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Multiple mating by females in the Chesapeake Bay blue crab *Callinectes sapidus* population

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ABSTRACT: Mature females of the Chesapeake Bay (USA) blue crab *Callinectes sapidus* population mate multiple times. Microsatellite DNA analysis of sperm stored in the spermathecae has shown that virtually all (~97%, 104 out of 107) females collected in a 2009–2010 study mated with at least 2 males. Enumeration of stored sperm revealed that despite multiple mating, some females did not have enough sperm to fulfill their lifetime reproductive potential, suggesting that female blue crabs may experience varying levels of sperm limitation. This could result from multiple factors. The average body size of males in the population has decreased, which reduces the amount of sperm that can be produced at a given time. The highly female-biased sex ratio of the population means that more females must be successfully mated per male than historically, placing greater demand on present-day males. Both of these factors are likely to have resulted from fishing mortality and sex-based management of the population. While much attention has been given to the management of female blue crabs, it is imperative to assess these organisms as a reproductive population in order to assure the continued health and maintenance of this ecologically important species.

KEY WORDS: Blue crab · Reproduction · Microsatellite DNA · Mating · Sperm limitation

INTRODUCTION

Reproductive strategies used by organisms can vary based on population changes and reproductive success. Mate guarding is a strategy utilized by many organisms such as birds, mammals, insects, and crustaceans (Birkhead 1979, Grafen & Ridley 1983, Poole 1989, Alcock 1994). By preventing other males' access to the guarded female, males benefit by ensuring paternity, while the female is protected (Birkhead 1979, Grafen & Ridley 1983, Poole 1989, Alcock 1994). In brachyuran crustaceans, guarding of a recently inseminated female by her mate is practiced when the female has mated following the pubertal molt, and is therefore especially vulnerable to predation (Grafen & Ridley 1983, Christy 1987) and insemination by other males. In populations where sperm limitation is a factor, a shift in reproductive strategy from female monogamy to female multiple mating has been observed (Kellogg et al. 1995, Zeh & Zeh 1997, Jennions & Petrie 2000). This shift allows for a greater likelihood of full sperm reserves, and the potential for greater genetic variation of the offspring with multiple sperm donors (Kellogg et al. 1995, Zeh & Zeh 1997, Zane et al. 1999, Jennions & Petrie 2000). Increased multiple mating can also result in a change in ejaculate allocation by males, due to competition (Wedell et al. 2002). As many populations experience declining numbers and imbalanced sex ratios, modification of reproductive strategies may be important for sustaining healthy populations.

The blue crab *Callinectes sapidus* population in Chesapeake Bay (USA) declined in the mid-1990s and remained low until 2009. This decline has been attributed to poor recruitment in combination with

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high fishing mortality (Lipcius & Stockhausen 2002). As fishing mortality has declined in the last few years due to changes in harvesting regulations, a rebound in total population abundance was reported (Miller et al. 2011).

A downward shift in male size was previously reported for this population (Carver et al. 2005). Intense preferential harvesting of males with a carapace width >127 mm resulted in a decrease in average size (Carver et al. 2005). There is a positive relationship between male size, ejaculate size, and the ability of males to successfully compete for mates (Jivoff 1997a,b, 2003, Kendall et al. 2002). Decreasing average male size has the potential to negatively affect male reproductive output at the population level, which may impact population sustainability.

Smaller males produce less ejaculate when initially mating, and males of all sizes recover sperm reserves slowly (Jivoff 1997a, Kendall et al. 2001, 2002, Wolcott et al. 2005). A decreased ability to recover sperm reserves with fewer sperm being released per ejaculation could lead to severe sperm limitation in mature females.

Mate guarding by male blue crabs provides females protection from predators and cannibalism, and also ensures the guard male's paternity. When male density is low, the occurrence and duration of mate guarding decreases (Jivoff 1997a, Kendall et al. 2002). While a decrease in mate guarding does free a male to mate with other females more quickly, it also prevents the successful buildup of sperm reserves. The full recovery time of sperm and seminal fluid is 9 to 20 d in larger males (Kendall et al. 2001). Lack of, or truncated, mate guarding may force females to find additional mates to increase sperm reserves through multiple copulations (Jivoff 1997b). Prior to the population decline in the mid-1990s, Jivoff (1997b) used a morphological technique and estimated that about 12% of females were multiply mated.

