ABSTRACT

Title of Document: POTENTIAL FOR SPERM LIMITATION IN BLUE CRABS OF CHESAPEAKE BAY

Sarah Ann Mellace Rains, Master of Science, 2014

Directed By: Associate Professor, Dr. Michael J. Wilberg, Marine Estuarine Environmental Sciences

Sperm limitation is a concern for blue crabs *Callinectes sapidus* in Chesapeake Bay due to their reproductive biology and sex specific fishing pressures from regulations. Our objectives were to 1) characterize differences in sperm quantity per female among six tributaries in Chesapeake Bay and evaluate if it is related to the tributaries mature male:female sex ratio and 2) develop an individual based model to simulate the effect of harvest on the reproductive sustainability of the blue crab fishery. We found that sperm quantity per female varied among tributaries, as did sex ratio, but were not related to each other. Additionally, all simulated fishing scenarios showed no significant differences in sperm per female except for when all mature males were fished at five times current fishing pressure and females were unfished. Our results suggest that sperm limitation is not a concern for blue crabs of Chesapeake Bay under current regulations.

POTENTIAL FOR SPERM LIMITATION IN BLUE CRABS OF CHESAPEAKE BAY

By

Sarah Ann Mellace Rains

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Advisory Committee:
Associate Professor, Dr. Michael J. Wilberg, Chair
Dr. Thomas Miller
Dr. Elizabeth North

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Introduction

Blue Crabs of Chesapeake Bay

Blue crab (*Callinectes sapidus*) supports one of the most important commercial fisheries in Chesapeake Bay. Their ex-vessel landings are valued at around $73 million dollars annually making them the highest valued commercial fishery in the Bay (Bunnell et al. 2010). Declines in harvest over the past two decades have caused concern about recruitment overfishing. This concern led to implementation of sex-specific regulations in 2008, which reduced the harvest of females by about 30% (Miller et al. 2011). These regulations limit daily catch allotments of mature females in the fall, with an early end to their season, and placed the winter dredge fishery in Virginia under a moratorium (Bunnell et al. 2010). Male regulations have remained the same, restricting the harvest of hard shell crabs under 127mm carapace width. During 2008-2010 abundance has increased substantially, particularly for females (Figure 1). Changes in management appeared to have been successful in increasing female abundance, but the ratio of males to females has become skewed to about 1:5, from a pre-regulation ratio of about 1:2 (Miller et al. 2011). This shift in sex ratio has been a cause of concern within the management community. Managing to protect a portion of egg production or female spawning biomass from harvest is very important for sustainability of most fisheries, but managing for adequate male abundance may be equally important for blue crabs.

Sperm limitation of blue crabs in Chesapeake Bay has been raised as a potential concern for the sustainability of the population (Hines et al. 2003, Kendall et al. 2002). The process of fertilization in blue crabs is the major reason for concern over male and female sex ratio and potential sperm limitation. Female blue crabs receive sperm during their terminal, or final molt.

During this time they both mate and mature. Female maturation is based on size with maturation occurring at about 111 mm (Rains Chapter 2). At this time, the female signals to local males that she is ready to mate (Jivoff et al. 2007). A male will cradle her underneath him while she molts and flip her over to permit insertion of sperm packages into her two sperm storage organs, the spermathecae. He will then protect her until her shell hardens and she becomes reproductively unavailable. This is the only time a female is assumed to receive sperm and the amount she receives will dictate how many eggs she will be able to fertilize in her lifetime (Jivoff et al. 2007, Hines et al. 2003, Jivoff 2003a). Most females are thought to mate only once.

Males, on the other hand, can mate an indefinite number of times. Male maturation is size dependent, with male blue crabs becoming fully mature by approximately 107 mm (Jivoff et al. 2007). However, males deplete a portion of their sperm storage during each mating and need approximately 20 days to fully recuperate (Wolcott et al. 2005, Hines et al. 2003, Jivoff 2003b, Kendall et al. 2001 & 2002). Males not given enough time to fully recuperate between matings transfer significantly less sperm to females with each consecutive coupling (Kendall et al. 2002). Sperm limitation due to low male abundance has been observed in other crustacean populations, most notably in field manipulation studies of Japanese stone crabs, *Hapalogaster dentate* (Sato and Goshima 2006) and laboratory studies of snow crabs, *Chionoectes opilio* (Rondeau and Sainte-Marie 2001).

Chesapeake Bay provides a natural experiment to test for effects of sperm limitation because the sex ratio varies spatially due to differential migration patterns between the sexes (Wenner 1989). After females have mated they begin a one-way migration to the mouth of Chesapeake Bay because their larvae require the high salinity waters of the Atlantic Ocean (Jivoff et al. 2007). Mature females remain in these high salinity waters near the mouth of the

Bay, while males remain in the tributaries in which they settled initially. This causes a male dominated sex ratio in the northern portion of Chesapeake Bay, while the lower Bay has a more female oriented sex ratio. These spatial gradients in sex ratio within the Bay could lead to differences in productivity of females.

The goal of my research is to determine whether sperm limitation is occurring within blue crabs of Chesapeake Bay and to understand the effects of fishery regulations on future stock abundance. Concerns have been raised that sperm limitation could be happening within the Bay's population, but Bay-wide studies have not been conducted to determine if this occurring (Hines et al. 2003, Jivoff 2003b, Kendall et al. 2002). In addition, mathematical models to determine how fishing effects sperm received per female and what induces sperm limitation in a population have not been developed. Understanding the potential for sperm limitation can have a positive impact on blue crab fisheries by determining whether current fishery management is sustainable and developing guidance for future management.

Blue crabs are one of the most economically important fisheries in Chesapeake Bay. Well-informed management to ensure sustainability of this fishery is crucial for its continued benefit to the region. The results of my research will determine whether there is evidence for sperm limitation in blue crabs of Chesapeake Bay. My study will also provide fishing mortality rates and sex ratios that are necessary to avoid decreased production due to sperm limitation. Additionally, blue crabs are a critical species to consider for ecosystem-based management in Chesapeake Bay because of their importance as a predator and prey species.

Objectives

The objectives of my thesis research are to:

1. *Characterize differences in sperm quantity per female among six tributaries and evaluate if sperm limitation is occurring in Chesapeake Bay.* I completed this objective with a field study, in which I collected mature female crabs from six tributaries that spanned the latitudinal gradient of Chesapeake Bay. I then compared the average number of sperm per female in each tributary by the sex ratio.

2. *Develop an individual based model to simulate the effect of male harvest on long-term reproductive sustainability of the blue crab fishery.* Using previous literature on blue crab biology, I created an individual based model that simulates mortality, growth, maturity, and mating of a population of blue crabs over a 2 year period. The model was tailored to represent conditions in Chesapeake Bay. I used this to compare average sperm received per female in different scenarios of fishing mortality to an unfished crab population.

Figures

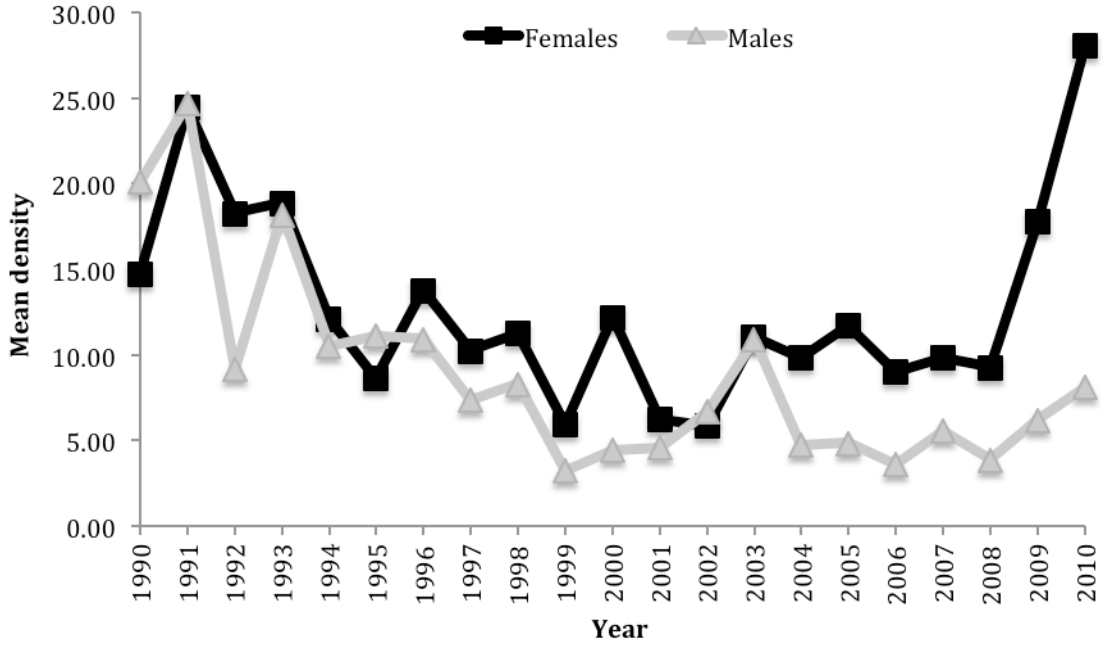

Figure 1.1. Mean density (crabs/1000 m^2) of age 1+blue crabs in Chesapeake Bay during 1990-2010 by sex. Data are from the Chesapeake Bay blue crab winter dredge survey.

Chapter 1: A field study comparing sex ratios and average sperm per female in six tributaries of Chesapeake Bay

Abstract

Sperm limitation has been a documented concern for several crustacean species around the world. It is of particular concern for blue crabs *Callinectes sapidus* in Chesapeake Bay due to their reproductive biology and sex specific fishing pressures from regulations. Our objectives were to characterize differences in sperm counts of females from tributaries in Chesapeake Bay and to determine if sperm quantity was affected by the ratio of males to females in each system. We collected adult female blue crabs from six tributaries of Chesapeake Bay. Each tributary was sampled 1-6 times on a biweekly schedule during September - November of 2011. We quantified sperm storage for each crab and compared the sperm counts of females among river systems to the adult male to female sex ratio using ANCOVA and linear regressions. Total sperm quantity per female ranged between a maximum of $1.3x10^9$ and a minimum of $9.1x10^7$ and varied among tributaries. Sex ratio per tributary was also variable but was not related to total sperm quantity per female. Total sperm quantity per female was negatively related to the development stage of the spermathecae ($F=68.93$; $df=1,123$; $p<0.0001$). Calculated sperm to egg ratios averaged from 153:1 to 4:1, but were always higher than 1:1. Our results suggest that sperm quantities are not affected by mature male to female sex ratios and that sperm limitation due to sex ratios is likely not a concern in tributaries similar to those included in our study.

Introduction

In the management of many fisheries, eggs are considered to be the limiting resource for reproductive output (Quinn and Deriso 1999). However, sperm has been found to be the limiting resource of reproduction in some free-spawning marine species (Levitan and Petersen 1995) and

has also been a concern for crustaceans and protogynous fishes that have internal fertilization (Alonzo and Mangel 2005; Hines et al. 2003). Sperm limitation could occur by changing the average size of males, where smaller males may not have as much sperm as larger ones, or the male abundance, where a low abundance of males are required to fertilize females too often and are not able to effectively restore sperm storages between each one (Hines et al. 2003; Kendall et al. 2002). In crustaceans, sperm limitation has been observed for both small male size and low male abundance, most notably in field manipulation studies of Japanese stone crabs, *Hapalogaster dentate* (Sato and Goshima 2006).

Because eggs are, in most cases, the limiting reproductive resource, fisheries are often managed to protect a portion of egg production or female spawning biomass from harvest, but managing for adequate male abundance to avoid sperm limitation may be equally important for some species, particularly blue crabs (*Callinectes sapidus*; Jivoff et al. 2007). The blue crab supports the highest valued commercial fishery in Chesapeake Bay, with ex-vessel landings valued at around $73 million annually (Bunnell et al. 2010). Declines in harvest over the past two decades have caused concern about recruitment overfishing. This concern led to implementation of sex-specific regulations in 2008, which reduced the harvest of females by about 30% (Miller et al. 2011). These regulations limit daily catch allotments of mature females in the fall, with an early end to their season, and placed the winter dredge fishery in Virginia under a moratorium (Miller et al. 2011). Male regulations have remained the same, which generally restrict harvest under 127mm. Since implementation of these regulations, abundance has increased substantially, particularly for females (Figure 1).

Changes in management appear to have succeeded in increasing female abundance, but the ratio of males to females has become skewed to about 1:5, from a pre-regulation ratio of

about 1:2 (Miller et al. 2011; Figure 1). Due to the change in sex ratio and reproductive biology of the blue crab, sperm limitation is a potential concern in maintaining a sustainable population. Female blue crabs are thought to mate once and store sperm from that mating to produce multiple broods of eggs. Females receive sperm when they mate during their terminal, or final, molt. This is thought to be the only time a female mates and the amount of sperm she receives will dictate how many eggs she will be able to fertilize in her lifetime (Hines et al. 2003, Jivoff 2003a). The average female is thought to produce about three broods over her lifetime (Hines et al. 2003) with an average of $3.3x10^6$ eggs per brood (Prager et al. 1990). In contrast with females, males can mate an indefinite number of times. However, males deplete their sperm stores by about half during each mating and need approximately 9-20 days to fully recuperate (Kendall et al. 2002, Hines et al. 2003, Jivoff 2003b, Wolcott et al. 2005). Males not given enough time to fully recuperate between matings give females approximately 50% less sperm with each consecutive coupling (Kendall et al. 2002). Therefore, a male to female sex ratio skewed towards females could cause males to mate more often with shorter periods to recover their sperm storages. The transferring of reduced amounts of sperm per mating could potentially lead to sperm limitation. Sperm limitation in blue crabs has been observed in lab settings, where females have created broods of eggs that were unfertilized, presumably due to lack of sperm (Hines et al. 2003, S. Chung, Institute of Marine and Environmental Technology, personal communication).

In most mating systems, multiple sperm are associated with mature eggs, such that the optimal fertilization success occurs at a ratio of sperm to eggs much greater than 1:1 (Hines et al. 2003). Many studies, particularly those on decapod crustaceans, have used the ratio of sperm to eggs to indicate the presence of sperm limitation in a population (Sato and Goshima 2006; Hines

et al. 2003; Rondeau and Sainte-Marie 2001). Knowledge of the sperm:egg ratio required for optimal reproduction success is especially important for management because it would permit, when combined with field estimations of total sperm counts in females, direct estimation of potential reproductive impairment. Calculation of the optimal sperm:egg ratio in blue crabs, however, is complicated because females only mate once, meaning the amount of sperm transferred at mating must support her lifetime egg production. Moreover, there is great uncertainty over the number of broods a female produces in her lifetime, even though three has been the assumed average (Darnell et al. 2009; Hines et al. 2003). To address this question of sperm:egg ratios in blue crabs, we compared estimates of total sperm counts per female to a range of egg production estimates to determine the variability in these sperm:egg ratios.

Previous studies have raised concerns over sperm limitation in blue crabs of Chesapeake Bay by comparing the number of sperm per female from laboratory matings (Kendall et al. 2002) and less fished areas (Indian River Lagoon, FL: Hines et al. 2003) with field collected samples of Chesapeake Bay. Hines et al.'s results showed that females in the Indian River Lagoon, FL had a much higher average sperm received per female than Chesapeake Bay (Chesapeake Bay: $5.0x10^8$, Indian River Lagoon: $1.2x10^9$) and concluded that females in Chesapeake Bay were receiving half of those in a less fished Indian River Lagoon population. Kendall et al. (2002) compared their laboratory data on sperm numbers received per female of consecutively mated males (First mate: $3.35x10^9$, Third (final) mate: $9.31x10^8$) with numbers seen in field females collected in the Rhode River, MD, to conclude that most females within the tributary were receiving amounts of sperm closer to that of laboratory females mated with depleted males (Field average: approx. $9.0x10^8$). Neither of these studies, however, directly evaluate if the reason for

differences in amount of sperm per female is due to the abundance of available males within the populations or other factors.

A reason that these differences between numbers of sperm may not be directly related to the sex ratio of a population is that, in most crustaceans, the operational sex ratio is often skewed toward males. Though the sex ratio of a population is usually defined as the abundance of mature individuals of one sex relative to the other, studies on sexual competition usually refer to the operational sex ratio of a population, or the number of mature males to fertilizable females (Rondeau and Sainte-Marie 2001, Kendall et al. 2001). Because blue crab females are only fertilizable during short windows of time, finding a mate within this time frame is crucial for successful fertilization (Rondeau and Sainte-Marie 2001). If the operational sex ratio of these crustacean species reveals low male abundance relative to females, sperm limitation could occur because either some females will not be able to find mates, or available males will not have sufficient sperm to fertilize all the receptive females. However, female blue crabs mature asynchronously and are only thought to mate once, so that the pool of receptive males is usually larger than receptive females (Jivoff et al. 2007). This should make the operational sex ratio almost always skewed toward males, even if the total population is skewed toward females (Rondeau and Sainte-Marie 2001). Additionally, males mature at a smaller size than females (Jivoff et al. 2007). Unfortunately, it is very difficult to determine the ratio of receptive males and females because it is difficult to identify a female preparing to mature during her next molt.

Our goal was to determine whether the amount of sperm per female varied spatially in Chesapeake Bay and if differences were related to differences in sex ratios among tributaries. Although concerns have been raised that sperm limitation could be happening within the Chesapeake Bay blue crab population (Hines et al. 2003, Jivoff 2003b, Kendall et al. 2002), no

direct comparisons have been made between the amount of sperm observed in field collected females and the male:female sex ratio of that population. Furthermore, Bay-wide studies have not been conducted to test this hypothesis in Chesapeake Bay. We examined the amount of sperm per female among six major tributaries of Chesapeake Bay to evaluate if sperm storage per female differed among systems and was related to the local mature sex ratio. We hypothesized that females in tributaries with higher male:female sex ratios would receive more sperm per female than tributaries with lower sex ratios because males should have longer times between pairings to recover sperm stores. We also calculated sperm:egg ratios for a range of assumed brood production schedules. Because blue crabs support an economically important fishery, it is important to have a metric in which managers can effectively evaluate the productivity of a tributary. Using the male:female sex ratios, or sperm:egg ratios, seen in these tributaries may be a good metric to help managers decide the best regulations for the Chesapeake Bay blue crab fishery.

Methods

During fall of 2011, we collected mature female crabs from commercial watermen near the mouth of six tributaries that spanned the latitudinal gradient of Chesapeake Bay: the Chester, Choptank, Patuxent, Potomac, York, and James rivers (Figure 2). Maturity was determined by the shape of each blue crab's ventral hood, which is dome shaped on mature females but triangular shaped on immature ones. Blue crabs were collected 1-6 times per tributary in September, October, and November with an average catch of 135 females per collection from local watermen of that area. Collection sites were chosen based on close proximity to the mouth of each tributary in order to collect mature females as they migrated out of each tributary to the

spawning grounds at the mouth of Chesapeake Bay. Blue crabs were labeled by location and date and frozen for subsequent examination in the laboratory.

We dissected 21 females per tributary to quantify the abundance of sperm per female crab in each river system (total n=126). Individual samples were thawed in cool water and the carapace width was measured from lateral spine to lateral spine. During dissection we recorded spermathecae development stages based on color and size. This scale categorizes crabs from recently mated crabs with spermathecae at 100% fullness to crabs ready to spawn at 0% fullness (Hines et al. 2003, Wolcott et al. 2005). Six females in our sample had differences in the percent fullness between their right and left spermathecae; for these individuals we calculated overall percent fullness by averaging the values of the two spermathecae. The spermathecae were then removed, and their wet weight was recorded after resting both sides on a lens tissue to remove excess water.

The methods used to quantify the amount of sperm in each crab were modified from the methods of Hines et al. (2003). In particular, our study was modified to use both spermathecae, as there is significant variability in number of sperm between the left and right spermatheca of the same female, even though the weight of the pair of spermathecae was often similar (Rains unpublished). The spermathecae from one crab were placed in a graduated cylinder with 2-5 mL of full strength artificial seawater (ASW), and the volume of the sample was recorded. The ASW and spermathecae were then added to a Dounce homogenizer and ground for 30 minutes. Two 50 μL subsamples were diluted with 1500 μL of ASW. Preliminary studies indicated that this dilution made counting easier and more efficient. We added 7.5μL of 1% aqueous crystal violet stain to aid in identifying sperm. A 10 μL subsample of this 15,575μL solution was injected into a hemocytometer for counting. We counted the number of sperm under 400x

magnification using a compound microscope in five of the 25 hemocytometer grid squares, the four corners and the middle. Four 10 μL subsamples were counted for each crab, giving a total of 20 counted grid squares for each sample. The counts were averaged to provide a mean abundance of sperm per square, and then scaled up by the initial sample volume to estimate total sperm quantity for the crab,

$$TSC = \left(\frac{a}{0.004 * 1000 * 1.5575}\right) \times \left(\frac{s}{0.05}\right)$$

where *TSC* is the total sperm count, *a* is the average sperm count per hemocytometer grid, and *s* is the sample volume. The parameters reflect the total sample volume (1500μL+50μL+75μL=1.5575mL).

