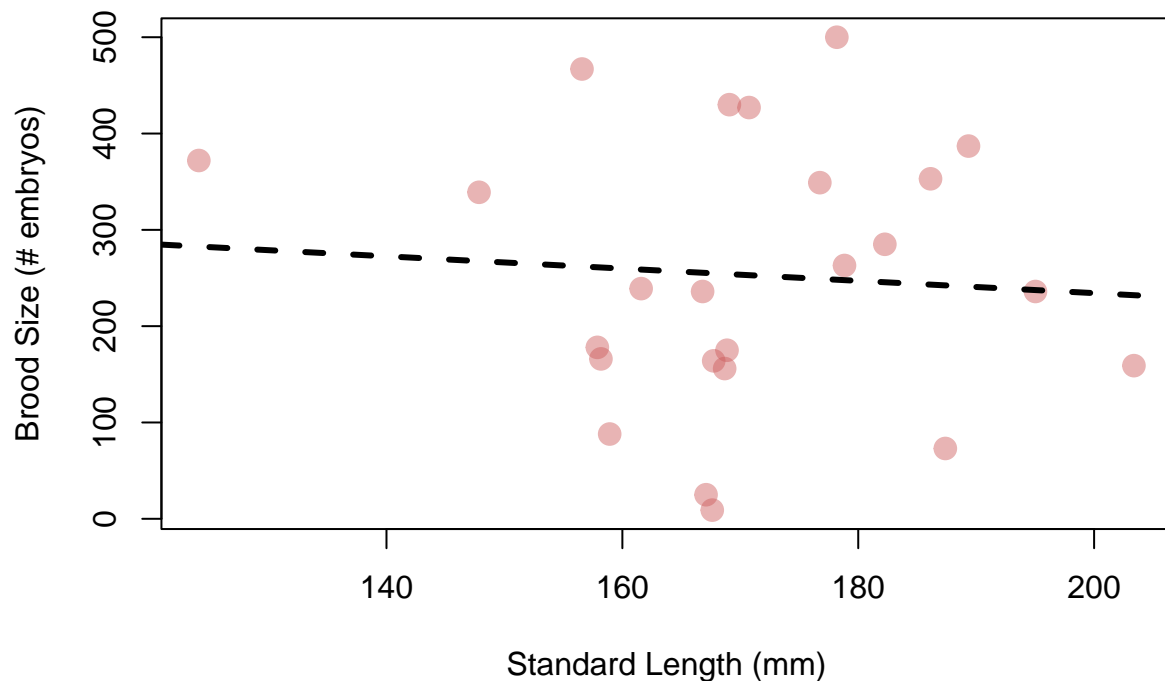


Figure 2: Scatterplot of the relationship between standard length (mm) and the number of embryos a male had.

```
##
## Pearson's product-moment correlation
##
## data: as.numeric(mated_mal$length) and mated_mal$totalEggs
## t = -0.35647, df = 22, p-value = 0.7249
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.4649642 0.3379463
## sample estimates:
##      cor
## -0.07578142
```


There appears to be no significant correlation between standard length and the number of eggs in males, unlike what has been found in the species previously (Fig. 2). This is unsurprising as we didn't find any hint of a relationship in the brood pouch size metrics and there was a considerable number of males that did not have a full brood pouch.

Females

Across all 7 trials and 56 total females, there were 21 females that mated at least one time, 5 females that mated twice, and 2 that mated 3 times.

Looking across all females, including the ones that did not mate, this is what we find as the mean, sd, and se for the total number of embryos transferred from each female (across all of her mates if applicable) and how many of those developed versus didn't:

	mean	SD	SE	max	min
Number of Embryos	108.4821429	195.2328206	26.089083	916	0
Developed Embryos	94.1785714	177.0691808	23.6618646	785	0
Undeveloped Embryos	14.3035714	29.7033329	3.9692748	131	0

These values will be influenced by the number of 0s coming from females who did not mate. So let's look at the same thing, but this time for only females who had at least one successful mating:

	mean	SD	SE	max	min
Number of Embryos	289.2857143	223.3819919	48.745947	916	35
Developed Embryos	251.1428571	211.7324457	46.2038076	785	7
Undeveloped Embryos	38.1428571	38.360508	8.3709491	131	0

We can see from the bottom table that even when we only include females who mated there is still a wide range in the number of eggs transferred. I want to see what relationship there may be between female body size (in terms of standard length, depth, and SVL) and the number of eggs she transferred. I also want to see on average how many eggs were transferred per mating. I'm going to calculate this by taking the total number of eggs and dividing it by the number of mates.

[1] 202.3254

[1] 14.68782

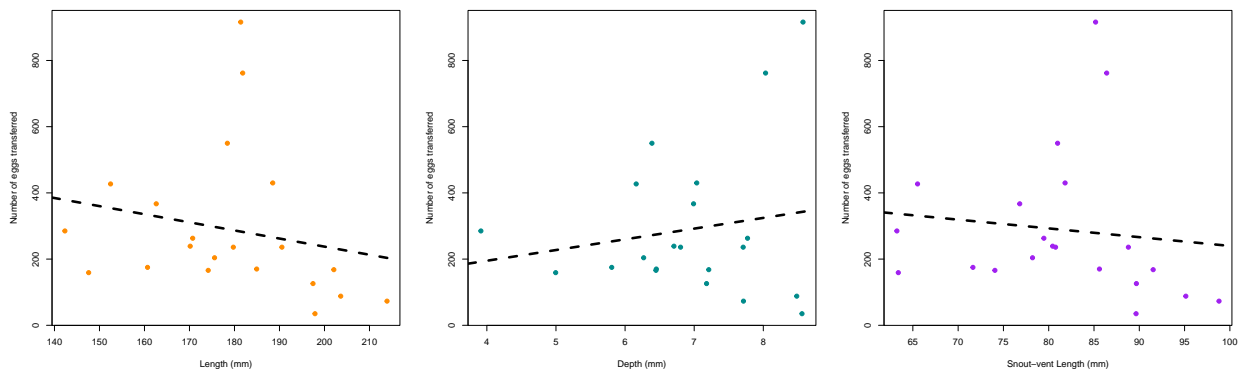


Figure 3: Scatterplot of the relationship between female size metrics and the number of eggs transferred.

