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ARTICLE

Sex Ratios and Average Sperm per Female Blue Crab *Callinectes sapidus* in Six Tributaries of Chesapeake Bay

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Abstract

Sperm limitation has been a concern for several crustacean species around the world. It may be of particular concern for blue crabs *Callinectes sapidus* in Chesapeake Bay due to the species' reproductive biology and the sex-specific fishery regulations in place. Our objectives were to characterize the differences in sperm counts in mated females from six tributaries of the Chesapeake Bay and to determine whether sperm quantity was affected by the ratio of males to females in each system. Mature females were sampled 1–4 times in each tributary on a biweekly schedule from September to November 2011. We quantified sperm storage for each crab by microscopic examination and compared the sperm counts of females among river systems with the adult male : female sex ratios using ANOVA and linear regression. Total sperm quantity per female varied 16-fold ($0.9\text{--}13.0 \times 10^8$) among tributaries. The sex ratio was also variable among tributaries. Total sperm quantity per female was not significantly related to sex ratio, tributary, or month but was negatively related to the development stage of the spermathecae. Estimated sperm : egg ratios varied from 153:1 to 2:1 but were always higher than 1:1. Our results suggest that sperm quantities are not affected by male : female sex ratios and that sperm limitation caused by low sex ratios is likely not a concern in tributaries similar to those in our study.

Eggs are considered to be the limiting resource for reproductive output in many exploited species (Quinn and Deriso 1999). However, sperm may be the limiting resource in some free-spawning marine species (Leviton and Petersen 1995), particularly crustaceans (Rondeau and Sainte-Marie 2001; Hines et al. 2003; Sato and Goshima 2006; Ogburn et al. 2014) and protogynous fishes that have internal fertilization (Alonzo and Mangel 2005). Fisheries can induce such limitation by reducing the abundance of adult males and/or their average size (Sato and Goshima 2006). A reduction in the number of males available for mating could cause an Allee effect, in which females cannot find mates or males mate too frequently to recuperate adequate sperm.

Managing fisheries to provide adequate male abundance may be important for species susceptible to sperm limitation. Many crustacean fisheries, such as those for Dungeness crab *Cancer magister* and snow crab *Chionoecetes opilio*, are managed with sex-specific regulations to conserve the number of females and thus to protect egg abundance (Rondeau and Sainte-Marie 2001). However, focusing the harvest on males may have the unintended consequence of inducing sperm limitation (Alonzo and Mangel 2005). Several authors have suggested that the blue crab *Callinectes sapidus* is one species in which this occurring (Hines et al. 2003; Jivoff et al. 2007; Ogburn et al. 2014). The blue crab supports one of the highest-valued commercial fisheries in Chesapeake Bay, with exvessel

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landings valued at around US\$73 million annually (Bunnell et al. 2010). Declines in harvest in the 1990s and 2000s caused concern about recruitment overfishing. This concern led to the implementation of sex-specific regulations in 2008, which reduced the harvest of females by about 30% (Miller et al. 2011). These regulations limited the daily catches of mature females in the fall (imposing an early end to their season) and placed a moratorium on the winter dredge fishery in Virginia, which primarily caught mated female crabs (Miller et al. 2011). The regulations pertaining to males remained the same, restricting the harvest of blue crabs by means of minimum size limits that vary by season and jurisdiction. Following the implementation of these regulations abundance increased substantially, particularly for females, but these gains have since been eroded (Figure 1). The immediate increase in adult females was expected because of reduced fishing mortality (Miller et al. 2011), but the cause of the decline in more recent years remains unclear.

During 2008–2011 changes in management succeeded in increasing female abundance, but they also shifted the ratio of males to females from about 1:2 before the regulations were changed to 1:5 in recent years (Miller et al. 2011; Figure 1). Ogburn et al. (2014) have suggested that sperm limitation is a potential concern in maintaining a population. The blue crab has a complex life history in which pelagic larvae occur primarily in the coastal ocean while adults live in coastal bays and estuaries. Females receive sperm only when they mate during their final molt. Although blue crabs do not have a “terminal” molt as some crustaceans do, subsequent molting in females seems to be bioenergetically or physiologically restricted (Jivoff et al. 2007). Thus, the amount of sperm the female receives during this mating determines how many eggs she will be able to fertilize in her lifetime (Hines et al. 2003; Jivoff 2003a). Darnell et al. (2009) found that female blue crabs in North Carolina produced up to seven broods over 1–2 spawning seasons following mating.

Although a female may produce that many broods, with an average of 3.3×10^6 eggs per brood (Prager et al. 1990), most of the reproductive output is concentrated in the first few (<4) clutches (Darnell et al. 2009). Unlike females, males can mate an indefinite number of times (Jivoff et al. 2007). However, males deplete their sperm stores by about one-half during each mating and need approximately 9–20 d to fully recuperate before the next mating (Kendall et al. 2002; Hines et al. 2003; Jivoff 2003b). Males that do not have time to fully recuperate between matings transfer approximately 50% less sperm to females with each consecutive coupling (Kendall et al. 2002). A female-skewed sex ratio, such as that induced by the recent changes to the management of blue crabs in Chesapeake Bay, could cause males to mate more frequently, thereby leading to sperm limitation through the transfer of reduced amounts of sperm per mating. Sperm limitation in blue crabs has been observed in laboratory settings, where females have created broods of eggs that were unfertilized, presumably due to lack of sperm (Hines et al. 2003).