Sex ratio data for mature blue crabs during August-November of 2011 for each tributary were obtained from trawl surveys conducted by the Maryland Department of Natural Resources (for the Chester, Choptank, and Patuxent rivers) and Virginia Institute of Marine Science (for the York and James rivers) and from commercial harvest records from the Potomac River Fisheries Commission for the entirety of each tributary. Mature females from the surveys were visually identified by the shape of their abdomen, which transforms from a triangular shape as an immature female to a domed shape once mature (Jivoff et al. 2007). Males larger than 107mm, the mean size of maturity, were considered mature, because there are no external differences between immature and mature male blue crabs (Jivoff et al. 2007). For the Potomac River, the sex ratio was estimated from the harvest data (in number of bushels, approximately 35.2 liters, per sex). These data only included males above the autumn minimum size limit of 127 mm. We converted from bushels to numbers by multiplying the number of bushels harvested by an average number of individuals per bushel (males – 75 per bushel; females – 135 per bushel) based on similar methods of Miller et al. (2011). To correct the Potomac River sex ratio for the

minimum size limit, we calculated mean ratio of males between 107mm and 126mm to mature males above 127mm for all Maryland tributaries for August through October. We then multiplied this ratio with the Potomac River landings records of males.

Data for sperm count per female were summarized over they months sampled by tributary system. We found no significant effect of month or female carapace width on the total sperm count per female so did not use these variables in our analysis. We conducted a one-way ANOVA with log-transformed sperm count per female as the dependent variable and tributary as the independent variable to test if sperm quantity differed among tributaries. Total sperm counts for each female were $\log_e$-transformed in order to satisfy the assumptions of normality and homogeneity of variances. A Tukey Honestly Significant Difference (HSD) multiple means comparison test was done to identify which rivers were significantly different from one another in terms of $\log_e$-transformed sperm counts.

We used an ANCOVA with the quantity of sperm per female as the dependent variable, sex ratio in the tributary as the independent variable, and percent fullness of the spermathecae as a covariate,

$$y_{ij} = \beta_0 + x_i\beta_1 + c_j\beta_2 + \varepsilon_{ij}$$

where y_{ij} is sperm quantity per female of the *i*th sex ratio and *j*th percent fullness, x_i is the sex ratio of the *i*th tributary, c_j is the *j*th percent fullness, β_0 is the y-intercept, β_1 is the slope of sex ratio, β_2 is the slope of percent fullness, and ε_{ij} is the error term for the *i*th sex ratio and *j*th percent fullness. We did not include the interaction term in our model because there was little contrast in percent fullness in some tributaries, which hindered estimation.

We used two approaches to account for differences in spermathecae fullness. First, we regressed TSC per female on sex ratio for females determined to have 0% spermathecae fullness

to test if sperm quantity was related to sex ratio to control for the effect of percent fullness. We also conducted a linear regression between sperm quantity per female and sex ratio for all individuals after correcting for the effect of percent fullness. We applied a proportional correction using the results from the regression of TSC on sex ratio. The correction is given by:

$$0\%\ Corrected\ Total\ Sperm\ Count = TSC - (TSC \times 0.30 + c)$$

where *TSC* is the sample's total sperm count, *0.30* is the average percent sperm decreased between average counts at 100% and 0% fullness (Eq. 1), and *c* is the percent fullness of the individual's spermathecae. This correction assumes that all spermathecae loose sperm at the same rate, and differ only in the amount of sperm transferred.

Sperm to egg ratios were calculated to evaluate how much sperm an average female was receiving per egg. The mean, maximum, and minimum sperm quantities at 0% full spermathecae in our study and the average amount of sperm a fully recovered male can give a female from Wolcott et al. (2005) were compared to different values of average eggs produced per lifetime. Because Wolcott et al. (2005) estimated TSC right after mating, their TSC value was corrected so that 50% of sperm were lost between mating and first brood production. The mean number of eggs produced per brood was $3.3x10^6$ (Prager et al. 1990). While female blue crabs may survive up to two years after maturity, most only live for less than one year, with an average number of broods per female in North Carolina of 1.4 (Darnell et al. 2009). The average number of eggs produced in 1.4 broods is $4.5x10^6$. We also calculated sperm:egg ratios using three broods per season from Hines et al. (2003) and the maximum amount of broods a female produced over her lifetime from Darnell et al. (2009), which is seven. The average number of eggs produced in three broods is $9.9x10^6$ and the average number of eggs produced in seven broods is $2.3x10^7$.

Results

The TSC per female blue crab was highly variable among individuals in each river system and ranged between $1.3x10^9$ (Choptank River) and $9.1x10^7$ (James River; Appendix I). The average TSC across all tributaries was $3.6x10^8$ (standard error (SE) $2.4x10^7$) with a median of $2.6x10^8$ and a standard deviation (SD) of $2.7x10^8$. The difference between the mean and the median indicates a right-skewed distribution. For samples corrected to 0% fullness, the average TSC was $3.1x10^8$ (SE $1.7x10^7$), with an SD of $1.9x10^8$. The minimum decreased to $7.9x10^7$ and the maximum decreased to $9.2x10^8$.

Log_e-transformed TSC differed among tributaries ($F=3.08$; $df=5,120$; $p = 0.01$; Figure 4). However, only two sets of tributaries were significantly different from one another; the James River had significantly lower TSC than the Potomac River ($t = -3.29$; $p = 0.02$) and the Choptank River ($t = -2.94$; $p = 0.04$).

Male to female sex ratios varied between 3.48 and 0.93 among tributaries (Figure 3). The highest sex ratio was observed in the Chester River (3.70), while the lowest sex ratio was in the Choptank River (0.66).

TSC was not related to male:female sex ratio ($F=0.01$; $df=1,123$; $p = 0.93$; Figure 5), but was positively related to the percent fullness of the spermathecae. Average TSCs were well described by a linear relationship with percent fullness, from 0% full to 100% full, with an increase of approximately 30% ($F=68.93$; $df=1,123$; $p <0.0001$; Figure 6). The non-zero intercept indicates that spermathecae scored as 0% full contained approximately $2.6x10^8$ sperm. When we restricted our analysis to individuals with 0% spermathecae fullness, sperm quantity was still not related to sex ratio ($F=2.69$: $df=1,52$; $p=0.11$; Figure 7). Correcting all of our

samples to 0% fullness also did not show a relationship between sperm quantity and sex ratio ($F=0.61$; $df=1,124$; $p=0.44$; Figure 8).

Sperm to lifetime egg ratios were all higher than 1:1 (Table 1). The mean sperm quantity in females with 0% full spermathecae relative to 1.4 broods of eggs gave an estimated sperm to egg ratio of 59:1. The mean for our study relative to Hines et al. (2003)'s three broods of eggs gave an estimated sperm to egg ratio of 26:1 and the maximum number of seven broods from Darnell et al. (2009) had a ratio of 11:1. Evaluating the ideal circumstances where fully recovered laboratory males of Wolcott et al. (2005) gave an average of $6.0x10^8$ sperm to a female, showed that 1.4 broods had a sperm to egg ratio of 134:1, three broods was 61:1, and the most extreme scenario of seven broods was 26:1. Calculating the highest sperm to egg ratio, using the Darnell et al. (2009)'s average of 1.4 broods with the maximum sperm quantity in our sample ($6.8x10^8$ sperm) gave a sperm to egg ratio of approximately 153:1. Calculating the lowest sperm to egg ratio, using the maximum number of broods a female can produce in her lifetime (seven broods) with the minimum sperm quantity in our sample ($9.1x10^7$ sperm) gave a sperm to egg ratio of approximately 4:1.

Discussion

Sperm quantity per female was not related to the sex ratio among six tributaries in Chesapeake Bay, which indicates that mature male:female sex ratio does not explain differences in sperm quantity per female and thus sperm limitation is not happening at this time. We hypothesized that if sperm limitation was occurring, we would see a positive relationship between male:female sex ratio and the amount of sperm a female has stored, at least over some portion of the range of observed sex ratios. However, we found no difference in the average amount of sperm stored between females in the Chester River (the tributary with the highest

male:female sex ratio – 3:1) and those in the Choptank River (male:female sex ratio – 1:2). All of the females we examined had been inseminated, which also indicates that females are able to find mates under the current sex ratios (i.e., Allee effects are not occurring).

The development stage of the spermathecae was significantly related to TSC per female. Our findings are similar to those of Wolcott et al. (2005) who found that an average of 49% of stored sperm are lost between insemination and brood production. We estimated a 30% average decrease in sperm quantity between the first and last stage of spermathecae development. Differences between the amount of sperm loss over time between our study and Wolcott et al. (2005) are likely due to differences in time since mating. Blue crabs in their the Wolcott et al. (2005) study had known dates of mating, whereas we did not know the date of mating for our samples. Development of the spermathecae usually progresses as a female blue crab is preparing to brood eggs, with 0% fullness assumed to be right before she creates her first brood (Jivoff et al. 2007). Therefore, a female can be expected to lose 30-50% of her sperm between insemination and production of her first brood.

Our study has some limitations, but we believe our conclusions about a lack of evidence of sperm limitation are robust. Our crab collections were over a limited period of time, with two tributaries only having one collection each. It is possible that the sperm quantity per recently mated female changes over the course of the season (Wolcott et al. 2005); our collections occurred in too narrow a frame of time to capture seasonal dynamics. Nevertheless, females are thought to remain in the tributary in which they mated until temperature cues signal their migration to the mouth of the Bay for spawning (Jivoff et al. 2007). The females in our samples likely mated at different times during the season, and therefore our samples capture variability over a large part of the breeding season. Lastly, our sample size was still relatively small given

the large amount of variation in TSC per female, which causes relatively low power for our statistical tests. Our sample collection and sperm counting methods are, however, similar to other studies we have compared our results to (Kendall et al. 2002, Hines et al. 2003, Wolcott et al. 2005) with comparable sample sizes.

Our observed female TSCs were also in the same range as those from the laboratory studies done by Carver et al. (2005) and Wolcott et al. (2005), but the differences are likely caused by time since mating. Wolcott et al. (2005) found that the average number of sperm transferred differed with mating history, with unmated males transferring an average of $1.2x10^9$ sperm and males mated three times without recovery transferring an average of $4.1x10^8$. However, these numbers are recorded from right after mating, and correcting these numbers to 0% fullness gives them lower numbers of $6.0x10^8$ for unmated males and $2.1x10^8$ for fully depleted (mated twice consecutively) males. The corrected values from Wolcott et al. (2005) are within the same range as corrected counts found in our study. Kendall et al. (2002)'s laboratory and field comparison study had TSCs outside of the range of our study and the experimental studies of Carver et al. (2005) and Wolcott et al. (2005).

Estimated sperm to lifetime egg production ratios from our study were, in some cases, lower than those observed for other crustacean species, but the ratio of sperm to eggs necessary for fertilization is unknown for blue crabs. Prior studies have relied on information from crustacean species with different mating strategies (Wolcott et al. 2005; Hines et al. 2003). Sperm to egg ratios necessary for full fertilization are highly variable in other crustacean species, ranging from mud crab *Eurypanopeus depressus* at 3,700:1 (Rodgers et al. 2011) to snow crab *Chionoecetes opilio* at 70:1 (Sainte-Marie and Lovrich 1994). By comparison our sperm to egg ratios are, on average, lower than the range of other studied crustaceans.

Previous studies that have used sperm:egg ratios to conclude sperm limitation have assumed that females can create seven broods of eggs over a two-year lifetime after maturity (Hines et al. 2003). However, seven broods is likely a maximum estimate because annual survival is estimated to be quite low in Chesapeake Bay blue crabs (15%, Miller et al. 2011). This has been confirmed by Darnell et al. (2009)'s caged field experiments, where 69% (74 out of 107) of his brooding female blue crabs did not reach their second clutch and less than 1% (1 out of 107) made it to a seventh brood. Because Darnell et al. (2009) conducted a caged field study done in North Carolina, we also calculated the annual proportion of females that survive to the next age for Chesapeake Bay blue crabs using the equation:

$$N_{t+1} = N_t * e^{-Z}$$

where t is year, N is the abundance at time t (initially starting at 1), and Z is the instantaneous mortality rate (1.95; Miller et al. 2011). According to this model, 15% of the population survive to their second year, potentially creating about two broods of eggs, 2% survive to their third year to create up to five broods of eggs, and only 0.2% survive long enough to produce up to seven full broods. These numbers are similar to those found in Darnell et al. (2009), where 27% survived to two broods of eggs, 5% survived to five broods of eggs, and 0.9% survived to seven broods of eggs. The average female only creates 1.5 broods in her lifetime according to the model, which also coincides with the average of 1.4 broods per female from Darnell et al. (2009).

The ratio of mature males to mature females is only a proxy of the operational sex ratio for mating. Ideally, we would use the ratio of mature males to females that are ready to mate, which we should be substantially higher because males mature at a smaller size than females and can mate multiple times. However, calculation of this ratio is challenging because females that

will mature on their next molt cannot be differentiated from those that will need multiple molts to mature until they are very close to molting. We estimated operational sex ratios from the Maryland Trawl Survey (MDTS) at nine sites (Chester River, Patuxent River, Choptank River, Eastern Bay, Tangier Sound, Little Choptank River, Fishing Bay, Nanticoke River and Pocomoke Sound) to test our hypothesis that operational sex ratios are higher than male:female sex ratios. To do this, we calculated the number of males over 107mm (assumed size at maturation; Jivoff et al. 2007) and the number of females between 95-130mm (sizes with a 1-98% chance of molting to maturity using the female maturation probability equation from Rains Chapter 2) during the mating season (May – October) to evaluate the operational sex ratio of each site in the 2011 mating season. The operational sex ratio of all MDTS sites remained above 1:1 with a mean value calculated at 2.2 (SE 0.54). The values for each were Chester River (6.4), Patuxent River (2.0), Choptank River (1.3), Eastern Bay (1.6), Tangier Sound (1.1), Little Choptank River (1.5), Fishing Bay (2.2), Nanticoke River (1.9) and Pocomoke Sound (1.7). This, again, is a rudimentary examination of the operational sex ratio of these sites. Nevertheless, they confirm that even if a male:female sex ratio of a blue crab population is skewed toward females, the operational sex ratio of the population can remain skewed toward males.

To conclude, our results suggest that blue crabs in Chesapeake Bay are not experiencing sperm limitation at this time. Blue crabs support the largest commercial fishery in Chesapeake Bay, making it important for management to sustain a healthy population for not only ecological but economic reasons. We believe that, based on our results, management should continue to focus on conserving females unless substantially less restrictive regulations are being considered for males.

Tables and Figures

Table 2.1. Compared scenarios of sperm to eggs for different estimations of eggs produced and sperm transferred to females. The studies used for eggs produced were Prager et al. 1990 (for average number of sperm per brood), Darnell et al. 2009 and Hines et al. 2003. The values used for number of sperm per female were Rains Chapter 1 0% full spermathecae samples and Wolcott et al. 2005 corrected for sperm loss that was calculated in the same study.

Egg Scenario	# of Broods	# of Eggs	Sperm Scenario	# of Sperm	Sperm:egg Ratio
Darnell Average	1.4	4.47E+06	Rains Maximum	6.84E+08	152.9
			Wolcott Average	6.00E+08	134.2
			Rains Average	2.61E+08	58.5
			Rains Minimum	9.08E+07	20.3
Hines Average	3	9.90E+06	Rains Maximum	6.84E+08	69.0
			Wolcott Average	6.00E+08	60.6
			Rains Average	2.61E+08	26.4
			Rains Minimum	9.08E+07	9.2
Darnell Maximum	7	2.31E+07	Rains Maximum	6.84E+08	29.6
			Wolcott Average	6.00E+08	26.0
			Rains Average	2.61E+08	11.3
			Rains Minimum	9.08E+07	3.9

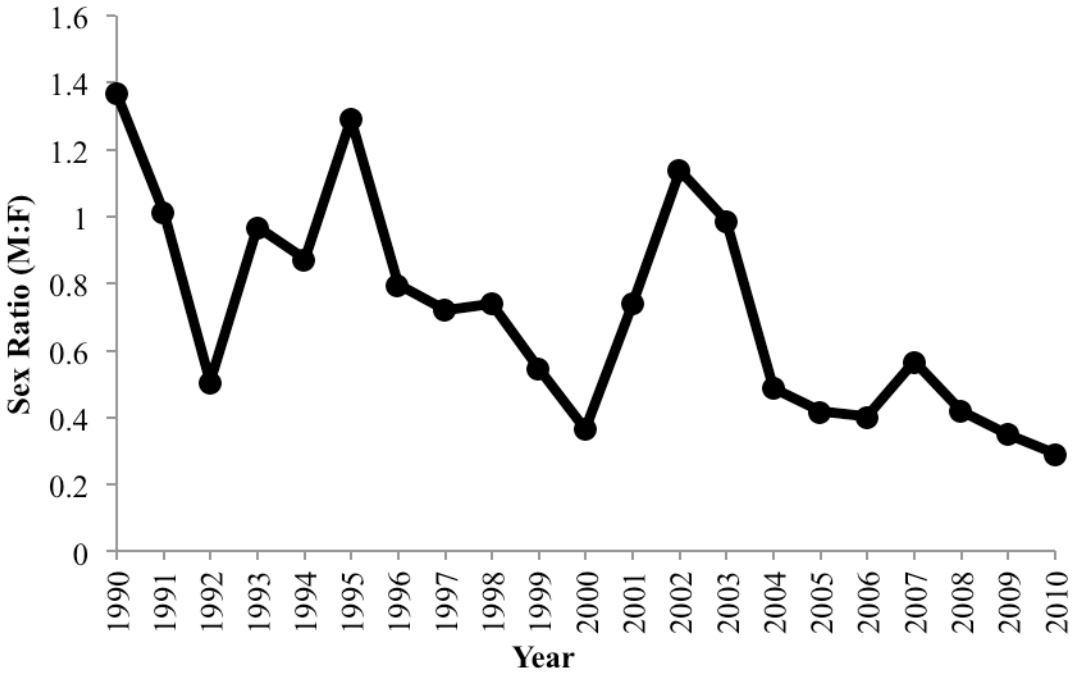

Figure 2.1. Sex ratio of male to female (M:F) age-1+ blue crabs during 1990-2010 from the Chesapeake Bay blue crab winter dredge survey.

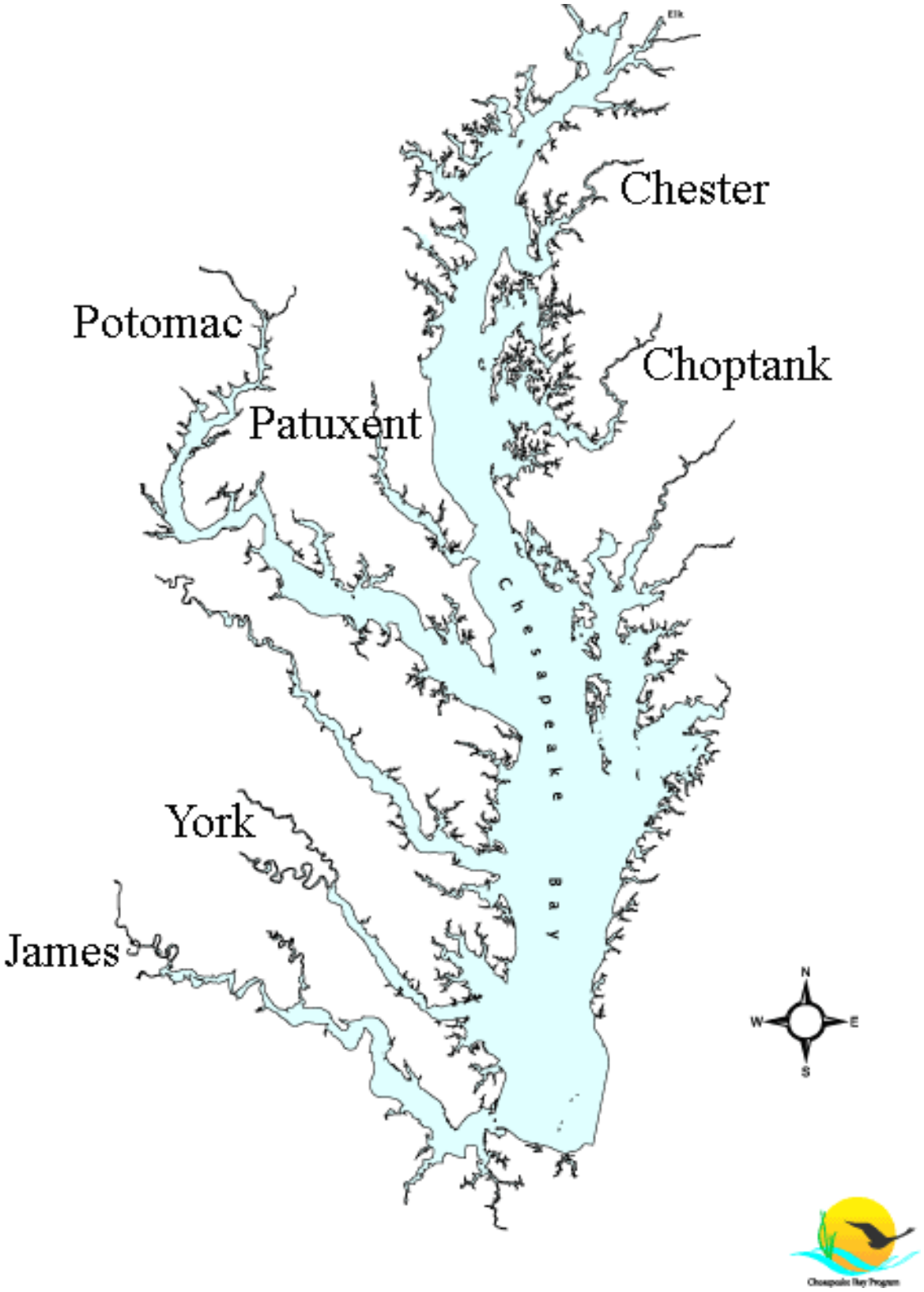

Figure 2.2. Map of Chesapeake Bay with tributaries used in this study labeled. Image from the Chesapeake Bay Program.