There may be some correlation happening here, but it doesn't look particularly strong (Fig. 3). Let's run some correlations tests to see what they say.

```
##
## Pearson's product-moment correlation
##
## data: mated_fem$length and as.numeric(mated_fem$totalEggs)
## t = -0.92304, df = 19, p-value = 0.3676
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.5864091 0.2465727
## sample estimates:
## cor
## -0.2071652
```

```
##
## Pearson's product-moment correlation
##
## data: mated_fem$depth and as.numeric(mated_fem$totalEggs)
## t = 0.74454, df = 19, p-value = 0.4657
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.2839543 0.5593989
## sample estimates:
## cor
## 0.1683713
```

```
##
## Pearson's product-moment correlation
##
## data: mated_fem$svl and as.numeric(mated_fem$totalEggs)
## t = -0.5112, df = 19, p-value = 0.6151
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.5219227 0.3318958
## sample estimates:
## cor
## -0.1164796
```

There is not a significant correlation between the number of eggs and size of the female in terms of standard length, depth, or snout-vent length. Interestingly, however, there is a negative correlation for length and SVL and a positive correlation for depth (but they are all overall weak).

Differences between mated individuals and unmated individuals

I want to now see if there are any significant differences in the sizes of individuals who mated vs individuals that didn't mate in males and females.