Previous studies of blue crabs have raised concerns about sperm limitation based on comparisons of the number of sperm per female among those collected in Chesapeake Bay with the number from laboratory matings (Kendall et al. 2002) and crabs from less-fished areas (Hines et al. 2003). Females in the less heavily exploited Indian River Lagoon, Florida, had a higher average number of sperm per female than females in the more heavily exploited Chesapeake Bay (Indian River Lagoon: 1.2×10^9 ; Chesapeake Bay: 5.0×10^8 ; Hines et al. 2003). Kendall et al. (2002) relied on laboratory data on the number of sperm received per female when they were mated consecutively (first mating: 3.35×10^9 ; third/final mating: 9.31×10^8). Kendall et al. (2002) suggested that the amount of sperm per female within the Rhode River, Maryland (field average: $\sim 9.0 \times 10^8$), was closest to that observed in laboratory females mated with depleted males. Neither of these studies, however, directly evaluated whether the differences in the amount of sperm per female were due to the abundance of available males within the populations or to other factors.

Although the sex ratio of a population is usually defined as the abundance of mature individuals of one sex relative to that of the other, studies of sexual competition usually refer to the operational sex ratio of a population, or the number of available adult males to fertilizable females (Kendall et al. 2001; Rondeau and Sainte-Marie 2001). Female blue crabs mature asynchronously and are thought to mate only once, so that the pool of receptive males is usually larger than that of receptive females (Jivoff et al. 2007). Additionally, males mature at a smaller size than females (Jivoff et al. 2007). Both factors should skew the operational sex ratio toward males, even if the sex ratio of the total population is skewed toward females (Rondeau and Sainte-Marie 2001). In the Rhode River, the operational sex ratio of reproductively active males to prepubertal females ranged from 2.38 to 15 over the course of the mating season. Furthermore, the lowest number of sperm per

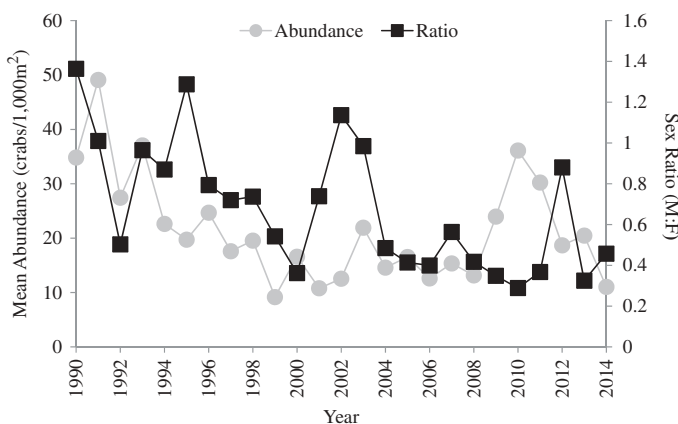


FIGURE 1. Mean abundance and male : female sex ratio of age-1 and older blue crabs during 1990–2010 Chesapeake Bay winter dredge surveys.

female coincided with periods when the operational sex ratio was lowest (Ogburn et al. 2014). Unfortunately, although females close to molting can be identified, quantifying the operational sex ratio is challenging because it can be difficult to identify a female preparing to mature during her next molt (Jivoff et al. 2007), and this information is not commonly recorded in surveys in Chesapeake Bay.

Finally, in most mating systems, fertilization 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 infer the presence of sperm limitation in a population, with low ratios being cited as evidence for such limitation (Rondeau and Sainte-Marie 2001; Hines et al. 2003; Sato and Goshima 2006; Ogburn et al. 2014). Knowledge of the sperm : egg ratio required for optimal reproductive success is especially important for management because it permits direct estimation of the potential reproductive impairment when combined with field estimates of total sperm counts in females.

In this study, we sought to evaluate the potential for fisheries-induced sperm limitation in Chesapeake Bay blue crabs. We compared sperm per female across a gradient of sex ratios from a range of tributaries from the bay to determine whether the number of sperm per female was related to the sex ratio. If fishery-induced sperm limitation is occurring, we would expect to see the lowest number of sperm per female in tributaries with the lowest male : female sex ratios. We hypothesized that females in tributaries with higher sex ratios would receive more sperm because males would have longer times between pairings to recover sperm stores. Further, we compared estimates of total sperm counts per female with a range of assumed brood production schedules.

METHODS

During the fall of 2011, mature female blue crabs were collected by commercial watermen near the mouths of six large tributaries of Chesapeake Bay: the Chester, Choptank, Patuxent, Potomac, York, and James rivers (Figure 2). These tributaries were selected because they span much of the latitudinal range of Chesapeake Bay and were expected to yield a gradient of sex ratios because of differential migration patterns for males and females and differences in fishing mortality rates. Blue crabs were collected 1–4 times per tributary in September, October, and November, with an average catch of 135 females per collection. Blue crab regulations allow all mature females to be harvested, with no size limit. The collection sites were chosen based on their proximity to the mouth of each tributary in order to sample mature females as they migrated to their overwintering and spawning grounds at the mouth of Chesapeake Bay. Our goal was to sample females that were assumed not to have spawned yet because they had not reached high-salinity waters, thereby avoiding females that had already depleted their sperm stores by producing broods of eggs. Captured crabs were labeled by location and date and frozen for subsequent examination in the laboratory.