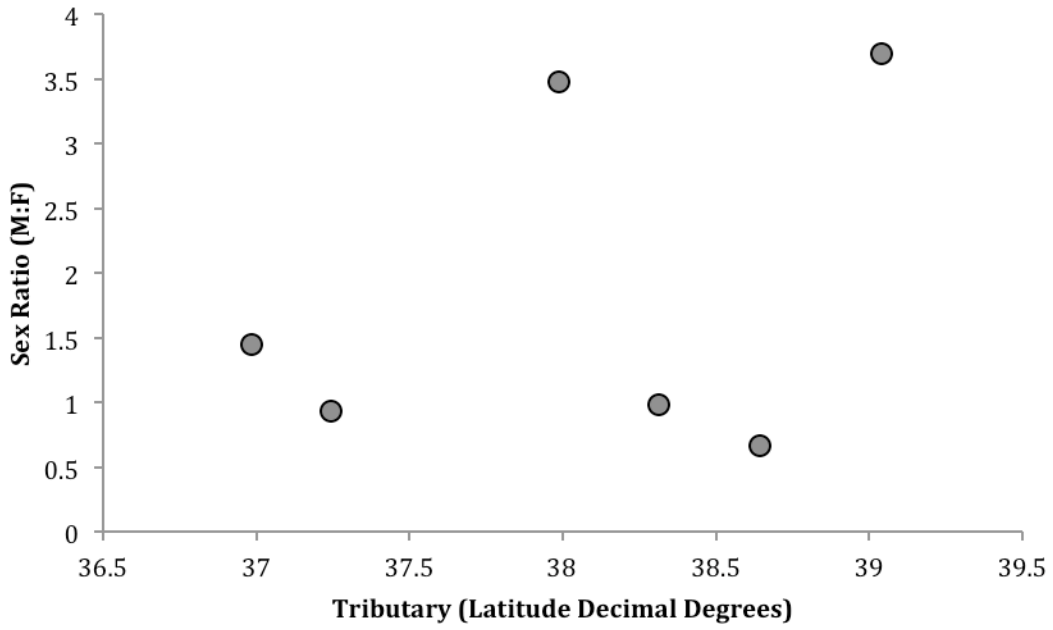

Figure 2.3. Male to female (M:F) sex ratio of mature blue crabs by tributary.

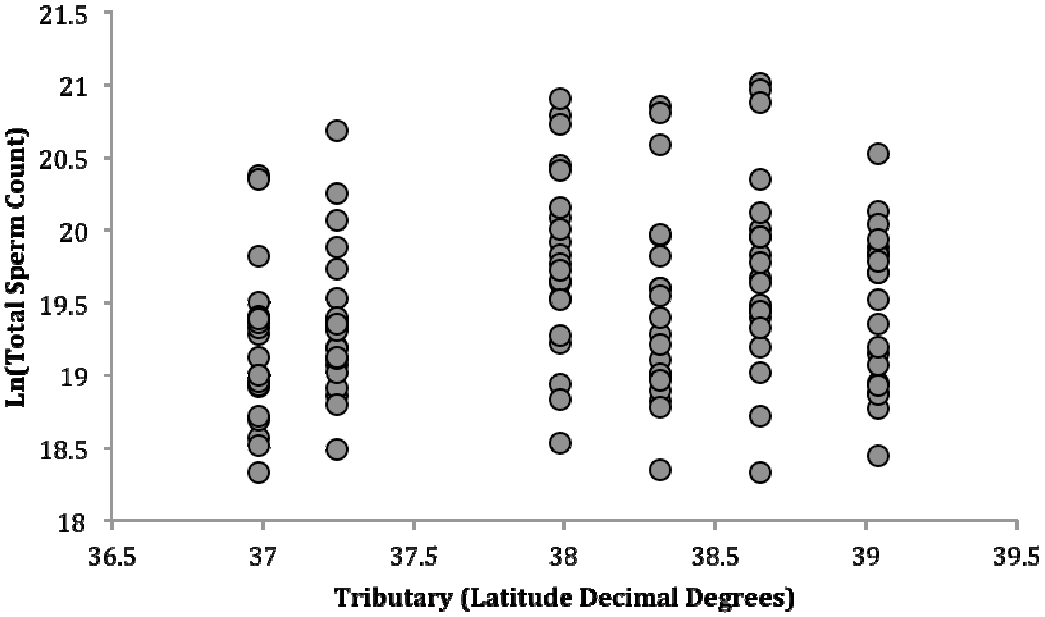

Figure 2.4. Log transformed sperm counts per female versus tributary (F=3.08; df =5,120; p = 0.01).

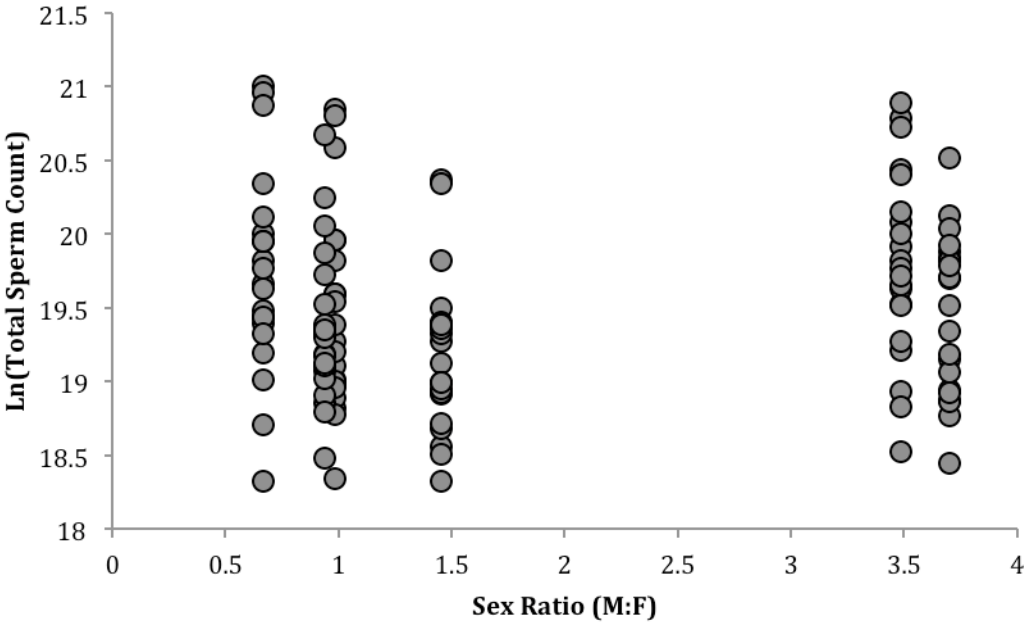

Figure 2.5. Total sperm count of female blue crabs versus the sex ratio (males to females).

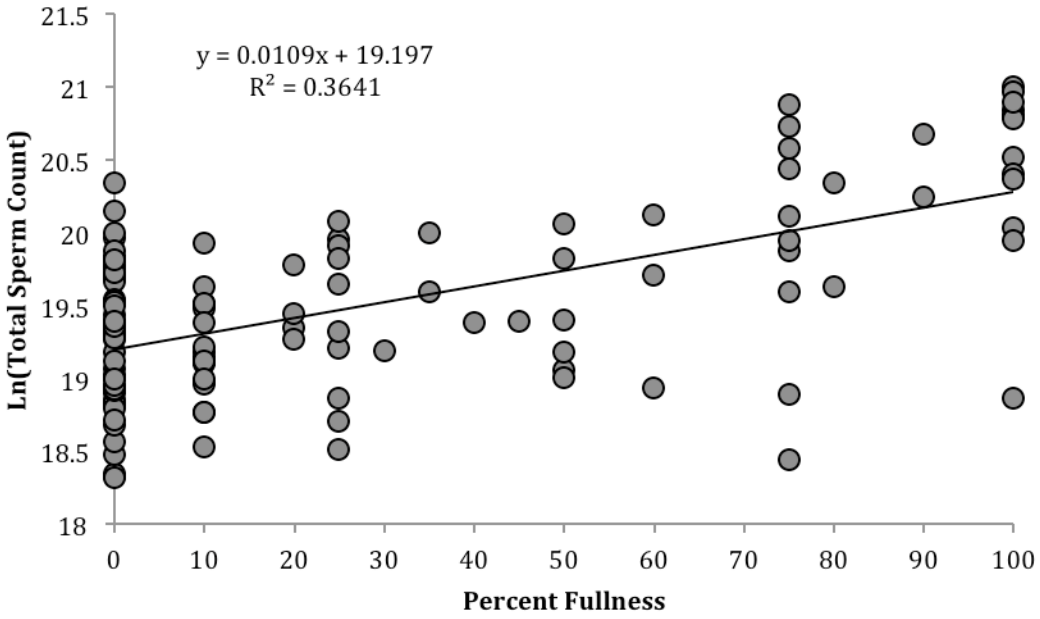

Figure 2.6. Relationship between total sperm count of female blue crabs and percent fullness of the female's spermatheca ($F=68.93$, $df=1,123$, $p<0.0001$). At 100% full a female has recently been inseminated and at 0% full she is preparing for her first brood of eggs.

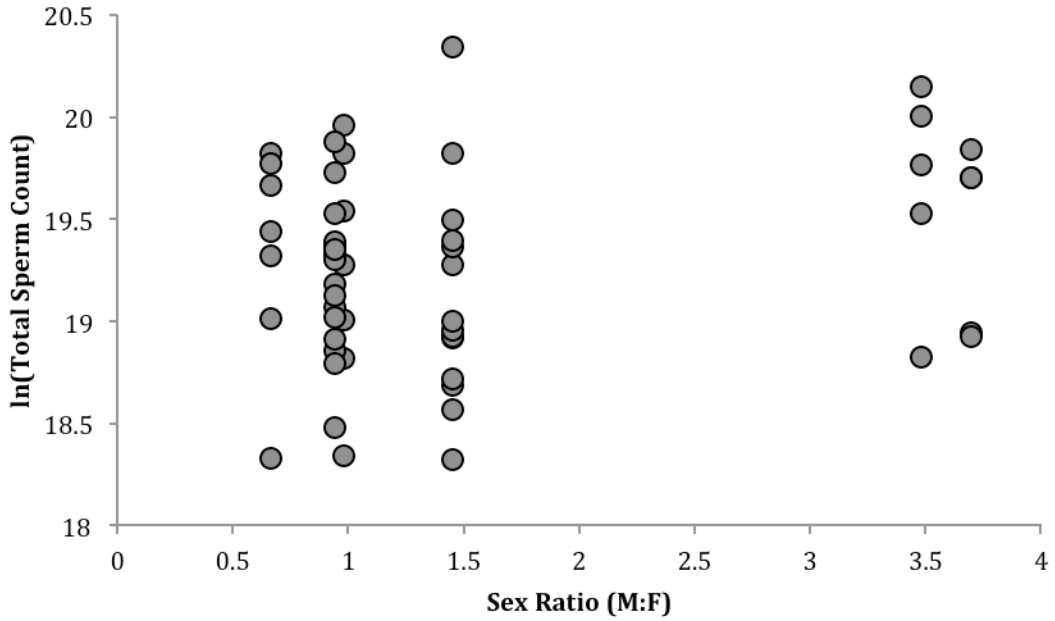

Figure 2.7. The total sperm count of female blue crabs with 0% fullness spermathecae plotted against the sex ratio (males to females).

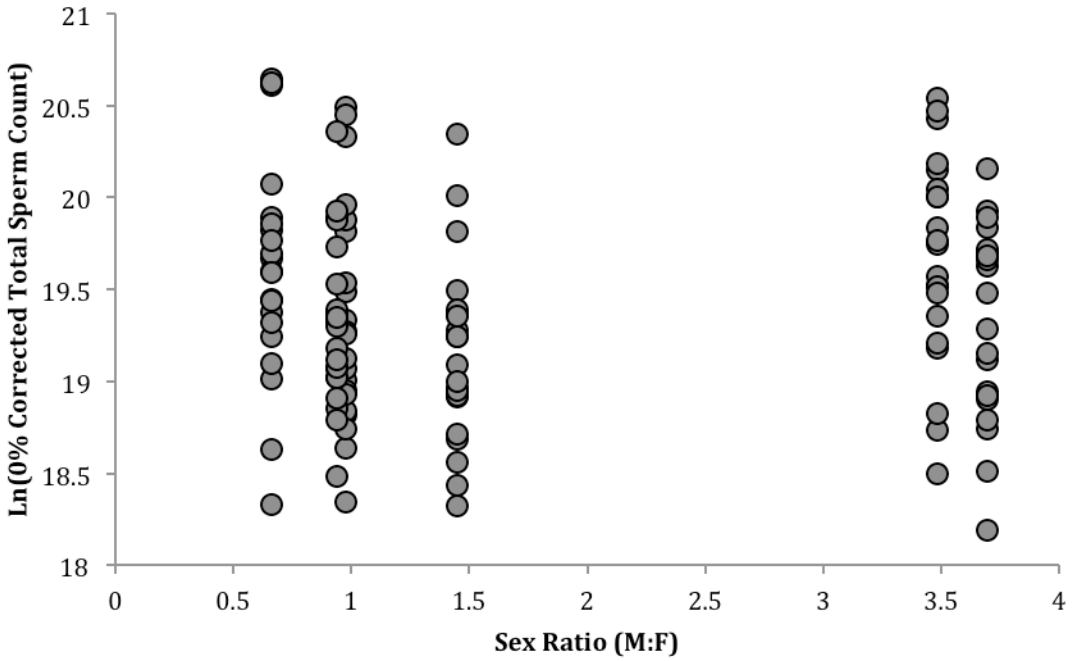

Figure 2.8. Total sperm count of female blue crabs, corrected to 0% fullness, versus the sex ratio (males to females).

Chapter 2: An individual based model simulation study comparing sex ratios and average sperm per female in a blue crab population under different fishing scenarios

Abstract

Sperm limitation, when the reproductive output of a population is restricted by its sperm production, is a concern for several crustacean species around the world including blue crabs in Chesapeake Bay. Our objective was to use a simulation study to test the effects of different fishing pressures and regulations on the male to female sex ratio of a blue crab population and the average number of sperm received per female. We created an individual based model that included sex-specific growth, maturity, and mating of a closed blue crab population. The model was run daily over a two year period in which an incoming cohort of immature females were allowed to grow and mate in a population of immature and mature males. We monitored sperm storage for each mature female and compared the sperm counts of both sexes between different sex ratios and fishing pressure scenarios. Average sperm counts for females and male:female sex ratio of the population varied among scenarios, but were not related to each other. Average sperm per males, however, was positively related to male:female sex ratio. Fishing pressure scenarios also had a significant negative effect on average sperm per female but only when five times the current fishing pressure was placed on all mature males and females were unfished. All other scenarios showed no significant differences in average sperm per female, although a broad range of fishing pressures and regulations were simulated. Our results suggest that sperm quantities should not be directly related to mature male to female sex ratios and that sperm limitation does not appear to be a main concern for blue crabs of Chesapeake Bay under current regulations.

Introduction

In the management of many fisheries, females are considered to be the limiting resource for reproductive output (Quinn and Deriso 1999). Less attention is usually given to male abundance because sperm limitation is not thought to occur very often, particularly in internally fertilizing species (Levitan and Petersen 1995). However, in populations where large males are the primary targets of exploitation, such as in many decapod crustaceans and protogynous fishes, male abundance may become low enough to limit population growth through low availability of sperm (Wenner 1989, Alonzo and Mangel 2005, Brooks et al. 2008). For example, sperm limitation has been observed in field manipulation studies of Japanese stone crabs, *Hapalogaster dentate* (Sato and Goshima 2006) and laboratory studies of snow crabs, *Chionoectes opilio* (Rondeau and Sainte-Marie 2001).

Many studies on decapod crustaceans use the number of sperm received per female compared to female fecundity as indicators of sperm limitation in a population (Sato and Goshima 2006; Hines et al. 2003; Rondeau and Sainte-Marie 2001; MacDiarmid and Butler 1999; Hankin et al. 1997; Sainte-Marie and Lovrish 1994). This indirect measure helps to compare the amount of sperm a female receives to how many eggs she will be able to fertilize. However, ratios of sperm to egg within internally fertilizing, decapod crustaceans are both difficult to measure and highly variable, ranging from mud crab *Eurypanopeus depressus* at 3,700:1 (Rodgers et al. 2011) to snow crab at 70:1 (Sainte-Marie and Lovrich 1994). This variability makes it difficult to use the sperm:egg ratio to compare among species or exploitation levels.

The sex ratio of a population has also been thought to be an indicator of sperm limitation, particularly if it becomes too skewed toward females (Rankin and Kokko 2006; Sato and

Goshima 2006; Kendall et al. 2001). The sex ratio of a population is often defined as the abundance of mature individuals of one sex relative to the other. Studies on sexual competition usually refer to the operational sex ratio of a population, or the number of mature males to fertilizable females (Rondeau and Sainte-Marie 2001, Kendall et al. 2001). Most female crustaceans are only fertilizable during short windows of time, which can mean that finding a mate within this time frame is crucial for successful fertilization (Rondeau and Sainte-Marie 2001). If the operational sex ratio of these crustacean species reveals low male abundance relative to females, sperm limitation could occur because either some females will not be able to find mates, or available males will not have sufficient sperm to fertilize all the receptive females. However, in many species females mature asynchronously, causing the pool of receptive males to be larger than receptive females and making the operational sex ratio almost always skewed toward males (Rondeau and Sainte-Marie 2001).

The process of mating is the major reason for concern about potential sperm limitation in blue crab. Female blue crab are thought to only mate once and with only one male, when they undergo their maturation molt (Jivoff 2007). The female stores sperm in sperm storage organs termed spermathecae. The amount of sperm a female receives will dictate how many eggs she will be able to fertilize in her lifetime (Hines et al. 2003, Jivoff 2003a). Males, in contrast, can mate an indefinite number of times. However, males deplete a portion of their sperm storage during each mating and need approximately 20 days to fully recuperate (Kendall et al. 2001). In a population with an operational sex ratio skewed toward females, males may have to mate more frequently and accordingly could pass less sperm to each mate, which could cause sperm limitation. Management of blue crabs in Chesapeake Bay is presently more concerned with

sustaining female abundance than male abundance (Miller et al. 2011), but calls to protect males to avoid sperm limitation have been made (Hines et al. 2003).

Previous studies have compared sperm quantities of both males and females in the field to observations in either laboratory or less fished populations to determine whether sperm limitation is occurring (Carver et al. 2005, Wolcott et al. 2005, Hines et al. 2003, Kendall et al. 2002). However, this comparative approach assumes that blue crabs did not evolve to be sperm limited, so that unfished populations would provide an indication of the maximum amount of sperm a blue crab population could produce. This comparative approach also assumes that if sperm limitation were an issue in fished blue crab populations, that small decreases in male abundance should cause disproportionately large decreases in the average sperm per female (Brooks et al. 2008). It is argued that by comparing populations experiencing different fishing pressure scenarios to the unfished population, we are able to interpret whether sperm is the limiting factor in reproductive output. Yet, neither of these assumptions are frequently stated, and are tested even less frequently.