Males

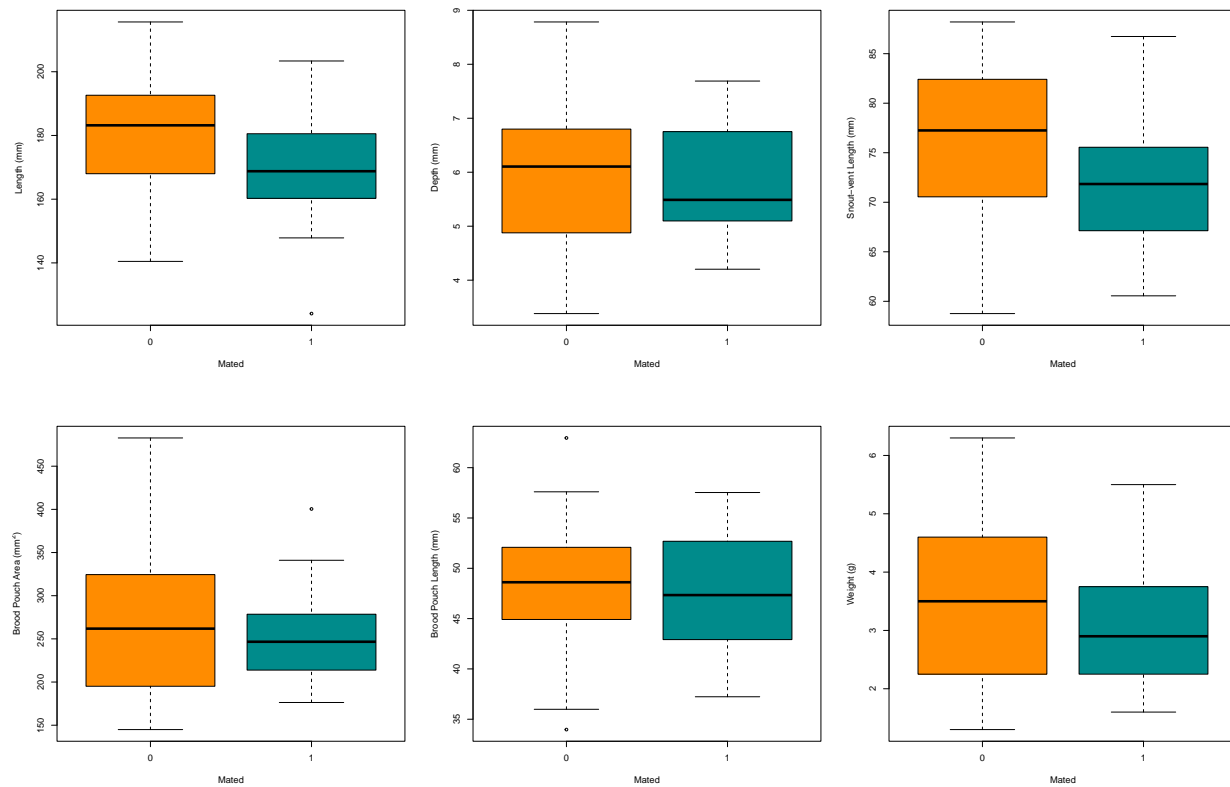


Figure 4: Six different morphometrics compared between males who successfully mated versus those that didn't. Orange represents unmated and blue represents mated males.

```
##
## Wilcoxon rank sum exact test
##
## data: mal_succ$length by mal_succ$preg_status
## W = 521, p-value = 0.02297
## alternative hypothesis: true location shift is not equal to 0

##
## Wilcoxon rank sum test with continuity correction
##
## data: mal_succ$depth by mal_succ$preg_status
## W = 413, p-value = 0.637
## alternative hypothesis: true location shift is not equal to 0

##
## Wilcoxon rank sum exact test
##
## data: mal_succ$svl by mal_succ$preg_status
## W = 525, p-value = 0.01914
## alternative hypothesis: true location shift is not equal to 0
```

```

##
## Wilcoxon rank sum exact test
##
## data:  as.numeric(mal_succ$bp_area) by mal_succ$preg_status
## W = 382, p-value = 0.5525
## alternative hypothesis: true location shift is not equal to 0

##
## Wilcoxon rank sum exact test
##
## data:  mal_succ$bp_length by mal_succ$preg_status
## W = 387, p-value = 0.4947
## alternative hypothesis: true location shift is not equal to 0

##
## Wilcoxon rank sum test with continuity correction
##
## data:  mal_succ$weight by mal_succ$preg_status
## W = 448.5, p-value = 0.289
## alternative hypothesis: true location shift is not equal to 0