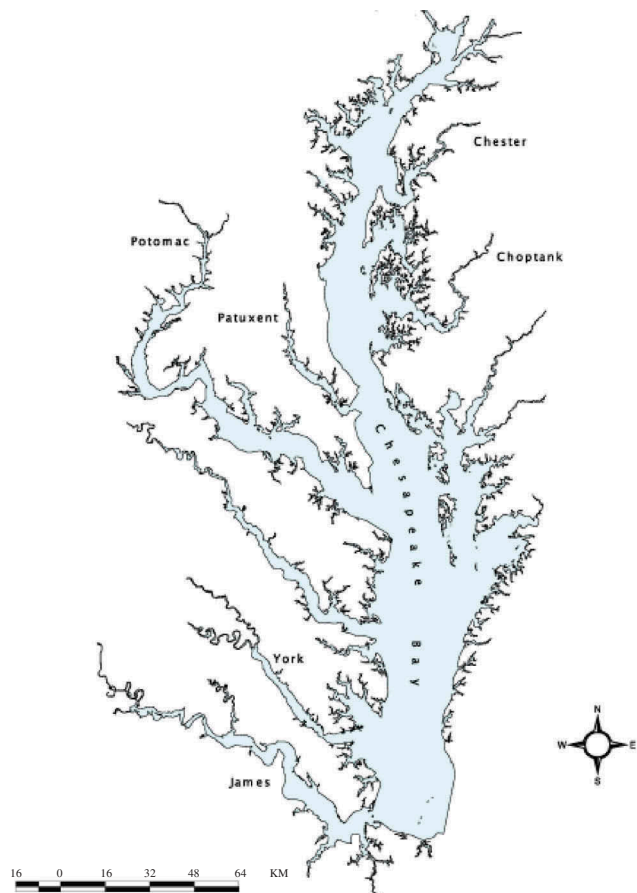


FIGURE 2. Tributaries of Chesapeake Bay from which blue crab samples were obtained during 2011.

In the laboratory, 21 females per tributary were dissected to quantify the abundance of sperm per female crab in each river system (total $n = 126$). Females were subsampled from all of the frozen samples by randomly choosing an equal number from each sampling event within a tributary to achieve a sample size of 21 per tributary. In the laboratory, frozen samples were thawed in cool water and the carapace width (point to point) was measured with Vernier calipers. During dissection we recorded spermathecal development stages based on color and size (Wolcott et al. 2005). Spermathecae were categorized on a scale from 0% full (void of seminal fluid, flaccid, small, and white) and ready to spawn to 100% full (full of seminal fluid, swollen, large, and pink) and recently mated (Hines et al. 2003; Wolcott et al. 2005). Six females in our sample had right and left spermathecae that differed in percent fullness; 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 recorded after the removal of excess water.

We used a procedure modified from Hines et al. (2003) to quantify the amount of sperm in each female blue crab. In particular, our study used both spermathecae because preliminary

investigations (Rains, unpublished data) indicated significant variability in the number of sperm observed in the left and right spermathecae of the same female, even though their weights or estimated fullness were similar. The spermathecae from one blue 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 transferred to a Dounce homogenizer and ground for 30 minutes. Two 50- μ L subsamples were each diluted with 1,500 μ L of ASW, and 7.5 μ L of 1% aqueous crystal violet stain was added to aid in identifying sperm. Preliminary studies indicated that this dilution made counting more efficient and reliable (Rains, unpublished data).

A 10- μ L subsample of this solution was injected into a hemocytometer for counting. We counted the number of sperm under 400 \times magnification in 5 of the 25 hemocytometer grid squares (the four corners and the middle). Each hemocytometer grid square contained 4 μ L of solution. Four 10- μ L 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, i.e.,

$$\text{TSC} = \left(\frac{a}{0.004 \cdot 1,557.5} \right) \cdot \left(\frac{s}{0.05} \right) \quad (1)$$

where TSC is the total sperm count, a is the average sperm count per hemocytometer grid, and s is the sample volume. The numerical values derived from this equation are converted from microliters to milliliters by adjusting for the volume of each hemocytometer grid square (0.004 mL), the total volume of the diluted sample (1,557.5 mL), and the volume for each subsample (0.05 mL).

Preliminary analyses indicated that the total sperm count per female was not related to carapace width or spermathecal weight, so we did not use these variables in later analyses. The total sperm counts for each female were \log_e transformed to satisfy the assumptions of normality and homogeneity of variance. We calculated the average sperm count per female for each month and tributary from the individual TSC estimates.