In response to the reduced ejaculate of males, females may be capable of and may need to mate multiple times to increase the amount of sperm in the spermathecae to maximize fertilization success (Kendall et al. 2001, 2002, Wolcott et al. 2005). This phenomenon has also been observed in another heavily fished decapod species, *Paralithodes brevipes* (Sato et al. 2005, 2006). In 2008, fishery managers began to enact specific regulations to preferentially decrease the harvest of mature female blue crabs in Chesapeake Bay. This reduction in female harvest has since increased the number of females available for the male population to inseminate. There were no comparative regulations for males, contributing to a more female-biased sex ratio than already observed in this population (Miller et al. 2011), which could also result in an increase in multiple matings (Jivoff 1997a, Kendall et al. 2002, Carver et al. 2005). With the decline in total population size and individual crab size observed in the 2000s, we anticipated that multiple mating by mature females is currently greater than the previous estimate of 12% (Jivoff 1997b). The goal of this study was to assess the rate of multiple mating by females using microsatellite DNA analysis of sperm stored in the spermathecae to detect contributions from multiple sires, enumerate stored sperm in an effort to determine the number of broods each female could produce, and to ascertain whether sperm-limited females were present in our samples.

MATERIALS AND METHODS

Sample collection

Mature females were collected from a Hampton, Virginia, lower Chesapeake Bay crab-processing house in April and November of 2009, and in April of 2010 over the course of 2 d mo^{-1}. Watermen that fish throughout the lower portion of the mainstem of the Chesapeake Bay deliver their catch to this location. Since all females migrate to the lower Bay to spawn, these animals are assumed to represent a cross-section of the female population in the Bay. During the early spring and fall, most mature females have mature oocytes in the ovaries, and have been inseminated (S. Wells, pers. obs.). The spermathecae of mature non-ovigerous females were dissected and frozen at −80°C for sperm enumeration and genetic analysis. At the time of dissection, it was noted whether a sperm plug was present, as evidenced by hardened seminal fluid in the spermathecae.

Sperm enumeration

A subset of all spermathecae collected was used for sperm enumeration (n = 113). The left and right spermatheca were processed separately initially to determine if there were differences in sperm number between the two. If there were no differences, 1 spermatheca was used for sperm enumeration and the other for multiple mating analysis. Differences between the sperm numbers stored in the spermathecae of individual females were detected, and samples

were collected separately for enumeration and microsatellite analysis. Each spermatheca was dissected and homogenized in artificial seawater of a known volume (following the protocols of Kendall et al. 2002 and Carver et al. 2005). Homogenization ruptured each of the spermatophores, releasing all of the sperm. Membranous spermatophore material was removed by filtration through a 35 µm filter, leaving only sperm in the sample. Sample volume was then brought to 50 ml with artificial seawater. Three subsample replicates of 1 ml per spermatheca were removed from the homogenized sample, filtered on a black polycarbonate 0.4 µm filter, and stained with DAPI. Counting was performed via fluorescence microscopy (1000×, oil immersion). Ten random fields were counted from each filter and then averaged. The total number of sperm was calculated from the average number in each of the 3 replicates.