```

Females

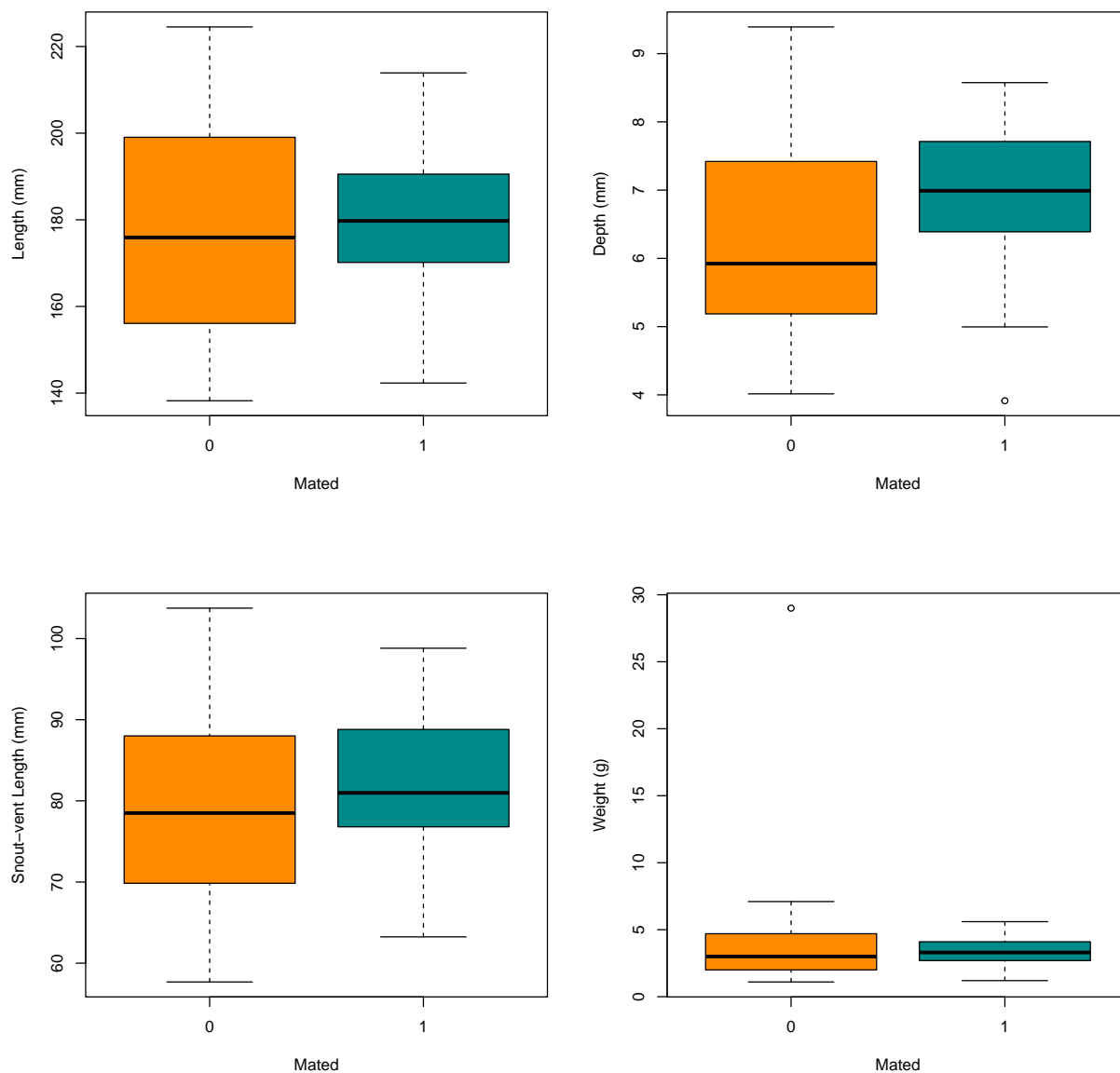


Figure 5: Four different morphometrics compared between females who sucessfully mated versus those that didn't. Orange represents unmated and blue represents mated females.

```
##
## Wilcoxon rank sum exact test
##
## data: fem_succ$length by fem_succ$mated
## W = 353, p-value = 0.8143
## alternative hypothesis: true location shift is not equal to 0
##
```

```
## Welch Two Sample t-test
##
## data: fem_succ$length by fem_succ$mated
## t = -0.26553, df = 50.1, p-value = 0.7917
## alternative hypothesis: true difference in means between group 0 and group 1 is not equal to 0
## 95 percent confidence interval:
## -13.20003 10.11733
## sample estimates:
## mean in group 0 mean in group 1
## 177.3453 178.8867

##
## Wilcoxon rank sum exact test
##
## data: fem_succ$depth by fem_succ$mated
## W = 261, p-value = 0.07263
## alternative hypothesis: true location shift is not equal to 0

##
## Welch Two Sample t-test
##
## data: fem_succ$depth by fem_succ$mated
## t = -1.7518, df = 50.502, p-value = 0.08589
## alternative hypothesis: true difference in means between group 0 and group 1 is not equal to 0
## 95 percent confidence interval:
## -1.35211388 0.09217102
## sample estimates:
## mean in group 0 mean in group 1
## 6.284743 6.914714

##
## Wilcoxon rank sum exact test
##
## data: fem_succ$svl by fem_succ$mated
## W = 325, p-value = 0.4805
## alternative hypothesis: true location shift is not equal to 0

##
## Wilcoxon rank sum test with continuity correction
##
## data: fem_succ$weight by fem_succ$mated
## W = 338.5, p-value = 0.6292
## alternative hypothesis: true location shift is not equal to 0
```

Episode of Sexual Selection

Partitioning the Total Opportunity for Selection (I)

```
#Create a dataframe to store all of the intermediate values of fitness in
fem_succ_fitness <- data.frame(matrix(ncol = ncol(fem_succ) + 9,
```

```

                                nrow = 0))
colnames(fem_succ_fitness) <- c(colnames(fem_succ),
                                "w1", "w1_squared",
                                "W2", "W2_bar", "w2",
                                "W3", "W3_bar", "w3", "i3")

#Create a dataframe to store the final calculations of I in
opp_selection_episodes_fem <- data.frame(matrix(ncol = 12,
                                                nrow = 0))
colnames(opp_selection_episodes_fem) <- c("trial_num", "I_1", "I_1per", "I_2", "I_2per",
                                          "I_3", "I_3per", "I_12", "I_12per",
                                          "I", "Iper", "I_s")

for (trial in unique(fem_succ$trial_num)) {

  #Subset the overall dataframe to work with an individual trial
  tmp <- fem_succ[fem_succ$trial_num == trial, ]

  #Calculate the absolute pre-copulatory fitness (Eq. 14 Arnold & Wade 1984)
  tmp$w1 <- tmp$MatingSuccess/mean(tmp$MatingSuccess) #Relative mating success
  tmp$w1_squared <- (tmp$w1)^2

  I_1 <- var(tmp$w1) #Variance in relative mating success

  #Post-copulatory selection event 1 (Number of eggs transferred) (Eq. 15 Arnold & Wade 1984)
  tmp$W2 <- ifelse(tmp$MatingSuccess > 0,
                  tmp$totalEggs/tmp$MatingSuccess,
                  0) #Number of eggs per mate
  tmp$W2_bar <- tmp$W2 * (tmp$w1/nrow(tmp)) #Number of eggs per mate adjusted by the # of individuals w
  tmp$w2 <- tmp$W2/sum(tmp$W2_bar)

  I_2 <- (sum((tmp$w1 * (tmp$w2)^2))/nrow(tmp) - 1) * nrow(tmp)/(nrow(tmp) - 1)

  #Post-copulatory selection event 2 (Number of eggs developed) (Eq. 16 Arnold & Wade 1984)
  tmp$W3 <- ifelse(tmp$totalEggs > 0,
                  tmp$NumDeveloped/tmp$totalEggs,
                  0) #Proportion of transferred eggs that developed
  tmp$W3_bar <- tmp$W3 * ((tmp$totalEggs/mean(tmp$totalEggs))/nrow(tmp)) #Prop. of eggs developed adjus
  tmp$w3 <- tmp$W3/sum(tmp$W3_bar)
  tmp$i3 <- ((tmp$totalEggs/mean(tmp$totalEggs))/nrow(tmp)) * ((tmp$w3 - 1)^2)

  I_3 <- sum(tmp$i3) * nrow(tmp)/(nrow(tmp) - 1)

  I_12 <- var(tmp$totalEggs)/(mean(tmp$totalEggs)^2)

  #Total selection
  I <- var(tmp$NumDeveloped)/(mean(tmp$NumDeveloped)^2)

  I_s <- var(tmp$MatingSuccess)/(mean(tmp$MatingSuccess)^2)

  #Calculating percentages for each selection event
  I_1per <- (I_1/I)*100
  I_2per <- (I_2/I)*100