Sex ratio data for mature blue crabs from each tributary during August–November of 2011 were obtained from trawl surveys conducted by the Maryland Department of Natural Resources (for the Chester, Choptank, and Patuxent rivers) and the Virginia Institute of Marine Science (for the York and James rivers) and from commercial harvest records from the Potomac River Fisheries Commission. These months were chosen because females that mated earlier in the year likely migrated out of the system in late summer (Jivoff et al. 2007). Mature females from the surveys were visually identified by the shape of their abdomen. Males larger than 107 mm (the mean size of maturity) were considered mature (Jivoff et al. 2007). For the Potomac River, the sex ratio was estimated from the

harvest data (number of bushels [~35.2 L] 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 Miller et al. (2011). To correct the Potomac River sex ratio for the minimum size limit, we calculated the mean ratio of males between 107 and 126 mm to mature males above 127 mm for all Maryland tributaries for August through October. We then multiplied the Potomac River landings records of males by this ratio.

We tested for the effect of sex ratio on the average amount of sperm per female using multiple linear regression. We modeled the \log -transformed quantity of sperm per female as a linear function of the sex ratio in the tributary and percent fullness of the spermathecae, i.e.,

$$\log_e \text{TSC} = \beta_0 + \beta_1 x + \beta_2 c + \varepsilon \quad (2)$$

where x is the sex ratio, c is the percent fullness of an individual's spermathecae, and ε is a normally distributed random error. We included the effect of percent fullness because the number of sperm per female declines after mating (Wolcott et al. 2005). 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 also estimated a linear regression between sperm quantity per female and the sex ratio for all individuals after correcting for the effect of percent fullness because females had unknown mating times and sperm is lost after mating (Wolcott et al. 2005). We applied a proportional correction using the results from the regression of TSC on sex ratio. The correction is given by

$$0\% \text{ corrected total sperm count} = \exp(\log_e \text{TSC} - \beta_2 c). \quad (3)$$

This correction assumes that all spermathecae lose sperm at the same rate, cease losing sperm once they reach 0% fullness, and differ only in the initial amount of sperm present.

We used the 0% corrected total sperm counts to conduct a two-way ANOVA with \log_e transformed corrected sperm count per female as the dependent variable and tributary and month as independent variables. We used these sperm counts to account for the factor of time since mating while testing to see whether sperm quantity differed among months or tributaries. We did not include an interaction term in our model because there was little contrast in months for most tributaries, which hindered estimation. Tukey's honestly significant difference multiple-means comparison test was used to examine differences among the levels of the independent variables.

Sperm : egg ratios were calculated under a range of values of sperm received and lifetime egg production to evaluate the potential for sperm limitation at the individual level. The mean, maximum, and minimum sperm quantities from this study and

the average amount of sperm a fully recovered male can give a female from Wolcott et al. (2005) were compared with different values of average eggs produced per lifetime. We adjusted the sperm quantities for both our study and the Wolcott et al. (2005) study to 0% fullness because we assumed that this represents a value is closer to the state of a female preparing to fertilize her first brood. Because Wolcott et al. (2005) estimated TSC right after mating, their TSC value was corrected to reflect the amount of sperm lost between mating and first brood production in their own samples, which they calculated at 49%. This is different from the value in our samples, which we calculated as 66% (equation 2) and used in the correction of our own samples (equation 3). We used the Prager et al. (1990) estimate of the number of eggs produced per brood, 3.3×10^6 . Although female blue crabs may survive for up to 2 years after maturity, most live for less than 1 year in Chesapeake Bay (Miller et al. 2011). In North Carolina, an average female that survived the full study period had 4.14 broods (Darnell et al. 2009). However, this number does not take into consideration the substantial number of females that did not survive the full study period. Accordingly, we adjusted the Darnell et al. (2009) results for the average mortality of mature females in Chesapeake Bay (Miller et al. 2011) using a constant mortality rate over a 3-year period to calculate an average number of broods per female in Chesapeake Bay of 1.4. The expected lifetime egg production per female is thus $1.4 \times 3.3 \times 10^6 = 4.5 \times 10^6$. We also calculated sperm : egg ratios using three broods per season from Hines et al. (2003) and the maximum number 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 9.9×10^6 , and the average number produced in seven broods is 2.3×10^7 .

RESULTS

The TSC per female blue crab was highly variable among individuals in each river system, ranging from 9.1×10^7 (James River) to 1.3×10^9 (Choptank River; Table 1). The average TSC

across all tributaries was 3.6×10^8 , with a median of 2.6×10^8 and a standard deviation (SD) of 2.7×10^8 . Male : female sex ratios varied between 0.66 and 3.70 among tributaries (Table 1). The highest sex ratio was observed in the Chester River, while the lowest was observed in the Choptank River.

Total sperm count was positively related to the percent fullness of the spermathecae ($t = 8.30$; $df = 1, 123$; $P < 0.0001$; Figure 3) but not to the male : female sex ratio ($t = 0.08$; $df = 1, 123$; $P = 0.93$). Average TSC values were well described by a linear relationship with percent fullness; the average TSC values at 0% fullness were only 34% of those at 100% fullness. The intercept indicated that spermathecae with 0% fullness contained approximately 2.2×10^8 sperm on average.