The total number of sperm stored per female was compared with previously determined fecundity estimates (Wells 2009) to identify the number of broods that could be potentially fertilized. A ratio of 20:1 (sperm:oocyte) was used as an indicator for complete fertilization success (Hines et al. 2003). While blue crab fertilization experiments have not been performed to determine the exact ratio needed, a previous study found that females may have ≥400 sperm oocyte^{-1} available for a first brood, but as few as 20 sperm oocyte^{-1} for successive broods (Hines et al. 2003). Here we assumed that 20 is the minimum number of sperm necessary for fertilization throughout the reproductive lifetime of the female. The biological limit is a 1:1 ratio, but decapods generally have a wide variety of ratios necessary for fertilization success that all exceed 1:1 (Sainte-Marie & Lovrich 1994, Rodgers et al. 2011). This study assumes that the blue crab exceeds the biological limit similar to other decapods. Fecundity estimates (Wells 2009) were determined for the 2002 to 2006 spawning seasons, using the dry weight method outlined by Prager et al. (1990). Egg masses were collected from spawned females by removing the eggs from the pleopods. A subsample of 200 eggs was weighed from each egg mass. The entire egg mass and the subsample were dried to a constant weight at 60°C. The total number of eggs was calculated using the mean weight of the counted eggs and the total dry weight of the egg mass. The average determined over the 5 spawning seasons was 8.7×10^5 (SE = 0.21×10^5, n = 617 females) eggs brood^{-1} (Wells 2009). The mean number of eggs has increased since the study began in 2002 (2002 average 3.7×10^5, SE = 0.25×10^5; 2006 average 13.1×10^5, SE = 0.65×10^5), but the mean number of eggs produced per brood annually is 27% of the previously reported value of 3.2×10^6 (Prager et al. 1990, Wells 2009). This 5-season average was used in our calculation to determine sperm limitation.

Nonlinear regression analysis was used to assess whether a relationship exists between the total number of sperm stored per female and carapace width. Large differences in sperm numbers between the 2 spermathecae of an individual female were detected initially in the study, so we analyzed the spermathecae separately for total number of sperm. Pairwise *t*-tests were used to determine if there was a significant difference in the number of sperm stored in the left and right spermatheca. The sperm number data were also examined for seasonal and yearly effects using ANOVA. All analyses were conducted using the SPSS package.

Multiple mating analysis

To determine the number of males transferring sperm into spermathecae, additional females (n = 107) were dissected to remove all spermatophores. The dissection was done under a dissecting microscope so that the spermatophores could be removed without taking any of the maternal tissue from the spermathecal wall. Spermatophores from left and right spermathecae were processed separately. All efforts were made to ensure that there was no contamination between sperm samples. All glassware was acid-washed in a 10% HCl bath, and dried in a 60°C oven between samples. Metal dissecting instruments that were not disposable were cleaned between samples using isopropyl alcohol. Spermatophore DNA was extracted using the Qiagen DNeasy Kit. For each sample, 3 sets of microsatellite primers (CSC-001, CSC-004, CSA-035) were used (Table 1, adapted from Steven et al. 2005) for PCR (denature 2 min at 94°C, then 40 cycles of 94°C for 30 s, then relevant annealing temperature in Table 1 for 30 s, then 72°C for 1 min, followed by 10 min of elongation at 72°C). Samples were analyzed with an ABI 3730XL automated sequencer (Applied Biosystems; genotyping conducted by the Centre for Applied Genomics, Toronto, ON, Canada). Females were scored as having mated multiple times if there were more than 2 different alleles for at least 2 of the genotyped loci (Bilodeau et al. 2005, Gosselin et al. 2005). A conservative estimate of the number of sperm donors was determined by dividing the number of alleles by 2 per locus (Bilodeau et al. 2005). Since sperm are hap-

Table 1. Blue crab *Callinectes sapidus* microsatellite loci adapted from Steven et al. (2005). F: forward, R: reverse

Locus	Primer sequence	Product size range (bp)	Number of alleles	Annealing temp. (°C)
CSC-001	F:attgggtggttgcttcat R:acgaggagaaagttgagattgc	307–366	42	48
CSC-004	F:aaacaacggtaattgtacgagaaa R:aggctaatgccaccatcatc	156–252	49	50
CSA-035	F:gactggagaaacgataggtg R:gaacaaggagattacacggattc	145–252	64	51

loid, heterozygosity was assumed for potential sires, which results in a conservative estimate of actual paternity. Correlation analysis was used to determine if there was a relationship between the number of mates and female carapace width.