Our goal for this study was to determine the effect of sex-specific regulations and fishing pressures on sperm received per female blue crab in a modeling environment, which allowed us to evaluate potential assumptions regarding the effects of mating behavior and fishing pressure on reproductive success directly. To address our goal, we created an individual-based model (IBM) to simulate the effect of harvest regulations and mating strategies on the average amount of sperm received by females. The IBM included a range of sex-specific fishing pressures and regulations as well as several mate preference strategies to determine their effect on sperm per female. It also included size-dependent maturity, molt cycle growth, and natural mortality following Bunnell and Miller (2005) and Bunnell et al. (2010). We compared sperm quantities

received by females under different fishing scenarios to those of unfished conditions to estimate the potential for sperm limitation.

Methods

The IBM simulated a population of 4500 blue crabs using a daily time step over the course of two years (Fig.1; Appendix II), which is the life span of an average blue crab in Chesapeake Bay. Based on estimated mortality rates, only 2% of individuals in the Chesapeake Bay blue crab population are estimated to live to a third year (Miller et al. 2011). The model simulated crabs distributed in two-hectare area. The resultant crab density ($225/1000m^2$) is close to the average density observed in the Chesapeake Bay blue crab winter dredge survey (CBWDS) in 2010. Evidence suggests blue crabs move roughly 5-15m $hour^{-1}$ (Hines 2007) making it possible for crabs to cross the entire area within about one day. Within this model domain crabs grew, matured, mated, and died according to stochastic functions based on previously published data (Fig. 1) over a two-year period started on January 1.

The IBM was run for 39 scenarios that included combinations of overall fishing mortality, sex-specific regulations, and mate preference strategies. We compared the amount of sperm per female under scenarios of fishing mortality and fishing regulation to both a no fishing scenario for three different assumed mating strategies and to data collected by the field and laboratory studies of Rains (Chapter 1), Carver et al. (2005), Wolcott et al. (2005), and Hines et al. (2003).

Initial Conditions

All females began as age-0 juveniles on January 1 and represented a cohort who had settled the previous summer. Males were apportioned between age-0 and age-1+ based on

estimates from CBWDS data, with 69% of the males as age-0 juveniles and 31% as age 1+. We assumed that all mature females from the following year would have migrated out of the system to spawn the previous fall (Aguilar et al. 2005).

The initial size distribution of each sex was based on carapace widths collected from the CBWDS in 2008-2010. Female sizes at the beginning of the year were drawn from a lognormal distribution with a back-transformed mean of 23.2mm and a log-scale standard deviation (SD) of 0.4 based on the size distribution of an incoming cohort from the CBWDS. Males included in the model came from both incoming and established cohorts, which was modeled using a mixture distribution with 69% of the males in age-0 category and 31% in age 1+. Carapace widths for the age-0 males were drawn from a lognormal distribution with a back-transformed mean of 13.6 mm and a log-scale SD of 1.0. Carapace widths for age-1+ males were drawn from a normal distribution with a mean of 124.2mm and an SD of 25.4 (CBWDS, unpublished data).

Growth

Growth was represented using a temperature-dependent molt process model (Brylawski and Miller 2006). The model tracked each crab's maturity, shell status (hard or soft), number of growing degree-days accumulated, time until next molt, sperm number, and number of mates (for males only). First, the model would determine if a crab survived for the day. The model tracked individual, cumulative degree-day exposure. Once a critical degree-day threshold had been exceeded, the model used a stochastic function on each subsequent day to determine whether an individual molted.

The molt process model recognized growth per molt (GPM) and intermolt periods (IP; Bunnell and Miller 2005). GPM was stochastic and was modeled using normal distributions with sex-specific mean GPMs and SDs. Results from blue crab growth studies by Newcombe et

al. (1949) and Tagatz (1968) were averaged by sex to calculate the mean GPM. On average, male carapace width increased 24% per molt with an SD of 7%, and the mean GPM for females was 25% with an SD of 6%, except for the maturation molt. The mean GPM for the maturation molt for females was 32% with an SD of 6% (Tagatz 1968). These GPM values are similar to those used by Bunnell and Miller (2005) and Smith and Chang (2007).

We adopted the approach of Bunnell and Miller (2005) to model the IP as a stochastic function of accumulated growing degree-days, with parameters derived from Tagatz (1968). At day 1 and after each molting event, the value for the next IP was drawn from a shifted exponential distribution,

$$f(IP) = \left(\frac{1}{\beta}\right) e^{-\frac{IP-\gamma}{\beta}}, IP \geq \gamma,$$

where γ is a power function of carapace width (CW) and represents the minimum amount of growing degree days necessary for molting

$$\gamma = 69.70 \times (1.0149)^{CW}.$$

The β parameter describes the variability of the IP distribution and is also a function of CW,

$$\beta = (166.39 * (1.0115)^{CW}) - \gamma.$$

For each day above 8.9 C, the physiological minimum temperature for blue crab growth (Smith and Chang 2007), degree-days are accumulated by subtracting 8.9 from the day's temperature value. Once the number of accumulated degree-days exceeds the IP value of a given crab, that crab will molt, grow based on their assigned GPM, become a soft shell crab for 2 days, and a new IP and GPM is drawn for the next molt. Average daily temperature estimates from the Patuxent River during 1985-2011 were used.

Mortality

Mortality was a stochastic process and depended on the size, sex, shell status, and fishing mortality scenario. Fishing (*F*) and natural (*M*) mortality were modeled as simultaneous and additive processes,

$$S = e^{-(M+F)},$$

where S is the daily survival rate. Natural mortality was set at 0.9 year^{-1} following Bunnell et al. (2010) and Miller et al. (2011). The annual rate was converted to a daily rate by dividing it by the number of days in a calendar year, so that the daily *M* was 0.0025 day^{-1}. During soft shell status, crabs had a natural mortality of twice the daily rate, as in Bunnell and Miller (2005). Fishing mortality depended on the size and sex of the crab as well as the fishing scenario. The annual rate was converted to a daily rate by dividing it by the number of days in the Maryland blue crab season for that sex (205 for females, 258 for males). During days outside of the fishing season, *F* was set equal to zero. For each crab on each day, a number was drawn from a uniform (0,1) distribution; if that number was greater than *S*, then the crab died.

Maturity and mating

For immature individuals, maturation was a process that could occur when they molted, but maturation was handled differently for males and females. Male maturation followed a knife-edge function with all males maturing at 107 mm (Jivoff 2007). At maturation a male is assigned a maximum number of sperm from a lognormal distribution with a back-transformed mean of 2.1x10^{9} and log-scale SD of 0.56 based on vas deferens counts from Kendall et al. (2001) and Carver et al. (2005). Multiple studies have shown that there is no relationship between sperm per male and male carapace width, so males retained their maximum number of sperm over the rest of their lifetime (Carver et al. 2005, Kendall et al. 2001). Once a male

matures and its carapace hardens it is then eligible to mate. Maturation in male crabs did not prevent further growth.

The maturation probability for females followed a logistic function of CW, similar to the approach of Bunnell and Miller (2005),

$$P(Maturity) = \frac{1}{1+\left(\frac{CW}{111}\right)^{-\text{steep}}}.$$

where the mean CW for the maturation molt was 111 mm. The mean CW for the maturation molt was estimated by back calculation of the average CW of mature females collected in Chesapeake Bay during 2011 (Chapter 1), assuming that females' CWs grew 32% with their maturation molt (Tagatz 1968). The determination of whether an individual female crab molted relied on comparing a uniform random (0,1) to the P(Maturity) for each female crab on each day of the simulation. Once a female matured, she no longer grew.

Mating occured at a female's maturation molt with the male randomly chosen from a multinomial distribution given their relative probability (RP) of mating,

$$P(Mate_i) = \frac{RP_i}{\sum_j RP_j}.$$

The relative probability for each mature male depended on the mate choice scenario: random, size selective (Jivoff 1997b), or previous mating history (Kendall and Wolcott 1999), which are described subsequently. When a female matured, all males in the population that were alive, mature, not already mating with another individual, hard shell, and above a minimum sperm threshold were considered as potential mates.

Once a mating pair was determined, the female received half of her mate's sperm stores and the male's sperm was reduced by half. The amount of sperm transferred was based on studies that counted the average sperm decrease between recuperated males and males having mated twice consecutively, causing an approximate 75% reduction in sperm stores of males

(Kendall et al. 2001, Wolcott et al. 2005). The sperm a female received was further reduced by 50% to account for sperm degradation between mating and a female's first brood of eggs (Wolcott et al. 2005; Chapter 1). Although, in reality, this reduction is a gradual process, we included it in the model as an initial process of mating in order to simplify computations.

The model also tracked sperm stores of males. All hard shell, non-mating males accumulated sperm daily at approximately 6% per day (Kendall et al. 2001),

$$Sperm_t = Sperm_{t-1} + e^{0.057},$$

where $Sperm_t$ is the sperm a male has at day t. Once the crab reached its maximum amount of sperm, it would stop producing until it mated again. Males were also given a minimum sperm threshold below which their sperm stores would become too low and they would stop mating in order to replenish them. The minimum sperm threshold was calculated as the average amount of sperm a male would have after three consecutive mates, about 3.0×10^8. When a male would reach a sperm quantity below this threshold, the model would not include the male in the pool of potential mates until he had replenished above the threshold. Males cease mating after three consecutive mating events (Wolcott et al. 2005, Hines et al. 2003, Kendall et al. 2002 & 2001) and the minimum sperm threshold was implemented to replicate this pattern.

Scenarios

The model was run under 39 scenarios, made up of combinations of fishing mortality, fishery regulations, and mate preference scenarios. Four fishing mortality scenarios included no fishing (F=0 year^{-1}) where 41% of population should survive to the next year, present fishing (F=1.05 year^{-1}) where 14% of population should survive to the next year, twice present fishing (F=2.1 year^{-1}) where 5% of population should survive to the next year, and five times present fishing (F=5.25 year^{-1}) where 0.02% of population should survive to the next year. Scenarios

were chosen to create a control situation where no fishing occurs, a situation to evaluate model performance where present fishing occurs, twice present fishing was the estimated F from the 1970-1980s in Chesapeake Bay, and an extreme case of fishing at five times present fishing. Regulations from the Maryland portion of Chesapeake Bay were used to simulate harvest. Current Maryland blue crab regulations for males include a minimum legal size of 127mm for hard shell and 89mm for soft shell. The male season extends April 1st to December 15th, 258 days. For females, harvest of all hard shell mature females and soft shell females above 89mm is legal from April 1st to October 23rd, 205 days. Five alternative fishery regulation scenarios were developed to test different effects of male and female fishing on average sperm per female. The alternative regulation scenarios included current regulations on one sex with a moratorium on fishing for the other sex, all year fishing on males with current regulations on females, and a minimum size of 107 mm on males with either current regulations or a moratorium on females.

We included three mate choice scenarios: random, size selective, and previous mating history. The random mate choice scenario had all pooled males given the same relative probability of being chosen. The size selective scenario is developed from field observations of a linear relationship between coupled blue crabs in the Rhode River (Jivoff 1997b). To simulate this scenario, males have a higher probability of being selected the more similar they are to the maturing female's size,

$$RP = e^{\frac{(CW_F - mp)^2}{2 \cdot var}},$$

where CW_F is the carapace width of the molting female, *mp* is the mean preferred size from the linear relationship between a pre-copulatory female and its mates carapace width (CW_M; $mp=73.33+(0.255\times CW_M)$; Jivoff 1997b), and *var* is the residual variance of the linear relationship from Jivoff 1997b (approximately, 72.2 mm^2).

The previous mating history scenario is based on Kendall and Wolcott (1999), who found that males that had recently mated have a higher probability of mating again in a laboratory study. They suggested this was mainly due to experienced males being more able to control females. Because Kendall and Wolcott (1999) did not know the full mating history of each mature male crab, they allowed males 20 days to recuperate sperm storages; we assumed that only matings within the most recent 20-day window would affect the relative probability of mating. Therefore, each male's number of mates over the previous 20 days was summed to calculate each male's relative mating probability, with the RP equal to the number of mates in the last 20 days plus one. This meant that a male with 0 mates had an RP of 1, a male with 1 mate had an RP of 2, and a male with two mates had an RP of 3. Males that had mated two times previously were three times as likely to successfully pair with a female than males that had not mated at all in mating experiments (Kendall and Wolcott 1999). Males with three or more mates in the 20 day span had a zero probability of being chosen because experiments indicated that males would not mate after three consecutive pairings (Wolcott et al. 2005, Hines et al. 2003, Kendall et al. 2002 & 2001).

Analysis

For each of the 39 scenarios, a variety of performance metrics was calculated. Sex ratio for each scenario was calculated as the surviving males divided by the surviving females on October 31 (i.e., the end of the mating season) during the second year of the simulation. Operational sex ratio for each scenario was calculated as the ratio of mature available (alive, non-molting, above sperm threshold) males divided by the maturing females on each day averaged over the two years. The mean and standard deviations of sperm produced by males,

number of mates per mature male, and sperm per female over the simulation period were calculated for each scenario. The 95% confidence intervals were calculated by the equation

$$CI = \bar{x} \pm \left(\frac{SD}{\sqrt{n}}\right) \times 1.96.$$

where $\bar{x}$ is the sample mean and *n* is the sample size. Saturation curves were fit to the relationships of both the average number of sperm per female and average number of sperm per male to male:female sex ratio using maximum likelihood estimations of the parameters. The relationships between average number of sperm per female by male to female sex ratio, operational sex ratio, and average number of mates per male were estimated using linear regressions to see if there were linear relationships between them. An ANCOVA was performed with fishing pressure scenario (the combination of F and regulations placed on the population) as the independent variable and average sperm per female as the dependent variable, while accounting for each mate choice scenario as a factor, in order to determine if there were differences in average sperm per female among scenarios. Lastly, a Tukey honest significant difference (HSD) multiple means comparison test was performed when a significant p-value was found for the ANCOVA.

Results

Average sperm per female was variable and depended on the fishing pressure and mate selection scenario (Table 1 and Figure 2). In general, females received an average of 4.8×10^8 sperm (SE 1.67×10^7). Among the three mate choice scenarios, the random mate choice had the greatest differences in average sperm per female among the fishing pressure scenarios and no fishing. For all mate choice scenarios, the only fishing pressure scenarios that differed more than 25% from the no fishing scenario were the five times fishing on all mature males only scenarios (AM5). The minimum average sperm per female was found in the size mate choice and five

times present fishing pressure on all mature males only scenario (AM5), with an average sperm count of $2.78x10^8$. The maximum was found in the random mate choice and no fishing pressure scenario (NO0), with an average sperm count of $6.21x10^8$.

The random mate scenarios, where females chose mates randomly, seemed to follow the expected pattern with average sperm per female values under all fishing pressure scenarios lower than the no fishing scenario (Figure 2). Most fishing scenarios had average sperm per female values that differed by less than 10% from the unfished scenario. The only three that differed more were in scenarios where males were fished at five times current fishing pressure (MF5: 13%, MO5: 22%, and AM5: 56%).

In the size mate scenarios, where females preferred males closer to their own size, average sperm per female values were both lower and higher than in the no fishing scenario (Figure 2). Again, the average sperm per female values were within 10% of the unfished scenario for most of the fishing scenarios. In the twice (MF2: 3%) and five (MF5: 8%) times fishing pressures on both males and females scenarios and the twice fishing pressure on females only scenario (FO2: 9%), average sperm per female was higher than in the no fishing scenario. The current fishing on both males and females (MF1: 0.7%) and five times fishing on males only (MO5: <0.01%) were less than 1% different than the unfished scenario. The scenario where all mature males were fished at five times current fishing (AM5) resulted in a 27% lower sperm per female than the unfished scenario.

Average sperm per female was higher than in the no fishing scenario in the history mate scenarios, in which females preferred males that had mated previously (Figure 2). As with the other mate choice scenarios, average sperm per female differed by less than 10% from the unfished scenario for most fishing scenarios. Only two scenarios had decreases larger than 10%

in average sperm per female relative to the unfished scenario: five times fishing pressure on males only (MO5: 11% lower) and five times fishing on all mature males only (AM5: 46% lower).

All scenarios had 100% of mature females finding mates, except for the three scenarios that had five times fishing pressure on all mature males only (AM5); 15-25% of females in the AM5 scenarios did not find mates and received no sperm.

Average sperm produced by males was fairly consistent across scenarios (Figure 3). Males stored an average of 2.08×10^9 sperm with an SE of 9.04×10^6 (Table1). The minimum average sperm per male was found in the history mate choice and five times fishing pressure on males only scenario (MO5), with an average sperm count of 1.93×10^9. The maximum was found in the history mate choice and twice present fishing pressure on females only scenario (FO2), with an average sperm count of 2.16×10^9. Across mate choice scenarios, average sperm per male only decreased noticeably when males were fished at five times current fishing pressure and there was a moratorium on females (MO5, AM5). But even then, it was never less than 8% of the no fishing pressure scenario for the same mate preference strategy.

Both the sex ratio of mature males to females on the end of the mating season in the second year and the operational sex ratio were variable, but were not affected by mate preference scenarios (Figure 4&5). This is to be expected because the fishing scenario was the primary driver of the sex ratio. Mature sex ratios (male:female) had a mean of 1.36 (SE = 0.22), minimum of 0.06, and maximum of 5.52. Sex ratios under most of the other scenarios ranged from 1.5 to 0.5 with a few exceptions and followed expected patterns from the fishing scenarios. Operational sex ratios (male:female) had a mean of a 143.89 (SE = 14.72), a minimum of 10.70,

and a maximum of 315.46. Operational sex ratios ranged considerably throughout the scenarios but followed expected patterns with the fishing scenarios.

The mean number of mates per male was variable and depended on mate preference scenario (Figure 6). The mean number of mates per male was 0.22 with an SE of 0.003 (Table 1). In the random mate preference scenario, almost all scenarios were less than 5% different than the unfished scenario, except for when females were fished at two or five times current fishing (MF2: 10%, MF5: 19%, FO2: 13%, FO5: 14%) or all mature males were fished (AM1: 10%, AM5: 23%), which resulted in lower mates per male. The size mate preference scenarios showed that all except for the current fishing pressure on males only (MO1: 4%) were between 10-20% lower than the unfished scenario. However, the size mate preference scenarios had very wide and overlapping standard errors per scenario. The history mate preference scenarios were all less than 10% lower than the unfished scenario, except for the five times fishing pressure on all mature males only and the five times current regulations on males only scenarios (AM5: 26% lower, MO5: 5% higher). The minimum mean number of mates per male was seen in the history mate preference scenario and five times fishing pressure on all mature males only at 0.17 mates per male. The maximum was found in the random mate choice and current fishing on males only scenario (MO1), with a number of 0.25 mates per male.

The average number of sperm per female was not linearly related to the male:female sex ratio at the end of the spawning season in the second year (R^2=0.03; p=0.16; Figure 7). The average number of sperm per female and average number of sperm per male were best fit to male:female sex ratio with saturation curves

$$S = \frac{S_{max} \times R}{B + R}$$

Where S is the average number of sperm, S_{max} is the maximum number of sperm, R is the male to female sex ratio, and B is the male to female sex ratio where sperm is at half of S_{max}. The average number of sperm per female plotted against male to female sex ratio had an S_{max}, or maximum number of sperm received, of 5.23×10^8 and a B of 0.06 (Figure 7). The average number of sperm per male plotted against male to female sex ratio had an S_{max}, or maximum number of sperm produced, of 2.11×10^9 and a B of 0.007 (Figure 8). There was also a positive linear relationship between the average number of sperm per female and the average number of mates per male in each scenario, but again the relationship did not explain much of the variation and was driven primarily by the low values of both average sperm per female and average number of mates per male in the five times fishing pressure on all mature males only scenarios for each mating preference (R^2=0.19; p=0.006; Figure 9). The average sperm per female was also not related to operational sex ratio of available mature males to maturing females and only showed a significant decrease in average sperm per female at the lowest operational sex ratios around 10:1 male:female in the he five times fishing pressure on all mature males only scenarios for each mating preference (R^2=0.07; p=0.06; Figure 10).

Average sperm number per female was significantly different among fishing pressure scenarios and mate preference scenarios (F=17.9; df=14,24; p<0.0001; Figure 2). However, a Tukey HSD test comparing all scenarios to each other showed that only the scenario of five times fishing pressure on all mature males (AM5) had significantly lower average number of sperm per female than all other scenarios.