Log_e transformed 0% corrected total sperm counts per female were not related to the male : female sex ratio ($F = 0.06$; $df = 1, 124$; $P = 0.80$; Figure 4). Additionally, they did not differ among tributaries ($F = 1.86$; $df = 5, 118$; $P = 0.11$; Figure 5) or across months ($F = 2.01$; $df = 2, 118$; $P = 0.14$). For samples corrected to 0% fullness, the average TSC was 2.5×10^8 , with an SD of 1.2×10^8 . The minimum decreased to 5.2×10^7 and the maximum decreased to 6.8×10^8 .

Sperm-to-lifetime-egg ratios were all higher than 1:1 (Table 2). The mean sperm quantity in females with 0% full spermathecae relative to 1.4 broods of eggs gave an estimated sperm : egg ratio of 57:1. The mean ratio was 26:1 for three broods of eggs and 11:1 for seven broods (the maximum observed by Darnell et al. 2009). If females received 6.0×10^8 sperm (fully recovered males; Wolcott et al. 2005) and produced 1.4 broods, the sperm : egg ratio would be 134:1. If females produced three and seven broods from a mating with a fully recovered male, the ratios would be 61:1 and 26:1, respectively. The highest sperm : egg ratio (assuming 1.4 broods and the maximum sperm quantity in our sample [6.8×10^8 sperm]) produced a ratio of approximately 153:1. The minimum sperm : egg ratio, which was calculated using the maximum number of broods (seven; Darnell et al. 2009) and the minimum sperm quantity in our sample (5.2×10^7 sperm) gave a ratio of approximately 2:1.

TABLE 1. Summary of collected data by river system during 2011 for blue crabs in Chesapeake Bay, including the number per month and timing of sampling, the male : female sex ratio calculated from the Maryland Department of Natural Resources and Virginia Institute of Marine Science trawl surveys (or Potomac River Fisheries Commission landings data), the mean carapace width, the uncorrected mean, minimum, and maximum number of sperm per female, and the average percent fullness of the spermathecae.

River	Sex ratio	Months sampled (no. of samples)	Mean carapace width (mm)	Sperm per female			
				Mean	Minimum	Maximum	% Fullness
Chester	3.7	September (1), October (2)	155.3	3.2×10^8	1.0×10^8	8.1×10^8	34.5
Choptank	0.7	September (1), October (1)	150.1	4.7×10^8	9.1×10^7	1.3×10^9	37.1
Patuxent	1.0	October (2), November (2)	141.7	3.6×10^8	9.3×10^7	1.1×10^9	30.5
Potomac	3.5	October (2), November (1)	161.2	4.8×10^8	1.1×10^8	1.2×10^9	38.1
York	0.9	November (1)	141.4	3.0×10^8	1.1×10^8	9.5×10^8	13.8
James	1.5	November (1)	141.8	2.5×10^8	9.1×10^7	7.0×10^8	11.0

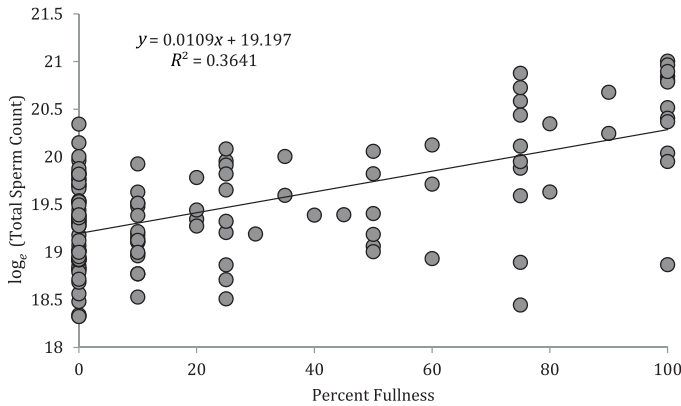


FIGURE 3. Estimated relationship between total sperm count of female blue crabs and percent fullness of the female's spermathecae ($F = 68.93$; $df = 1, 123$; $P < 0.0001$) in six tributaries of Chesapeake Bay during 2011. At 100% fullness a female has recently been inseminated, and at 0% fullness it has been several months since she mated.

DISCUSSION

Sperm quantity per female was highly variable, but not related to the sex ratio in the fall months among the six tributaries of Chesapeake Bay. These results indicate that the male : female sex ratio of mature blue crabs does not explain differences in average sperm quantity per female. We hypothesized that if fisheries-induced sperm limitation were occurring we would find a positive relationship between the male : female sex ratio and the amount of sperm a female had stored, at least over some portion of the range of observed sex ratios (as was the case for the sex ratios calculated in Ogburn et al. 2014). 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 [11:3]) and those in the Choptank River (the tributary with the lowest ratio [2:3]). Indeed, all of the females that we examined

had been inseminated, which indicates that females were able to find mates under the current sex ratios and which is consistent with the results of other studies (Hines et al. 2003; Ogburn et al. 2014).