A subset of mature females (n = 9) was collected to ensure that there was no evidence of cross-contamination of the spermathecal extraction from the female DNA. Backfin muscle tissue was collected from each female along with the full spermathecae. All tissues were extracted, amplified, and analyzed as above. The raw data from the maternal muscle was used to determine whether maternal microsatellite DNA appeared in the spermathecae. Lack of maternal microsatellites in the spermathecae would demonstrate no contamination. These samples were also used to estimate allelic error for the microsatellites, and automatic, computer-generated genotypes were compared to manually scored peaks through PeakScanner for mis-scores (Hoffman & Amos 2005). Controlled mating experiments between single males and single females would definitively show the expected microsatellite DNA pattern in the spermathecae for a single mating, and would be important to conduct in the future, but were not done for this study.

RESULTS

Sperm enumeration of the pooled right and left spermathecae averaged 1.13×10^8 (SE = 0.07×10^8) total sperm female^{-1}. The minimum number of sperm detected in both spermathecae of a female was 0.11×10^8, and the maximum was 4.8×10^8. A previous study found that females may have a ratio of ≥400 sperm oocyte^{-1} available for a first brood, but as few as 20 sperm oocyte^{-1} for successive broods (Hines et al. 2003). Assuming that *Callinectes sapidus* females from Chesapeake Bay can produce as many as 6 broods over a 2 yr reproductive lifetime (Hines et al. 2003), and using the average fecundity per brood determined by Wells (2009) with the conservative sperm:oocyte ratio of 20:1, there is evidence of sperm limitation in the wild population sampled (Fig. 1). Only 54% (61 of 113) of the females examined had sufficient sperm reserves to produce 6 or more broods in their lifetime (Fig. 1b), which would be likely over 2 reproductive seasons. Many females do not survive multiple seasons, but there is still evidence of varying levels of sperm limitation in the population. Of the females sampled, only 2.7% (3 of 113) had enough sperm to produce a single brood. If we used higher sperm to oocyte ratios as suggested in other studies, sperm limitation in this population would be more prevalent.

There was a significant relationship between female carapace width and the total amount of sperm

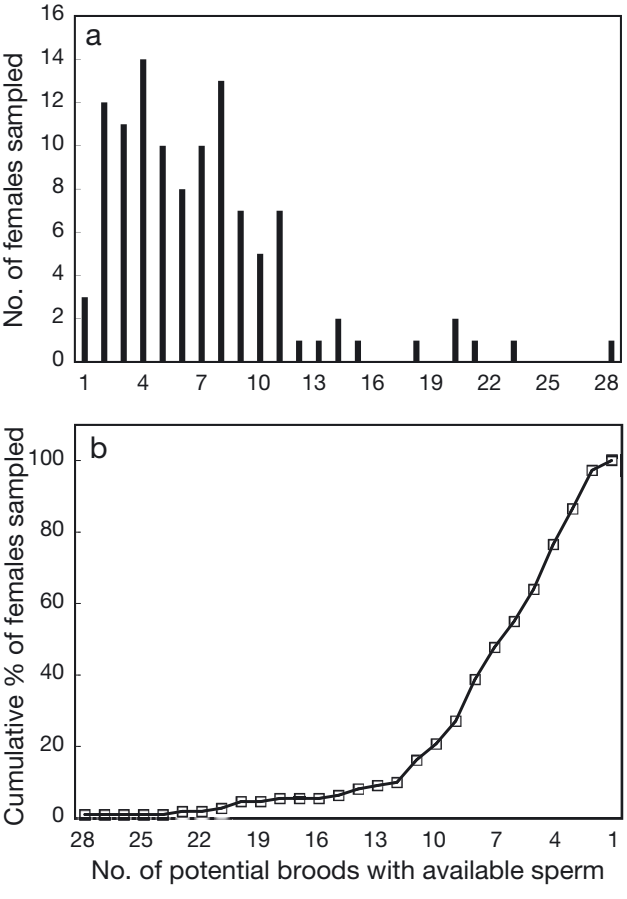

Fig. 1. (a) Number and (b) cumulative percentage of sampled female blue crabs *Callinectes sapidus* that could potentially produce a given number of fertilized broods based on sperm stored in the spermathecae