```

```

I_3per <- (I_3/I)*100
I_12per <- (I_12/I)*100
Iper <- (I/I)*100

#Combining all of the selection values (Is) and saving the output
trial_num <- trial
selection <- cbind(trial_num, I_1, I_1per, I_2, I_2per, I_3, I_3per,
                   I_12, I_12per, I, Iper, I_s)

opp_selection_episodes_fem <- rbind(opp_selection_episodes_fem, selection)

#Save the intermediate values
fem_succ_fitness <- rbind(fem_succ_fitness, tmp)
}

#Create a dataframe to store all of the intermediate values of fitness in
mal_succ_fitness <- data.frame(matrix(ncol = ncol(mal_succ) + 9,
                                     nrow = 0))
colnames(mal_succ_fitness) <- c(colnames(mal_succ),
                                "w1", "w1_squared",
                                "W2", "W2_bar", "w2",
                                "W3", "W3_bar", "w3", "i3")

#Create a dataframe to store the final calculations of I in
opp_selection_episodes_mal <- data.frame(matrix(ncol = 12,
                                               nrow = 0))
colnames(opp_selection_episodes_mal) <- c("trial_num", "I_1", "I_1per", "I_2", "I_2per",
                                           "I_3", "I_3per", "I_12", "I_12per",
                                           "I", "Iper", "I_s")

for (trial in unique(mal_succ$trial_num)) {

  #Subset the overall dataframe to work with an individual trial
  tmp <- mal_succ[mal_succ$trial_num == trial, ]

  #Calculate the absolute pre-copulatory fitness (Eq. 14 Arnold & Wade 1984)
  tmp$w1 <- tmp$MatingSuccess/mean(tmp$MatingSuccess) #Relative mating success
  tmp$w1_squared <- (tmp$w1)^2

  I_1 <- var(tmp$w1) #Variance in relative mating success

  #Post-copulatory selection event 1 (Number of eggs transferred) (Eq. 15 Arnold & Wade 1984)
  tmp$W2 <- ifelse(tmp$MatingSuccess > 0,
                  tmp$totalEggs/tmp$MatingSuccess,
                  0) #Number of eggs per mate
  tmp$W2_bar <- tmp$W2 * (tmp$w1/nrow(tmp)) #Number of eggs per mate adjusted by the # of individuals w
  tmp$w2 <- tmp$W2/sum(tmp$W2_bar)

  I_2 <- (sum((tmp$w1 * (tmp$w2)^2))/nrow(tmp) - 1) * nrow(tmp)/(nrow(tmp) - 1)

  #Post-copulatory selection event 2 (Number of eggs developed) (Eq. 16 Arnold & Wade 1984)
  tmp$W3 <- ifelse(tmp$totalEggs > 0,
                  tmp$NumDeveloped_Calc/tmp$totalEggs,

```



```

                                0) #Proportion of transferred eggs that developed
tmp$W3_bar <- tmp$W3 * ((tmp$totalEggs/mean(tmp$totalEggs))/nrow(tmp)) #Prop. of eggs developed adjus
tmp$w3 <- tmp$W3/sum(tmp$W3_bar)
tmp$i3 <- ((tmp$totalEggs/mean(tmp$totalEggs))/nrow(tmp)) * ((tmp$w3 - 1)^2)

I_3 <- sum(tmp$i3) * nrow(tmp)/(nrow(tmp) - 1)

I_12 <- var(tmp$totalEggs)/(mean(tmp$totalEggs)^2)

#Total selection
I <- var(tmp$NumDeveloped_Calc)/(mean(tmp$NumDeveloped_Calc)^2)

I_s <- var(tmp$MatingSuccess)/(mean(tmp$MatingSuccess)^2)

#Calculating percentages for each selection event
I_1per <- (I_1/I)*100
I_2per <- (I_2/I)*100
I_3per <- (I_3/I)*100
I_12per <- (I_12/I)*100
Iper <- (I/I)*100

#Combining all of the selection values (Is) and saving the output
trial_num <- trial
selection <- cbind(trial_num, I_1, I_1per, I_2, I_2per, I_3, I_3per,
                  I_12, I_12per, I, Iper, I_s)

opp_selection_episodes_mal <- rbind(opp_selection_episodes_mal, selection)

#Save the intermediate values
mal_succ_fitness <- rbind(mal_succ_fitness, tmp)
}

```

Decomposition of selection differentials (s)

```

#Create a dataframe to store all of the intermediate values of fitness in
fem_succ_select_diff <- data.frame(matrix(ncol = ncol(fem_succ) + 6,
                                          nrow = 0))
colnames(fem_succ_select_diff) <- c(colnames(fem_succ),
                                   "fit1", "eggs_per_mate", "fit2", "prop_dev", "fit3", "StdLength")

#Create a dataframe to store the final calculations of I in
select_diff_fem <- data.frame(matrix(ncol = 11,
                                     nrow = 0))
colnames(select_diff_fem) <- c("trial", "s1", "s2", "s3", "s12", "s123",
                              "s1_prime", "s2_prime", "s3_prime", "s12_prime", "s123_prime")

for (trial in unique(fem_succ$trial_num)) {

  #Subset the overall dataframe to work with an individual trial
  tmp <- fem_succ[fem_succ$trial_num == trial, ]

  #Calculate fitness relating to pre-cop. selection (#matings)