The developmental 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 3 months later. We estimated a 66% average decrease in sperm quantity between the first and last stages of spermathecae development. The differences between our study and Wolcott et al. (2005) are likely due to differences in the time since mating. The blue crabs in the Wolcott et al. (2005) study had known dates of mating and were sacrificed at known intervals until their first brood of eggs 3 months after mating, whereas we did not know the date of mating for our samples. The development of the spermathecae usually progresses as a female blue crab is preparing to brood eggs, with 0% fullness assumed to occur right before she creates her first brood (Jivoff et al. 2007). In one of their scenarios, Ogburn et al. (2014) calculated sperm loss at a constant 20% monthly rate over the lifetime of the female. However, our study assumed that females lose a fixed amount of sperm between insemination and the production of their first brood, with no more being lost after that except through the fertilization of eggs. Our assumption is based on the fixed 49% decrease found by Wolcott et al. (2005). Additionally, Wolcott et al. (2005) looked at the change in viable versus dead sperm in recently mated females and found that the decrease was mainly attributable to dead sperm, while the amount of viable sperm remained approximately the same. Therefore, it is likely that losses in sperm are primarily of dead sperm cells and that viable sperm do not degrade over at least the first 3 months, which suggests that sperm degradation ends after all dead sperm cells are shed.

The ratio of mature males to mature females calculated for this study was also highly variable. However, this ratio is only a proxy for the operational sex ratio for mating. Ideally, we would have used the ratio of mature males to females that were ready to mate, which may be substantially more male biased because females mature asynchronously, males mature at a smaller size than females, and males can mate multiple times. However, calculation of this ratio is challenging because it is difficult to tell the difference between females that will mature on their next molt 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 such ratios are higher than male : female sex ratios. To conduct this test, we calculated the number of males over 107 mm (the assumed size at maturity; Jivoff et al. 2007) and the number of females between 95 and 130 mm (sizes with a 1–98% chance of molting to maturity using a

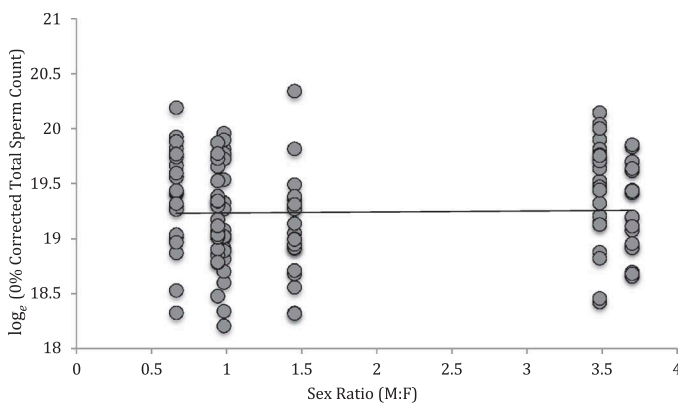


FIGURE 4. \log_e transformed sperm counts of female blue crabs in Chesapeake Bay during 2011, corrected to 0% fullness, versus the male : female sex ratio. The estimates in each "column" are from crabs collected in the same tributary and thereby having the same sex ratio.