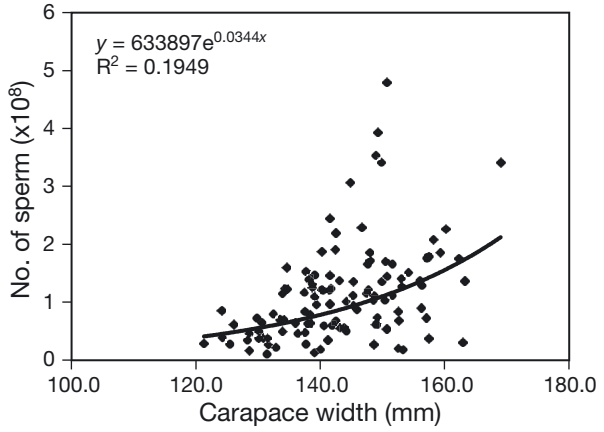

Fig. 2. Relationship between the carapace width of mature female blue crabs *Callinectes sapidus* and the amount of sperm stored in spermathecae. Larger females have more sperm available, and while the relationship is significant ($p < 0.001$), the low R^2 indicates that there are other factors that determine how much sperm individual females receive

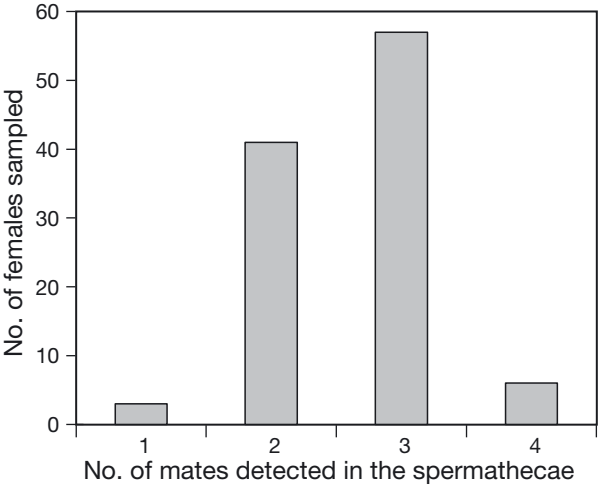

Fig. 3. Estimated number of mates in the sampled female blue crab *Callinectes sapidus* population based on microsatellite analysis. The majority of females had sperm from multiple males stored in their spermathecae, indicative of the prevalence of multiple mating in this population

stored (nonlinear regression analysis, $p < 0.001$; Fig. 2). While larger females tend to have more sperm than smaller ones, the R^2 for this relationship is very low (0.1949).

The left and right spermathecae had average sperm numbers of 5.4×10^7 (SE = 0.4×10^7) and 5.8×10^7 (SE = 0.5×10^7), respectively. Of individual females examined, 80% (90 of 113) had significant differences in the number of sperm for the left and right spermathecae. A pairwise *t*-test indicated that there was no preference between the left or right spermatheca ($p = 0.369$).

Sperm numbers calculated for the spring and fall seasons differed significantly (ANOVA, $F = 10.144$, $p = 0.002$). The average sperm number for individual females for the spring season was 1.3×10^8 (SE = 0.11×10^8), and the fall season was lower at 9.6×10^7 (SE = 1.04×10^7). There was also a significant difference between the 2 years when comparing the spring seasons (ANOVA, $F = 28.49397$, $p < 0.0001$). The average total sperm for 2009 was 3.3×10^7 (SE = 0.55×10^7), and for 2010 it was 1.5×10^8 (SE = 0.12×10^8).

Analysis of the 3 microsatellite loci demonstrated that the majority of females mated with multiple males. Only 2.8% (3 of 107) of the females sampled had stored sperm from only 1 male (i.e. all loci tested had no more than 2 alleles at each of the 3 loci). Most females (97.2%, 104 of 107) had sperm from at least 2 males (i.e. there were 6 alleles at 2 or more loci; Fig. 3). A small proportion of the sampled population (5.6%, 6 of 107) had 4 sperm donors, requiring at least 8 alleles at 2 or more of the loci tested.