```

```

tmp$fit1 <- tmp$MatingSuccess/mean(tmp$MatingSuccess) #Relative mating success

#Calculate fitness relating to post-mating selection (#eggs transferred)
tmp$eggs_per_mate <- tmp$totalEggs/tmp$MatingSuccess
tmp$fit2 <- ifelse(tmp$MatingSuccess > 0,
                  tmp$eggs_per_mate/mean(tmp$eggs_per_mate, na.rm = TRUE),
                  0) #Relative eggs transferred

#Calculate fitness relating to post-mating selection (eggs that developed)
tmp$prop_dev <- (tmp$NumDeveloped/tmp$MatingSuccess)/tmp$eggs_per_mate
tmp$fit3 <- ifelse(tmp$MatingSuccess > 0,
                  tmp$prop_dev/mean(tmp$prop_dev, na.rm = TRUE),
                  0)

#Standardizing the trait value to have a mean of 0 and sd of unity
tmp$StdLength <- (tmp$svl - mean(tmp$svl))/sd(tmp$svl)

#Calculating the absolute selection differentials (s)
s1 <- cov(tmp$svl, tmp$fit1)
s12 <- cov(tmp$svl, tmp$fit2)
s123 <- cov(tmp$svl, tmp$fit3)
s2 <- s12 - s1
s3 <- s123 - s12

#Calculating the standardized selection differentials (s')
s1_prime <- cov(tmp$StdLength, tmp$fit1)
s12_prime <- cov(tmp$StdLength, tmp$fit2)
s123_prime <- cov(tmp$StdLength, tmp$fit3)
s2_prime <- s12_prime - s1_prime
s3_prime <- s123_prime - s12_prime

#Combining all of the selection differentials (s, s') and saving the output
selection <- cbind(trial, s1, s2, s3, s12, s123,
                  s1_prime, s2_prime, s3_prime, s12_prime, s123_prime)

select_diff_fem <- rbind(select_diff_fem, selection)

#Save the intermediate values
fem_succ_select_diff <- rbind(fem_succ_select_diff, tmp)
}

#Create a dataframe to store all of the intermediate values of fitness in
mal_succ_select_diff <- data.frame(matrix(ncol = ncol(mal_succ) + 6,
                                         nrow = 0))
colnames(mal_succ_select_diff) <- c(colnames(mal_succ),
                                   "fit1", "eggs_per_mate", "fit2", "prop_dev", "fit3", "StdLength")

#Create a dataframe to store the final calculations of I in
select_diff_mal <- data.frame(matrix(ncol = 11,
                                     nrow = 0))
colnames(select_diff_mal) <- c("trial", "s1", "s2", "s3", "s12", "s123",
                              "s1_prime", "s2_prime", "s3_prime", "s12_prime", "s123_prime")

```

```

for (trial in unique(mal_succ$trial_num)) {

  #Subset the overall dataframe to work with an individual trial
  tmp <- mal_succ[mal_succ$trial_num == trial, ]

  #Calculate fitness relating to pre-cop. selection (#matings)
  tmp$fit1 <- tmp$MatingSuccess/mean(tmp$MatingSuccess) #Relative mating success

  #Calculate fitness relating to post-mating selection (#eggs transferred)
  tmp$eggs_per_mate <- tmp$totalEggs/tmp$MatingSuccess
  tmp$fit2 <- ifelse(tmp$MatingSuccess > 0,
                    tmp$eggs_per_mate/mean(tmp$eggs_per_mate, na.rm = TRUE),
                    0) #Relative eggs transferred

  #Calculate fitness relating to post-mating selection (eggs that developed)
  tmp$prop_dev <- (tmp$NumDeveloped_Calc/tmp$MatingSuccess)/tmp$eggs_per_mate
  tmp$fit3 <- ifelse(tmp$MatingSuccess > 0,
                    tmp$prop_dev/mean(tmp$prop_dev, na.rm = TRUE),
                    0)

  #Standardizing the trait value to have a mean of 0 and sd of unity
  tmp$StdLength <- (tmp$svl - mean(tmp$svl))/sd(tmp$svl)

  #Calculating the absolute selection differentials (s)
  s1 <- cov(tmp$svl, tmp$fit1)
  s12 <- cov(tmp$svl, tmp$fit2)
  s123 <- cov(tmp$svl, tmp$fit3)
  s2 <- s12 - s1
  s3 <- s123 - s12

  #Calculating the standardized selection differentials (s')
  s1_prime <- cov(tmp$StdLength, tmp$fit1)
  s12_prime <- cov(tmp$StdLength, tmp$fit2)
  s123_prime <- cov(tmp$StdLength, tmp$fit3)
  s2_prime <- s12_prime - s1_prime
  s3_prime <- s123_prime - s12_prime

  #Combining all of the selection differentials (s, s') and saving the output
  selection <- cbind(trial, s1, s2, s3, s12, s123,
                    s1_prime, s2_prime, s3_prime, s12_prime, s123_prime)

  select_diff_mal <- rbind(select_diff_mal, selection)

  #Save the intermediate values
  mal_succ_select_diff <- rbind(mal_succ_select_diff, tmp)
}

```

Mate success versus Reproductive success (Bateman Gradient)

I now want to look at any relationship that may exist between mating success and reproductive success for males and females. The Bateman gradient will be calculated, which is the slope of the weighted least-squares regression of relative reproductive success (number of offspring divided by the mean) on mating success.