Discussion

We developed a model that combined the current understanding of growth, maturation, and mating to determine when sperm limitation is likely to occur in blue crabs of Chesapeake

Bay using an IBM that included multiple mate selection and fishing scenarios. We built upon the models of Bunnell and Miller (2005) and Bunnell et al. (2010) and incorporated male maturity (Jivoff 2007), number of sperm per male (Carver et al. 2005; Kendall et al. 2001), mating preferences (Kendall and Wolcott 1999; Jivoff and Hines 1998a, 1998b; Jivoff 1997a, 1997b), sperm transferred to females during mating (Wolcott et al. 2005; Kendall et al. 2002), and sperm degradation of females between mating and brood production (Chapter 1; Wolcott et al. 2005). Because average sperm numbers per female remained, for the most part, close to the unfished conditions, we conclude that sperm limitation is very hard to induce in the model population, and by extension in the wild. Fishing pressure needed to remove approximately 99% of all of the mature males in the population in order to reduce the average number of sperm per female by more than 25%, which was true regardless of mating strategy scenario.

Although some variation between the model and field observations did exist, most differences were relatively small. Female blue crabs matured between May and October, with a majority them maturing during the months of July and August. Maturation of females ceased in late November when temperatures begin to drop. This follows the expected pattern seen in Chesapeake Bay (Jivoff 2007). Less than 1% of blue crabs, of either sex, survived each two-year simulation but failed to mature. The carapace width of mature females followed a normal distribution similar to the mature females reported in the Chesapeake Bay blue crab winter dredge survey (CBWDS), with our average crabs being slightly larger than the ones from the CBWDS (Model: 166mm; CBWDS: 142mm). Average amount of sperm per male for all scenarios was less than 1% different than average counts from laboratory studies (Carver et al. 2005; Kendall et al. 2001). Average sperm per female during present fishing pressure scenarios (MF1), for both the random and history mate preference scenarios, were within 10% of observed

average sperm per female from Hines et al. (2003; $5.0x10^8$) and Wolcott et al. (2005; $5.9x10^8$). Average sperm per female during present fishing pressure scenarios (MF1) for the size mate preference scenario was about 23% lower than the average sperm per female found in Hines et al. (2003), 23% higher than Rains (Chapter 1), and 33% lower than Wolcott et al. (2005). Male to female sex ratios were within the range of those observed in Chesapeake Bay during 2011 (Rains Chapter 1). Crab survival also followed patterns expected by population dynamic equations based on the natural and fishing mortalities experienced in the scenario. In the current fishing mortality scenarios, all females found mates, which closely followed field observations where less than 2% of mature females are unfertilized at current fishing pressures (Wolcott et al. 2005, Hines et al. 2003, Kendall et al. 2002).

The size and history preference mating preference scenarios had interesting results in that some of the fishing pressure scenarios had average sperm per female above what was seen in unfished conditions. This is most likely due to favored males being removed from the population, thereby spreading mating opportunities among more males. In all scenarios where average sperm per female was above unfished conditions, regulations did not harvest males until they reached 127mm. Scenarios that allowed harvest of all mature males (>107mm), regardless of fishing pressure and mate preference scenario, had a decrease in average sperm per female compared to the unfished scenario, albeit sometimes small. This seems to indicate that current regulations, with a 127mm minimum size limit on hard shell males, provides males at least one chance to mate before being susceptible to harvest. This would give females a consistent supply of mates throughout the mating season and lead to females receiving sperm numbers larger than if they had mated with previously mated males.

Furthermore, in scenarios where males were given more than one opportunity to mate, males would cease mating when sperm reserves hit a minimum threshold. When males cease mating at low sperm numbers, a biological control is established that stops sperm limitation from occurring in a the population. Studies by Carver et al. (2005), Wolcott et al. (2005), Hines et al. (2003), and Kendall et al. (2002 & 2001) showed that after three consecutive mates, a male would cease mating. In our model we simulated this by making males ineligible for mating once their sperm reserves were below the amount an average male crab would have after mating three times. This mechanism was a reason that our scenario with five times fishing pressure on males only (MO5), where over 99% of males above 127mm died, only had decreases in average sperm per female of less than 25% of unfished conditions in all mating scenarios (Random: 22%, Size: <0.1%, History: 11%), whereas when the same scenario of five times fishing pressure on males only was applied to all mature males (AM5), average sperm per female was closer to 50% less than unfished conditions (Random: 52%, Size: 28%, History: 46%).

We used a population size of 4500 crabs in our model. The population size was chosen based on average crab densities of the Chesapeake Bay blue crab winter dredge survey in 2010. We assumed that crabs within a two-hectare area could reasonably interact with one another during a 1-2 day period. Population size could affect our results if the area of interaction for blue crabs is substantially larger or smaller than what we included in the model. Little is known about the physical and chemical cues associated with blue crab mating and so the size of the area in which males will respond to pre-pubertal females is uncertain. However, female blue crabs are expected to release hormones for several days before their terminal molt, which we assume gives our entire population of males ample time within our two-hectare area to acquire the signal and reach the female before she molts (Jivoff et al. 2007; Shirley et al. 1990).

Sex ratio of males to females at the end of the second mating season, the mating season in which about 66% of the females would mature, was not significantly related to the average sperm number per female. Our population did not have incoming cohorts for the second year, making it difficult to predict if the lack of relationship is a property of the model. However, over 75% of females matured by the middle of the mating season in the second year, which is the time that an incoming cohort would have grown to sizes ready to mature. Since the incoming cohort of the second year only overlaps with a small portion of our maturing cohort, this should decrease the likelihood of sperm limitation since most of our cohort's females would have received sperm, making the exclusion of the next cohort less important for fertilization purposes. We believe the reason for a lack of relationship between sex ratio and average sperm per female is because females only mate once and mature asynchronously, skewing the operational sex ratio toward mature males, regardless of population sex ratio. This means that at any time a female is ready to mate, there is more than one male prepared to mate with her, regardless of what the population sex ratio is at that time. Other crustacean species are known to have operational sex ratios skewed toward males (e.g., Rondeau and Sainte-Marie 2001), and, according to our calculated operational sex ratios for each scenario, blue crab also follows this pattern.

Our results differ from those of several studies that have suggest that sperm limitation is a concern for blue crabs in Chesapeake Bay. Hines et al. (2003) also used the metric of sperm received per female to compare field collected female blue crabs of the heavily fished Chesapeake Bay and the less fished Indian River Lagoon, FL. Hines et al.'s results showed that females in Indian River Lagoon had a much higher average sperm received per female than Chesapeake Bay (Chesapeake Bay: 5.0×10^8, Indian River Lagoon: 1.2×10^9) and concluded that this showed females in Chesapeake Bay were receiving half of those in a less fished population.

However, the timing of crab collection relative to mating is an important variable when comparing sperm per female because sperm degradation of up to 50% occurs between insemination and first spawning event (Rains Chapter 1; Wolcott et al. 2005). Also, due to the differences in mating seasons between Chesapeake Bay (summer and early fall) and the almost year round season of Indian River Lagoon, FL, females may have mated more recently in the Florida site, causing less degradation to occur. The results of Hines et al. (2003) do not include corrections for sperm degradation or differences in the mating seasons between the two locations. Not taking into account sperm degradation or location differences could be a reason the average sperm per female is different between the two sites.

Studies have also used comparisons between amounts of sperm female received in laboratory matings to those in field observations to examine whether sperm limitation was an issue. Males not given sufficient time to recover between matings, gave roughly 50% less sperm to their following mates (Kendall et al. 2002). Kendall et al. compared their laboratory data on sperm numbers received per female (Fully-recovered: $3.35x10^9$, Depleted: $9.31x10^8$) with numbers seen in field collected females of Rhode River, MD, to conclude that most females within the tributary were receiving amounts of sperm closer to that of laboratory females mated with depleted males (Field average: approx. $9.0x10^8$). However, their sperm numbers per female are substantially higher than other laboratory and field studies from the same region (Carver et al. 2005, Wolcott et al. 2005, Kendall et al. 2001), and suggest that females are, in fact, receiving comparable amount of sperm to recovered males (Fully recovered: $1.2x10^9$; Carver et al. 2005).

Compared to previous studies on Chesapeake Bay blue crab that have suggested sperm limitation, we feel our study uses a more direct approach. Previous studies have compared average sperm per female in both laboratory settings and lightly fished field populations to

average sperm per female in field collected blue crabs of Chesapeake Bay in order to address the concern of sperm limitation (Hines et al. 2003; Kendall et al. 2002). While field and lab studies have their merits, they cannot evaluate a similar population over multiple fishing scenarios, or compare them to the same population in unfished conditions. Our simulation study, which is still indirect, is closer to this ideal situation. Our metric of evaluating different fishing pressure scenarios, under a variety of assumed mating strategies, evaluates the sperm output of a population in direct comparison with the same population in an ideal unfished condition.

Frequent mating by males has been the primary mechanism suggested for sperm limitation in blue crabs, where average sperm per female decreases when males are required to mate more often (Hines et al. 2003, Kendall et al. 2002). We did not see evidence of increased mating frequency with increased fishing mortality in our model. Additionally, the model predicted a positive relationship between average number of mates per male and average sperm per female (Figure 7), which is the opposite relationship of what other studies have suggested should happen if sperm limitation due to males mating more frequently were occurring (i.e. there would be a negative relationship between average number of mates per male and average sperm per female). Reductions in sperm per female in our study were due to a different type of sperm limitation, where females are not able to find mates, and was driven by the five times fishing pressure on all mature males only (AM5) scenarios within each mate preference scenario. The positive relationship between average number of mates per male and average sperm per female provides evidence that sperm limitation only happens when mates are unavailable in the models. To continue, the only scenarios in which average sperm per female was substantially reduced were the five times fishing pressure on all mature males (AM5). In these scenarios the reduction in average sperm per female was caused by females not finding mates rather than because mates

that were found had low sperm reserves. This is also true of our fitted equation for average sperm per female by mature male:female sex ratio, which shows that it takes sex ratios well below 0.06 (or 3 males for every 50 females) in order to reduce the sperm numbers females receive to half of the maximum sperm, or what Kendall et al. (2002) had predicted a male's second consecutive mate would receive (Figure 7).

An assumption of our analyses is that blue crabs are not sperm limited in unfished conditions. In other words, our comparisons assume that blue crabs are not naturally sperm limited. We base this assumption on theoretical literature about the physiological characteristics of a sperm limited population. The sex with the limiting gamete will allocate more resources to its production than the sex with the non-limiting gamete (Levitan and Petersen 1995). In blue crab reproduction, females allocate disproportionately more of their internal cavity and energy resources to the storage of sperm and creation of eggs, which would theoretically have evolved because eggs are the limiting factor in reproduction (Jivoff 2007). Other factors usually associated with egg limited populations are internal fertilization, male-male competition for fertilizable females, and high degrees of sexual dimorphism in the population (Levitan and Petersen 1995). Blue crabs exhibit all of these attributes, which suggests that blue crabs are not expected to be sperm limited under unfished conditions.

Our model suggests that it should be very difficult to induce sperm limitation in blue crabs and that the sex ratio of the population, at any single point in time, is likely not a good indicator of fertilization success for the population. Our results suggest that female blue crabs in Chesapeake Bay are not currently receiving significantly less sperm than they would in a moratorium scenario, which indicates that sperm limitation is not an issue at present. Additionally, current regulations of Maryland that protects mature males under 127mm likely

have a beneficial effect of maintaining a pool of available males for mating. Assuming that the population is not sperm limited in unfished conditions means that, as long as mature males are available, sperm limitation will not likely occur. We conclude that current regulations of Chesapeake Bay appear to be effective at avoiding sperm limitation.

Tables and Figures

Table 3.1. Results from all 39 simulations, grouped by mating strategy scenario showing different fishing pressure scenarios, separated by regulations and fishing mortality of each gender, and the associated statistics calculated for each.

	ID	Fishing Regulations	Fishing Mortality	Male: F	Female: F	Sex Ratio	Operational Sex Ratio	Mean sperm: Male	Mean Mate #/Male	# of Males that Mated	Mean sperm: Female	SD sperm: Female	# of Matured Females	# of Unfertilized Mature Females
Random	NO0	No Fishing	0	0	0	1.12	235.72	2.11E+09	0.23	363	6.21E+08	3.32E+08	585	0
	MF1	Current	Present	1.05	1.05	1.07	146.29	2.13E+09	0.22	301	5.59E+08	3.10E+08	550	0
	MF2	Current	2x Present	2.1	2.1	0.80	81.73	2.08E+09	0.21	276	5.96E+08	4.01E+08	525	0
	MF5	Current	5x Present	5.25	5.25	0.99	35.04	2.08E+09	0.19	219	5.42E+08	3.32E+08	476	0
	MO1	Current on Males Only	Present	1.05	0	0.65	147.95	2.12E+09	0.25	336	6.17E+08	3.43E+08	620	0
	MO2	Current on Males Only	2x Present	2.1	0	0.43	91.27	2.09E+09	0.25	326	5.74E+08	3.38E+08	614	0
	MO5	Current on Males Only	5x Present	5.25	0	0.34	37.90	1.98E+09	0.23	249	4.79E+08	3.34E+08	579	0
	FO1	Current on Females Only	Present	0	1.05	2.08	234.63	2.10E+09	0.23	372	5.55E+08	2.88E+08	586	0
	FO2	Current on Females Only	2x Present	0	2.1	2.95	257.45	2.09E+09	0.20	350	5.74E+08	2.77E+08	508	0
	FO5	Current on Females Only	5x Present	0	5.25	5.53	315.46	2.09E+09	0.20	355	5.62E+08	3.13E+08	499	0
	YR1	Current on Females/Current on Males but open all year	Present	1.05	1.05	1.04	117.09	2.09E+09	0.23	327	5.73E+08	3.12E+08	565	0
	AM1	Current on Females/All Mature Males	Present	1.05	1.05	0.84	124.77	2.10E+09	0.21	299	5.58E+08	3.03E+08	529	0
	AM5	No Female Fishing/All Mature Males	5x Present	5.25	0	0.09	10.70	1.97E+09	0.18	131	2.93E+08	3.61E+08	600	150
Size	NO0	No Fishing	0	0	0	1.17	291.91	2.12E+09	0.24	145	3.86E+08	3.31E+08	607	0
	MF1	Current	Present	1.05	1.05	0.85	148.17	2.09E+09	0.20	141	3.83E+08	3.33E+08	489	0
	MF2	Current	2x Present	2.1	2.1	0.82	102.36	2.11E+09	0.20	157	3.99E+08	3.43E+08	489	0
	MF5	Current	5x Present	5.25	5.25	1.68	54.88	2.02E+09	0.19	158	4.17E+08	3.58E+08	472	0
	MO1	Current on Males Only	Present	1.05	0	0.65	139.50	2.03E+09	0.23	158	3.47E+08	2.80E+08	583	0
	MO2	Current on Males Only	2x Present	2.1	0	0.46	76.11	2.06E+09	0.21	178	3.74E+08	3.03E+08	534	0
	MO5	Current on Males Only	5x Present	5.25	0	0.21	33.96	2.01E+09	0.22	175	3.86E+08	3.36E+08	544	0
	FO1	Current on Females Only	Present	0	1.05	1.90	253.52	2.10E+09	0.22	141	3.68E+08	2.98E+08	538	0
	FO2	Current on Females Only	2x Present	0	2.1	2.05	248.12	2.11E+09	0.20	144	4.21E+08	3.23E+08	512	0
	FO5	Current on Females Only	5x Present	0	5.25	5.47	270.58	2.13E+09	0.21	136	3.71E+08	2.93E+08	525	0
	YR1	Current on Females/Current on Males but open all year	Present	1.05	1.05	0.93	119.82	2.13E+09	0.22	146	3.70E+08	3.48E+08	540	0
	AM1	Current on Females/All Mature Males	Present	1.05	1.05	0.93	141.49	2.13E+09	0.22	154	3.51E+08	2.90E+08	549	0
	AM5	No Female Fishing/All Mature Males	5x Present	5.25	0	0.06	14.00	1.97E+09	0.20	122	2.78E+08	3.21E+08	593	97
History	NO0	No Fishing	0	0	0	1.21	259.57	2.12E+09	0.23	352	5.29E+08	3.29E+08	581	0
	MF1	Current	Present	1.05	1.05	1.09	158.97	2.10E+09	0.22	293	5.27E+08	3.21E+08	539	0
	MF2	Current	2x Present	2.1	2.1	0.89	93.48	2.12E+09	0.23	281	5.50E+08	3.52E+08	582	0
	MF5	Current	5x Present	5.25	5.25	1.13	52.14	2.10E+09	0.19	209	5.36E+08	3.96E+08	464	0
	MO1	Current on Males Only	Present	1.05	0	0.66	135.36	2.06E+09	0.23	323	5.40E+08	3.06E+08	569	0
	MO2	Current on Males Only	2x Present	2.1	0	0.46	83.68	2.04E+09	0.24	275	5.68E+08	3.58E+08	605	0
	MO5	Current on Males Only	5x Present	5.25	0	0.15	35.13	1.93E+09	0.23	225	4.72E+08	3.33E+08	580	0
	FO1	Current on Females Only	Present	0	1.05	2.04	275.27	2.11E+09	0.23	377	5.63E+08	3.30E+08	580	0
	FO2	Current on Females Only	2x Present	0	2.1	3.01	254.48	2.16E+09	0.21	355	5.93E+08	3.57E+08	529	0
	FO5	Current on Females Only	5x Present	0	5.25	5.32	286.42	2.10E+09	0.21	335	5.83E+08	3.40E+08	528	0
	YM1	Current on Females/Current on Males but open all year	Present	1.05	1.05	1.01	132.21	2.08E+09	0.22	308	5.91E+08	3.92E+08	558	0
	AM1	Current on Females/All Mature Males	Present	1.05	1.05	0.92	100.78	2.10E+09	0.22	250	5.26E+08	2.98E+08	551	0
	AM5	No Female Fishing/All Mature Males	5x Present	5.25	0	0.11	13.81	1.94E+09	0.17	115	2.83E+08	3.05E+08	508	79

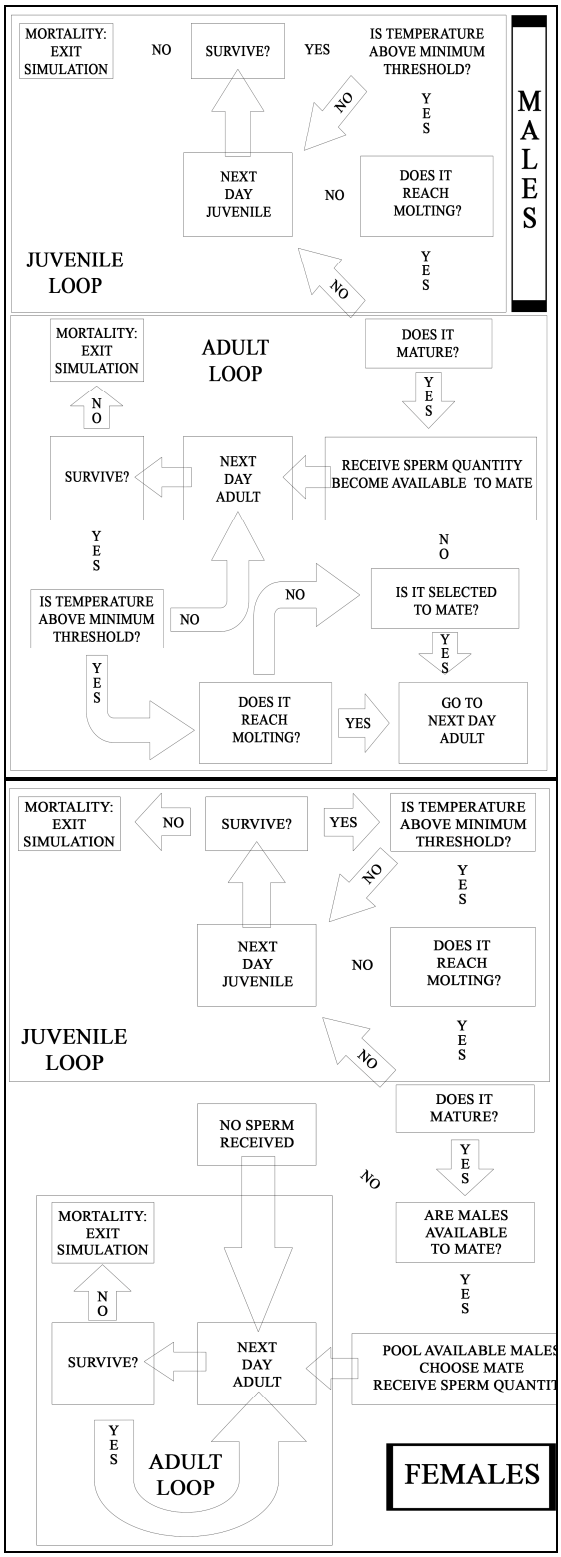

Figure 3.1. Conceptual diagrams of the individual based model simulation process separated by gender.