Of those samples tested for maternal cross-contamination of the spermathecae (n = 9), none demonstrated contamination by maternal microsatellite DNA: 33% were homozygous for primer CSC-001, 22% for primer CSC-004, and 14% for primer CSA-035. Based on the data collected, there are obviously common alleles between males and females, but by looking at multiple alleles and multiple primers we could exclude maternal DNA from the majority of samples. For example, a heterozygous female for primer CSC-004 had alleles 161/198 in the backfin muscle. Alleles 161/186/196/212 were found within the left spermatheca, and 161/186/191 within the right spermatheca. Allele 161 is common among all 3 samples, but does not indicate contamination by maternal DNA because of the lack of 198 from the female in either spermatheca. This is also reinforced by the absence of maternal alleles in the spermathecae of the same female, but using the other 2 primers. This particular example also shows evidence of multiple mating, and differences in apportionment between the right and left spermathecae. One can assume that 161/186 are from the same male in both spermathecae, but the 196/212 in the left spermatheca would be different from the male that provided the 191 in the right spermatheca. Of those females sampled for contamination analysis, 60% had multiply mated based upon the conservative assumption of all males being heterozygous. These data also show that not all organisms are heterozygous and that multiple mating is likely being underestimated.

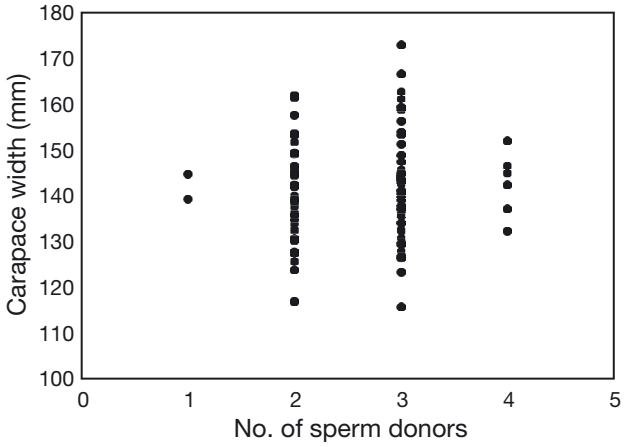

Fig. 4. Number of sperm donors identified for female blue crabs *Callinectes sapidus* of different sizes (carapace width) used in microsatellite DNA analysis. No relationship was detected between female size and number of mates, indicating that size is not a determining factor in male mate choice

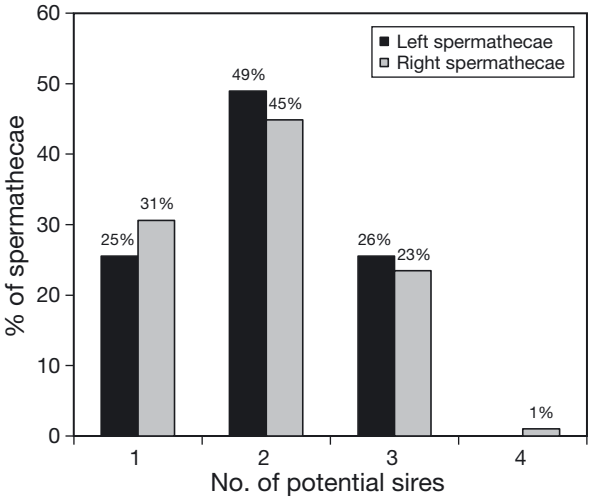

Fig. 5. Differences in the number of male blue crab *Callinectes sapidus* sperm donors identified between left and right spermathecae using microsatellite DNA (where each spermatheca sums to 100%)

When examining these samples for allelic error, 13% of the samples were scored differently when comparing the automated method to the manual method. Of this 13% error, 10.7% were mis-scores where the allele scored by the computer was different than the allele scored manually, and 14.3% were scored in the original automated genotyping, but were not included in the manual genotyping. Of those that were scored differently, 75% were not present in the original automated data, but were apparent alleles using the manual method. This shows that the majority of allelic error is attributed to the exclusion of alleles. Accounting for this error would result in an increase in multiple mating and potential sires greater than that observed because some males would be excluded as a consequence of the error.

Nonlinear regression analysis of carapace width and the number of mates per female indicated no relationship between the two ($p = 0.799$; Fig. 4). Large females do not seem to be more prone to multiple mating than small females, suggesting that female multiple mating is size independent.