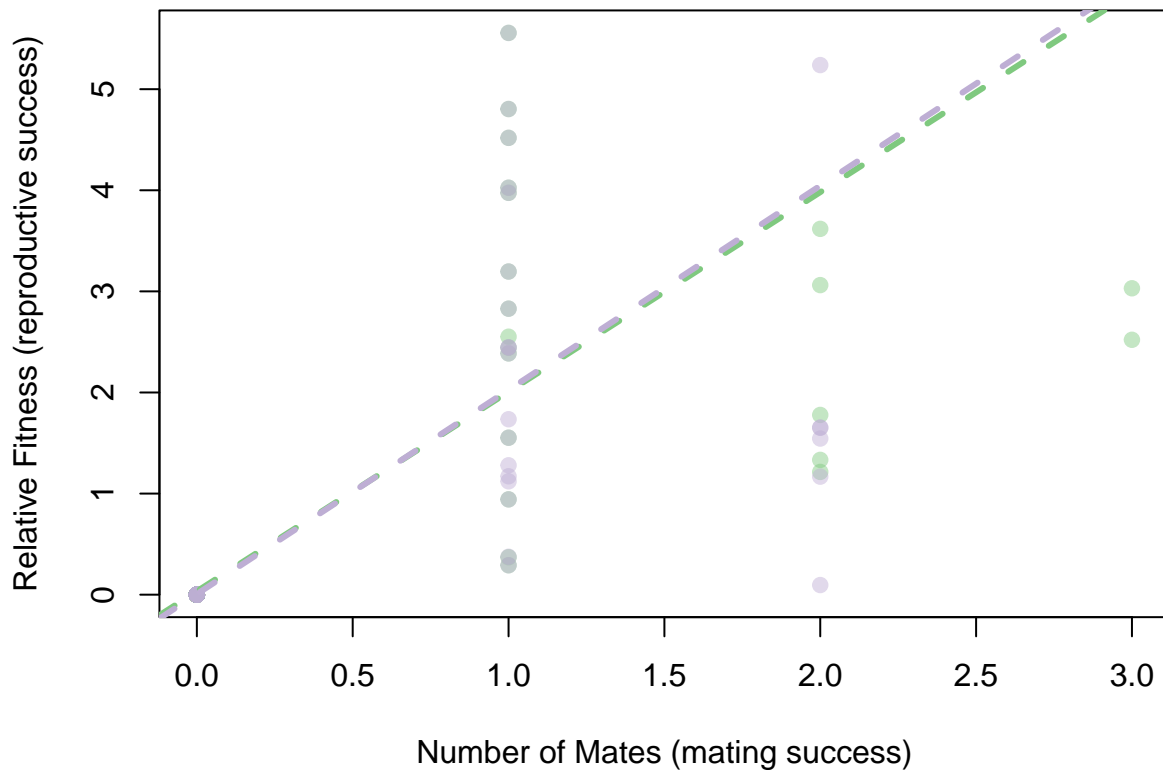


Figure 6: Relationship between reproductive success and mating success for male (purple) and female (green) *Syngnathus floridae*. Reproductive success is shown as relative fitness (i.e. number of offspring produced divided by the mean number of offspring produced). Bateman's gradient is shown as the weighted least-squares regression line (dashed) for males and females.

```
summary(wls_model_fem)
```

```
##
## Call:
## lm(formula = fem_bateman$rel_repo_fitness ~ fem_bateman$MatingSuccess,
##     weights = wt_fem)
##
## Weighted Residuals:
##      Min       1Q   Median       3Q      Max
## -1.7361 -0.0766 -0.0766 -0.0766  3.5023
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.03316   0.07395   0.448   0.656
## fem_bateman$MatingSuccess 1.97416   0.20935   9.430 5.24e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

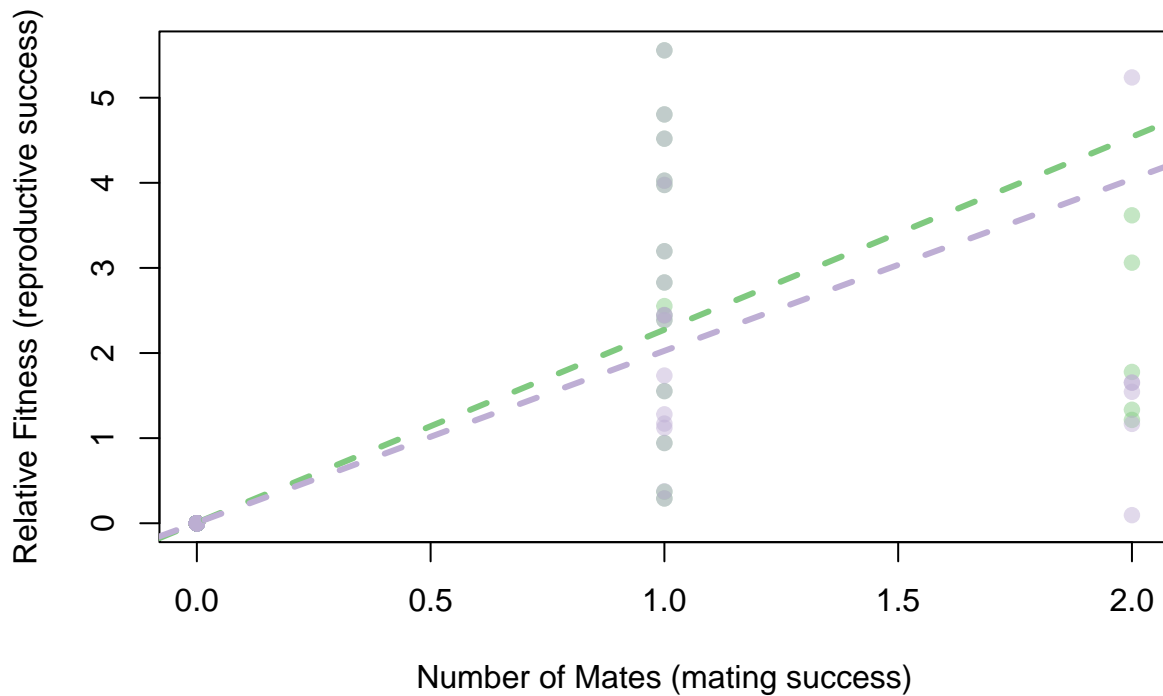
```
##
## Residual standard error: 1.016 on 54 degrees of freedom
## Multiple R-squared:  0.6222, Adjusted R-squared:  0.6152
## F-statistic: 88.92 on 1 and 54 DF,  p-value: 5.237e-13
```

```
summary(wls_model_mal)
```

```
##
## Call:
## lm(formula = mal_bateman$rel_repo_fitness ~ mal_bateman$MatingSuccess,
##     weights = wt_mal)
##
## Weighted Residuals:
##      Min       1Q   Median       3Q      Max
## -1.83508 -0.02358 -0.02358 -0.02358  2.91438
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.006378   0.041449   0.154   0.878
## mal_bateman$MatingSuccess 2.019383   0.210713   9.584 3.01e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8676 on 54 degrees of freedom
## Multiple R-squared:  0.6297, Adjusted R-squared:  0.6229
## F-statistic: 91.85 on 1 and 54 DF,  p-value: 3.015e-13
```

Omitting females with high mating

To make sure the two females that mated 3 times are not significantly affect the Bateman gradient I am re-plotting and re-running the model with those points omitted.