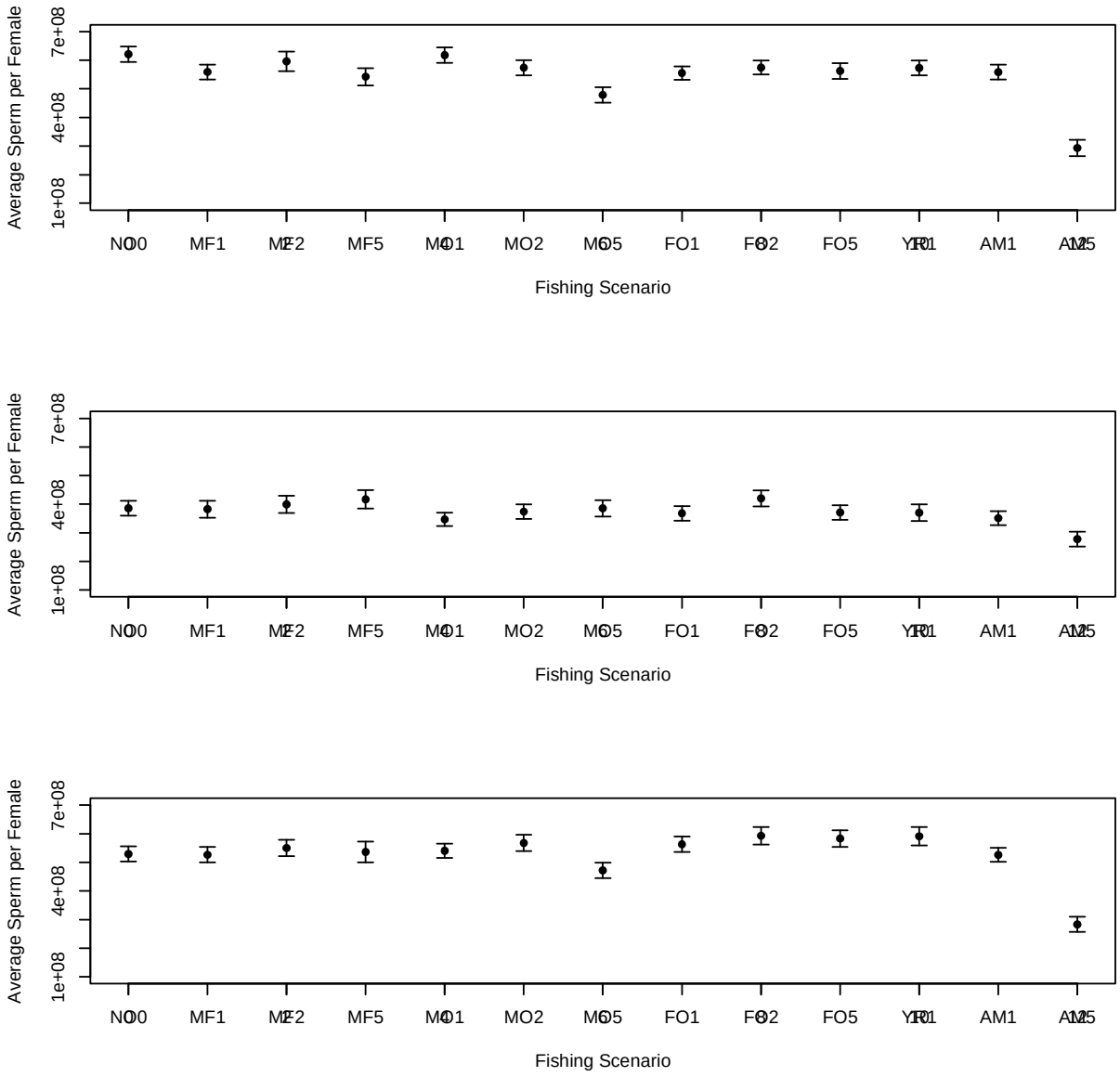

Figure 3.2. Average number of sperm per female for each fishing scenario, separated by mate choice scenario (Top panel: Random, Middle panel: Size, Bottom panel: History). Definitions of the fishing scenarios are in Table 1. Dots are the mean value in each scenario and whiskers are the 95% confidence intervals.

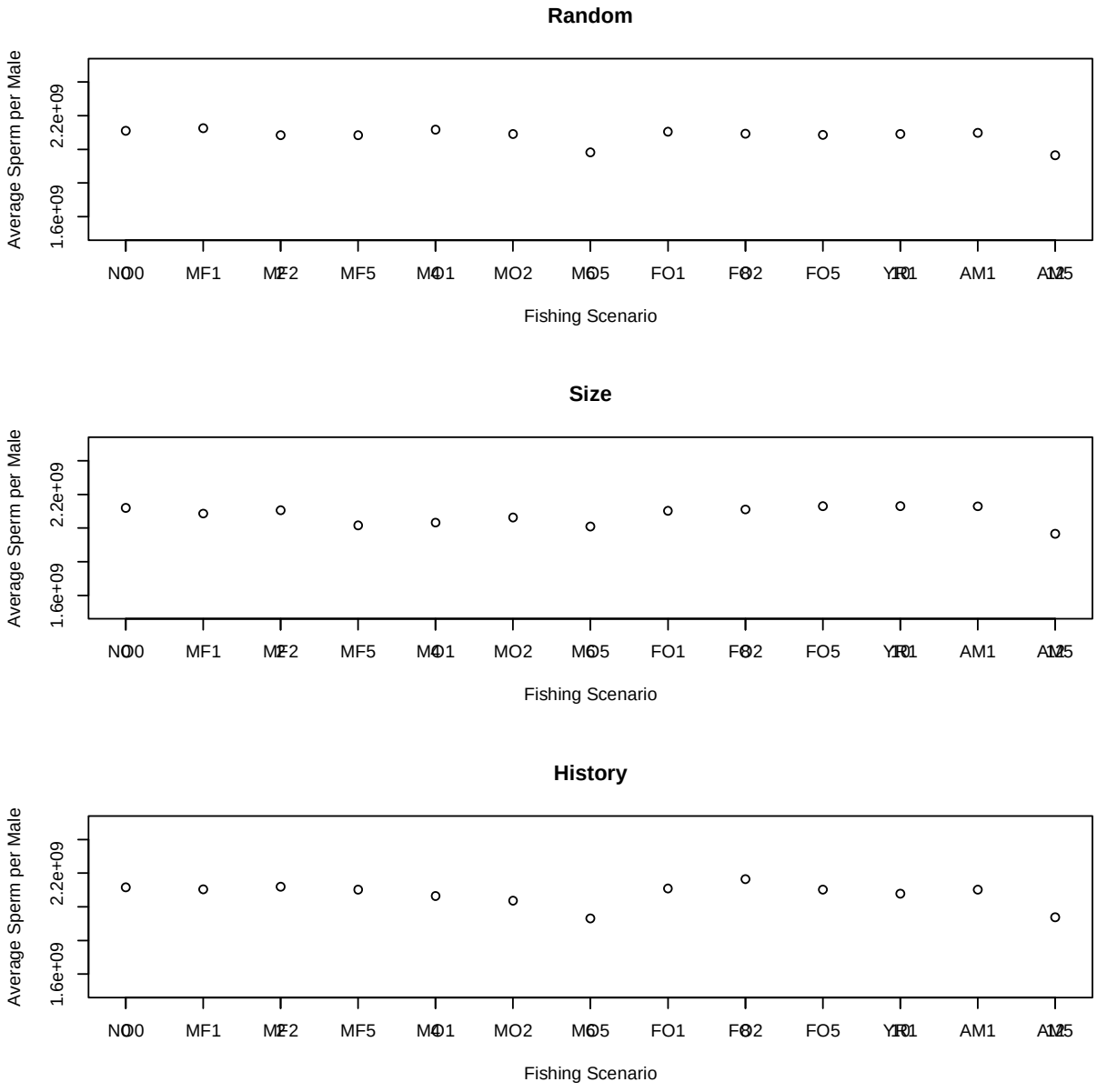

Figure 3.3. Average number of sperm per male for each fishing scenario, calculated as the geometric mean and separated by mate choice scenario.

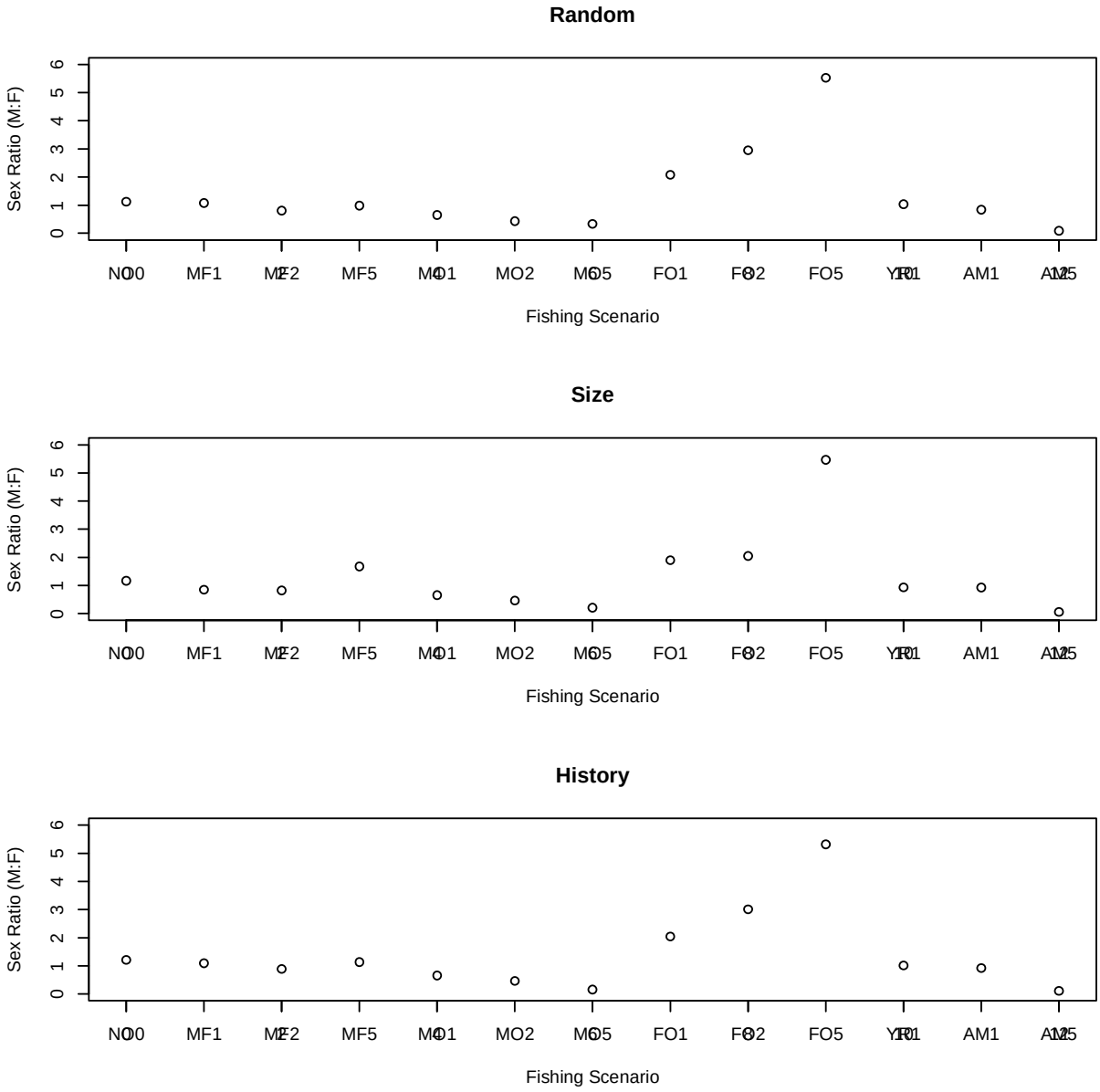

Figure 3.4. Male to female sex ratio at the end of the second year mating season for each fishing scenario, separated by mate choice scenario.

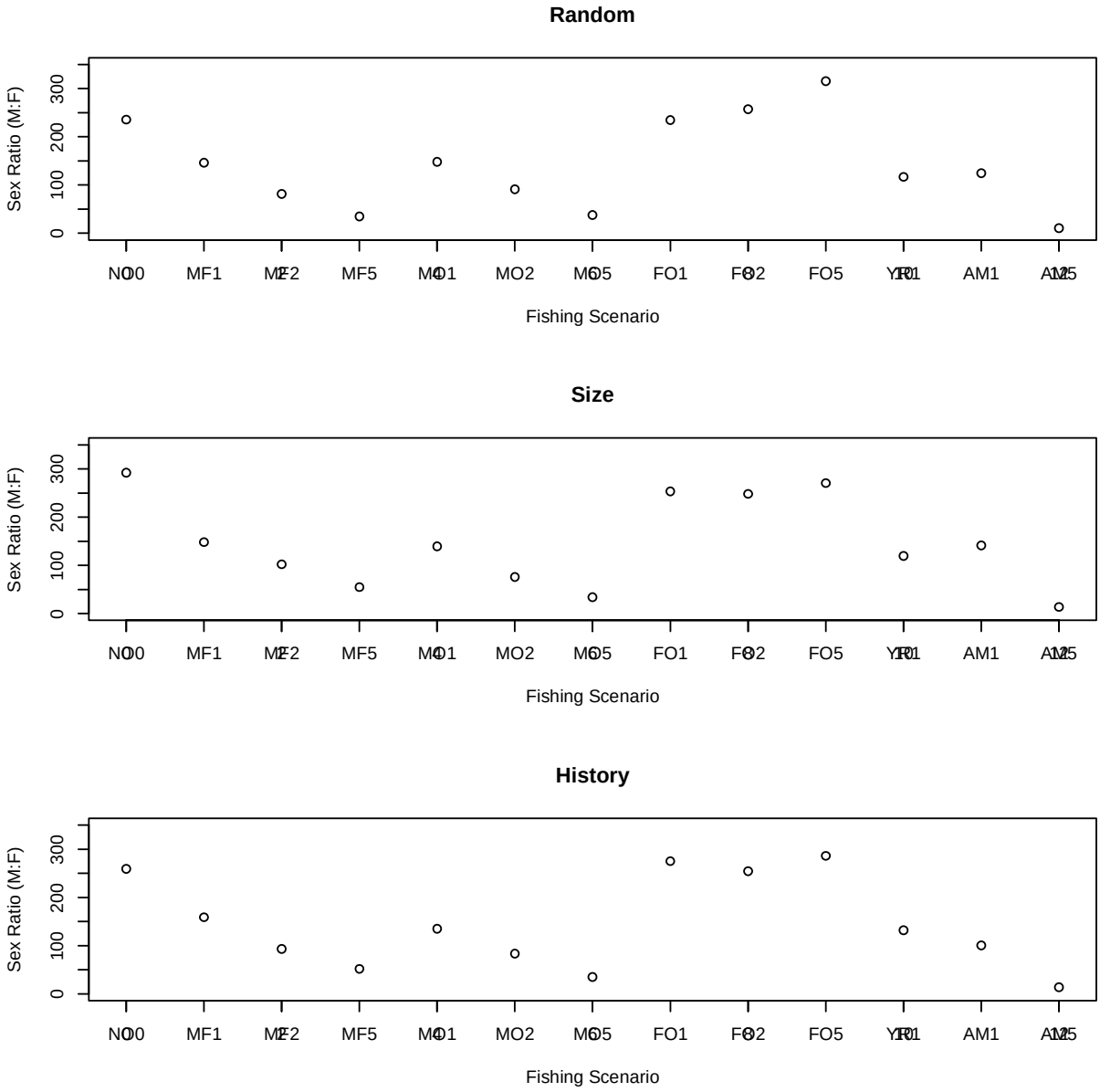

Figure 3.5. Average operational male to female sex ratio for each fishing scenario, separated by mate choice scenario.

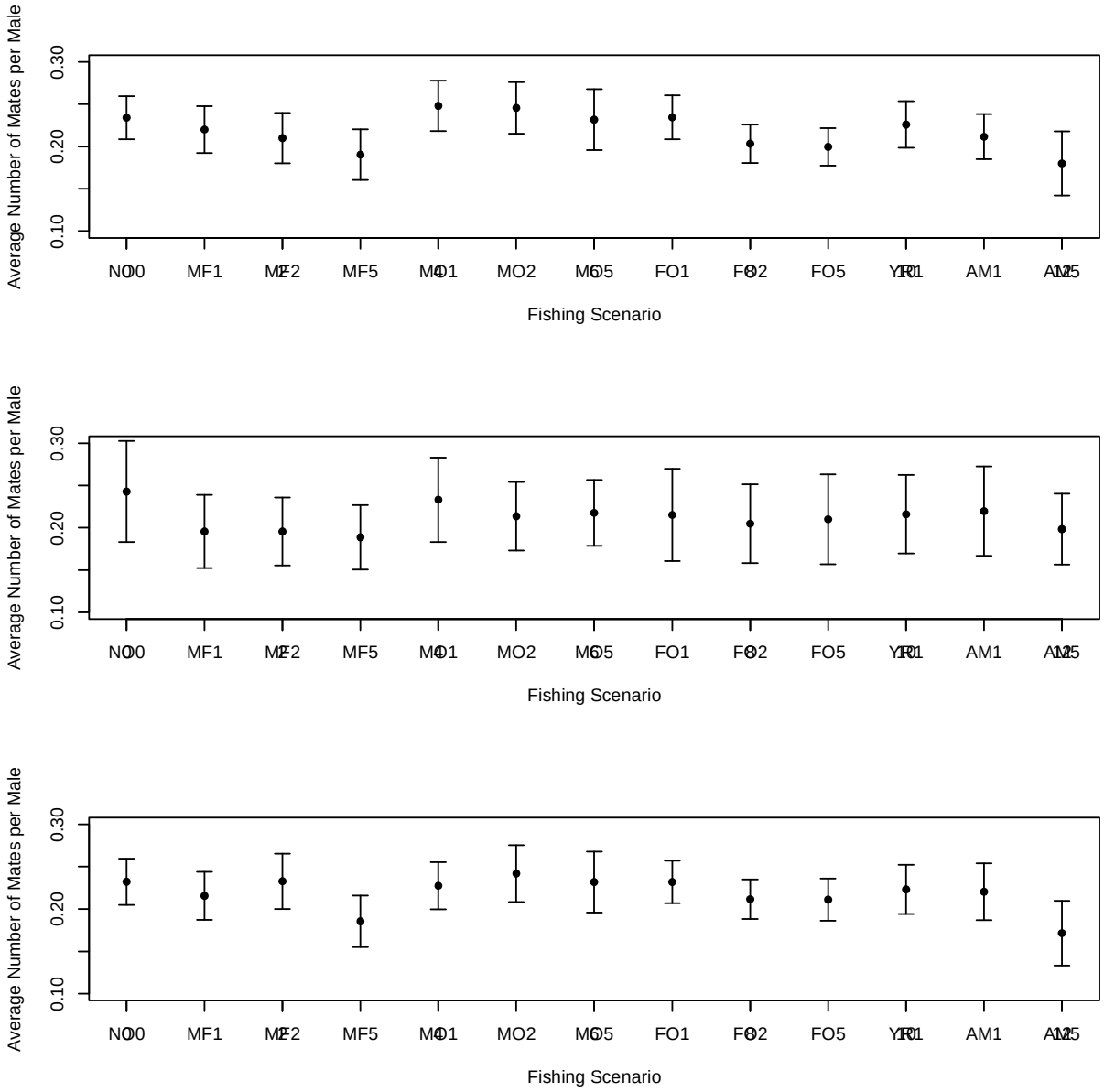

Figure 3.6. Average number of mates per male for each fishing scenario, separated by mate choice scenario (Top panel: Random, Middle panel: Size, Bottom panel: History). Dots are the mean value in each scenario and whiskers are the 95% confidence intervals.