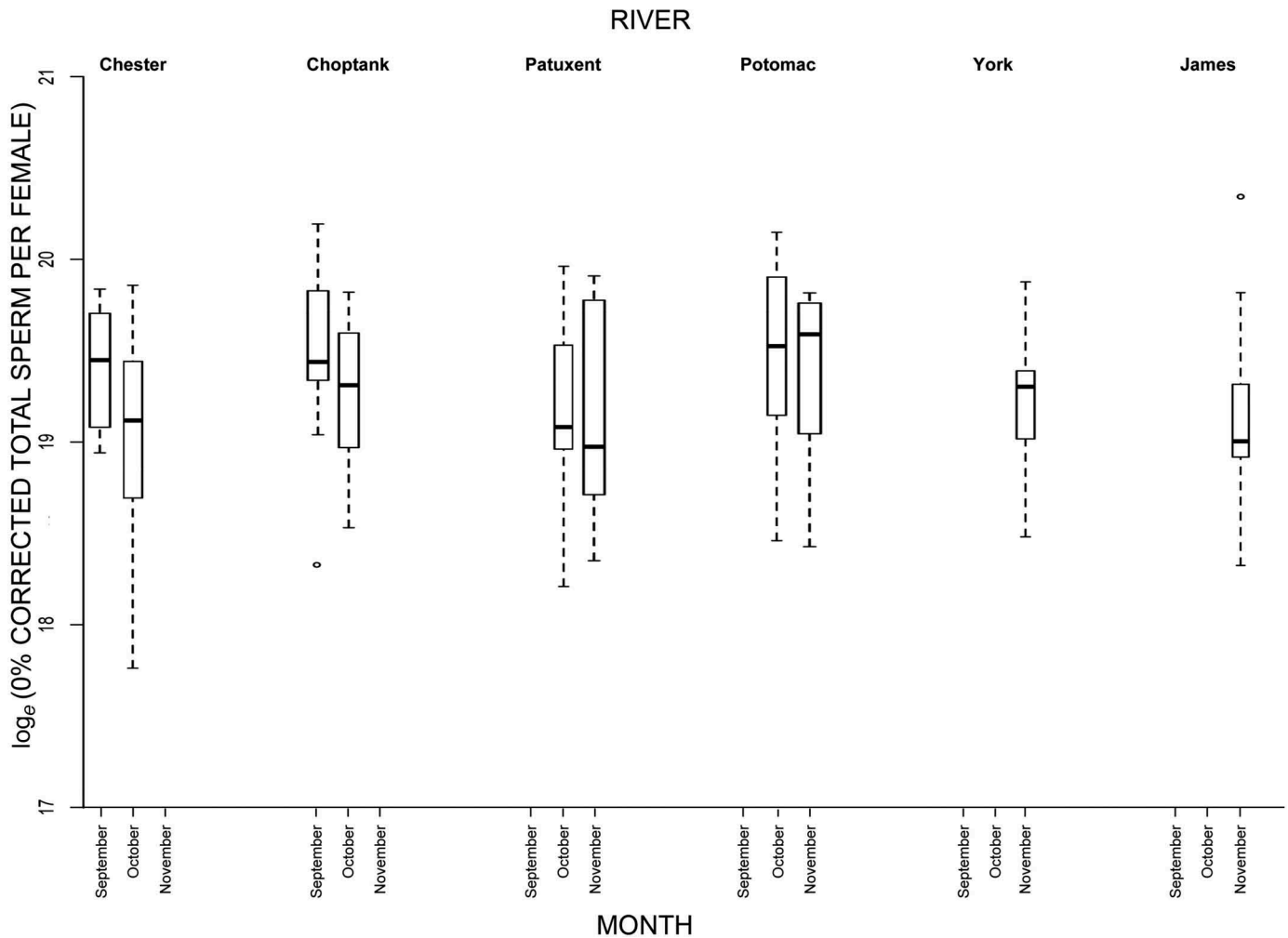


FIGURE 5. \log_e transformed sperm counts of female blue crabs from Chesapeake Bay, corrected to 0% fullness, by tributary and month. The dark lines within the boxes represent the medians, the lower and upper boundaries of the boxes the first and third quartiles, and the dashed lines 1.5 times the interquartile ranges; the circles represent outliers.

modified female maturation probability equation from Bunnell and Miller 2005) during the mating season (May–October) to evaluate the operational sex ratio of each site in the 2011 mating season. The operational sex ratios of all MDTs sites were above 1:1, with a mean value of 2.2 (SE, 0.54); the values for the individual tributaries were as follows: 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 is a rudimentary treatment of the operational sex ratios at these sites because it assumes that all females mature on the same day. Because only a small fraction of female blue crabs will mature on a given day, the actual operational sex ratios for individual females will be higher than those calculated using all potential females and males. Nevertheless, this analysis confirms that even if the 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.

The 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 (e.g., Hines et al. 2003; Wolcott et al. 2005) have relied on information from crustacean species with different mating strategies, particularly calculations of sperm : egg ratios from single mating instances in species that mate multiple times. The sperm : egg ratios necessary for full fertilization are highly variable in decapods, ranging from 70:1 for snow crabs (Sainte-Marie and Lovrich 1994) to 3,700:1 for mud crabs *Eurypanopeus depressus* (Rodgers et al. 2011). By comparison, our estimates of sperm : egg ratios for blue crabs fall below the range for other studied decapods. Our lifetime ratio ranged from a low of 2:1 to a high of 153:1, depending on the

TABLE 2. Sperm : egg ratio scenarios for different estimation methods of eggs produced and sperm transferred to female blue crabs in Chesapeake Bay. The studies used for the average number of eggs per brood were Prager et al. (1990), Darnell et al. (2009), and Hines et al. (2003). The values used for the number of sperm per female were corrected to 0% full spermathecae samples from this study and the average number of sperm per female from Wolcott et al. (2005) corrected for sperm loss.

Egg scenario	No. broods	No. of eggs	Sperm scenario	No. of sperm	Sperm : egg ratio
Darnell average	1.4	4.47×10^6	Rains maximum	6.84×10^8	152.9
			Wolcott average	6.00×10^8	134.2
			Rains average	2.55×10^8	57.0
			Rains minimum	5.18×10^7	11.6
Hines average	3	9.90×10^6	Rains maximum	6.84×10^8	69.0
			Wolcott average	6.00×10^8	60.6
			Rains average	2.55×10^8	25.7
			Rains minimum	5.18×10^7	5.2
Darnell maximum	7	2.31×10^7	Rains maximum	6.84×10^8	29.6
			Wolcott average	6.00×10^8	26.0
			Rains average	2.55×10^8	11.0
			Rains minimum	5.18×10^7	2.2

number of broods estimated, with the assumption that a 1:1 ratio is a biological limit. Evidence that blue crabs are receiving enough sperm per egg (i.e., above a 1:1 ratio) agrees with observations from the Chesapeake Bay spawning sanctuary, where spawning females exhibit fully fertilized broods of eggs (Ogburn et al. 2014).