```
##
## Call:
## lm(formula = fem_bateman$rel_repo_fitness[fem_bateman$MatingSuccess <
##      3] ~ fem_bateman$MatingSuccess[fem_bateman$MatingSuccess <
##      3], weights = wt_fem2)
##
## Weighted Residuals:
##      Min       1Q   Median       3Q      Max
## -1.7550 -0.0237 -0.0237 -0.0237  2.9057
##
## Coefficients:
##                                     Estimate Std. Error
## (Intercept)                        0.006598   0.040690
## fem_bateman$MatingSuccess[fem_bateman$MatingSuccess < 3] 2.267234   0.218687
##                                     t value Pr(>|t|)
## (Intercept)                        0.162    0.872
## fem_bateman$MatingSuccess[fem_bateman$MatingSuccess < 3] 10.367 2.95e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8657 on 52 degrees of freedom
## Multiple R-squared:  0.6739, Adjusted R-squared:  0.6677
## F-statistic: 107.5 on 1 and 52 DF,  p-value: 2.945e-14
```

It doesn't look like omitting those few individuals has any effect on the results of the Bateman gradient.

Visualizing post-copulatory selection

As a way to visualize selection acting AFTER the mating event (post-copulatory selection) I am plotting the proportion of eggs that survived against mating success. Hopefully this will tell us if acquiring more mates is having any affect on the ability for the eggs to develop.

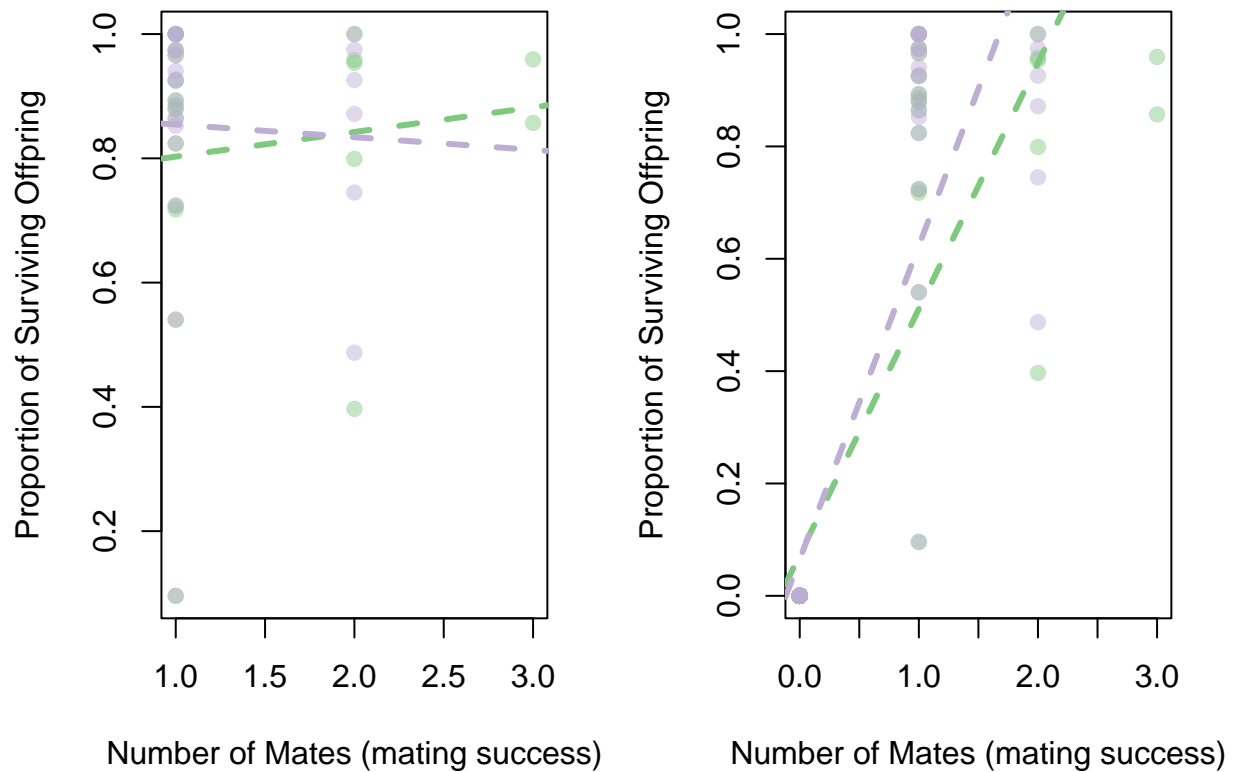


Figure 7: Plotting the relationship between the proportion of eggs that developed and the number of mates acquired for both males (purple) and females (green). This was done omitting the individuals that did not mate (left) and including those individuals (right).