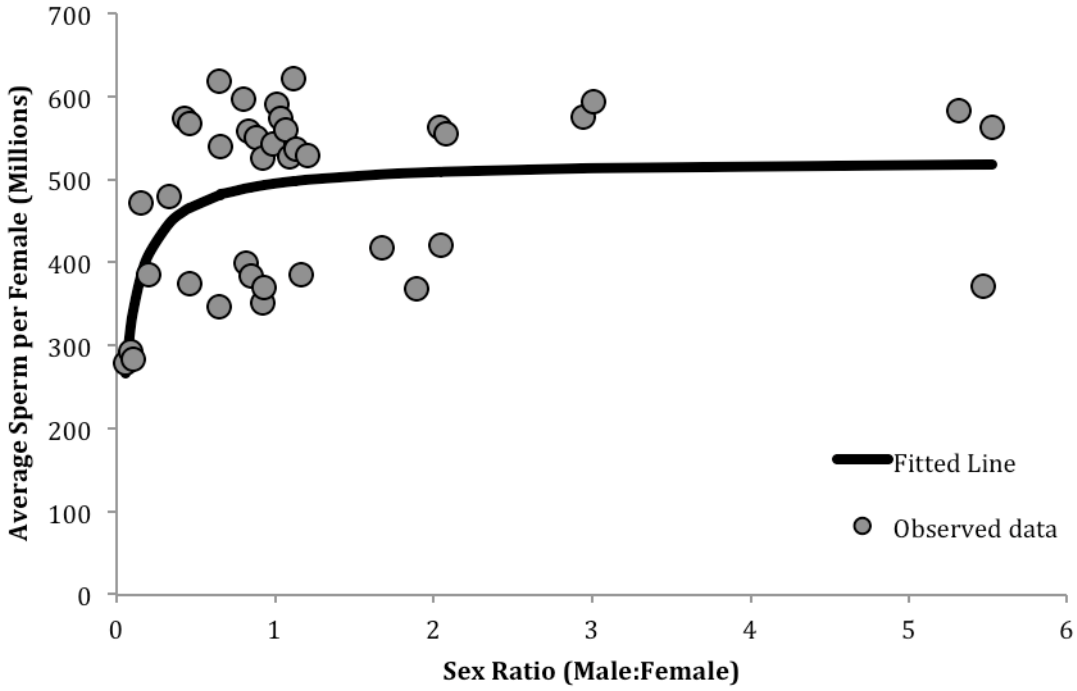

Figure 3.7. Average number of sperm per female by male to female sex ratio with fitted non-linear regression line.

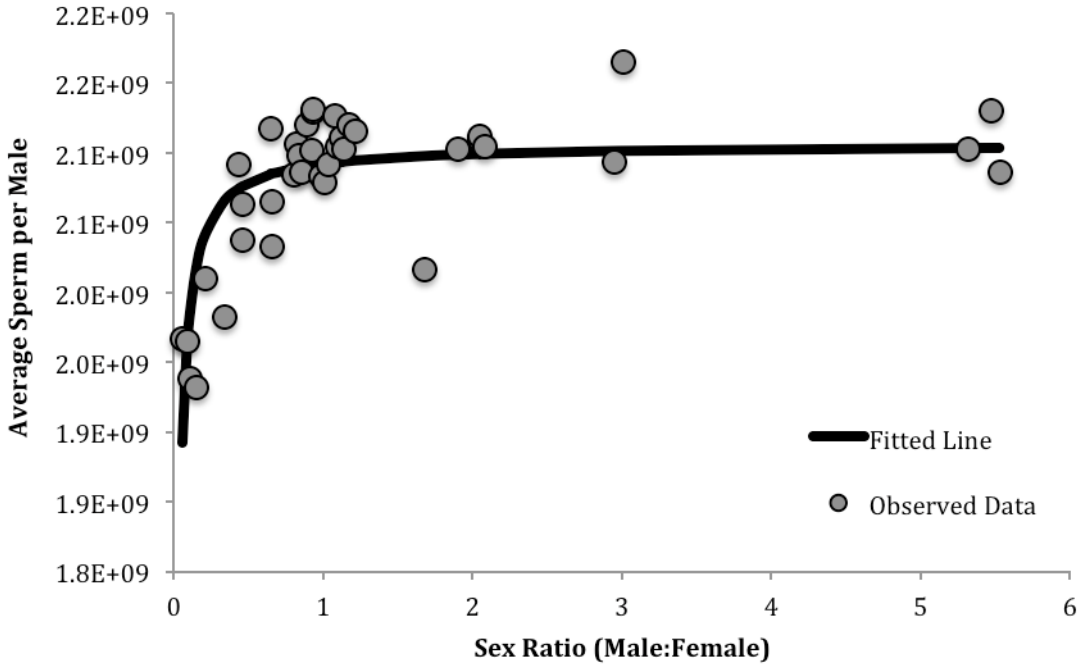

Figure 3.8. Average number of sperm per male by male:female sex ratio with fitted non-linear regression line.

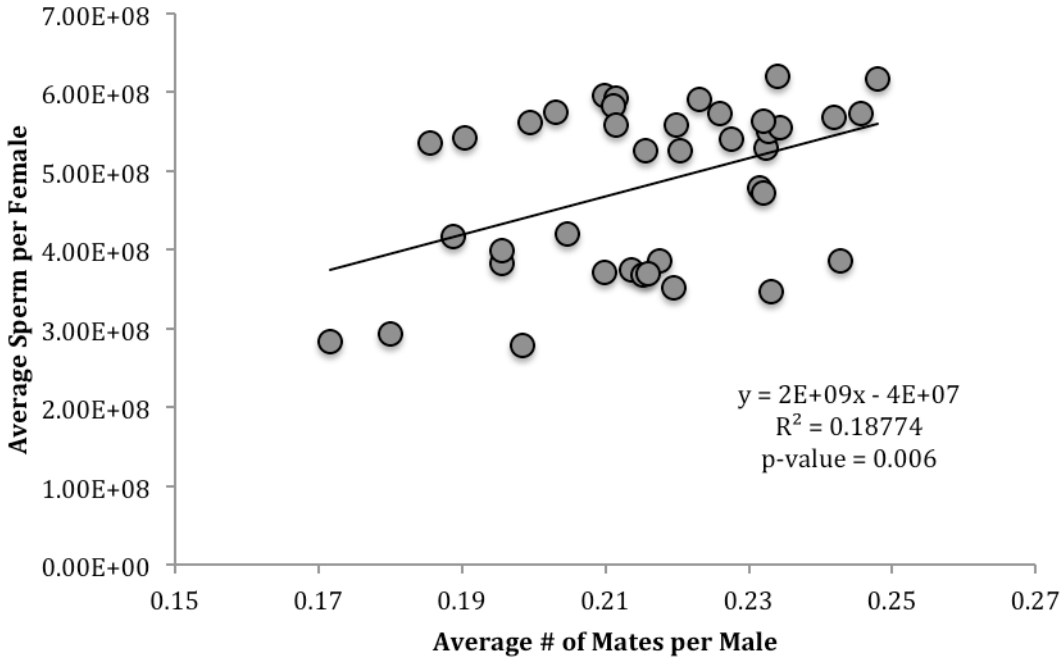

Figure 3.9. Average number of sperm per female by the average number of mates per male (F=8.552; df=1,37; p-value= 0.006).

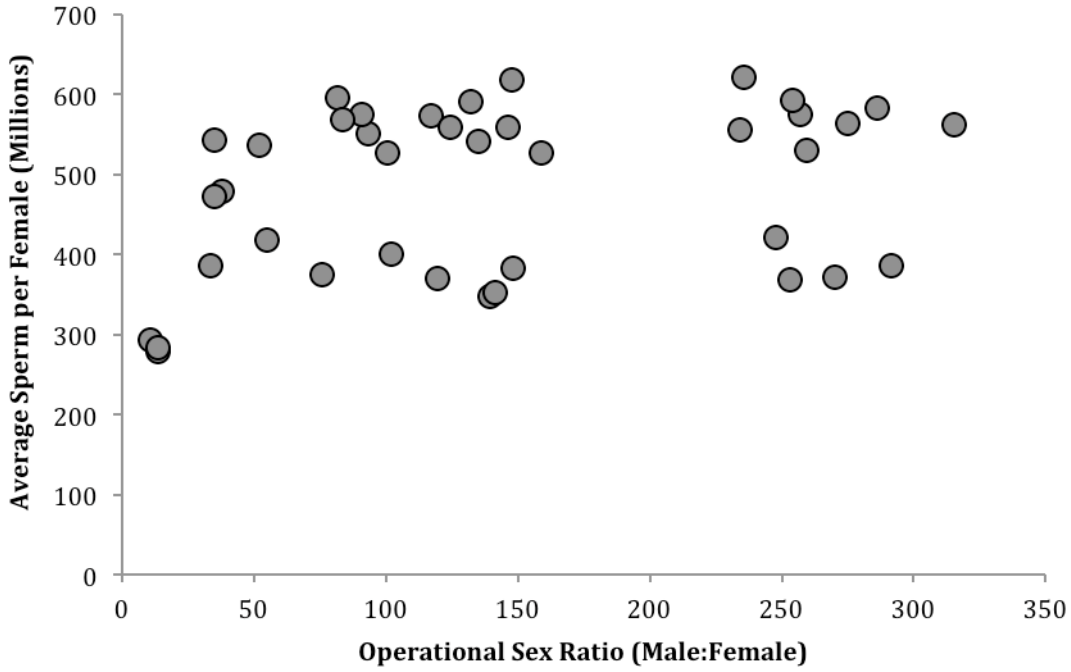

Figure 3.10. Average number of sperm per female by the operational male to female sex ratio.

Appendices

Appendix I: Total sperm count per female field data for Chapter 1. The latitude and mature male to female sex ratio are given for each tributary. For each female blue crab the month it was collected, carapace width, total sperm count, and percent fullness of the spermathecae are recorded.

River	Latitude	M:F Sex Ratio	ID	Month	Carapace Width	Total sperm/female	% Fullness
Chester	39.04	3.70	1	September	161	359159500	0
			2	September	141	430493000	75
			3	September	165	1682100000	0
			4	September	147	412114500	0
			5	September	155	360872750	0
			6	September	151	207147500	10
			7	October	146	549486000	60
			8	October	159	2521203125	20
			9	October	168	156450875	100
			10	October	171	141927187.5	10
			11	October	138	190015000	50
			12	October	149	156061500	25
			13	October	160	406273875	50
			14	October	135	215012875	10
			15	October	174	298105500	10
			16	October	168	390309500	20
			17	October	155	165172875	0
			18	October	145	102483500	75
			19	October	139	5046300000	100
			20	October	191	450506875	10
			21	October	144	813015000	100

River	Latitude	M:F Sex Ratio	ID	Month	Carapice Width	Total sperm/female	% Fullness
Choptank	38.64	0.66	1	October	146	277546500	20
			2	October	146	347439312.5	0
			3	October	155	334940375	10
			4	October	157	405183625	0
			5	October	150	133711375	25
			6	October	148	1806700000	0
			7	September	146	91113750	0
			8	September	153	287942812.5	10
			9	September	157	487186000	35
			10	September	142	461175750	75
			11	September	142	543178125	75
			12	September	141	264152000	45
			13	September	145	1324653750	100
			14	September	158	1272477500	100
			15	October	143	461993437.5	100
			16	October	162	215869500	30
			17	October	168	686546000	80
			18	September	145	276689875	0
			19	September	157	1165321500	75
			20	September	141	385091875	0
			21	October	150	246123937.5	0
Patuxent	38.31	0.98	1	October	139	323843187.5	35
			2	October	140	179891250	0
			3	November	144	322402500	75
			4	November	143	1131913125	100
			5	October	101	234676312.5	0
			6	October	152	197919312.5	10
			7	October	161	1490527.50	0
			8	October	157	175530250	10
			9	October	141	160266750	75
			10	October	136	404171250	0
			11	November	126	305815125	0
			12	November	137	179190375	50
			13	November	151	464446500	25
			14	November	127	171714375	10
			15	October	134	466860625	0
			16	October	141	263100687.5	40
			17	October	147	1085772188	100
			18	November	146	870486750	75
			19	November	157	142199750	10
			20	November	152	219140250	25
			21	November	144	925155000	0

River	Latitude	M:F Sex Ratio	ID	Month	Carapice Width	Total sperm/female	% Fullness
Potomac	37.99	3.48	1	October	169	3017266875	0
			2	October	160	221165000	10
			3	October	158	444899875	25
			4	October	160	527330562.5	25
			5	October	171	562452187.5	0
			6	October	173	4053783125	25
			7	November	171	335563375	80
			8	November	159	166652500	60
			9	November	164	1063928250	100
			10	November	152	342650000	25
			11	November	161	1187282250	100
			12	November	161	383768000	0
			13	November	127	364455000	60
			14	November	161	751961000	75
			15	October	164	727664000	100
			16	October	159	234598437.5	20
			17	October	177	149909375	0
			18	October	160	111361250	10
			19	October	158	298105500	10
			20	October	167	1002445938	75
			21	October	153	486563000	0
York	37.24	0.94	1	November	125	214857125	50
			2	November	134	302816937.5	0
			3	November	132	514130750	50
			4	November	145	191338875	0
			5	November	165	428740812.5	0
			6	November	155	246941625	0
			7	November	152	1062215000	0
			8	November	129	154542937.5	0
			9	November	133	213299625	0
			10	November	126	162758750	0
			11	November	149	181604500	0
			12	November	138	258700750	0
			13	November	151	241568250	0
			14	November	133	198737000	10
			15	November	136	370062000	0
			16	November	135	263412187.5	0
			17	November	136	619923937.5	90
			18	November	147	252704375	0
			19	November	147	954942187.5	90
			20	November	157	201501562.5	0
			21	November	145	144769625	0

River	Latitude	M:F Sex Ratio	ID	Month	Carapice Width	Total sperm/female	% Fullness
James	36.99	1.45	1	November	142	2019229875	10
			2	November	148	2927771062.5	0
			3	November	151	2675006625	50
			4	November	142	1152939375	0
			5	November	131	701653750	100
			6	November	142	683586750	0
			7	November	140	404327000	0
			8	November	132	1643551875	0
			9	November	142	235182500	0
			10	November	155	1781780000	10
			11	November	155	1653286250	0
			12	November	133	1302070000	0
			13	November	150	1093365000	25
			14	November	132	1701568750	0
			15	November	116	2466690625	25
			16	November	145	2559361875	0
			17	November	151	2638405000	0
			18	November	135	2621272500	10
			19	November	145	1781780000	0
			20	November	151	1343343750	0
			21	November	139	908022500	0