The number of sperm donors in left and right spermathecae differed within individual females (McNemar's test, $p < 0.001$; Fig. 5). While nearly all females in the study had multiple mates, just under half (43%) of the females examined had sperm from only 1 male in 1 spermatheca, and more than 1 donor contributing to the other spermatheca, while 57% of the females sampled contained sperm from multiple males in both left and right spermathecae. Although this demonstrates multiple mating, it also indicates that males are not evenly contributing sperm to individual females.

Sperm plugs were not observed for all females in both the enumeration and microsatellite DNA analysis groups. Of 220 female crabs, only 2 had plugs (0.9%): 1 female of the 113 sampled for sperm enumeration, and 1 female of the 107 sampled for microsatellite analysis. Sperm plugs are considered to be a normal part of blue crab mating, but dissolve after approximately 5 wk (Jivoff & Hines 1998, Wolcott et al. 2005). Early fall mating of females should result in a greater number of undissolved plugs from the sampled population, but only 1 of the females with a plug was obtained in the fall. While this has not been directly detected in blue crabs, other decapod crustaceans without plugs may experience sperm leakage and therefore loss of fertilization (Johnson 1980, Jivoff et al. 2007).

DISCUSSION

Deviations from historical accounts of the classically accepted reproductive behavior of *Callinectes sapidus* are evident from this study. Assuming that these are new patterns, it is likely that the reproductive output of the population has been altered. Literature on *C. sapidus* mating indicates that females mate with 1 male and that paternity is ensured through mate guarding and sperm plug formation (van Engel 1958, Millikin & Williams 1984, Jivoff 1997a,b). The microsatellite DNA data from this

study demonstrated that nearly all (97.2%) females in the study population were multiply mated, typically with 3 or more males. Further, based on the conservative assumption that all males were heterozygous, the result reported here is a conservative estimate of the actual number of mates. The observed Chesapeake Bay blue crab heterozygosity coefficients reported by Steven et al. (2005) for the microsatellites used in this study ranged from 0.5455 (CSC-001) to 0.9655 (CSA-035). This indicates that the majority of individuals tested may be heterozygous for each of these loci. Because CSC-001 had an observed coefficient of just over 50% from the study by Steven et al. (2005), if we assume 45% of the potential donors are homozygous in our study, it would indicate that the actual number of mates is potentially higher than what we detected by a range of 3 to 45%. Also, the analysis of allelic error demonstrated that some alleles could be excluded in the initial scoring, resulting in the exclusion of sires. Combined, these data suggest that nearly all females in the population are mating with multiple males.

Based on the high rate of multiple mating, our data suggest that mate guarding of females is either no longer occurring, is occurring at an extremely reduced duration, or is ineffective. A reduction or elimination of mate guarding would allow males to mate with more females in a given period of time, resulting in further depletion of limited sperm resources. Changes in the mating behaviors of males are often brought about by changes in the ratio of males to females, as well as increases or decreases in competition with other males (Rondeau & Sainte-Marie 2001, Jivoff 2003, Weir et al. 2011). Male depletion would exacerbate sperm limitation for this population, potentially resulting in further reductions of sperm transfer per mating.

In the Chesapeake Bay population, males experience fishing pressure, resulting in a strong female-biased sex ratio (Miller et al. 2011). In a population of reduced numerical abundance and a strong female sex bias, females likely benefit from insemination by multiple partners since multiple mating increases the total amount of sperm available for reproduction. Multiple partners may also increase the genetic variation of the females' progeny, which may increase female individual fitness. In 2008, regulations were put into place in both Virginia and Maryland to reduce the harvest pressure on females throughout Chesapeake Bay. Both states aimed for a 34% reduction in female harvest in the Bay. This further increases the harvesting pressure on males throughout Chesapeake Bay, possibly leading to further bias in the sex ratio throughout the population. Miller et al. (2011) reported that the average sex ratio from 1994 to 2006 was 3 females for every male based upon fishery-dependent data. This female bias occurred prior to the reduction in female harvest, so the ratio could be even more strongly skewed following the regulatory changes. A skewed sex ratio combined with females that are historically believed to only mate at 1 time period and usually with only 1 male could alter the effective population size and potentially reduce the total genetic variance in the population (Sugg & Chesser 1994, Zane et al. 1999, Martinez et al. 2000). However, multiple mating may increase fertilization, which in turn could improve sustainability and resilience (Sugg & Chesser 1994, Zane et al. 1999, Martinez et al. 2000).