Previous studies that used sperm : egg ratios to conclude that fishery-induced sperm limitation is occurring in Chesapeake Bay assumed that most females create up to seven broods of eggs over a 2-year lifetime after maturity (Hines et al. 2003; Darnell et al. 2009). However, seven broods is likely a maximum potential estimate that ignores the impact of mortality. The effect of mortality on expected lifetime egg production is substantial. In caged field experiments performed by Darnell et al. (2009), 27% of mature females survived to produce two broods of eggs, 5% to produce five broods of eggs, and only 0.9% to produce seven broods of eggs. Because Darnell et al. (2009) conducted a caged field study, the expected mortality that they observed is likely lower than that which blue crabs would experience in the wild; therefore, we also calculated the proportion of females expected to survive to a given age using an instantaneous total mortality rate of 1.95 per year from Chesapeake Bay (Miller et al. 2011). With this level of mortality, 15% of the population is expected to survive to the end of their first year (potentially producing about two broods of eggs), 2% to the end of their second year (producing up to five broods of eggs), and only 0.2% long enough to produce seven broods.

Our results do not support a conclusion of fisheries-induced sperm limitation from heavy harvesting of males in the blue crab population in Chesapeake Bay because there was no relationship between the number of sperm per female and

our calculated sex ratio. However, our crab collections were made over a limited period of time, and in two tributaries there was only one collection. It is possible that the quantity of sperm per recently mated female changes over the course of the season and that our collections occurred in too narrow a timeframe to capture the seasonal dynamics. Both Wolcott et al. (2005) and Ogburn et al. (2014) found seasonal differences in sperm counts, but the temporal patterns were different. Ogburn et al. (2014) found the lowest sperm counts in the Rhode River during the middle of the mating season (June–August, when large numbers of matings take place), and Wolcott et al. (2005) found the lowest sperm counts in North Carolina during the early mating season (February–April, when crabs come out of dormancy). Additionally, 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 an unknown part of the breeding season. Females that mate in July and August in Chesapeake Bay may not begin migration until fall (Jivoff et al. 2007). Given the migration patterns of mature females, we expect that none of those in our samples had yet produced a brood. Nevertheless, females from the York and James rivers could have spawned and migrated back up to those systems, although we think that this is unlikely. Lastly, our sample size was still relatively small given the large amount of variation in TSC per female, which causes our statistical tests to have relatively low power. The sample size would need to be about eight times as large to have an 80% chance of finding a significant slope given the variability and size of the effect estimated in our study. However, our sample

sizes, sample collection, and sperm counting methods are similar to those of the other studies with which we compared our results (Kendall et al. 2002; Hines et al. 2003; Wolcott et al. 2005; Ogburn et al. 2014).

Our observed female TSCs were also in the same range as those from the laboratory studies by Carver et al. (2005) and Wolcott et al. (2005) and are similar to the field data reported by Ogburn et al. (2014). Wolcott et al. (2005) found that the average number of sperm transferred differed with mating history, with previously unmated males transferring an average of 1.2×10^9 sperm and males that had mated three times without recovery transferring an average of 4.1×10^8 . However, these numbers were recorded immediately after mating, and correcting them to 0% fullness using the 49% decrease of Wolcott et al. (2005) reduces them to 6.0×10^8 for unmated males and 2.1×10^8 for males that mated twice consecutively. The corrected values from Wolcott et al. (2005) are within the same range as the corrected counts in our study. Ogburn et al. (2014) also reported TSC values similar to those reported here, with average sperm per female of 2.02×10^9 , which fell to 5.0×10^8 prior to fertilization of the first brood. Interestingly, the average sperm per female from Ogburn et al. (2014) suggests that females are receiving amounts of sperm similar to those from fully recovered males in the laboratory studies of Kendall et al. (2001) and Wolcott et al. (2005).

Although our study is similar to Ogburn et al. (2014), we reached different conclusions about sperm limitation. Both Ogburn et al. (2014) and our study sought to measure the effects of the sex ratio on sperm transfer from male to female blue crabs but did so at different temporal and spatial intervals. Ogburn et al. (2014) found significant differences in total sperm per female over the upper, middle, and lower portions of Chesapeake Bay. Our results showed no spatial trend in TSC among the sites in our study. Ogburn et al. (2014) accounted for sperm loss by using only females that had an intact sperm plug. Depending on the duration of sperm plug persistence in blue crabs, the time since mating could differ among systems. We also calculated sex ratios differently. We used the ratio of mature males to mature females as a proxy for the sex ratio when mating occurred, while Ogburn et al. (2014) used the ratio of mature males to prepubertal females identified by abdomen coloration. Neither of these perfectly represents the true operational sex ratio of the population at the time the females mated. Additionally, using either a mature male : mature female or mature male : immature female sex ratio assumes that every female matures at exactly the same time. Female blue crabs mature asynchronously (Jivoff et al. 2007), so the sex ratios used in our study and Ogburn et al. (2014) are underestimates of the true operational sex ratio at any given time.

To conclude, our results suggest that blue crabs in Chesapeake Bay are not experiencing decreases in sperm due

to lower male : female sex ratios and do not offer evidence of fisheries-induced sperm limitation in the bay, at least given recent sex ratios and fishing mortality rates. Either broader, more fine-scale field studies that match the local operational sex ratio with sperm per female or simulation modeling could provide more insight into the conditions under which we would expect sperm limitation in blue crabs. This would give us the ability to evaluate whether females are regularly receiving sperm from males with depleted sperm stores under current fisheries management.

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