Appendix II: The Individual Based Model Simulation code for R.

```
setwd("/location")
options(scipen=999)#no scientific notation

## parameters ##
ID <- 0 #scenario number
day <- 730 #days observed
Nf <- 2000 #Abundance Females
Nm <- 2500 #Abundance Males
M <- 0.9 #Nat mort
Fm0 <- 0 #Fishing mort males scenario: 0 fishing
Ff0 <- 0 #Fishing mort females scenario: 0 fishing
Fm1 <- 1.05 #Fishing mort males scenario
Ff1 <- 1.05 #Fishing mort females scenario
MatePref <- 1 #Mating preference scenarios 1-Random 2-Size 3-
History
#1.05 = Fishing mort: present fishing
tht <- 8.9 # temp threshold

## Functions ##
CalcZ <- function(F,s) #Calculate Z for each Fishing pressure and
sex
{
      return((M/365)+(F/s))
}

SoftZ <- function(SF,Ss) #Calculate Z for soft fishing pressure
{
      return(((2*M)/365)+(SF/Ss))
}
```

```
GPM <- function(init_cw,mu,sigma) #Calculate Growth per molt
based on size, maturity, and sex
{
      return(init_cw+(init_cw*rnorm(1,mean=mu,sd=sigma)))
}

IP <- function(cw) #Calculate Intermolt period for carapice width
{
      gamma = 69.70*(1.0149)^(cw)
      beta = (166.39*(1.0115)^(cw))-gamma
      z=runif(1,min=0,max=1)
      return(gamma-beta*log(1-z))
}

MateProb <- function(pref,female_cw,male_cw,mate_num)
#calculating relative probability for each male based on mate
preference scenario
{
      if (pref==1)
      {
            return(1)
      }
      if (pref==2) #if size based preference
      {
            mprob=73.33+(0.255*male_cw) #linear relationship
between male and female carapice width, based on male carapice
width
            var=72.2 #rate at which probability decreases
            return(exp(-(female_cw-mprob)^2/(2*var)))
      }
      if (pref==3) #if mate history preference
      {
            mprob=mate_num+1 #set relative probability at mates
plus 1
            for(i in 1:length(mate_num))
            {
                  if(mate_num[i]>=3) {mprob[i]=0} #if more than 3,
no probability (needs to rest)
            }
            return(mprob)
      }
}

## Matrices ##
t <- scan("PaxRiverTemps.txt") #temperatures
Fm <- array(NA, c(1,day))  #set up matrix of days for Fishing
pressure by season for males
Ff <- array(NA, c(1,day))  #set up matrix of days for Fishing
pressure by season for females
for (i in 1:90) #males
{
      Fm[i]=Fm0
}
for (i in 91:349)
{
      Fm[i]=Fm1
}
for (i in 350:455)
{
      Fm[i]=Fm0
}
```

```
for (i in 456:714)
{
      Fm[i]=Fm1
}
for (i in 715:730)
{
      Fm[i]=Fm0
}

for (i in 1:90) #females
{
      Ff[i]=Ff0
}
for (i in 91:296)
{
      Ff[i]=Ff1
}
for (i in 297:455)
{
      Ff[i]=Ff0
}
for (i in 456:661)
{
      Ff[i]=Ff1
}
for (i in 662:730)
{
      Ff[i]=Ff0
}

m <- array(NA, c(Nm,day,12)) #males (1=carapice,
width,2=live/dead,3=DegreeDays,4=Maturity,5=ShellStatus,6=Soft/sp
erm/mateDays,7=IPvalue,8=Sperm, 9=MaxSperm, 10=#ofMates, 11=Crab
ID, 12=mating relative probability)
for (r in 1:Nm) #set male carapice width by cohort (from WDS
data)
{
      x<-runif(1,min=0,max=1)
      if (x<0.69) #if random number is less than 70 percent crab
is from new cohort
      {
            m[r,1,1] <- rlnorm(1,meanlog=2.6,sdlog=1.0)
            if (m[r,1,1]>70||m[r,1,1]<10.0) #constrain crabs with
carapice widths above or below natural maximum to incoming cohort
            {
                  m[r,1,1]=18.8
            }
      }
      else #otherwise crab is from old cohort
      {
            m[r,1,1] <- rnorm(1,mean=124.2,sd=25.4)
            if (m[r,1,1]>210) #constrain crabs with carapice
widths above natural maximum
            {
                  m[r,1,1]=200
            }
      }
} #end Carapice Width designation loop
#m[,1,1] <- runif(Nm, min=2.2, max=174.7) #set initial carapice
widths on the first day males**#Size structure from WDS or other
Data not runif
```

```
m[,1,2] <- 0 #set each new crab to live
m[,1,3] <- 0 #set each new crab to 0 Degree Days
#m[,1,4] setup
for (r in 1:Nm) #Set maturity for each male
{
      if (m[r,1,1]<107)
      {
            m[r,1,4]=0 #If male is under 107mm, immature
      }
      else
      {
            m[r,1,4]=1 #If male is over 107mm, mature
      }
}#end maturity for loop
m[,1,5] <- 1 #set each new crab to Hard Shell
m[,1,6] <- 0 #set each new crab to 0 days as soft shell

m[,1,7] <- IP(m[,1,1])
#set max sperm for mature crabs
for (r in 1:Nm)
{
      if (m[r,1,4]==1)
      {
            m[r,1,9] <- rlnorm(1,meanlog=21.49,sdlog=0.56)
            if (m[r,1,9]<700000000||m[r,1,9]>6000000000)
#constrain crabs with sperm counts above or below natural maximum
            {
                  m[r,1,9]=1900000000
            }
            m[r,1,8] <- m[r,1,9]
      }
      else
      {
            m[r,1,9] <- 0
      }
} #End max sperm loop
m[,1,10] <- 0 #start all males at 0 mates
m[,1,11] <- 1:Nm #Give every crab an ID number


f <- array(NA, c(Nf,day,12)) #females (1=carapice
width,2=live/dead,3=DegreeDays,4=Maturity,5=ShellStatus,6=Soft/Ma
teDays,7=IPvalue,8=Sperm,9=Eggs, 10=MateID#, 11=crabID,
12=FertEggs)
for (r in 1:Nf) ##set initial carapice widths (mm) on the first
day females** (from WDS data)
{
      f[r,1,1] <- rlnorm(1,meanlog=3.1,sdlog=0.4)
      if (f[r,1,1]>70||m[r,1,1]<10.0) #constrain crabs with
carapice widths above or below natural maximum to incoming cohort
      {
            f[r,1,1]=23.2
      }
} #end carapice width designation loop
f[,1,2] <- 0 #set each new crab to live
f[,1,3] <- 0 #set each new crab to 0 Degree Days
f[,1,4] <- 0 #set each new crab to Immature
f[,1,5] <- 1 #set each new crab to Hard Shell
f[,1,6] <- 0 #set each new crab to 0 days as soft shell

f[,1,7] <- IP(f[,1,1]) #Intermolt period (IP) for first molt
```

```
f[,1,11] <- 1:Nf #Give every crab an ID number


#Code (Created: 6-25-2013)
for (c in 2:day) #loop over number of days, starting at day 2
{
#Male Crab Loop
      for (r in 1:Nm)
      {  #loop over the number of crabs
            m[r,c,11]=m[r,c-1,11] #Keep crab ID number
            if (m[r,c-1,2]==0) # if crab is alive
            {
                if (m[r,c-1,5]<2) #If shell is hard
                {
                  x=runif(1,min=0,max=1) #draw random uniform
number
                  if (m[r,c-1,1]>107) #If crab is legal limit and
in fishing season
                        {
                              Z=CalcZ(Fm[c],258)
                              S=exp(-Z)
                        }
                        else #If crab is under legal limit for
fishing, apply only natural mortality
                        {
                              Z=CalcZ(Fm0,258)
                              S=exp(-Z)
                        }
                  #print(c(x,S,Z))
                  if(x>S) #if crab dies
                  {
                        m[r,c,2]=1 #change the live flag to dead
                        m[r,c,1]=m[r,c-1,1] #display Carapice
width
                  } #end death if statement
                        else #if crab lives
                  {
                        m[r,c,2]=0 #Keep live flag as live
                        m[r,c,1]=m[r,c-1,1]
                        m[r,c,3]=m[r,c-1,3]
                        m[r,c,4]=m[r,c-1,4]
                        m[r,c,5]=m[r,c-1,5]
                        m[r,c,6]=m[r,c-1,6]
                        m[r,c,7]=m[r,c-1,7]
                        m[r,c,8]=m[r,c-1,8]
                        m[r,c,9]=m[r,c-1,9]
                        m[r,c,10]=0 #start the day with no mates
                        if (t[c]>tht) #is temperature threshold
reached
                        {
                              if (m[r,c,4]==0) #if crab is immature
                              {
                                    m[r,c,3]=m[r,c-1,3]+(t[c]-tht)
#Add another Degree Day
                                    if (m[r,c,3]>m[r,c,7]) #If the
crab reach growth threshold
                                    {
                                          if (m[r,c,1]<107) # If
male does not mature but molts
                                          {
```

```
                                                m[r,c,1]=GPM(m[r,c-
1,1],0.24,0.07) #How much does the crab grow
                                                m[r,c,3]=0 #reset
Degree Days to 0
                                                m[r,c,5]=2 #Set
shell to soft
                                                      m[r,c,7] <-
IP(m[r,c,1]) #Draw new IP number based on size change
                                          } # End immature growth
if statement
                                          else # If male does
mature
                                          {
                                                #print("matures!")
                                                m[r,c,1]=GPM(m[r,c-
1,1],0.24,0.07) #How much does the crab grow

                  #print(f[r,c,4])
                                                m[r,c,3]=0 #Set
degree days to 0
                                                m[r,c,4]=1 #change
immature male to mature

      #print(c(r,c,f[r,c,4]))
                                                m[r,c,5]=2 #set
shell to soft
                                                m[r,c,6]=0 #resest
soft shell days to 0
                                                m[r,c,7] <-
IP(m[r,c,1]) #Draw new IP number based on size change

      m[r,c,9]=rlnorm(1,meanlog=21.49,sdlog=0.56) #set max number
of sperm for male
                                                      if
(m[r,1,9]<700000000||m[r,1,9]>6000000000) #constrain sperm counts
above or below natural maximum
                                                      {

      m[r,1,9]=1900000000
                                                      }
                                                      m[r,c,8] <-
m[r,c,9] #Begin sperm count at max
                                          }     # End maturity
growth if statement
                                          } #End growth threshold
if statement
                                          #print(f[r,c,4])
                                    } # End immature if statement
                                    else #if male is mature
                                    {
                                          m[r,c,3]=m[r,c-
1,3]+(t[c]-tht) #Add another Degree Day
                                          m[r,c,4]=1 #keep male as
mature
                                          if (m[r,c,3]>m[r,c,7])
#if crab reaches growth threshold
                                          {
                                                m[r,c,1]=GPM(m[r,c-
1,1],0.24,0.07) #How much does the crab grow
                                          m[r,c,3]=0 #reset Degree
Days to 0
```

```
                                        m[r,c,5]=2 #Set shell to
soft
                                        m[r,c,6]=0 #reset day
counts
                                        if (m[r,c,1]<210) #if too
large to molt again
                                        {
      m[r,c,7]=99999999999
                                        }
                                              else
                                              {
                                                    m[r,c,7] <-
IP(m[r,c,1]) #Draw new IP number based on size change
                                              }
                                        }
                                        else #If crab does not
molt
                                        {
                                              if
(m[r,c,8]<300000000) #if crab reaches low sperm quantities
                                              {
                                                    m[r,c,5]=0
#Place in hard shell, non-mate category
                                                    m[r,c,8]<-
m[r,c,8]*exp(0.057) #increase sperm
                                                    #print("1")
                                              }
                                              else  #if above low
sperm threshold
                                              {
                                                    if
(m[r,c,8]<m[r,c,9]) #If have lower than sperm max
                                                    {
                                                          m[r,c,8]
<- m[r,c,8]*exp(0.057) #increase sperm
      m[r,c,5]=1 #place back in mating pool
      #print("2")
                                                    }
                                                    else #If max
sperm is reached
                                                    {
                                                          m[r,c,8]
<- m[r,c,9] #Keep max sperm number
      m[r,c,5]=1 #place back in mating pool
      #print("3")
                                                    }
                                              } #end sperm
recuperate
                                              #flush.console()
                                        } #end sperm increase
                                  }     #end mature if statement

                        } #end temperature if statement
                  } #end hard shell live if statement
                  } #end hard shell if statement
```

```
                  else # If shell is soft or mating
                  {
                        w=runif(1,min=0,max=1)
                              if (m[r,c-1,1]>89) #If crab is legal
limit and in fishing season
                        {
                              SZ=SoftZ(Fm[c],258)
                              Soft=exp(-SZ)
                        }
                        else #If crab is under legal limit for
fishing, apply only natural mortality
                        {
                              SZ=SoftZ(Fm0,258)
                              Soft=exp(-SZ)
                        }
                        #print(c(w,Soft,SZ))
                  if (w>Soft)  #If crab dies while soft shell
                        {
                              m[r,c,2]=1 #set crab to dead
                              m[r,c,1]=m[r,c-1,1]
                        } #end soft shell death if statement
                        else  #If crab lives while soft shell
                        {
                              m[r,c,2]=0 #set crab to live
                              m[r,c,3]=m[r,c-1,3]
                              m[r,c,8]=m[r,c-1,8]
                              m[r,c,9]=m[r,c-1,9]
                              m[r,c,10]=0 #no mates for the day
                              if (m[r,c-1,6]<2) #If crab lives but
shell doesn't harden
                              {
                                    m[r,c,1]=m[r,c-1,1]
                                    m[r,c,4]=m[r,c-1,4] #keept
maturity status
                                    m[r,c,6]=m[r,c-1,6]+1 #Add day
to soft shell count
                                    m[r,c,5]=2 #keep shell soft
                                    m[r,c,7]=m[r,c-1,7] #Keep IP
number until next molt
                              }
                              else # If crab lives and shell
hardens
                              {
                                    m[r,c,1]=m[r,c-1,1]
                                    m[r,c,4]=m[r,c-1,4] #keep
maturity status
                                    m[r,c,5]=1 #change shell to
hard
                                    m[r,c,6]=0 #reset soft shell
days to 0
                                    m[r,c,7]=m[r,c-1,7] #keep IP
number until next molt
                              }
                        } #End live soft shell if statement
                  } #End soft shell if statement
            } #end live if statement
            else #if crab is dead
            {
                  m[r,c,2]=1
            }
      } #end male crab for loop
```

```
#Female Crab Loop
      for (r in 1:Nf)
      {  #loop over the number of crabs
            f[r,c,11]=f[r,c-1,11] #Keep crab ID Number
            f[r,c,12]=f[r,c-1,12] #Keep amount of fertilized eggs
            if (f[r,c-1,2]==0) # if crab is alive
            {
                if (f[r,c-1,5]<2) #If shell is hard
                {
                  x=runif(1,min=0,max=1) #draw random uniform
number
                        if (f[r,c-1,4]==1) #If crab is mature
                        {
                              #print("1")
                              Z=CalcZ(Ff[c],205) #apply fishing
mortality in Z
                              S=exp(-Z)
                        }
                        else #If crab is immature, apply only
natural mortality
                        {
                              #print("2")
                              Z=CalcZ(Ff0,205)
                              S=exp(-Z)
                        }
                        #print(c(x,S,Z))
                  if(x>S) #if crab dies
                  {
                        f[r,c,2]=1 #change the live flag to dead
                        f[r,c,1]=f[r,c-1,1] #display Carapice
width
                  } #end death if statement
                        else #if crab lives
                  {
                        f[r,c,2]=0 #Keep live flag as live
                        f[r,c,1]=f[r,c-1,1]
                        f[r,c,3]=f[r,c-1,3]
                        f[r,c,4]=f[r,c-1,4]
                        f[r,c,5]=f[r,c-1,5]
                        f[r,c,6]=f[r,c-1,6]
                        f[r,c,7]=f[r,c-1,7]
                        f[r,c,8]=f[r,c-1,8]
                              f[r,c,9]=f[r,c-1,9]
                              f[r,c,10]=f[r,c-1,10]

                        if (t[c]>tht) #is temperature threshold
reached
                        {
                              if (f[r,c,4]<1) #if crab is immature
                              {
                                    f[r,c,3]=f[r,c-1,3]+(t[c]-tht)
#Add another Degree Day
                                    if (f[r,c,3]>f[r,c,7]) #If the
crab reach growth threshold
                                    {
                                          y = runif(1,min=0,max=1)

                                          MatProb = 1/(1+(f[r,c-
1,1]/111)^-28.51) #Maturity probability based on carapice width
```

```
      #print(c(y,MatProb,y>MatProb))
                                        if (y>MatProb) # If
female does not mature but molts
                                        {
                                              f[r,c,1]=GPM(f[r,c-
1,1],0.25,0.06) #How much does the crab grow
                                              f[r,c,3]=0 #reset
Degree Days to 0
                                              f[r,c,5]=2 #Set
shell to soft
                                                    f[r,c,7] <-
IP(f[r,c,1]) #Draw new IP number based on size change
                                        } # End immature growth
if statement
                                        else # If female does
mature
                                        {
                                              #print("matures!")
                                              f[r,c,1]=GPM(f[r,c-
1,1],0.32,0.06) #How much does the crab grow

                                              f[r,c,3]=-99 #Set
degree days to ended
                                              f[r,c,4]=1 #change
immature female to mature
                                              f[r,c,5]=2 #set
shell to soft
                                              f[r,c,6]=0 #reset
soft shell days to 0
                                              f[r,c,7]=0 #Do not
run through molt cycle
                                              f[r,c,9]=(-
2.248+(0.377*f[r,c,1]))*100000 #Calculate female's amount of eggs
based on CW
                                                    Mates <-
m[m[,c,2]==0 & m[,c,4]==1 & m[,c,5]==1 &
m[,c,8]>=300000000,c,1:12] #Select all available males

      #print(dim(Mates))
                                                    #print(Mates)
                                                    flag=0 #set
flag as no mates available
                                                    matdim<-
dim(Mates)

      if(is.null(matdim)==TRUE) #if only one or no mates are
available
                                                    {

      if(Mates[1]>0) #check to make sure there is one mate
                                                          {

      flag=2 #keep flag as only one mate available
                                                          }
                                                    }
                                                    else #more
than one potential mate
                                                    {
```

```
      if(matdim[1]!=0) {flag=1} #flag as more than one mate
available
                                                        }
                                                        if(flag==0)
#if mates are not available
                                                        {

      #print("flag 0")

      f[r,c,8]=0 #no sperm for poor crab

      f[r,c,10]=0 #no mate for her either

                                                        }
                                                        if(flag==1)
#if more than one mate is available and mate preference scenario
is size dependent
                                                        {

      #print("flag 1")

      if(MatePref==3) #if mate history male preference
                                                                {

      #print(m[Mates[,11],c,10])

      Mates[,10]<-rowSums(m[Mates[,11],(c-20):c,10]) #sum all
mates within past 20 days
                                                                }

      #print(Mates[,10])

      Mates[,12]<-
MateProb(MatePref,f[r,c,1],Mates[,1],Mates[,10])

      #print(Mates)


      TotalProb <- sum(Mates[,12]) #sum all mate probabilities
                                                                Prob <-
array(Mates[,12], c(1,nrow(Mates))) #create a vector of all mate
probabilities

      if(sum(Prob)==0) #if mates are not available
                                                                {

      #print("0")

      f[r,c,8]=0 #no sperm for poor crab

      f[r,c,10]=0 #no mate for her either

                                                                }
                                                                else #if
mates available
                                                                {

      Prob[] = Prob[]/TotalProb #scale mate probabilities against
each other
```

```
      Winner <-
Mates[sample(1:nrow(Mates),1,replace=TRUE,Prob[]),11] #select
mating partner's crab ID

      #print(Winner)

      #print(m[Winner,c,8])

      f[r,c,12] <- m[Winner,c,8]/2 #female recieves half of
mate's sperm

      #print(f[r,c,12])

      m[Winner,c,8] <- m[Winner,c,8]/2 #male mate looses half of
sperm

      #print(m[Winner,c,8])

      m[Winner,c,5]=2 #Male must spend 2 days mating

      m[Winner,c,10]=1 #Add a mate to male's # of mates

      f[r,c,10]=Winner #select Mate's crab ID

      f[r,c,8] <- f[r,c,12]-(f[r,c,12]*0.5) #decrease female
sperm by 50 percent

      #print(f[r,c,8])
                                                          }
                                                    }
                                                    if(flag==2)
#if only one mate is available
                                                    {

      #print("flag 2")
                                                          Winner
<- Mates[11] #select mating partner's crab ID

      #print(Winner)

      #print(m[Winner,c,8])

      f[r,c,12] <- m[Winner,c,8]/2 #female recieves half of
mate's sperm

      #print(f[r,c,12])

      m[Winner,c,8] <- m[Winner,c,8]/2 #male mate looses half of
sperm

      m[Winner,c,5]=2 #Male must spend 2 days mating

      m[Winner,c,10]=1 #Add a mate to male's # of mates

      f[r,c,10]=Winner #select Mate's crab ID
                                                          f[r,c,8]
<- f[r,c,12]-(f[r,c,12]*0.5) #decrease female sperm by 50 percent


      #print(f[r,c,8])
```

```
                                                }#End mating
if statement

      #flush.console()
                                          }     # End maturity
growth if statement
                                          } #End growth threshold
if statement
                                          #print(f[r,c,4])
                                    } # End immature if statement
                                    else #if crab is mature
                                    {
                                          f[r,c,1]=f[r,c-1,1]
                                          f[r,c,4]=1 #keep female
as mature
                                          f[r,c,5]=1 #set shell to
remain hard
                                          f[r,c,8]=f[r,c-1,8] #keep
sperm storages
                                          f[r,c,9]=f[r,c-1,9] #keep
egg storages
                                          f[r,c,10]=f[r,c-1,10]
#keep Mate's crab ID
                                    }     #end mature if statement

                              } #end temperature if statement
                        } #end hard shell live if statement
                        } #end hard shell if statement
                        else # If shell is soft
                        {
                              w=runif(1,min=0,max=1)
                                    if (f[r,c-1,1]>89) #If crab is legal
limit and in fishing season
                              {
                                    SZ=SoftZ(Ff[c],205)
                                    Soft=exp(-SZ)
                              }
                              else #If crab is under legal limit for
fishing, apply only natural mortality
                              {
                                    SZ=SoftZ(Ff0,205)
                                    Soft=exp(-SZ)
                              }
                              if (w>Soft)  #If crab dies while soft
shell
                              {
                                    f[r,c,2]=1 #set crab to dead
                                    f[r,c,1]=f[r,c-1,1]
                              } #end soft shell death if statement
                              else  #If crab lives while soft shell
                              {
                                    f[r,c,2]=0 #set crab to live
                                    f[r,c,3]=f[r,c-1,3]
                                    f[r,c,8]=f[r,c-1,8]
                              f[r,c,9]=f[r,c-1,9]
                              f[r,c,10]=f[r,c-1,10]

                                    if (f[r,c-1,6]<2) #If crab lives but
shell doesn't harden
                                    {
                                          f[r,c,1]=f[r,c-1,1]
```

```
                                    f[r,c,4]=f[r,c-1,4] #keept
maturity status
                                    f[r,c,6]=f[r,c-1,6]+1 #Add day
to soft shell count
                                    f[r,c,5]=2 #keep shell soft
                                    f[r,c,7]=f[r,c-1,7] #Keep IP
number until next molt
                              }
                              else # If crab lives and shell
hardens
                              {
                                    f[r,c,1]=f[r,c-1,1]
                                    f[r,c,4]=f[r,c-1,4] #keep
maturity status
                                    f[r,c,5]=1 #change shell to
hard
                                    f[r,c,6]=0 #reset soft shell
days to 0
                                    f[r,c,7]=f[r,c-1,7] #keep IP
number until next molt
                              }
                        } #End live soft shell if statement
                  } #End soft shell if statement
            } #end live if statement
            else #if crab is dead
            {
                  f[r,c,2]=1
            }
      } #end female crab for loop

} #end day for loop

#Create Results spreadsheet
results <- array(NA, c(1,20))
colnames(results, do.NULL=TRUE, prefix="col")
colnames(results) <- c("ID","Mate Scenario","Male:
Fishing","Female: Fishing","Male: Mortality","Male: Mean
CW","Male: SD CW","Male: Average Sperm","Male: SD sperm","Male:
Mean Mate Number","Female: Mortality","Female: Mean CW","Female:
SD CW","Female: Average Sperm","Female: SD Sperm","Female:
Average Egg","Female: SD Egg","Sperm Min","Sperm Max","Sperm
Median")

results[1] <- ID
results[2] <- MatePref
results[3] <- Fm1
results[4] <- Ff1

#Male Stats
results[5] <- sum(m[,day,2]) #Male mortality
results[6] <- mean(m[,day,1],,na.rm=TRUE) #Male mean CW
results[7] <- sd(m[,day,1],na.rm=TRUE) #Male sd CW
MeanMSperm <- rowMeans(m[,,8],na.rm=TRUE) #calculate mean sperm
number for each male crab
results[8] <- exp(mean(log(MeanMSperm[]),,na.rm=TRUE)) #Male
total mean sperm number
results[9] <- sd(log(MeanMSperm[]),na.rm=TRUE) #Male total sd
sperm number
Mate_num <- apply(m[,,10],1,sum) #sum each male crab's mates
results[10] <- mean(Mate_num[],,na.rm=TRUE) #Mean mate number,
incorrect so did by hand
```

```
#Female Stats
results[11] <- sum(f[,day,2]) #Female Mortality
results[12] <- mean(f[,day,1],,na.rm=TRUE) #Female mean CW
results[13] <- sd(f[,day,1],na.rm=TRUE) #Female sd CW
MeanFSperm <- rowMeans(f[,,8],na.rm=TRUE) #calculate mean sperm
number for each female crab
results[14] <- mean(MeanFSperm[],,na.rm=TRUE) #Female total mean
sperm received
results[15] <- sd(MeanFSperm[],na.rm=TRUE) #Female total sd sperm
received
MeanEgg <- rowMeans(f[,,9],na.rm=TRUE) #All females sperm numbers
(regardless of mortality)
results[16] <- mean(MeanEgg,,na.rm=TRUE) #Mean egg numbers of
female population
results[17] <- sd(MeanEgg,na.rm=TRUE) #sd egg numbers of female
population
results[18] <- min(MeanFSperm[],na.rm=TRUE)
results[19] <- max(MeanFSperm[],na.rm=TRUE)
results[20] <- median(MeanFSperm[],na.rm=TRUE)

write.table(results[], file="Results.csv", append=TRUE, sep=",",
col.names=FALSE)

#Write new excel spreadsheet for scenario's daily timestep:
number is same as ID in results spreadsheet
write.table(m[,,1], file="Males.csv",sep=",")
write.table(f[,,1], file="Females.csv",sep=",")
for (e in 2:12) #create outputs
{
      write.table(m[,,e], file="Males.csv", append=TRUE, sep=",")
      write.table(f[,,e], file="Females.csv", append=TRUE,
sep=",")
}
```

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