Females will only survive 1 to 2 reproductive years, but the Virginia portion of the Chesapeake Bay population can be harvested upon reaching maturity (Millikin & Williams 1984). This means that females from this population are likely to be harvested before they have the opportunity to produce 6 broods. Based upon the number of sperm stored in the mature females examined in this study, sperm limitation can potentially play a role in reproduction even if females are unable to fulfill their lifetime potential. Of the females sampled, 23% could only produce a maximum of 3 fertilized broods, which is approximately what they can produce within a reproductive season in Chesapeake Bay (Hines et al. 2003). While sperm limitation may not be a defining factor in the reproductive output of the blue crab, it is likely still affecting reproduction and reducing the number of fertilized eggs that females can produce in their lifetime. Larger females have more sperm than smaller ones, but female size is a small factor in determining how much sperm an individual female receives based upon the weak correlation observed in this study. The more important factor is likely the amount of sperm available for transfer from the male.

There are now fewer total males in the population than historically reported, and a greater fraction of these males have small body sizes (Miller et al. 2011). Combined with a female-biased sex ratio, this can exhaust sperm resources (Jivoff 1997a, Kendall et al. 2002, Carver et al. 2005). Males are thought to be mating with more females than they have historically (Kendall et al. 2001, 2002, Wolcott et al. 2005), which may result in less time between mating encounters for sperm recharge. This results in lower sperm reserves and less energy available for the production of seminal fluid which is the major component of sperm plugs (Kendall et al. 2001, 2002, Wolcott et al. 2005).

While multiple mating may increase the number of fertilized eggs a female can produce, it may also result in male reproductive exhaustion, and in lower levels of seminal fluid being transferred during mating (Kendall et al. 2001). Seminal fluid is used to form a sperm plug in the spermathecae of females, which serves the functions of reducing sperm competition with other males, reducing the potential for sperm leaking out of the spermathecae, and possibly to enhance sperm viability (Ryan 1964, Hartnoll 1969, Subramoniam 1993, Kendall et al. 2001). Less than 1% of the females sampled in this study had sperm plugs in a population in which mate guarding and sperm plugs are expected as a routine part of normal mating activity (Jivoff & Hines 1998). Generally, sperm plugs dissolve after approximately 5 wk, so we would not expect to see them in every female throughout this study but would expect to see plugs in many of the females sampled in November based upon early fall mating expectations (Wolcott et al. 2005). Only 1 of the females sampled in November had a sperm plug, indicating that less seminal fluid is being transferred, or the historically accepted timing of mating has changed. The lack of sperm plugs may result in sperm leakage and suggests a lack of seminal fluid, which in turn suggests that males are putting fewer resources into assuring their paternity (Ryan 1964, Hartnoll 1969, Kendall et al. 2001). This is most likely because they simply do not have the needed resources. In this study, the low sperm numbers compared to the number of oocytes that would likely need to be fertilized, the absence of sperm plugs, and prevalence of multiple mating supports the hypothesis that sperm limitation is occurring.

Changes in some of the historically accepted reproductive norms for this Chesapeake Bay population of blue crabs are occurring, as seen in this study. Our results strongly suggest that the reproductive strategy of mate guarding by males has been replaced with multiple mating of females in the population. Multiple mating of mature females may positively benefit the population by increasing genetic diversity, and therefore increasing the effective population size. Reductions in mate guarding and the absence of sperm plugs indicate that males are allocating fewer resources to ensuring paternity than in the past. Differences in allocation of sperm number and the number of mates detected in opposite spermathecae strongly suggest that males are not transferring sperm equally. From an ecological perspective, retaining more, large reproductive males would benefit the population, as would assessing these organisms as a reproductive population in order to assure the continued health and maintenance of this ecologically important species.

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